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Medicine & Science IN Sports & Exercise
The Official Journal of the American College of Sports Medicine
www.acsm-msse.org

. . . Published ahead of Print

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Accepted for Publication: 15 October 2019

Medicine & Science in Sports & Exercise, Publish Ahead of Print
DOI: 10.1249/MSS.0000000000002200

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We would like to thank the Royal Society, Exeter University and Maastricht University who all contributed funding to support the completion of this work. Jonathan Fulford's salary was supported via an NIHR grant to the University of Exeter (CRF/2016/10027).

Conflicts of interests. No conflicts of interest, financial or otherwise, are declared by the authors. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Abstract

Purpose: Musculoskeletal injuries necessitate periods of disuse (i.e. limb immobilization) during which rapid skeletal muscle atrophy occurs. The relative susceptibility of different muscles of the thigh to disuse atrophy remains uninvestigated. We assessed muscle disuse atrophy of individual thigh muscles throughout one week of unilateral knee immobilization. **Methods**: Thirteen healthy, young (20.2±0.6 y) men underwent 7 days of unilateral leg immobilization via knee bracing. MRI scans were performed bilaterally prior to, and following 2 and 7 days of immobilization to determine the volume and anatomical cross-sectional area (aCSA) of the individual muscle groups of the upper legs. **Results**: In contrast to the control leg, total thigh muscle volume had decreased by 1.7±0.3 (P<0.01) and 5.5±0.6% (P<0.001) in the immobilized leg after 2 and 7 days of disuse, respectively. Muscle loss was significantly greater in the *M. quadriceps* (day 2; 1.7±0.3 (P<0.05) and day 7; 6.7±0.6%) when compared with the *M. hamstrings* (day 2; 1.4±0.2% (P<0.01) and day 7; 3.5±0.3%) following 7 days of disuse (P<0.001). Individual muscles of the thigh exhibited different atrophy rates with the *M. vastus lateralis* aCSA showing the greater (2.6±0.4 and 7.2±0.8%), and the *M. gracilis* the lesser (1.1±0.7 and 2.3±1.0%) decline following 2 and 7 days of immobilization, respectively (P<0.01). **Conclusion**: Thigh muscle disuse atrophy occurs rapidly and is already evident within 2 days of leg immobilization and progresses at a similar rate over the next 5 days (~0.8% muscle loss per day). *M. quadriceps* muscle shows more atrophy when compared with the *M. hamstrings*.

Keywords: Atrophy, immobilization, MRI, skeletal muscle, strength.

Introduction

Periods of skeletal muscle disuse can occur for a variety of reasons, such as hospitalization that may mandate bed-rest or due to injury which may require limb immobilization. The average hospital stay is 5-7 days when admitted with acute illness (1) and most periods of disuse requiring home-based recovery (illness or mild injury) also last less than one week (2). Consequently, the clinical relevance of such short-term disuse has recently attracted considerable research attention (3, 4, 5). Studies have shown that 5-7 days of disuse results in marked declines in muscle size (6), muscle fiber cross sectional area (CSA) (4), strength (3, 6), and insulin sensitivity (3, 7) and induces anabolic resistance of skeletal muscle tissue (8, 9). This potent deconditioning effect of short-term disuse also lends support for previous suggestions that short periods of disuse may be additive over the lifespan and may act as the key driver of age-related muscle loss (10, 11).

To date, research on short-term disuse atrophy has mainly assessed limb or whole body fat free mass (FFM) using dual-energy x-ray absorptiometry (DEXA), particularly for whole body disuse (12, 13), or muscle anatomical CSA (aCSA) of large muscle groups (for example mid- thigh or *M. quadriceps*) using computed tomography (CT) generally when a unilateral limb immobilization model is used (6, 9). Applying magnetic resonance imaging (MRI) to obtain serial images of a muscle group allows for the quantification of muscle volume of an entire muscle group, and allows for the calculation of changes in the individual constituent muscles (14, 15). Furthermore, by utilizing MRI to calculate muscle volume and by assuming or calculating muscle density, the determination of muscle mass is possible (16). Therefore, MRI

may provide a more sensitive tool to assess time dependent changes in muscle mass during short periods of disuse.

In the present study we used serial MRI scans to provide a more detailed and temporal picture of upper leg muscle atrophy in combination with unilateral leg strength testing during short-term (one week) unilateral leg immobilization in thirteen healthy, young men. We hypothesized that a decrease in thigh, *M. quadriceps* and *M. hamstrings* muscle volume would already be evident after two days of immobilization, and that further declines would be seen after a further five days of immobilization. Furthermore, we hypothesized that individual muscle groups (e.g. *M. quadriceps*) and constituent muscles (e.g. *M. vastus medialis* and *M. vastus lateralis*) that perform more of a role in habitual physical activities would experience significantly greater reductions in muscle volume (of *M. hamstrings*) and aCSA when compared with muscles that undertake less habitual loading (e.g. the *M. biceps femoris short head*, *M. adductor* group, *M. sartorius* and *M. gracilis* muscles). In line with this, we hypothesized that *M. quadriceps* strength would decline to a greater extent than *M. hamstrings* strength after seven days of unilateral leg immobilization.

Methods

Subjects' characteristics

Thirteen young, healthy males (age = 20±1 y; body mass index (BMI) = 23.4±0.9 $kg \cdot m^{-2}$; body fat % = 15±2; lean mass = 63±2 kg) volunteered to take part in this study. Prior to inclusion in the study all subjects underwent a routine medical screening to ensure no adverse health

conditions were present and that they met the eligibility criteria for the study. Only young males were included in the present study since both age (17) and sex (18) can influence the rate of muscle disuse atrophy, and our goal was to maintain a homogenous population for the current study. Exclusion criteria included; a personal or family history of thrombosis, musculoskeletal/metabolic disorders (e.g. type 2 diabetes), BMI <18.5 or >28, any musculoskeletal injury of the legs in the 12 months before starting the study, any contraindications to MRI scanning (e.g. metallic implants), participation in a structured resistance type exercise training program within 6 months prior to the study, consumption of any nutritional supplements prior to and during the study. During the screening visit subjects completed a general health questionnaire, body composition was assessed by air displacement plethysmography (BODPOD; Life Measurement, Inc. CA, USA), height, weight and blood pressure were measured. Subjects were also familiarized to the unilateral one-repetition maximum (1-RM) strength testing technique for each exercise with the dominant (i.e. stronger) leg being identified. All subjects were informed of the nature and possible risks of the experimental procedures before providing written informed consent. The study was approved by The Sport and Health Science Ethics committee of the University of Exeter (150221/B/02), in accordance with the guidelines set out in the Declaration of Helsinki. This study was registered as a clinical trial with clinicaltrials.gov (NCT02984332).

Experimental protocol

A schematic of the experimental design is shown in Figure, Supplemental Digital Content 1, http://links.lww.com/MSS/B824. After screening, eligible subjects reported to the laboratory at ~0900 h in the fasted state for a pre-testing visit where 1-RM strength testing was performed.

One week after the pre-testing visit subjects returned to the laboratory (subjects were instructed to refrain from vigorous exercise during this period) and underwent a pre-immobilization magnetic resonance imaging (MRI) scan of both thighs at ~0800 h before starting the immobilization period at ~0900 h. After 2 days of immobilization the subjects returned to the laboratory and underwent a second identical MRI scan at ~0900 h, during which the brace was removed by the experimenter for the MRI scan and was re-fitted in the exact same position immediately post-MRI. The subject was assisted onto the MRI bed using a wheelchair to ensure no weight bearing of the immobilized leg occurred. After 7 days of immobilization subjects completed the final study visit, which consisted of a final MRI scan at ~0900 h followed immediately by post-immobilization unilateral 1-RM strength testing. Subjects were only allowed to bear weight on the immobilized leg following completion of the strength testing.

Immobilization protocol

The model of immobilization used was a unilateral leg brace (X-ACT Donjoy brace, DJO global, Vista, CA, USA) with the subject ambulating on crutches (after receiving instructions) during the immobilization period. The immobilized leg was counterbalanced for leg dominance and the non-immobilized leg acted as a within-subject control. Using the hinge of the brace the knee was fixed at an angle of 40° flexion (i.e. full knee extension being considered as 0°) to ensure no weight bearing occurred. Subjects were instructed that all ground contact and muscle contraction (except for ankle rotation exercises twice per day to activate the venous muscle pump) in the immobilized leg were forbidden. Adhesive tape with the experimenter's signature inscribed was placed around the straps of the brace and breaking of the tape would indicate tampering and result in exclusion from the study (17). Subjects were provided with a plastic cover to wear over

the brace when showering. Daily contact was maintained with the subjects throughout the entire study to ensure compliance.

Determination of muscle volume and anatomical cross-sectional areas (aCSA)

A 1.5 tesla (T) MRI scanner (Intera, Phillips, The Netherlands) was used to obtain images of both thighs in the axial plane over the full length of the femur. A T1-weighted 3D turbo spin echo sequence was used (field of view 500 x 500 mm, reconstructed matrix 512 x 512 mm, echo time 15 ms, repetition time 645 ms, slice thickness 5 mm, slice gap 5 mm) with the subject lying still in the supine position. A 4-element sense body radiofrequency (RF) coil was wrapped around both thighs. During the pre-immobilization scan a specified distance from a bony landmark (femoral condoyle) on the immobilized leg in the frontal plane was used to center the axial plane images (19). This same distance was used on all subsequent MRI scans to ensure the axial images were in the same location along the length of the thigh on all scans.

Philips on-line MRI software was used to analyze the images obtained in the axial plane (the same experimenter performed all manual segmentation of the images). Starting at the most distal image (approximately mid patella) where each muscle group could be fully delineated, the muscle of interest was manually segmented to calculate slice anatomical cross-sectional area (aCSA). Moving proximally every third image was analyzed until complete delineation of the muscle groups of interest could no longer be identified (the femoral head for the *M. quadriceps* and thigh muscle groups and the ischial tuberosity (*M. hamstrings* origin) for the *M. hamstrings*). This was on average 16, 15 and 14 images for the thigh, *M. quadriceps* and *M. hamstrings* volume measurements, respectively (see **Figure 1** for representative images). This method for

calculating muscle volume has previously been reported to give accurate and reliable results (20). We also internally validated this approach where we compared data from analyzing every image (i.e. 45 images) along the length of the muscle with the analyzes of every third image (i.e. 15 images; for 3 subjects) which elicited equivalent calculations of muscle volume (bias = -21.6 cm^3, standard deviation = 7.7, lower limits of agreement = -36.7 cm^3, upper limits of agreement -6.4 cm^3). Muscle volume was calculated using a previously published method (16) where the total aCSA for all images was calculated and multiplied by the slice thickness plus the distance between slices (linear interpolation) (in this case a total of 3 cm, 5 mm slice thickness, 25 mm slice gap), summarized by the following equation:

$$muscle\ volume = \sum_{aCSA} \cdot (slice\ thickness + slice\ gap)$$

Mid *M. quadriceps* aCSA was calculated by segmenting the *M. quadriceps* muscle in a single MR image 15 cm above the top of the patella to aid comparisons to previous studies (9, 19, 21). aCSA was calculated for all the individual muscles of the thigh that could be completely delineated (eleven individual muscles; *M. rectus femoris*, *M. vastus lateralis*, *M. vastus intermedius*, *M. vastus medialis*, *M. semitendinosus*, *M. semimembranosus*, *M. biceps femoris short head*, *M. biceps femoris long head*, *M. adductor* group (3 *M. adductor* muscles combined), *M. sartorius*, *M. gracilis*) of both the immobilized and control legs (muscles such as the *M. pectineus*, *M. psoas* and *M. iliopsoas* were not analyzed as they could not be adequately visualized for quantification). The single axial MR image that had the largest aCSA for each muscle was located (at a specific location along the length of the thigh) and the muscle of interest was manually segmented as described above. This same corresponding image (on the same location of the thigh) was analyzed on scans 2 and 3 to assess for changes in aCSA over the immobilization period.

Calculations of muscle mass and muscle atrophy related to anatomical longitude

Muscle volumes calculated from the MRI scans were used to calculate muscle mass to understand the weight of muscle tissue lost during immobilization. Muscle mass was calculated by multiplying muscle volume by 1.04 $g \cdot cm^{-3}$ (the reported density of leg muscle tissue) (22) in line with previous work (16). To assess if different longitudinal sections of a muscle group atrophied to differing extents, the length of each muscle group (*M. quadriceps*, *M. hamstrings*, thigh) was first calculated by summing the thickness of all the slices and slice gaps between the most proximal and distal images analyzed. Subsequently, starting from the knee (the most distally analyzed image = 0%) the distances at 10% intervals up to the femoral head (the most proximally analyzed image = 100%) were calculated for each muscle and each subject to allow for comparisons between subjects of different heights and leg lengths. Then the aCSA analyzed at each 10% distance along the length of the muscle (or the aCSA analyzed that was closest to each 10% distance) for each muscle group and each subject was compared between pre- and post- immobilization (i.e. day 0 and 7) for the immobilized leg.

Strength testing

Unilateral leg extension, leg curl, leg press and calf raise exercises were performed in the stated order and for both legs separately using standard gym equipment (Life Fitness, Cambridge, UK). 1-RM strength testing was assessed using an incremental multiple repetition testing procedure that was carried out individually for each leg with the leg identified to be immobilized always being tested first. After two warm up sets of 8 and 4 repetitions at self-determined 25% and 50% of 1-RM, respectively, single repetitions at 1-RM were attempted. The weight was increased incrementally until no further weight could be lifted, with each attempt separated by a 2-min rest.

The final 1-RM lift was taken as the heaviest repetition that was successfully completed with correct technique where full range of motion was achieved.

Statistical analyses

All data are presented as means±SEM and all statistical analyses were conducted in GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA). Two-way repeated measures ANOVAs with leg (immobilized and control) and time (pre and post (for strength); or day 0, day 2 and day 7 (for MRI)) as within subjects factors were used to compare differences in strength, muscle volume and aCSA. For the immobilized leg only, a two-way ANOVA with muscle group and time as within subject factors was used to assess if different muscle groups responded differently to immobilization. Pearson's correlation analyses were used to assess the relationship between initial muscle size (*M. quadriceps* and *M. hamstrings*) or pre-immobilization muscle strength (leg extension and leg curl) with the absolute amount of muscle volume lost after 7 days for each muscle group. A Pearson's correlation was also used to assess the relationship between pre-immobilization aCSA along the longitude of each muscle (thigh, *M. quadriceps* and *M. hamstrings*) and the aCSA lost along the longitude of each muscle after 7 days of immobilization. For all ANOVAs, when a significant interaction was found Bonferroni post-hoc tests were applied to locate individual differences. Statistical significance was set at $P<0.05$.

Results

Muscle group volumes and mass

Muscle volume of the thigh, *M. quadriceps*, and *M. hamstrings* muscle groups as computed from MRI scans are displayed in **Figure 2**. There were no differences in muscle volume between the legs pre-immobilization for any of the muscle groups ((thigh: control leg = 4651±222 cm^3, immobilized leg = 4697±237 cm^3 (P>0.05); *M. quadriceps*: control leg = 2315±120 cm^3, immobilized leg = 2342±129 cm^3 (P>0.05); *M. hamstrings*: control leg = 833±40 cm^3, immobilized leg = 830±38 cm^3 (P>0.05)). In the control leg muscle volume remained unchanged at all time points in all muscle groups (all P>0.05). Significant time *x* leg interactions were detected for thigh (P<0.001), *M. quadriceps* (P<0.001) and *M. hamstrings* (P<0.001) such that the immobilized leg thigh muscle volume decreased by 1.7±0.3% (to 4617±236 cm^3, P<0.001), comprising a 1.7±0.3% decline in *M. quadriceps* volume (to 2301±127 cm^3; P<0.01) and 1.4±0.2% decline in *M. hamstrings* muscle volume (to 818±37 cm^3; P<0.001)) after 2 days. Similarly, after 7 days of immobilization thigh, *M. quadriceps* and *M. hamstrings* muscle volumes had decreased by a further 3.9±0.4, 5.0±0.6 and 2.1±0.2%, respectively (to 4438±223, 2186±117 and 801±37 cm^3, respectively). This resulted in a total muscle volume loss of 5.5±0.6, 6.7±0.6 and 3.5±0.3% from thigh, *M. quadriceps* and *M. hamstrings*, respectively, over the entire week. The contribution of *M. quadriceps*, *M. hamstrings* and remaining (e.g. *M. adductor* group, *M. sartorius*, *M. gracilis*) muscle volume lost to total thigh muscle volume lost after 7 days is shown in **Figure 3a**. The relative loss of muscle volume was not different between the *M. hamstrings* and *M. quadriceps* during the first 2 days of immobilization, but was significantly greater in the *M. quadriceps* between 2 and 7 days (P<0.01) and over the entire week (P<0.001).

When expressed as muscle mass, similar changes in the leg muscles were observed with immobilization. Specifically, there were no differences in thigh (4837±231 g and 4885±246 g; $P>0.05$), *M. quadriceps* (2407±124 g and 2435±134 g; $P>0.05$) or *M. hamstrings* (866±41 g and 863±40 g; $P>0.05$) muscle mass between control and immobilized legs, respectively, and no significant changes were observed with time in any of the muscle groups in the control leg. In the immobilized leg thigh, *M. quadriceps,* and *M. hamstrings* muscle groups lost 83±14 g *(*$P<0.01$), 42±8 g ($P<0.01$) and 12±2 g ($P<0.001$) of muscle mass, respectively, after 2 days of immobilization. After 7 days of immobilization the immobilized leg had lost 269±32 g from the thigh muscle ($P<0.001$), which was attributed to a *M. quadriceps* muscle mass loss of 162±19 g ($P<0.001$) and *M. hamstrings* muscle mass loss of 30±3 g ($P<0.001$). After 7 (day 7 *M. quadriceps* to *M. hamstrings* mass difference = 132 g, $P<0.001$) but not 2 (day 2 *M. quadriceps* to *M. hamstrings* mass difference 30 g, $P>0.05$) days of immobilization the *M. quadriceps* lost more muscle mass than the *M. hamstrings*.

Mid- M. quadriceps and individual muscle anatomical cross-sectional areas (aCSA)

There were no differences in mid- *M. quadriceps* aCSA at the pre-immobilization timepoint (control leg = 7940±306 mm^2, immobilized leg = 8068±326 mm^2 ($P>0.05$)). In the control leg mid- *M. quadriceps* aCSA remained unchanged at all time points (all $P>0.05$). Significant time *x* leg interactions were detected for mid- *M. quadriceps* aCSA ($P<0.001$) such that the immobilized leg mid- *M. quadriceps* aCSA decreased after 2 days by 1.6±0.3% (to 7935±331 mm^2, $P<0.05$) and 7 days by 6.3±0.5% (to 7556±300 mm^2, $P<0.001$).

Individual muscle aCSA for the control and immobilized legs during immobilization are presented in **Figure 4a and b**. For all the individual muscles there were no differences between the control and immobilized leg pre-immobilization ($P>0.05$). No changes were observed in any individual muscle aCSA during immobilization in the control leg ($P>0.05$) with the exception of the adductor muscle group which decreased after 7 days (1.3±0.5%, $P<0.05$). Significant time *x* leg interactions were found for all individual muscles (all $P<0.001$ except adductor group $P<0.01$) except the sartorius ($P=0.056$) and gracilis ($P>0.05$). After two days of immobilization the following individual muscles' aCSA decreased; *M. vastus lateralis* by 2.6±0.4% ($P<0.001$), *M. vastus medialis* by 2.0±0.5% ($P<0.01$), *M. vastus intermedius* by 2.5±0.4% ($P<0.05$), *M. rectus femoris* by 1.4±0.4% ($P<0.05$), *M. semitendinosus* by 1.7±0.5% ($P<0.01$), and *M. adductor* group by 1.4±0.4% ($P<0.05$). After 7 days of immobilization all individual muscles except the *M. gracilis* and *M. sartorius* had decreased in the immobilized leg; *M. vastus lateralis* by 7.2±0.8%, *M. vastus medialis* by 7.0±0.7%, *M. vastus intermedius* by 6.9±1.2%, *M. rectus femoris* by 4.5±0.6%, *M. biceps femoris long head* by 5.4±0.6%, *M. biceps femoris short head* by 2.9±0.6%, *M. semimembranosus* by 5.0±0.6%, *M. semitendinosus* by 4.6±0.6%, *M. adductor* group by 3.5±0.5% (all $P<0.001$). The contribution of each of the 11 individual muscle CSA lost to total thigh muscle CSA lost after 7 days is shown in **Figure 3b.** There were differences in relative muscle loss between individual muscles over the entire week of immobilization ($P<0.001$). Specifically, the *M. vastus lateralis* lost significantly more relative aCSA than the *M. adductor* group ($P<0.05$), *M. biceps femoris short head* ($P<0.01$), *M. sartorius* ($P<0.001$) and *M. gracilis* ($P<0.001$). The *M. vastus intermedius* reduced in aCSA to a greater degree than the *M. biceps femoris short head* ($P<0.05$), *M. sartorius* ($P<0.01$) and the *M. gracilis* ($P<0.001$)

muscles. The *M. vastus medialis* lost significantly more aCSA compared with the *M. biceps femoris short head* ($P<0.01$), the *M. sartorius* ($P<0.01$) and the *M. gracilis* ($P<0.001$) muscles.

Muscle atrophy related to anatomical longitude

The data presented in Figure, Supplemental Digital Content 2, show the absolute and relative muscle volume for the thigh (a and b), *M. quadriceps* (c and d) and *M. hamstrings* (e and f) muscle groups for the immobilized leg expressed as declines in aCSA along the length of the muscle (i.e. separated into 10% segments from knee to hip), http://links.lww.com/MSS/B825. After 7 days of immobilization the thigh, *M. quadriceps* and *M. hamstrings* experienced heterogenous absolute reduction in aCSA along the length of the muscle (Figure, Supplemental Digital Content 2a, c and e, http://links.lww.com/MSS/B825; all $P<0.001$) which was still the case for the thigh and *M. quadriceps* when expressed as relative loss ($P<0.001$), but not the *M. hamstrings* ($P>0.05$) (Figure, Supplemental Digital Content 2b, d and f, http://links.lww.com/MSS/B825). For the thigh, the central portion of the muscle (middle 10%, 50-60%) lost aCSA to a greater absolute extent (50% = 972±139 mm^2 and 60% = 1141±144 mm^2) compared with the most proximal 10% (90-100% of thigh length, hip joint. 90% = 335±126 mm^2 and 100% = -28±82 mm^2) and the most distal 10% (0-10% of thigh length, knee joint. 0% = -1±21 mm^2, 10% = 84±103 mm^2) sections of the thigh muscle. All 10% increments from 30% to 70% along the length of the thigh muscle reduced in aCSA in comparison to 0% (all $P<0.001$ except 30% compared with 0%, $P<0.05$) and 100% (all $P<0.001$, except 30% compared to 100%, $P<0.01$) where no change in aCSA was found between 0 and 7 days of immobilization ($P>0.05$). When looking at the relative change in aCSA along the length of the thigh muscle, the only differences were 50% (-6.0±0.8%, $P<0.05$) and 60% (-6.5±0.7%, $P<0.05$) sections which

declined to a greater extent than the 0% section (1.1±2.5%). Similar to the thigh, the *M. quadriceps* also experienced largest absolute reduction in aCSA in the central portion of the muscle (Figure, Supplemental Digital Content 2c, http://links.lww.com/MSS/B825), where 40-70% area above the knee reduced in aCSA significantly more than 0 and 100% (all $P<0.001$). Although there were fewer significant differences in relative *M. quadriceps* decreases in aCSA (Figure, Supplemental Digital Content 2d, http://links.lww.com/MSS/B825), again the central area of the *M. quadriceps* (40-70%) decreased in aCSA more than 0% ($P<0.05$) but not 100% ($P>0.05$). In contrast, the *M. hamstrings* experienced less variation in absolute reductions in aCSA (Figure, Supplemental Digital Content 2e, http://links.lww.com/MSS/B825) where only 40% and 50% areas above the knee declined more than 0 and 100% (both $P<0.05$), and no differences were found along the length of the *M. hamstrings* muscle when relative declines in aCSA was assessed (Figure, Supplemental Digital Content 2f, http://links.lww.com/MSS/B825).

Leg strength

Unilateral 1eg strength data are presented in Table 1. Maximum strength decreased for leg extension (by 18.7±1.8%, $P<0.001$), leg press (by 21.0±3.5%, $P<0.001$) and calf raises (by 8.3±1.5%, $P<0.001$) in the immobilized leg, with no changes in the control leg. Neither the immobilized nor control leg changed in maximum strength for the leg curl exercise after 7 days of immobilization ($P>0.05$).

Correlational analyzes

Correlational analyzes are shown in **Figure 5**. Pre-immobilization muscle volume showed a moderate but significant negative correlation with the absolute (but not relative) amount of

muscle volume lost for both the *M. quadriceps* (absolute, r=-0.679, $P<0.01$; relative, r=-0.15, $P>0.05$) and *M. hamstrings* muscle groups (r=-0.592, $P<0.05$; relative, r=-0.10, $P>0.05$), with the thigh muscle showing a trend for the same response (r=-0.501, $P=0.081$; relative, r=-0.06, $P>0.05$). The absolute change in *M. quadriceps* volume had a moderate positive correlation with the absolute change in leg extension strength (r=0.563, $P<0.05$), though no significant correlation was observed between the absolute loss of *M. hamstrings* volume and absolute change in leg curl strength after 7 days of immobilization (r=-0.592, $P<0.05$). Pre-immobilization aCSA along the length of the thigh, *M. quadriceps* and *M. hamstrings* muscles all had a positive correlation with aCSA lost along the length of each muscle respectively after 7 days of immobilization (thigh, r=0.718 $P<0.001$, *M. quadriceps*, r=0.741 $P<0.001$, *M. hamstrings*, r=0.596 $P<0.001$) (**Figure 5**).

Discussion

The present study demonstrates that thigh muscle atrophy occurs rapidly (within 2 days) following the onset of muscle disuse (limb immobilization via leg brace) and continues at a similar rate for at least one week (by approximately 1% per day). The majority of thigh muscle loss during one week of immobilization was mainly attributed to the loss of *M. quadriceps* volume rather than loss of *M. hamstrings* volume, and was reflected by a greater decline in leg extension compared with leg flexion strength. Of the 11 individual thigh muscle groups, the *M. vastus lateralis* was the most susceptible to short-term disuse atrophy.

The study of short-term (< one week) muscle disuse atrophy has direct applications to clinical practice and rehabilitation where physical inactivity is mandated (21, 23). Here, we report a

detailed characterization of thigh muscle atrophy during one week of leg immobilization. We report a basic rate of upper leg muscle atrophy over one week in line with previous comparable studies. For example, we observed thigh muscle aCSA at the mid-part of the upper leg (15 cm above the patella) atrophied by ~6%, largely explained by a ~6% loss of *M. quadriceps* aCSA (with only a ~3% loss of *M. hamstrings* aCSA), which is in keeping with previous reports of upper leg muscle atrophy (generally *M. quadriceps* only) determined by single slice CT scan during one week of leg immobilization (leg cast) (i.e. ~6% atrophy) (24, 25). The use of MRI in the present study also allowed us to extend on these findings by taking multiple images (~45, of which ~15 were manually segmented) across the whole upper leg allowing us to report thigh muscle, *M. quadriceps* and *M. hamstrings* volumes temporally across one week of unilateral leg immobilization. This approach allowed the striking observation that muscle volume of the thigh, *M. quadriceps* and *M. hamstrings* had already decreased substantially (by 1.7±0.3%, 1.7±0.3% and 1.4±0.2%, respectively) following merely 2 days of immobilization. The *M. quadriceps* had atrophied to a greater extent (6.7 vs 3.5 %) when compared with the *M. hamstrings* after seven days (see Figure 2 b and c). Given that the *M. quadriceps* muscle comprises the largest muscle group of the upper leg, it is clear that *M. quadriceps* atrophy largely explains thigh disuse atrophy during short term immobilization (see Figure 3a). Indeed, the susceptibility of the *M. quadriceps* to atrophy to a greater extent than the *M. hamstrings* could, at least in part, be explained by its larger mass (15, 9). In support, we report both initial *M. quadriceps* and *M. hamstrings* volumes (Figure 5 b and c), as well as the larger longitudinal areas of the muscle (Figure 5 e and f), correlate with the volume of muscle lost and the aCSA lost along the longitude of each muscle, after 7 days of immobilization, respectively. However, it is also likely that the degree of atrophy experienced by the two constituent muscle groups of the thigh is also

influenced by other factors such as the brace model utilized, where the *M. quadriceps* are immobilized at a longer length (of the muscle fascicles and overall muscle length e.g. distance between origin and insertion) than the *M. hamstrings*. Previous research has shown that the length at which a muscle is immobilized affects its rate of atrophy (26), which also impacts upon its basal tone throughout disuse (27) which may also modulate the rate of muscle atrophy. Furthermore, other factors such as differing muscle fiber composition (28, 29), differing 'training' status (i.e. habitual gravitational loading) (30), or differential expression of regulatory genes/proteins across muscle groups (28, 31), may contribute to the differential atrophy observed across muscle groups. Worthy of note is that this divergent disuse atrophy response of the *M. quadriceps* and *M. hamstrings* may be a transient phenomenon given that longer term (14 days) studies (albeit during bedrest) do not report any differences between the rate of *M. quadriceps* (-6.5%) and *M. hamstrings* volume (-6.1%) decline (29).

We also used our muscle volume measurements to estimate muscle mass. This allowed for the quantification of actual muscle tissue lost as a result of disuse, which amounted to 83 g from the thigh muscle after 2 days of immobilization (*M. quadriceps* = 42 g and *M. hamstrings* = 12 g). Over the full week of disuse the thigh muscles atrophied by 269 g, consisting of 162 g loss specifically from the *M. quadriceps* and 30 g from the *M. hamstrings.* Interestingly, these measurements slightly exceed previous estimations extrapolated from computed tomography (CT) approaches (33) which report ~220 g lost from a whole leg, or estimated changes of mass based on alterations in muscle protein turnover from stable isotope experiments which have estimated 150-200 g (9, 21). These data highlight the rapid *and* substantial muscle mass loss consequent with disuse, and underlines the importance of understanding the underlying

mechanisms and the development of effective interventions (34, 35, 36), for combatting functional and metabolic decline in multiple populations.

The resolution of MRI allows for the assessment of the aCSA of the individual muscles of the thigh, *M. quadriceps* and *M. hamstrings*. In this respect, we present the first full assessment of how each of the constituent individual muscles of the thigh temporally respond to one week of immobilization (see Figure 4 a and b). The rapid muscle disuse atrophy seen at two days of immobilization at the level of the whole thigh was detectable by the majority of the individual muscles, with the exceptions of the *M. biceps femoris long head*, *M. biceps femoris short head*, *M. semimembranosus*, *M. gracilis* and *M. sartorius* (see Figure 4b). After 7 days of immobilization, only the *M. gracilis* and *M. sartorius* did not exhibit decreased aCSA. The *M. biceps femoris short head*, *M. adductor* group, *M. sartorius* and *M. gracilis* typically atrophied to a lesser extent than the *M. vastii* muscles (Figure 4b). These data are in line with previous (longer term) bed-rest studies (15, 37) which also report that the *M. gracilis* and *M. sartorius* are more resistant to disuse atrophy, though this has not been seen in other studies (38), however due to the complexity of bed rest studies a limited number of participants (n=6) were included. Pooling data from multiple bed rest or leg immobilization studies may lead to better determination of possible atrophy resistant muscle groups (particularly of smaller muscle groups e.g. *M. gracilis*). Overall evidence suggests that the *M. gracilis* and *M. sartorius* are more resistant to atrophy. This is likely to be due to the low gravitational load placed upon these muscles (30) and the low activation of these muscles during common habitual activities e.g. walking (39).

The heterogeneous rates of muscle disuse atrophy that we observed throughout the thigh were reflected by divergent effects on muscle strength when activating those muscle groups. Specifically, leg extension strength, during which the force generation is provided almost exclusively by the activation of the *M. quadriceps*, declined considerably more when compared with the leg curl (where the *M. hamstrings* is the primary agonist) (Table 1). This mirroring supports the contention that the rate of muscle loss is a primary factor determining the loss of function during disuse (17). Indeed, we also observed a correlation between the amount of mass lost from the *M. quadriceps* and the magnitude of functional decline (Figure 5g). Though not determined in the present study, it is also clear that such a decline in muscle strength would also impact on muscle power, as has previously been noted (17). This provides an additional challenge specifically for the injured athlete when considering the multifactorial rehabilitation that may be required to return to full functional capacity. Though previous research has repeatedly shown unilateral leg immobilization results in a reduction in *M. quadriceps* strength (21, 17, 41), fewer studies have assessed the impact on *M. hamstrings* strength. However, two weeks of bed rest has been shown to reduce *M. quadriceps* and *M. hamstrings* strength (isometric MVC) to a similar degree (12-13%) (42) implying that our data showing *M. hamstrings* to be remarkably resistant to disuse induced declines in function may be attributable to the model of disuse which necessitates that the two muscle groups are immobilized at different positions (e.g. *M. quadriceps* at a longer length than the *M. hamstrings*).

The rapid (i.e. within two days) and muscle (group) specific decline in mass and function that we report during immobilization has important practical implications. For example, following a sports injury or in a more clinical setting, these data should encourage practitioners to begin

appropriate interventions (e.g. neuromuscular electrical stimulation (NMES) (35, 43) or higher protein intakes; to counteract muscle decline as soon as it is safe to do so. If an injury or illness is sustained and a subsequent period of non-weight bearing is mandated, interventions should focus on larger muscle groups (e.g. *M. quadriceps*) as these are more susceptible to atrophy and functional decline (partly due to greater habitual gravitational loading). In immobilization (not bed rest) the *M. quadriceps* seems uniquely resistant to declines in function with the *M. hamstrings* being resistant after one week. Such interventions may be beneficial since the data (44) indicate that reducing muscle loss and the metabolic decline of muscle tissue during disuse will aid in a faster return to pre-injury strength level and return to play in sports (44). A limitation of the present study is that only males were included. While this allowed for a more homogenous population to allow us to precisely measure and describe temporal, muscle specific disuse atrophy, it prevents the data being generalizable to females. Given that sex-based differences in the rate of muscle disuse atrophy likely exist (18), it is of importance that future work assesses whether similar results are demonstrable in females.

In conclusion, we report in this study that thigh muscle atrophy occurs rapidly (within just 2 days) and at a sustained rate (by approximately 0.8% per day) during one week of immobilization, and this atrophy is mainly attributed to the loss of *M. quadriceps* tissue mass. Furthermore, the constituent muscles of the thigh atrophy during immobilization at markedly differing rates (~0.4%-1.0% per day). The preponderance towards *M. quadriceps* rather than *M. hamstrings* atrophy during immobilization is accompanied by functional declines manifesting distinctly within leg extension movements.

Acknowledgements

We would like to thank the Royal Society, Exeter University and Maastricht University who all contributed funding to support the completion of this work. Jonathan Fulford's salary was supported via an NIHR grant to the University of Exeter (CRF/2016/10027).

Conflicts of interests

No conflicts of interest, financial or otherwise, are declared by the authors. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

References

1 Fisher SR, Kuo Y, Graham JE, Ottenbacher KJ, Ostir G V. Early Ambulation and Length of Stay in Older Adults Hospitalized for Acute Illness. Archives of Internal Medicine. 2010 Nov;170(21):1942.

2 Tully MA, Bleakley CM, O'Connor SR, McDonough SM. Functional management of ankle sprains: what volume and intensity of walking is undertaken in the first week postinjury. British Journal of Sports Medicine. 2012 Sep;46(12):877–82.

3 Dirks ML, Wall BT, Van De Valk B, Holloway TM, Holloway GP, Chabowski A, et al. One week of bed rest leads to substantial muscle atrophy and induces whole-body insulin resistance in the absence of skeletal muscle lipid accumulation. Diabetes. 2016;65(10):2862–75.

4 Suetta C, Frandsen U, Jensen L, Jensen MM, Jespersen JG, Hvid LG, et al. Aging Affects the Transcriptional Regulation of Human Skeletal Muscle Disuse Atrophy. PLoS ONE. 2012;7(12):e51238.

5 Wall BT, Dirks ML, Snijders T, Stephens FB, Senden JMG, Verscheijden M-L, et al. Short-term muscle disuse atrophy is not associated with increased intramuscular lipid deposition or a decline in the maximal activity of key mitochondrial enzymes in young and older males. Experimental Gerontology. 2015 Jan;61:76–83.

6 Dirks ML, Weerts DHJM, Wall BT, Verdijk LB, Nilwik R, van Loon LJC. Skeletal Muscle Disuse Atrophy Is Not Attenuated by Dietary Protein Supplementation in Healthy Older Men. The Journal of Nutrition. 2014;144(8):1196–203.

7 Stuart CA, Shangraw RE, Prince MJ, Peters EJ, Wolfe RR. Bed-rest-induced insulin resistance occurs primarily in muscle. Metabolism: clinical and experimental. 1988 Aug

[cited 2019 Mar 29]. ;37(8):802–6.

8 Drummond MJ, Dickinson JM, Fry CS, Walker DK, Gundermann DM, Reidy PT, et al. Bed rest impairs skeletal muscle amino acid transporter expression, mTORC1 signaling, and protein synthesis in response to essential amino acids in older adults. American Journal of Physiology-Endocrinology and Metabolism. 2012;302(9):E1113–22.

9 Wall BT, Fritsch M, Verdijk LB, Snijders T, Dirks ML, van Loon LJC, et al. Short-term muscle disuse lowers myofibrillar protein synthesis rates and induces anabolic resistance to protein ingestion. American Journal of Physiology-Endocrinology and Metabolism. 2015;310(2):E137–47.

10 Bell KE, von Allmen MT, Devries MC, Phillips SM. Muscle Disuse as a Pivotal Problem in Sarcopenia-related Muscle Loss and Dysfunction. The Journal of frailty & aging. 2016;5(1):33–41.

11 Wall BT, Dirks ML, Van Loon LJC. Skeletal muscle atrophy during short-term disuse: Implications for age-related sarcopenia. Ageing Research Reviews. 2013;12(4):898–906.

12 English KL, Mettler JA, Ellison JB, Mamerow MM, Arentson-lantz E, Pattarini JM, et al. Leucine partially protects muscle mass and function during bed rest in. Am J Clin Nutr 103: 465–473. 2016;465–73.

13 Paddon-Jones D, Sheffield-Moore M, Urban RJ, Sanford AP, Aarsland A, Wolfe RR, et al. Essential amino acid and carbohydrate supplementation ameliorates muscle protein loss in humans during 28 days bedrest. Journal of Clinical Endocrinology and Metabolism. 2004;89(9):4351–8.

14 Akima H, Kawakami Y, Kubo K, Sekiguchi C, Ohshima H, Miyamoto A, et al. Effect of short-duration spaceflight on thigh and leg muscle volume. Medicine and Science in

Sports and Exercise. 2000;32(10):1743–7.

15 Belavý DL, Miokovic T, Armbrecht G, Richardson CA, Rittweger J, Felsenberg D. Differential atrophy of the lower-limb musculature during prolonged bed-rest. European Journal of Applied Physiology. 2009;107(4):489–99.

16 Maden-Wilkinson TM, Degens H, Jones DA, McPhee JS. Comparison of MRI and DXA to measure muscle size and age-related atrophy in thigh muscles. Journal of musculoskeletal & neuronal interactions. 2013;13(3):320–8.

17 Suetta C, Hvid LG, Justesen L, Christensen U, Neergaard K, Simonsen L, et al. Effects of aging on human skeletal muscle after immobilization and retraining. Journal of Applied Physiology. 2009 Oct;107(4):1172–80.

18 Callahan DM, Tourville TW, Miller MS, Hackett SB, Sharma H, Cruickshank NC, et al. Chronic disuse and skeletal muscle structure in older adults: sex-specific differences and relationships to contractile function. American Journal of Physiology-Cell Physiology. 2015 Jun;308(11):C932–43.

19 Glover EI, Phillips SM, Oates BR, Tang JE, Tarnopolsky MA, Selby A, et al. Immobilization induces anabolic resistance in human myofibrillar protein synthesis with low and high dose amino acid infusion. Journal of Physiology. 2008;586(24):6049–61.

20 Tracy BL, Ivey FM, Metter EJ, Fleg JL, Siegel EL, Hurley BF. A more efficient magnetic resonance imaging-based strategy for measuring quadriceps muscle volume. Medicine and Science in Sports and Exercise. 2003;35(3):425–33.

21 Gibson JNA, Murdoch G, Hornsby GA, Watt PW, Stoward PJ, Halliday D, et al. Decrease in human quadriceps muscle protein turnover consequent upon leg immobilization. Clinical Science. 1987;72(4):503–9.

22 Snyder WS, Cook MJ, Nasset ES, Karhausen LR, Parry Howells G, Tipton IH. Report of the task group on reference man. Oxford: International Commission on Radiological Protection. 1975

23 Psatha M, Wu Z, Gammie FM, Ratkevicius A, Wackerhage H, Lee JH, et al. A Longitudinal MRI Study of Muscle Atrophy During Lower Leg Immobilization Following Ankle Fracture. J Magn Reson Imaging. 2012;35:686–95.

24 Backx EMP, Hangelbroek R, Snijders T, Verscheijden M-L, Verdijk LB, de Groot LCPGM, et al. Creatine Loading Does Not Preserve Muscle Mass or Strength During Leg Immobilization in Healthy, Young Males: A Randomized Controlled Trial. Sports Medicine. 2017 Aug;47(8):1661–71.

25 Horstman AMH, Backx EMP, Smeets JSJ, Marzuca-Nassr GN, van Kranenburg J, de Boer D, et al. Nandrolone decanoate administration does not attenuate muscle atrophy during a short period of disuse. PLOS ONE. 2019 Jan;14(1):e0210823.

26 Booth FW. Time course of muscular atrophy during immobilization of hindlimbs in rats. Journal of Applied Physiology Respiratory Environmental and Exercise Physiology. 1977 [cited 2019 Sep 5]. ;43(4):656–61.

27 Hník P, Vejsada R, Goldspink DF, Kasicki S, Krekule I. Quantitative evaluation of electromyogram activity in rat extensor and flexor muscles immobilized at different lengths. Experimental neurology. 1985 Jun [cited 2019 Mar 28]. ;88(3):515–28.

28 Evangelidis PE, Massey GJ, Ferguson RA, Wheeler PC, Pain MTG, Folland JP. The functional significance of hamstrings composition: is it really a “fast” muscle group? Scandinavian Journal of Medicine and Science in Sports. 2017;27(11):1181–9.

29 Staron RS, Hagerman FC, Hikida RS, Murray TF, Hostler DP, Crill MT, et al. Fiber Type

Composition of the Vastus Lateralis Muscle of Young Men and Women. Journal of Histochemistry & Cytochemistry. 2000;48(5):623–9.

30 Anderson FC, Pandy MG. Individual muscle contributions to support in normal walking. Gait & Posture. 2003 Apr;17(2):159–69.

31 Carroll CC, Fluckey JD, Williams RH, Sullivan DH, Trappe TA. Human soleus and vastus lateralis muscle protein metabolism with an amino acid infusion. Am J Physiol Endocrinol Metab. 2005;288:479–85.

32 Zange J, Mester J, Heer M, Kluge G, Liphardt AM. 20-Hz whole body vibration training fails to counteract the decrease in leg muscle volume caused by 14 days of 6° head down tilt bed rest. European Journal of Applied Physiology. 2009;105(2):271–7.

33 Dirks ML, Backx EMP, Verdijk LB, Wall BT, van Loon LJC. May bed rest cause greater muscle loss than limb immobilization? Acta Physiologica. 2016;10–2.

34 Dirks ML, Wall BT, van Loon LJC. Interventional strategies to combat muscle disuse atrophy in humans: focus on neuromuscular electrical stimulation and dietary protein. Journal of Applied Physiology. 2018 Sep;125(3):850–61.

35 Dirks ML, Wall BT, Snijders T, Ottenbros CLP, Verdijk LB, Van Loon LJC. Neuromuscular electrical stimulation prevents muscle disuse atrophy during leg immobilization in humans. Acta Physiologica. 2014;210(3):628–41.

36 Wall BT, van Loon LJ. Nutritional strategies to attenuate muscle disuse atrophy. Nutrition Reviews. 2013 Apr;71(4):195–208.

37 Akima H, Kubo K, Imai M, Kanehisa H, Suzuki Y, Gunji A, et al. Inactivity and muscle: Effect of resistance training during bed rest on muscle size in the lower limb. Acta Physiologica Scandinavica. 2001;172(4):269–78.

38 Akima H, Ushiyama J, Kubo J, Fukuoka H, Kanehisa H, Fukunaga T. Effect of unloading on muscle volume with and without resistance training. Acta Astronautica. 2007 Apr;60(8–9):728–36.

39 Ivanenko YP, Poppele RE, Lacquaniti F. Five basic muscle activation patterns account for muscle activity during human locomotion. Journal of Physiology. 2004;556(1):267–82.

40 Wall BT, Dirks ML, Snijders T, Senden JMG, Dolmans J, Van Loon LJC. Substantial skeletal muscle loss occurs during only 5 days of disuse. Acta Physiologica. 2013;210(3):600–11.

41 Bamman MM, Clarke MSF, Feeback DL, Talmadge RJ, Stevens BR, Lieberman SA, et al. Impact of resistance exercise during bed rest on skeletal muscle sarcopenia and myosin isoform distribution. Journal of Applied Physiology. 1998;84(1):157–63.

42 Gerovasili V, Stefanidis K, Vitzilaios K, Karatzanos E, Politis P, Koroneos A, et al. Open Access Electrical muscle stimulation preserves the muscle mass of critically ill patients: a randomized study. 2009 DOI: 10.1186/cc8123

43 Taradaj J, Halski T, Kucharzewski M, Walewicz K, Smykla A, Ozon M, et al. Clinical Study The Effect of NeuroMuscular Electrical Stimulation on Quadriceps Strength and Knee Function in Professional Soccer Players: Return to Sport after ACL Reconstruction. BioMed Research International. 2013;2013. DOI: 10.1155/2013/802534

Figure Legends

Figure 1. Representative MR images in the middle of the upper leg with delineations of; a) *M. quadriceps* muscle, b) *M. hamstrings* muscle, c) thigh muscle and d) 11 individual muscles.

Figure 2. Muscle volume of a) thigh, b) *M. quadriceps* and c) *M. hamstrings* muscle groups of both the control and immobilized legs before (day 0) (white bars), after 2 days (grey bars) and after 7 days (black bars) of unilateral knee immobilization. Muscle volume was measured using a 1.5 T MRI scanner. Lines represent individual subject responses. A two-way repeated measures ANOVA (leg x time) was conducted for each muscle group, time, condition and interaction effects are displayed above each graph. Bonferroni post tests were conducted; * denotes $P<0.01$ significant difference from day 0 within the same leg, # denotes $P<0.001$ significant difference from day 2 within the same leg. Data presented are means±SEM, $n=13$.

Figure 3. Individual muscle anatomical cross-sectional area of 11 muscles of the thigh of both the control leg (a) and the immobilised leg (b) before (day 0) and after 2 and 7 days of immobilisation, individual muscle cross sectional area was measured using a 1.5 T MRI scanner. The initials of each muscle are noted along the x axis (RF = rectus femoris, VL = vastus lateralis, VI = vastus intermedius, VM = vastus medialis, BF(L) = biceps femoris long head, BF(S) = biceps femoris short head, SM = semimembranosus, ST = semitendinosus, ADD = adductor group, SAR = sartorius, GRA = gracilis. A two-way repeated measures ANOVA (leg x time) was conducted for each muscle group, where a significant interaction was detected a Bonferroni post hoc test was conducted, * denotes $P<0.05$ significant difference from day 0 within the same

leg, # denotes $P<0.05$ significant difference from day 2 within the same leg. Data presented are means±SEM, $n=13$.

Figure 4. a) Pie chart depicting the contribution of absolute *M. quadriceps*, *M. hamstrings* and other (e.g. *M. adductor* group, *M. sartorius*, *M. gracilis* volume) muscle group volume lost as a proportion of absolute thigh volume lost in the immobilised leg after 7 days of immobilisation. Note absolute total thigh volume lost expressed as 100%, and *M. quadriceps*, *M. hamstrings*, and other muscle volume contributions expressed as percentages (means±SEM, displayed on each segment). b) Pie chart depicting the contribution of each of the 11 measured individual muscles absolute aCSA lost as a proportion of absolute thigh aCSA lost after 7 days of immobilisation in the immobilised leg. Note absolute total thigh aCSA lost expressed as 100% and the contribution of each individual muscle are expressed as percentages, as follows; *M. rectus femoris* contribution = 5.8±0.8%, *M. vastus lateralis* contribution = 20.0±2.6%, *M. vastus intermedius* contribution = 17.7±2.5%, *M. vastus medialis* contribution = 17.1±1.5%, *M. biceps femoris long head* contribution = 6.7±0.8%, *M. biceps femoris short head* contribution = 2.1±0.6%, *M. semimembranosus* contribution = 6.3±0.7%, *M. semitendinosus* contribution = 4.8±0.6%, *M. sartorius* contribution = 1.0±0.3%, *M. gracilis* contribution = 1.1±0.5%, *M. adductor* group contribution = 17.4±2.1%. For both figure 4a and b, dark grey segments represents *M. quadriceps* volume and *M. quadriceps* individual muscles respectively, light grey segments represent *M. hamstrings* volume and individual *M. hamstrings* muscles respectively, and white segments represents other/remaining muscle volume and individual muscles respectively. Data that make the segments represent means, $n=13$. Data in the legend represents means±SEM, $n=13$.

Figure 5. Correlational analyses. Left column of the panel shows correlations between initial muscle volume and muscle volume lost after 7 days of leg immobilization; for a) thigh, b) *M. quadriceps* and c) *M. hamstrings*, muscle groups, respectively. The middle column shows atrophy (by aCSA) after 7 days of immobilization along the longitude of the following muscle groups; d) thigh, e) *M. quadriceps* and f) *M. hamstrings*, respectively, correlated with pre-immobilization muscle aCSA along the longitude of each of the muscle groups. The column on the right are correlations between; g) *M. quadriceps* volume lost vs leg extension strength lost and h) *M. hamstrings* volume lost vs leg curl strength lost, both after 7 days of immobilization. For each individual graph data were analyzed using a Pearson's correlation coefficient, *P* and r values for each correlation are displayed on each graph respectively. Statistical significance was set at $P<0.05$. Data presented are means±SEM, $n=13$.

Supplemental Digital Content

SDC 1: TIME_MRI_paper_sup_fig1_schematic1000.tif

SDC 2: TIME_MRI_paper_sup_fig2_csa_length_leg1000.tif

Figure 1

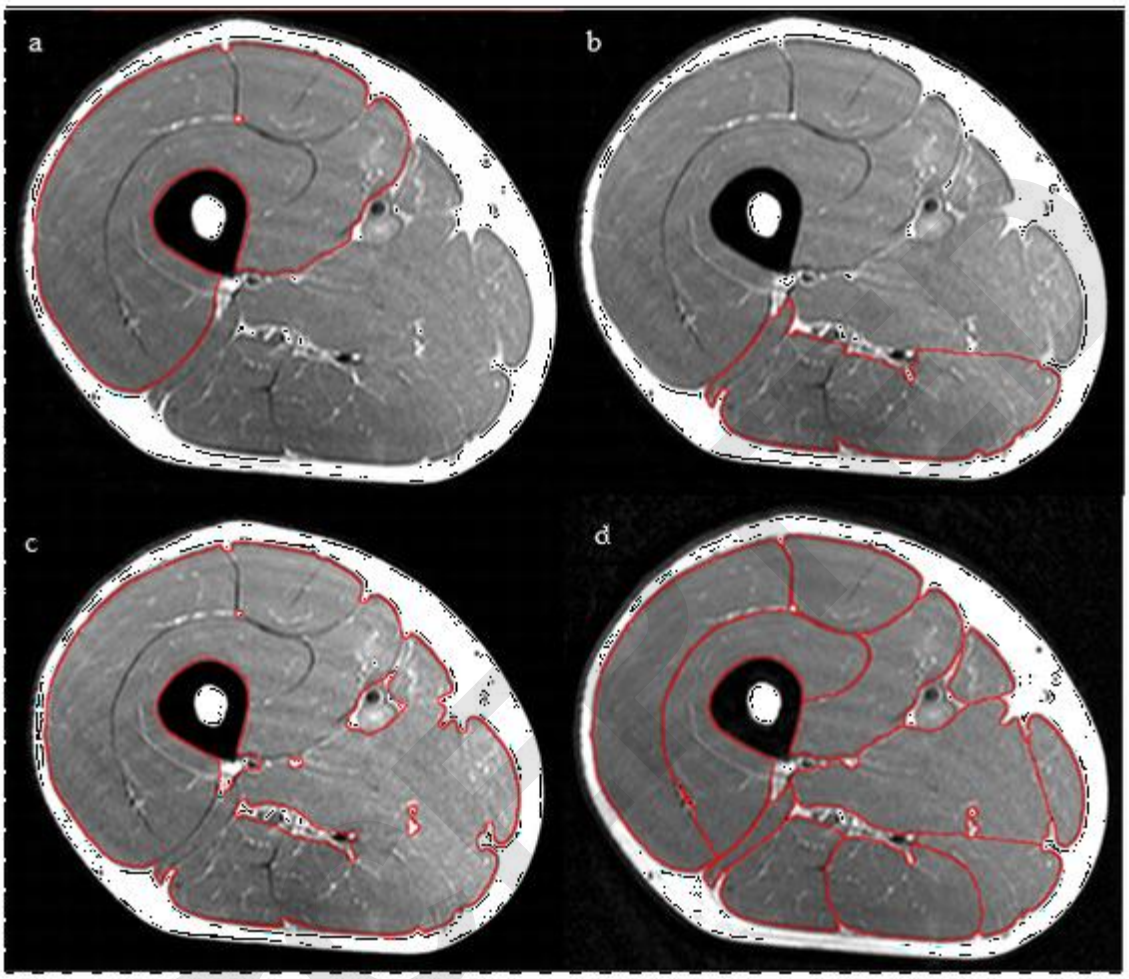

Figure 2

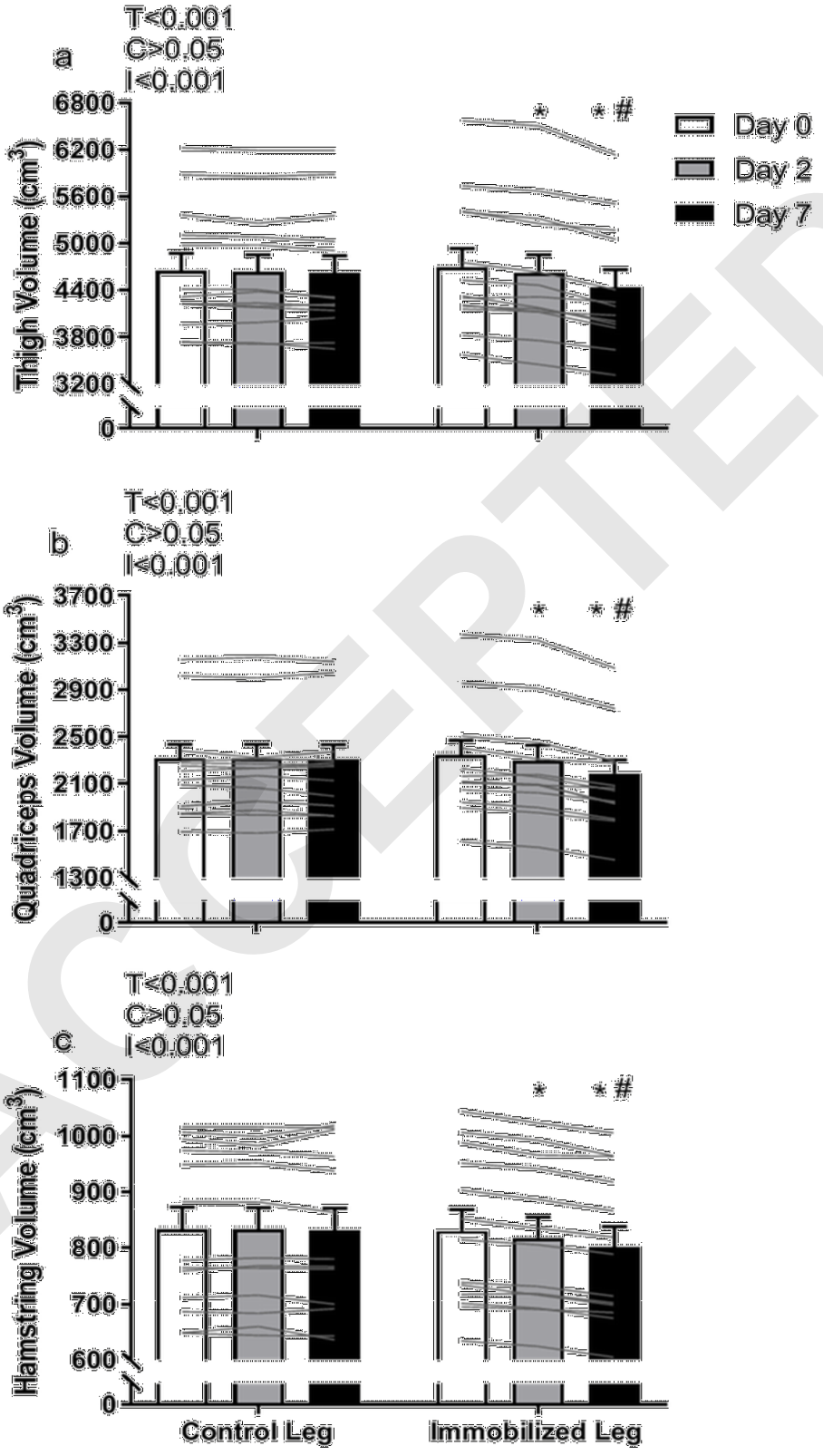

Figure 3

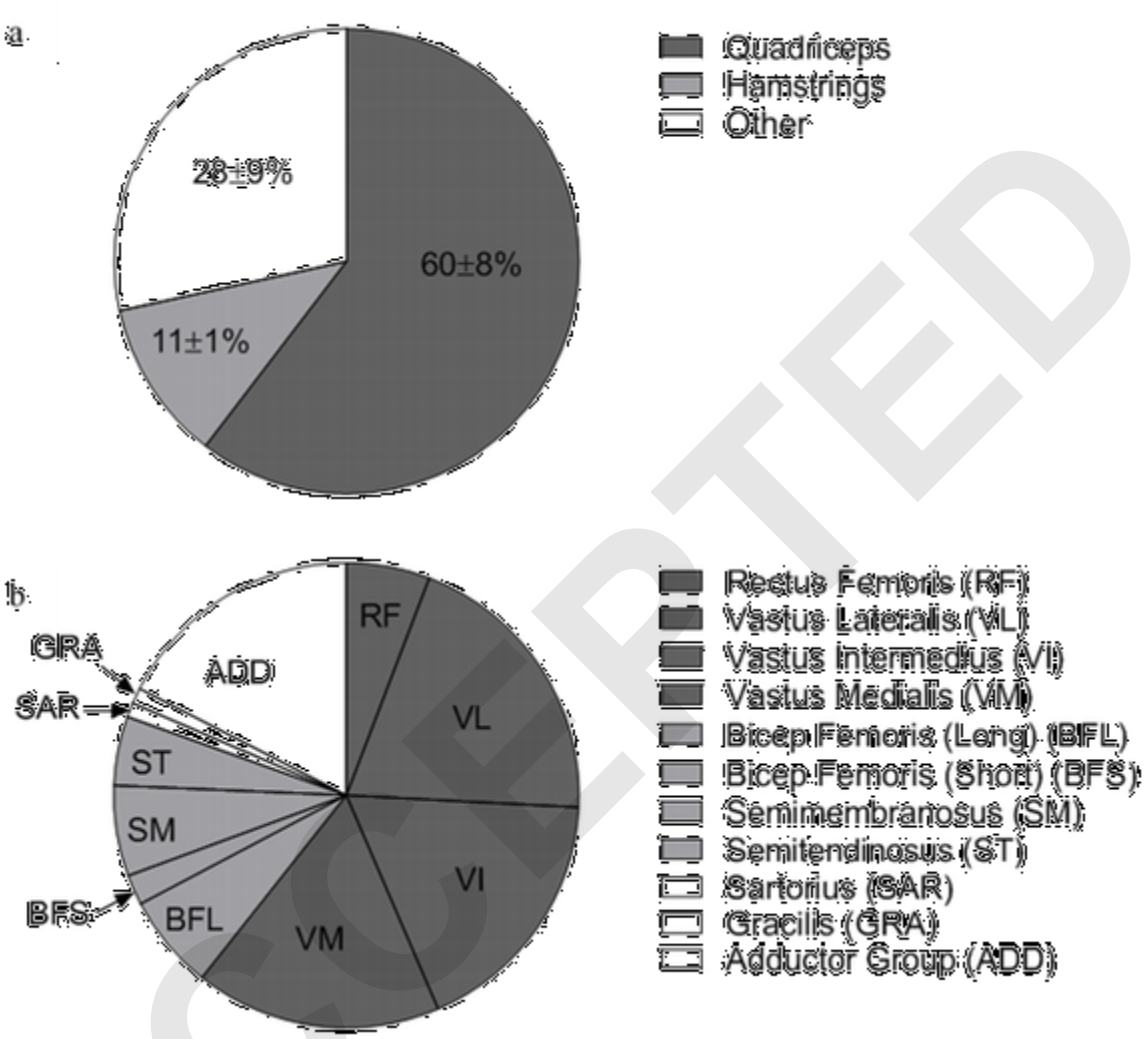

Figure 4

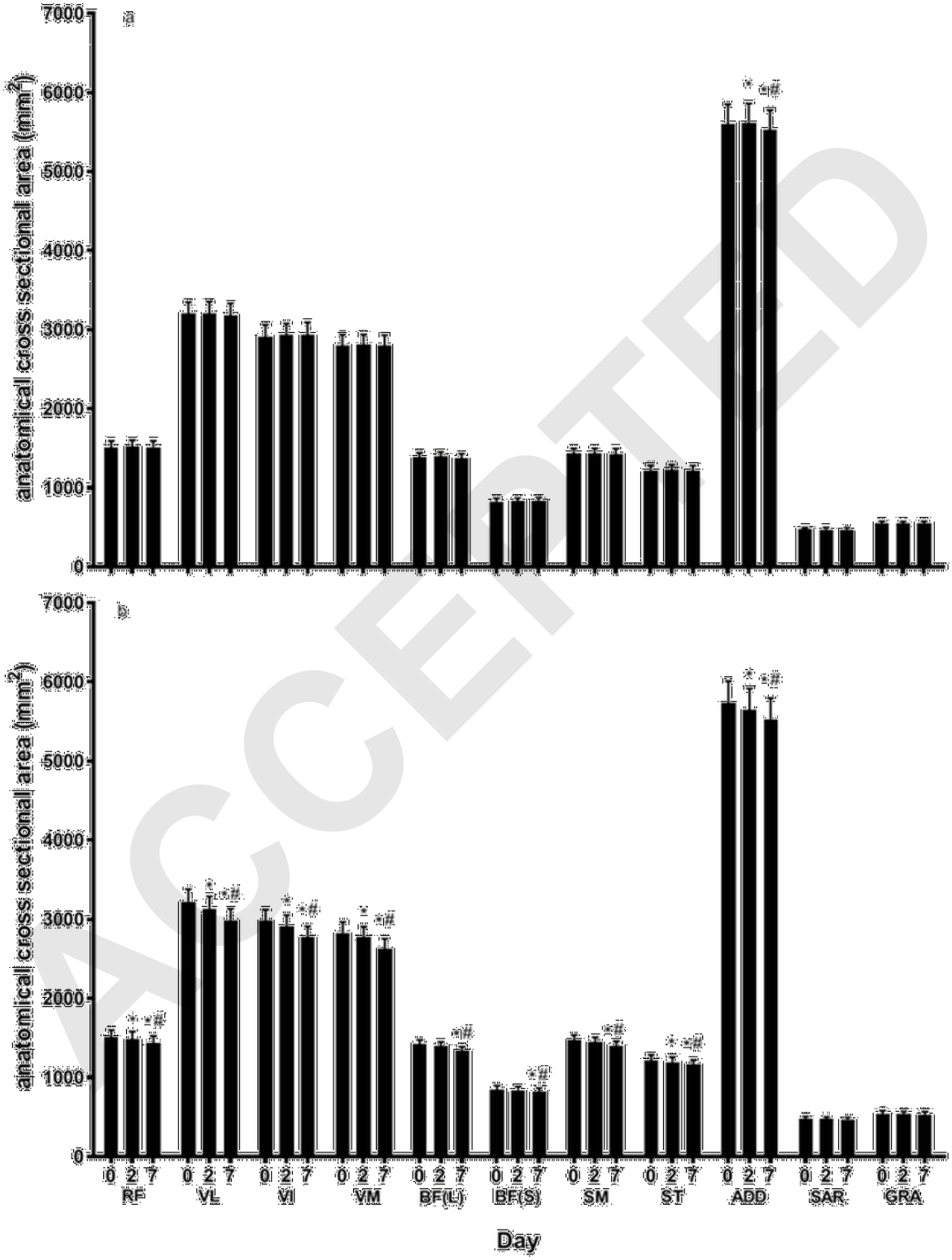

Figure 3

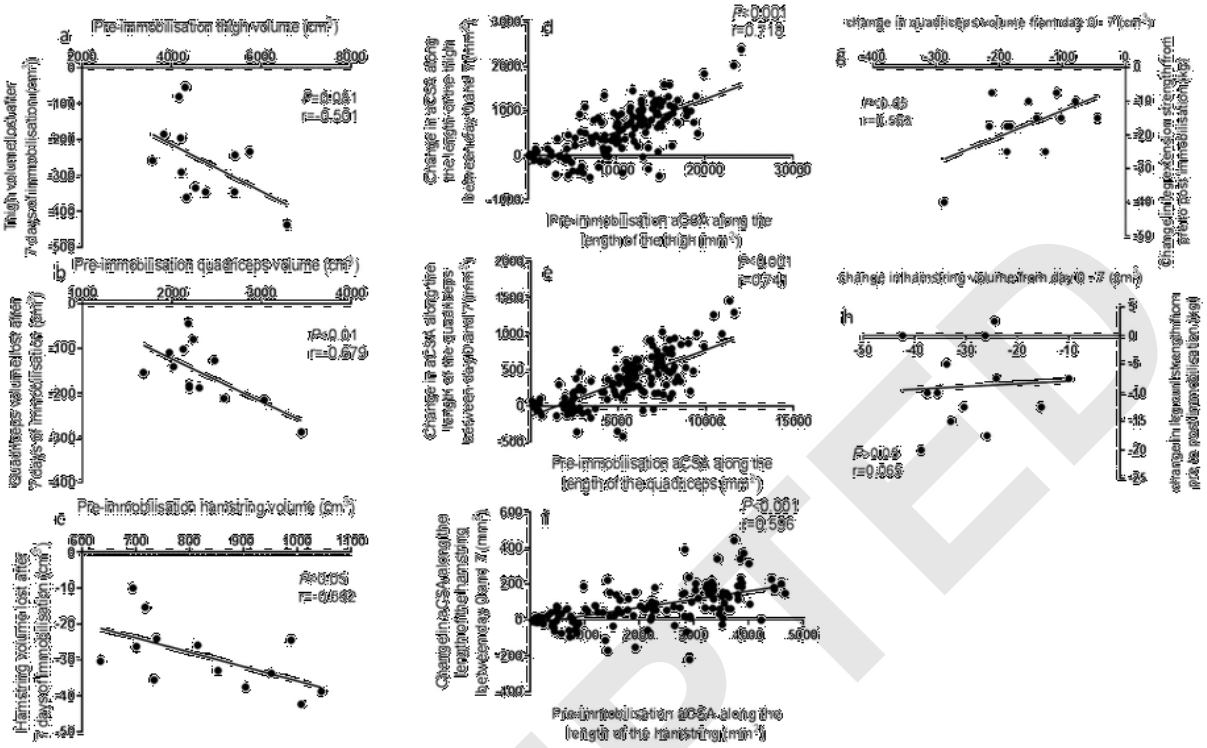

Table 1: Unilateral leg strength.

Leg	Exercise (single leg)	Pre Immobilization (kg)	Post Immobilization (kg)	Percentage change (%)
Control	Leg Extension	89±5	88±5	-1±2
	Leg Press	133±8	138±9	+3±2
	Leg Curl	49±3	50±3	+0.4±2
	Calf Raise	106±6	104±6	-2±2
Immobilized	Leg Extension	92±6	74±4*	-19±2[b]
	Leg Press	139±10	109±9*	-21±4[b]
	Leg Curl	49±3	47±3	-4±2
	Calf Raise	106±7	97±7*	-9±2[a]

Values represent means±SEM, n=13. *=significant difference from pre-immobilization value, P<0.001. [a]=significant difference from control leg for same exercise P<0.05, [b]=significant difference from control leg for same exercise P<0.001.

Figure Supplemental Data Content 1

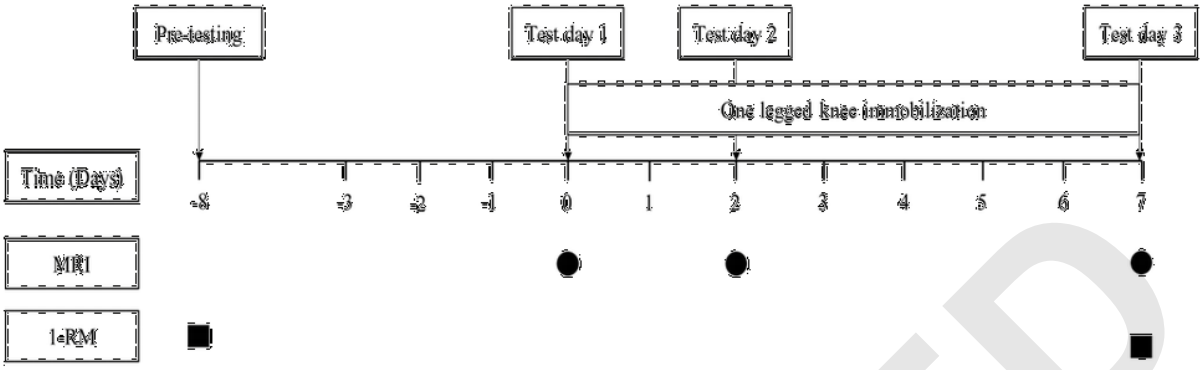

Figure Supplemental Digital Content 1. Schematic representation of the experimental design. Thirteen healthy, young males underwent 7 days of unilateral leg immobilization via knee brace. MRI, magnetic resonance imaging. 1-RM, unilateral 1-repetition maximum strength testing for a variety of exercises.

Figure Supplemental Data Content 2

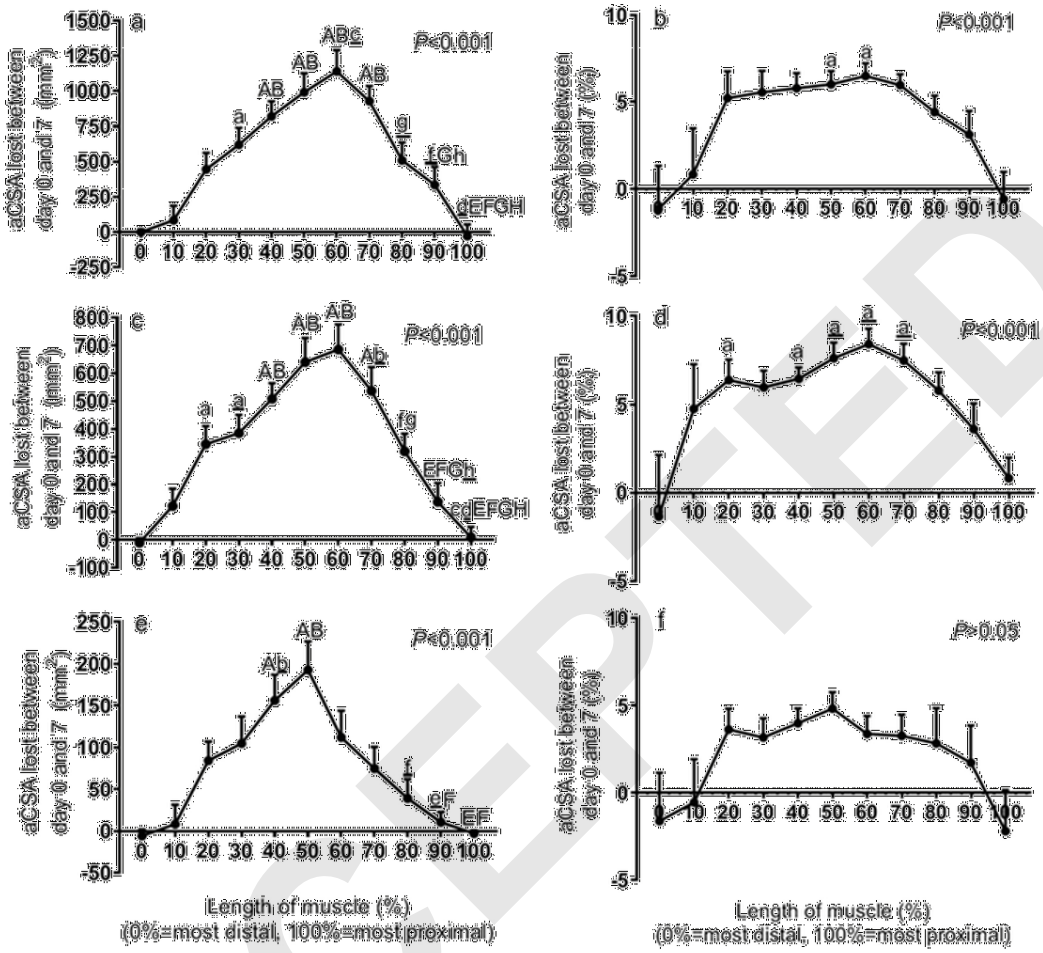

Figure Supplemental Digital Content 2. Muscle atrophy related to anatomical longitude of the muscle groups measured between days 0 and 7 of immobilization in the immobilized leg. 0% represents the most distal MRI image along the length of the leg that was analyzed e.g. lateral femoral condyle at the knee joint. 100% represents the most proximal MRI image along the length of the leg that was analyzed e.g. the greater trochanter at the hip joint. Graph a) = absolute aCSA lost along the length of the thigh muscle, graph b) = % aCSA lost along the length of the thigh muscle, graph c) = absolute aCSA lost along the length of the M. quadriceps muscle, graph d) = % aCSA lost along the length of the M. quadriceps muscle, graph e) = absolute aCSA lost along the length of the M. hamstrings muscle, graph f) = % aCSA lost along the length of the M. hamstrings muscle. A one-way ANOVA was used for each muscle group to assess if different locations along the length of the thigh atrophied more than others. P values represented above each graph, where a significant test was found Bonferroni post hoc tests were used to assess where differences occurred, these are displayed on the graph. a, a and A denote P<0.05, P<0.01, P<0.001 significant difference from 0%. b and B denote P<0.01, P<0.001 significant difference from 10%. c and c denote P<0.05 and P<0.01 significant difference from 20%. d denote P<0.01 significant difference from 30%. e and E denote P<0.01 and P<0.001 significant difference from 40%. f, f and F denote P<0.05, P<0.01, P<0.001 significant difference from 50%. g, g and G denote P<0.05, P<0.01, P<0.001 significant difference from 60%. h, h and H denote P<0.05, P<0.01, P<0.001 significant difference from 70%. Data presented are means±SEM, n=13.