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# Role of tissue perfusion, muscle strength recovery and pain in rehabilitation after acute muscle strain injury: A randomized controlled trial comparing early and delayed rehabilitation

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## Abstract

Muscle strain injuries disrupt the muscle-tendon unit, early rehabilitation is associated with a faster return to sports (RTS), but the time course of tissue healing remains sparsely described. The purpose was to examine tissue regeneration and the effectiveness of early versus delayed rehabilitation onset on functional and structural recovery after strain injuries. 50 recreational athletes with a severe acute strain injury in their thigh or calf muscles were randomized to early or delayed rehabilitation onset. Magnetic resonance imaging (MRI) was obtained initially, 3 and 6 months post injury and dynamic contrast-enhanced MRI (DCE-MRI) estimated tissue inflammation initially and after 6 months. Muscle strength was determined 5 weeks, 3 and 6 months post injury and a questionnaire determined soreness, pain and confidence. DCE-MRI microvascular perfusion was higher in the injured compared to an uninjured muscle acutely ($p< 0.01$) and after 6 months ($p< 0.01$), for both groups ($p> 0.05$) and unrelated to RTS ($p> 0.05$). Total volume of the injured muscle decreased from the acute to the 3 months scan, and to the 6 months scan ($p< 0.01$) in both groups. Muscle strength was similar in both groups at any time. There was a non-significant trend ($p\leq 0.1$) towards less pain and higher confidence with early rehabilitation. One re-injury was recorded. In conclusion, our data showed prolonged tissue repair with the initial response linked to muscle atrophy but did not explain why early rehabilitation onset accelerated recovery considering that structural and functional recovery was similar with early and delayed rehabilitation.

## Introduction

Muscle strain injuries are classified as traumatic injuries that require substantial clinical rehabilitation to return to normal pre-injury sports activity. These injuries have a high

incidence and a significant re-injury rate [1–4]. Re-injuries are particularly common in the early phase after return to sports (RTS)[1], which suggests that there is a discrepancy between the time allowed for tissue healing and the ability of the tissue to withstand high sport specific loading. Muscle strain injuries affect primarily the muscle-tendon interface, which implies that the tissue damage will involve both parts of the contractile muscle and the connective tissue of tendon and/or aponeurosis. The myotendinous junction (MTJ) can withstand considerable forces and strain before failure [5] and can adapt to changes in loading history [6], but it is the weakest link of the muscle-tendon unit [7]. Consequently, the healing of a strain injury will likely incorporate regeneration of the MTJ and connective tissue formation and re-connection between muscle fibers and collagen fibers. Given its low turnover and rather poor healing capacity [8], the connective tissue may be a limiting factor in the rehabilitation of strain injuries, but this has not been investigated. Previously, it has been demonstrated in animal models [9] and electrically stimulated humans muscle [10] that tissue healing involves extracellular matrix (ECM) regeneration. Although experimentally induced focal muscle damage is not directly comparable to strain injuries, it is worth noting that connective tissue regeneration can take place for many weeks to months after the injury.

Recently, we have demonstrated that early rehabilitation onset after muscle injury is crucial for a faster clinical recovery to sports [11]. This supports earlier findings that immobilization impairs both muscle and tendon tissue [12,13] and has a detrimental effect upon connective tissue structure and cells in *in vitro* system [14]. The mechanisms for such a disadvantageous outcome with delayed loading are unclear and rarely investigated in detail in humans. Therefore, the present study sought to investigate if *A*) tissue perfusion measured by dynamic contrast-enhanced MRI, *B*) tissue structure determined by conventional MRI, and *C*) muscle function examined by strength tests differed in athletes with a rehabilitation onset of 2 compared to 9 days post injury. Additionally, we wanted to assess subjective measures of pain, symptoms related to the injury and confidence in the injured limb.

Previous studies of acute muscle strain injuries have shown that tissue morphology at the injury site is abnormal for several weeks and months after injury [2,15]. It is, however, unknown how dynamic this process is and whether the initial severity of tissue damage yields long-term pathophysiological changes. Further, it is unknown whether these tissue changes are of clinical relevance, and if they play a role in re-injuries. To avoid invasive

procedures, the present study made use of dynamic contrast-enhanced MRI (DCE-MRI) to quantitatively measure microvascular permeability[16] and estimate tissue inflammation. This technique can serve as a surrogate marker of inflammation as shown previously using histology as a reference[17]. Damage to the microvascular barrier is a pathological consequence following trauma [18], and in the context of strain injuries, the Gadolinium contrast uptake in the injured tissue may represent microvascular leakage, cell injury and inflammatory processes.

Thus, in the present study we aimed to examine tissue perfusion as an indirect marker of inflammation over time and examine the relation between tissue perfusion and morphological changes of the muscle. Muscle strength tests were performed to study muscle function and perceived readiness of patients for RTS were registered. All parameters were analyzed in relation to either an early initiated or a more protracted rehabilitation regimen. The study goal was to investigate whether a shorter time to RTS is reflected in the injured tissue structures and function. We hypothesized that the early therapy group would be superior to the delayed therapy group in function measured as muscle strength. Further, we expected that the DCE-MRI parameters determining the rate and magnitude of contrast uptake by the tissue is significantly increased in the acute phase post injury. At the 6 months follow up scan, we hypothesized that contrast uptake would be normalized in the injured, compared to an uninjured muscle, reflecting resolution of inflammation and restoration of the tissue integrity. At this late time point, we did not expect a difference between the rehabilitation groups.

## Materials and Methods

### Study design, participants and rehabilitation

This study was a parallel design, two-arm RCT using a computer-generated minimization randomization procedure with a follow-up of 12 months. Minimization was based on muscle group and gender and was performed by the principal investigator. The primary outcome of the study was time to return to sports (RTS), which has been reported earlier together with a detailed outline of the clinical study design [11]. Briefly, patients were clinically examined

and diagnosed before inclusion (sudden onset of pain during explosive movement, palpation pain and a clear defect at the muscle-connective tissue interface visible on an ultrasound scan). Inclusion criteria are listed in the supplementary table S2. All patients gave written informed consent, the study was approved by the local ethical committee (The Regional Ethical Committee, ref. H-1-2014-005) and registered at clinical trials.gov. The rehabilitation protocols were identical for both groups, lasting for 12 weeks with a gradual increase in load over time focusing on the injured leg [11]. For all exercises, patients were instructed not to exceed the pain level of > 5 on the NRS pain scoring scale. The onset of rehabilitation for the early therapy group was two days and for the delayed therapy group nine days after the injury. Patients were cleared for return-to-sport when symptom-free during rehabilitation and pain-free during and after repeated maximal sprints and single-leg jumps (pain ≤ 1 on the NRS pain rating scale).

**Magnetic Resonance Imaging**

MRI of the injured thigh or calf was performed in the first week, and 3 and 6 months post injury. All scans were performed using a 1.5T (Ingenia Stream; Philips Healthcare, Best, the Netherlands), all patients were scanned in supine position using a 32 channel torso coil. The following MRI protocol was used: 3 plane localizer; TR 3.2ms; TE 1 ms; FA 90$^{o}$; Field of View (FOV) 530mm; Slice Thickness (ST) 10mm. Sagittal STIR (Short Tau Inversion Recovery) (TR 4.4 sec; TE 20ms; FA 90$^{o}$; FOV 440mm; ST 5mm; Matrix 218x218; TI 150ms). Sagittal T1W TSE; TR 503ms; TE 15ms; FA 90$^{o}$; FOV 440mm; ST 5mm; FOV 440mm; Matrix 218x218). Axial STIR (TR 6.2; TE 20ms; Flip Angle (FA) 90$^{o}$; FOV 250mm; ST 5mm; Matrix 128x128). Axial T1W TSE (TR 532ms; TE 20ms; FA 90$^{o}$; FOV 250mm; ST 5mm; Matrix 128x128). During an intravenous gadolinium injection (0.2ml/kg body weight using a power injector 2ml/s) an axial T1W FFE Dynamic Contrast Enhanced (DCE) sequence was performed (TR 4.1ms; TE 20ms; FA 12$^{o}$; FOV 440mm; ST 4mm; Matrix 268x268; Temporal res 31sec). Finally, a post contrast T1W SPIR (TR 590 sec; TE 20ms; FA 90$^{o}$; FOV 440mm; ST 5mm; Matrix 206x206; TI 150ms). The two last sequences were only performed at the first and last time point. Dynamic contrast-enhanced MRI is based on a fast MRI sequence performed of a given anatomy with few seconds between each acquisition before, during and the first 5 minutes

after injection of Gadolinium contrast. This MRI sequence allows extraction of time intensity perfusion curves in each image volume element (voxel) as the signal intensity in the voxels of the target tissue changes over time[19].

Renal function was tested before the MRI scan as an estimated glomerular filtration rate (eGFR) <60ml/min/1.73m$^2$ is a contraindication for administration of IV contrast medium. The DCE sequence was always performed as the second last sequence, i.e. a minimum of 20 min into the scan assuming a relaxed state of the patient with normalization of potential perfusion changes induced by movement and increased heart rate. In the early therapy group, 16 participants were included in the DCE-MRI analysis (non-participation due to personal reasons n= 1, technical issues n= 3). In the delayed therapy group, 17 patients were included in the DCE-MRI analysis (personal reasons n=4, technical issues n= 1), supplementary figure 1. Prior to the MRI, all participants were asked to mark the area of maximal pain and its distal and proximal extension so that the length of the painful area was defined and measured.

**Image analysis**

Images were interpreted by an experienced radiologist, who was blinded to group allocation and clinical details other than the suspected muscle injury. The radiologist recorded the presence or absence of abnormal intra- and inter-muscular STIR hyperintensity and potential changes on T1 weighted images and determined the severity of the strain injury based on the classification system where grade 3 strains are defined as minor/ moderate partial muscle tears and grade 4 as total/ sub-total muscle tears[20]. The muscles involved and the location of the abnormality in each muscle was determined and the muscle with the most extensive STIR hyperintensity was noted Injury volume was calculated by assuming that the injury had a shape of a rotational ellipsoid, that is, volume ≈ length × width × depth × 0.5 [21].

Muscle volume was quantified using the Osirix Software (Osirix Lite V.9.0) by manual segmentation by a blinded investigator. The muscle boundaries were identified and outlined on the 2D axial T1-weighted images synchronized to the axial STIR images to differentiate

between muscle tissue and the intra- and intermuscular hematoma. Two-dimensional muscle areas were measured on 20 consecutive images starting at the most distal site of injury moving 20 slices with a slice thickness of 5 mm in the proximal direction and summed to a muscle volume. The exact same location was used for the follow up scans measured as distance from anatomical landmarks; the apex of the patella or the femoral head for thigh injuries and the patella plateau for calf injuries. The investigator analyzed the muscle volume of 10 randomly chosen patients 2 times for reproducibility measurements. Three separate regions of the muscles were chosen (the most distal, the mid potion and the most proximal part) and here the cross-sectional area of the injured muscle, the uninjured agonist and the uninjured antagonist were measured. The re-test (second measurement) was more than one week apart following the first measurement. Typical error % for replicate measurements and the correlation coefficient r for the three regions and the three muscles are listed in table S1, supplementary information.

Dynamic contrast enhanced-MRI slices were analyzed using the computer software Dynamika® enterprise version 2.4.6 (Image Analysis LTD, London, http://www.imageanalysis.org.uk). Motion correction between temporal slices was applied on all the available axial DCE-MRI slices before regions of interest (ROIs) were drawn around areas with visible contrast-enhancement in the injured muscles (figure 1A). Vascular branches were avoided when drawing the ROIs which was facilitated by using the initial rate of enhancement (IRE) MAP superimposed on the grayscale images (figure 1A, B). ROIs were then combined to form a single volume. A ROI containing uninjured muscle in the same compartment and another ROI applied in the antagonist muscle region in the two most proximal and the two most distal slices of DCE-MRI dataset (figure 1A) served as references. For all voxels in the ROI, the analysis of contrast-enhancement included the mean of maximal enhancement (ME) selecting the height of the perfusion curves from baseline, the initial rate of enhancement (IRE) reflecting the steepness of the perfusion curve as percent contrast intensity increase over time from baseline. Finally the total number of voxels with a high enhancement reaching plateau or washout referred to as Nvoxel were determined [22]. The composite IRE*ME is a surrogate marker of the area under the perfusion curve [23]. The parameters IRE, ME and IRE*ME are expressed as the ratio of the ROIs at the site of injury and the reference ROIs. The first MR scan in the days after the injury was used as a baseline,

and ROIs were drawn into the corresponding slices on the 6 months follow up scan using the femoral head or the tibia plateau as anatomical landmark on the T1-weighted sagittal image.

**Maximal isometric muscle strength**

To evaluate mechanical muscle function, a maximal isometric muscle strength test determined peak muscle strength (maximal voluntary contraction, MVC) after the first rehabilitation phase, 5 weeks after the injury. The same test was repeated 3 as well as 6 months post injury. Isometric tests were chosen to avoid forceful isokinetic muscle contractions in the early phase post injury. Isometric strength was measured in newton 5 weeks, 3 months (13 weeks) and 6 months post injury with the Good Strength device (Version 3.14 Bluetooth; Metitur Ltd, Finland) as described in detail elsewhere[24]. Measurements were preceded by 10 minutes of warmup on a Monark cycle ergometer. Participants with an injury in the hamstring were seated with hips flexed at 90° and knees flexed at 30°, 60° and 80° from full extension (technical limitations did not allow 90°). Participants with a quadriceps injury were seated with hips flexed at 90°, knees flexed at 70° and 90° from full extension. Knee angles were measured with a hand-held goniometer. Stabilization belts were placed across the waist and distally across the ipsilateral thigh, and the transducer was placed 5 cm above the malleoli for injuries in the thigh [25]. Participants with a calf injury were seated with hips flexed at 90°, knees fixed at 0° knee ankle, ankle joints in a 90˚ position and the force transducer was placed beneath the metatarsal bones. The same placements were used for all time points. The participants were instructed to perform the contraction as fast and powerful as possible. Each contraction lasted 4 s and was separated by a rest period of 30 seconds. Three measurements were made at each time point, the trial with the highest MVC was used in the data analysis. For thigh measurements, forces were corrected for the lower leg weight distal to the knee joint for each knee angle and for the ankle isometric strength, the passive force against the transducer was subtracted from the active MVC.

**Maximal isokinetic muscle strength**

Maximal concentric (con) and eccentric (ecc) strength of hamstring (H) and quadriceps (Q) muscle was measured during isokinetic knee extension and flexion movements 3 and 6 months post injury. A Kin-Com dynamometer (Chattecx Corp., Chattanooga, Tennessee) was used for the measurements. Participants were seated and reclined 10°, their hips and thighs were firmly strapped to the seat of the dynamometer. The axis of rotation of the dynamometer lever arm was visually aligned with the lateral femoral condyle, and the lower leg was attached to the lever arm of the dynamometer 5 cm above the lateral malleolus. Measurements were preceded by warm-up and the isometric muscle strength measurement. For each specific contraction mode, two pre-conditioning trials were performed followed by 3 maximal contractions at the angular velocity of 60°/s, the interval of rest between trials was 30 to 90 seconds. Recorded forces were corrected for the weight of the lower leg distal to the knee joint. Range of motion was 10° to 90° (0° indicating full knee extension). The knee extension range of motion was altered 2-3˚ for a few patients that could not extend their knee to 0°. Tests were always in the same order starting with hamstring con then hamstring ecc, followed by quadriceps con then quadriceps ecc on their healthy leg followed by the injured leg. Strength was reported as the maximal torque and the angle at which maximal torque was recorded. The trial with the highest peak torque was used in the data analysis. In addition, we determined the H:Q ratio, which determines the muscle strength properties around the knee joint [26]. The conventional H:Q ratio is determined as the maximal hamstring concentric torque divided by the maximal quadriceps concentric torque ($H_{con}$:$Q_{con}$). The functional H:Q ratio representative for knee extension is the maximal hamstring ecc torque divided by maximal quadriceps con torque ($H_{ecc}$:$Q_{con}$). Lastly, the functional H:Q ratio representative for knee flexion was calculated by the maximal hamstring con torque divided by maximal quadriceps ecc torque ($H_{con}$:$Q_{ecc}$).

**Dynamic calf muscle function test**

The heel-rise test is a measure of repeated concentric and eccentric muscle contractions and was only carried out in patients with calf muscle strain injuries as isokinetic measurements of the calf on the KinCom dynamometer were not possible. The MuscleLab

(Ergotest Technology) system was used, which consists of a string connected to a sensor inside a linear encoder unit. When the string is pulled, the sensor outputs a series of digital pulses that corresponds to the distance travelled [27]. For balance, the participants were instructed to place two fingertips per hand against a wall at shoulder height. The participants were instructed to rise as high as possible on each heel-rise and then lower the heel to the starting position with concentric and eccentric phases each lasting 1 s guided by a metronome. Participants were asked to perform as many heel-rises as possible. The test was terminated when the participant was unable to raise the heel >5 cm at the correct pace.

**Questionnaire and return to sport**

The questionnaire to assess function scores of the injured muscle was previously validated by Engebretsen et al [28] and translated to Danish. It consists of five categories (symptoms, soreness, pain, function and activities and quality of life/ quality of sports performance), each category is scored separately. The total score is achieved by calculating the mean of the five categories in percent of the maximal score. The questionnaire was modified for calf and thigh injuries to be specific to these regions following the same principles as the Hamstring Outcome Score. Participants were asked to complete the questionnaire at 3 and 6 months as well as the one year follow up. RTS was defined as the time from injury until full, pain-free and confident participation in all sports activities on the pre-injury level could be performed.

**Statistics**

Two-way ANOVA with repeated measures in one factor (time) with post-hoc Holm-Sidak tests were used to detect statistical differences between groups and time points when the normality test was passed. Normality was tested with the Shapiro Wilk test. DCE-MRI parameters were tested on log transformed data, numbers are given as geometric means with the upper and lower SEM. Due to lack of normality and log normality, questionnaire items were tested with the Mann–Whitney U test for differences between therapy groups at each time point. Muscle volume changes also lacked normality and were tested with

Mann–Whitney U tests for differences between muscle and therapy groups at each time point. There was no difference between muscle and therapy groups and therefore time effects were evaluated by one-way repeated measures ANOVA on ranks (Friedman test, post-hoc Tukey) across intervention groups. Paired t-tests were used for the statistical analysis to determine whether there was a systematic difference between the first and second measurement in the reproducibility test of muscle volume quantification on 10 randomly chosen subjects. All correlations were performed using Spearman´s rank order correlation. The level of significance was set at $P< 0.05$. An a priori sample size calculation suggested that n= 21 was needed to detect a 30% difference in RTS at a $p<0.05$ level with a power of 80%. To account for drop-outs, a total of 25 patients in each of the studied groups were recruited and included in the study [11].

## Results

### Participants

Seventy-five recreational athletes with an acute strain injury within 48 hours prior to the medical examination were recruited. Of these, 50 patients fulfilled the inclusion criteria and were randomized to one of the two intervention groups, all of the patients had grade 3 or 4 muscle injuries [20]. The primary outcome of this clinical study was time until RTS, which has been published previously[11]. Briefly, the early therapy group had a significantly faster return to sport compared to the delayed therapy group (median days until RTS 62.5, interquartile range 48.8 to 77.8, and 83.0 days 64.5 to 97.3, respectively). According to Fuller et al[29], all injuries were classified as severe sports injuries. There was one re-injury registered in the early and none in the delayed therapy group. No statistical difference was detected between the groups in regards to age, height, weight, or the severity and location of the injury. An outline of patient characteristic and the injured muscles is found in the supplementary appendix, table S1) [11]. A total of 8 patients dropped out of the study during therapy due to reasons unrelated to this study, and 42 completed the study, and of these 23 had hamstring injuries (55%), 17 had calf injuries (40%) and 2 had quadriceps injuries (5%). There was no difference in acute pain levels, the dimensions of the edema and the severity of the injuries between the groups [11] (supplementary table S1).

**Dynamic contrast-enhanced-MRI**

There was no statistical difference between the two intervention groups for all DCE-MRI parameters acutely and 6 months post injury. Immediately after injury, there was a higher perfusion both for mean IRE and ME in the injured muscle compared to a healthy muscle when using the same compartment in an uninjured reference muscle ($p< 0.01$). This difference was still evident 6 months after injury (figure 1, $p< 0.01$). None of the DCE-MRI variables were normalized at 6 months post injury follow-up, but we did observe a significant reduction of all values in the injured area over time (figure 1, table 1, $p< 0.001$), a change that was independent upon rehabilitation group allocation. The data demonstrate a high correlation between the volume of the edema measured on STIR MRI images and the amount of maximally perfused voxels (Nvoxel) ($r= 0.74$, $p< 0.01$). Our data did not show any statistical correlation between the amount of highly perfused Voxels (Nvoxel), IRE and ME and the time until RTS, ($p= 0.2$, $p= 0.4$, $p= 0.4$, respectively, supplementary table S2). Likewise, there was no correlation between the change over time in all the DCE-MRI parameters included in the analysis and the time until full recovery (supplementary table S2, S3). In addition, the self-reported painful area was not correlated to the volume of the edema extracted from STIR images ($p= 0.3$, supplementary table S4) or the amount of maximally perfused voxels (Nvoxel), ($p= 0.7$, supplementary table S4) from the DCE-MRI. Further, no correlation was determined between the parameters mean IRE or ME ($p= 0.5$, $p= 0.9$, respectively, supplementary table S4). Finally, the acute pain score determined during the first rehabilitation session (data available in [11]) was not correlated to any static or dynamic MRI parameter.

**Muscle volume**

There was no difference between the early and delayed therapy group in the total muscle volume changes over time ($p= 0.9$), and no difference was observed in the relative changes between the thigh and calf muscle groups ($p= 0.5$). There was a decline in the volume of the injured muscle between the acute and the 3 months follow up scan (median reduction of 9%, figure 2, $p= 0.02$), which remained unchanged at the 6 months follow up scan (median change acute to 6 months follow up scan 9%, $p< 0.001$). There was no difference in the

muscle volume of an uninjured muscle chosen from the same compartment and no change in the uninjured antagonist muscle volume at any time point (figure 2).

There was a significant inverse correlation between the volume reduction of the injured muscle and the DCE-MRI parameter Nvoxel determined in the acute post injury scan, i.e. the more pronounced the perfusion, the greater the muscle loss. The association between Nvoxel and the measured volume changes from the acute scan to the 3 months follow up scan revealed a correlation coefficient r of -0.48, p= 0.003, and from the acute baseline scan to the 6 months follow up scan resulted in a correlation coefficient of -0.52 (figure 3, supplementary table S3, p= 0.001).

**Muscle isometric strength**

There were no statistical differences between the MVC in the injured leg compared to the contralateral uninjured leg at 5 weeks, 3 months and 6 months post injury, regardless of group allocation. The pattern of similar strength between the injured and healthy limb was the same for injuries in the thigh (hamstring and quadriceps) and calf muscles. Therefore, the collective MVC of the injured relative to the contralateral healthy limb is illustrated for all patients (figure 4 for all 3 time points. Isometric MVC between the injured and the healthy legs was similar for all knee angles tested in the thigh muscles.

**Muscle isokinetic strength of the thigh muscles**

There was no difference between the rehabilitation groups in the isokinetic muscle torque of the injured muscles at both 3 and 6 months in patients with hamstring injuries. The injured hamstrings performed a lower torque in comparison with the contralateral uninjured side for both the concentric and the eccentric muscle contractions 3 months post injury (p= 0.002 and 0.04, respectively, table 2, supplementary figure 2). On average, the hamstring concentric peak force was 12% lower, the eccentric peak force 5% lower in the injured leg . Quadriceps concentric and eccentric torque was similar between the legs. As a consequence, the calculated H:Q ratio was reduced in the injured compared with the

uninjured leg (p= 0.005) and this was also the case also for the functional $H_{con}$:$Q_{ecc}$, (p< 0.001). There was no difference in the functional $H_{ecc}$:$Q_{con}$ ratio (p= 0.2). Further, there was no difference between groups and between legs in the knee angle, at which the participants exerted their maximal concentric and eccentric hamstring strength (p= 0.48 and 0.53, respectively). The only group difference was manifested as a higher quadriceps con peak strength in the early therapy compared to the delayed therapy group (p= 0.03, table 2). Hamstring strength was recovered 6 months post injury, as there was no isokinetic deficit detectable at this time point (p= 0.23 for concentric, p= 0.86 for eccentric contractions). Quadriceps maximal torque was not different between the legs for both contraction forms and the H:Q ratios were similar between the injured and the uninjured leg. There was no difference between the legs in the angle at which peak hamstring strength was achieved (table 2).

**Test of calf muscle function**

There was no difference between the early and the delayed therapy group (figure 5, p= 0.37 for the number of repetitions and p= 0.61 for distance). Further, no statistical difference was detected between the injured and the contralateral uninjured calf for both the number of heel rises performed (p= 0.52) and the distance, i.e. the active range of motion of the calf muscles during the test (p= 0.51). This was found both 3 and 6 months post injury, (figure 5) and both the number of calf rises, and the range of motion were similar at 3 and the 6 months post injury (p= 0.17 and 0.42, respectively).

**Perception of symptoms and readiness**

There was no statistical difference in any of the subjective ratings of “symptoms”, “the level of pain”, “confidence in the injured leg”, “soreness” as well as “functional difficulties” between the groups. However, the participants in the early therapy group reported a trend towards reduced pain (p= 0.1) and greater “confidence in the injured leg” compared with the delayed group (p= 0.08, figure 6 A, B) 3 months post injury. The tendency towards a higher confidence in the injured leg for the early therapy group was similar 6 months post

injury (p= 0.06, figure 6 B), albeit not significant. There was no such trend seen for any of the other subcategories, soreness, symptoms and functional difficulties.

## Discussion

The main outcome of the present study is that tissue repair is a prolonged process that lasts for at least 6 months after a severe muscle strain injury. The tissue is not fully normalized at the time when athletes resume full physical activity at pre-injury level as illustrated by the increased DCE-MRI perfusion parameters in the injured area compared to the uninjured reference muscles. This finding supports the view that even after RTS following a musculoskeletal injury, the injured tissues still undergoes regenerative activities. The prolonged tissue regeneration in both rehabilitation groups is further underlined by the finding of a significant reduction in the volume of the entire injured muscle both after 3 and 6 months, which was correlated to the DCE-MRI parameters obtained acutely after injury. The tight relation between volume reduction at 3 and 6 months post injury and the increased tissue perfusion suggests a link between the magnitude of the trauma, the inflammatory response and muscle atrophy. This study cannot demonstrate that a faster RTS due to early onset of rehabilitation is directly related to any detectable differences either in static or dynamic maximal muscle strength or in structural analyses obtained by both conventional and DCE-MRI.

Muscle atrophy after the injury suggests that either the innervation or the mechanical tension, or the combination of both is severely altered following a strain injury. Since there is a disruption between the contractile elements and the force transducing connective tissue [30], we hypothesize that the change in mechanical tension is the major factor causing focal immobilization of muscle fibers and fascicles through detachment from the connective tissue. Even if the detachment is followed by re-attachment, the newly formed attachment site is most likely a mechanically weaker fibrotic tissue. Immobilization of limbs results in muscle atrophy [12,31], impairs connective tissue structure and function[13], can negatively change muscle architecture and evoke a decline in contractility of single muscle fibers [12,32]. Additionally, inflammatory processes are associated with skeletal muscle atrophy via apoptosis and proteolysis and could further negatively influence muscle regeneration[33].

Our data suggest a rapid loss in muscle volume manifested 3 months post injury with no improvement from 3 to 6 months. The lack of any increase in muscle mass is not the response of healthy muscle tissue as retraining of intact skeletal muscle after a period of immobilization reverses negative adaptations to unloading[31], and even low loads elicit increases in muscle cross-sectional area and adaptations at the fascicle level [34,35]. We interpret the lack of adaptation as persistent tissue damage, which is further supported by the persistent and increased tissue perfusion indicative of an inflammatory processes 6 months post injury. It is important to state that functional muscle innervation through neuromuscular junctions (NMJs) is indispensable for muscle structure and function. A deficit in nerve innervation would lead to muscle atrophy[36] and might therefore contribute to the observed muscle volume loss. However, denervation causes a decrease in capillaries and leads to de-vascularization[37], yet tissue perfusion measured by DCE-MRI was increased rather than reduced in the injured muscle, even 6 months post injury. However, it should be noted that despite these considerations, we did not directly determine NJMs and muscle innervation in the present study. Muscle atrophy as a result to strain injuries has also been reported by others [15,38], but unlike previous reports, the present study included an intermediate time point at 3 months post-injury at which time the volume loss was already present and unchanged at the later time point. It also noteworthy that the early start of rehabilitation did not counteract the significant reduction in muscle volume.

Further, the rehabilitation onset had no impact on the microvascular flow neither acutely nor 6 months post injury, which implies that there was no effect of loading onset on perfusion indicative of inflammatory processes in the healing phase up to 6 months after the injury. It cannot be excluded that there might have been a group difference in DCE-MRI parameters at an earlier time point when the delayed therapy group was lacking behind the early therapy group in RTS. In the period between the injury and RTS, elevated tissue inflammation might have been linked to pain perception as it was reported for other tissues in the musculoskeletal system [39,40].

There was a lack of a relationship between pain and DCE-MRI, or any of the MRI parameters and RTS, which indicate that the structural and dynamic parameters assessed by MRI has poor prognostic power with respect to RTS as reported previously [41]. Similar findings were reported by Reurink et al [42], with the majority of injured athletes showing persistent fluid

accumulation even when clinically recovered. Non-resolution of edema is due to increased capillary permeability and associated with prolonged inflammation[43]. In the present study with the help of DCE-MRI, we show that the persistent edema does not seem to be a passive remnant of the acute trauma, but rather a very active process, even several months after the injury. As Reurink et al[42], we find that these reparative processes do not hamper athletes in successful RTS but it can be speculated that ongoing repair weakens the tissue and renders it more prone to re-injuries. Whether this is the case should be explored in future studies and appears relevant as DCE-MRI offers predictive value in relation to muscle atrophy after a strain injury.

Re-injury rate was low in the present study despite the persistent tissue perfusion, which we interpret as a successful rehabilitation. There might be a critical time span during which the actively regenerating tissue should not see high loads to avoid re-ruptures, but this speculative since there is a lack of studies on tissue inflammation, mechanical properties of the regenerating tissue and neural innervation after human muscle strain injury. In our data we found that peak muscle strength between the injured and the contralateral healthy muscle was similar already at the first time point (figure 4). This picture was the same for different muscle lengths and for both therapy groups (figure 4). As the participants predominantly rehabilitated the injured leg only[11], the lack of a difference between the injured and the uninjured leg most likely reflects the adaptation to the training[44,45]. Further, our rehabilitation program was designed to stimulate the tissue frequently in the first phase, and in the later stages less often, but with heavy loading. The daily loading of the injured leg was based on findings indicating that mechanical loading has immediate though short lasting effects in healing connective tissue [46]. Heavy loading evoked local changes associated with remodeling at the MTJ [47]. In addition, the longer recovery time in our study compared to that in other studies [48–50] should be viewed in relation to the very low number of re-injuries found in our study.

The loss of muscle volume did not influence the potential for gain in strength in the hamstring muscle between the 3 and 6 months post injury tests as evidenced by the recovery of the strength deficit during this time span (table 2). The strength deficits at RTS were similar for both groups and did not increase the risk for re-injuries. This seems to corroborate the fact that even more pronounced strength deficits in elite football players

does not seem to be associated with any higher risk of re-injury [51]. Functional deficits in the calf muscles were not detected in our study, which may relate chosen functional test, which does not examine maximal strength per se, but rather the ability to perform repeated concentric and eccentric calf muscle movements. The heel rise test is, however, a widely used and reliable method to examine calf muscle function[27].

Besides the considerable difference in RTS, the two groups in this study were similar in every functional and structural parameter registered in the time after the injury. This leaves open the question of what factors were affected by the delay in rehabilitation, especially in relation to subjective pain, which ultimately caused the prolonged time to RTS in the delayed therapy group. Based on the suggestion that healing of strain injuries is governed by both muscle and connective tissue repair, it is interesting to note that isometric strength exercises may have analgesic effects in patients with painful tendons [52]. A study investigating immediate active motion following tendon transfer, reported a significantly earlier pain relief as a result to the intervention [53]. The same group found a shorter rehabilitation time after tendon transfer in a group subjected to early limb loading compared to immobilization [54]. A possible association between the immediate loading of the injured tissue and persistent hypoalgesia might have reduced pain avoidance behavior, which is often described following injuries [55,56] and caused higher confidence in the early therapy group as indicated by our data. Whether the lower pain perception was driven by positive adaptations on the tissue level remains speculative.

The authors acknowledge that there are some limitations to this study. First, we did not plan on including an MRI scan at the one year follow up and we lack therefore further insight into the development of both the muscle volume and tissue perfusion. Further, this study did not include any direct measurements of connective tissue synthesis within the injured tissues and therefore, the time course and the processes involved in the connective tissue repair remain somewhat speculative. Finally, we did not assess fascicle length and potential changes over the study period and lack EMG measurements of the different muscles to examine whether innervation of agonists and antagonists change after the injury. In conclusion, this study shows that the extent of the muscle strain injury is associated with persistent muscle atrophy and suggests that tissue repair is an ongoing process even after

successful RTS. The observed structural and functional changes were unable to explain the significantly shorter time to RTS in patients with early rehabilitation onset.

## Perspective

The data in this study show that tissue remodeling and muscle atrophy take place several months after an acute muscle strain injury. This underscores the prolonged time required for connective tissue repair to allow MTJ regeneration, which should be taken into account in the decision on RTS. Further, these data indicate strength training should likely be maintained as part of rehabilitation effort even after RTS. We think it is important that future studies on human muscle strains address the question related to the time course of tissue healing, the specific tissues involved, including the connective tissue, and if inflammatory processes are associated with the risk of re-injuries. We showed that amateur athletes returned to sports faster after early rehabilitation onset, but the mechanisms behind this remains elusive. Our data proposes further detailed studies on the interplay between tissue regeneration and pain improvement.

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**Figure captions**

Figure 1. Strain injury on DCE-MRI: A) Axial grey-scale DCE-MRI of an injured hamstring, orange ring points at injury ROI, green ring at reference ROI same compartment, pink ring reference ROI antagonist (not used). B) IRE map, C) ME map, D) individual data points of IRE measured in the injury ROI ("Injury") and reference ROI ("Reference"), acutely and 6 months post injury. Circles display ratings of the early group, squares display the delayed group. E) Individual data points of ME measured in injury ROI ("Injury") and reference ROI ("Reference"), acutely and 6 months post injury. Circles display ratings of the early group, squares display the delayed group. ** indicates $p < 0.01$ across muscles and time.

Figure 2. Muscle volume change towards initial muscle volume obtained from the acute post injury scan over time. Analyzed muscles include the injured muscle, a muscle from the same compartment and from the antagonist compartment. Data represented as median ± interquartile range. * indicates $p < 0.05$.

Figure 3. Scatter plot of the correlation analysis between the DCE-MRI parameter measure of perfused volume (Nvoxel) (x-axis) and the change in muscle volume. A) Volume change from acute scan to 3 months follow up scan, B) change from acute scan to 6 months follow up scan. Data presented on a logarithmic x-axis because Nvoxel was log transformed in the analysis. Note that the graph displays parametric data and a Pearson correlation for easier interpretation, but the statistical analysis applied a non-parametric Spearman correlation.

Figure 4. Isometric peak force difference between the injured leg compared to the contralateral uninjured leg of all 42 participants. Data are presented as % changes from peak force of the uninjured leg. Knee angle of hamstring isometric peak force was 30° and

for quadriceps 70°. Results from the early group are displayed as black bars, results of the delayed group as grey bars. Lines denote mean ± SEM.

Figure 5. Heel rise test of patients with a calf strain. A) Number of successful heel rises performed three and six months post injury and B) Maximum range of motion (distance in cm) of heel rises performed three and six months post injury. Results from the early group are displayed as black circles, results of the delayed group as grey squares. Lines denote mean ± SEM.

Figure 6. Participants perception and rating of A) pain, B) Quality of life/ confidence in the injured leg, C) levels of soreness, D) number of symptoms and E) scoring of functional difficulties in performing relevant activities at three, six and twelve months post injury. Scores are shown as percentage of the maximum score (100%). Maximum score translates to pain-free, fully confident, absence of soreness, symptoms and no functional difficulties, respectively. Circles display ratings of the early group, squares display the delayed group. Data represented as mean ± SEM.

Table 1. Overview of results obtained with DCE-MRI during the acute scan and the follow up scan 6 months post injury. Data are presented as geometric means ± geometric SEM.

| | **Acute scan** | **Six months follow up** | **P value** |
|---|---|---|---|
| | **A. Early therapy (n= 16)**<br>**B. Delayed therapy (n=17)** | **A. Early therapy (n=16)**<br>**B. Delayed therapy (n=17)** | |
| **Nvoxel**<br>*Volume of highest perfused voxels/ most perfused tissue* | A. 42369 (35298-50858)<br>B. 39909 (29532-53916) | A. 14251 (10661-19049)<br>B. 19117 (13896-26298) | Group 0.7<br>Time < 0.001<br>Group*time 0.4 |
| **Initial rate of Enhancement (IRE)**<br>*Mean relative increase in signal intensity per second from enhancement onset until ME is reached* | A. 2.59 (2.34-2.88)<br>B. 2.84 (2.56-3.15) | A. 1.44 (1.33-1.55)<br>B. 1.45 (1.31-1.60) | Group 0.8<br>Time < 0.001<br>Group*time 0.9 |
| **Maximal enhancement (ME)**<br>*Highest mean signal intensity relative to the baseline intensity* | A. 1.46 (1.42-1.50)<br>B. 1.41 (1.37-1.44) | A. 1.12 (1.09-1.14)<br>B. 1.11 (1.08-1.14) | Group 0.4<br>Time < 0.001<br>Group*time 0.2 |
| **IRE*Nvoxel**<br>*Composite parameter reflecting both the volume and degree of perfusion* | A. 369.3 (312-437)<br>B. 394.4 (289-538) | A. 81.6 (60-112)<br>B. 103.2 (70-152) | Group 0.8<br>Time < 0.001<br>Group*time 0.5 |
| **ME*Nvoxel**<br>*Composite parameter reflecting both the volume and degree of perfusion* | A. 77854 (64514-93954)<br>B. 70850 (51929-96664) | A. 20342 (15259-27117)<br>B. 26782 (19121-37511) | Group 0.8<br>Time < 0.001<br>Group*time 0.4 |
| **ME*IRE** | A. 3.79 (3.35-4.29)<br>B. 3.40 (3.35-4.5) | A. 1.60 (1.45-1.77)<br>B. 1.60 (1.41-1.82) | Group 0.9<br>Time < 0.001<br>Group*time 0.7 |

Table 2. Isokinetic peak torque of the injured and the uninjured contralateral leg of participants with a hamstring injury. Data are presented as means (SD). ***** indicates a difference between the injured and the uninjured leg across groups, p< 0.05, **$** indicates a difference between the groups across legs,p< 0.05.

| | **13weeks post injury** | | | | **26weeks post injury** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Early therapy group** | | **Delayed therapy group** | | **Early therapy group** | | **Delayed therapy group** | |
| **Hamstring con** | **Injured** | **Uninjured** | **Injured** | **Uninjured** | **Injured** | **Uninjured** | **Injured** | **Uninjured** |
| **Peak torque** | 122.1 (25)***** | 134.7 (23) | 106.7 (32)***** | 125.4 (39) | 133.6 (36) | 134.5 (21) | 114.5 (22) | 123.4 (34) |
| **Angle max** | 32.2 (11) | 31.8 (6) | 31.3 (13) | 35.3 (15) | 29.3 (7) | 28.1 (4) | 27.8 (7) | 29.5 (8) |
| **Hamstrings ecc** | **Injured** | **Uninjured** | **Injured** | **Uninjured** | **Injured** | **Uninjured** | **Injured** | **Uninjured** |
| **Peak torque** | 177.2 (31)***** | 185.3 (30) | 158.0 (41)***** | 165.8 (34) | 192.2 (36) | 186.5 (29) | 161.3 (37) | 165.7 (47) |
| **Angle max** | 22.4 (10) | 18.5 (7) | 25.3 (7) | 26.0 (13) | 23.4 (11) | 23.0 (13) | 18.0 (5) | 24.2 (12) |
| **Quadriceps con** | **Injured** | **Uninjured** | **Injured** | **Uninjured** | **Injured** | **Uninjured** | **Injured** | **Uninjured** |
| **Peak torque** | 247.3 (36) | 247.8 (50) | 201.9 (40)$^{\$}$ | 211.1 (47)$^{\$}$ | 247.2 (51) | 249.0 (55) | 205.0 (44) | 211.7 (41) |
| **Angle max** | 65.8 (5) | 64.9 (4) | 67.0 (7) | 66.1 (8) | 68.0 (5) | 62.3 (8) | 65.0 (6) | 65.8 (7) |

| **Quadriceps ecc** | **Injured** | **Uninjured** | **Injured** | **Uninjured** | **Injured** | **Uninjured** | **Injured** | **Uninjured** |
|---|---|---|---|---|---|---|---|---|
| **Peak torque** | 295.2 (37) | 273.5 (81) | 239.3 (81) | 244.7 (55) | 308.2 (51) | 289.9 (76) | 243.5 (48) | 254.1 (60) |
| **Angle max** | 61.6 (7) | 60.1 (7) | 57.5 (11) | 63.9 (11) | 60.4 (8) | 60.9 (8) | 61.9 (10) | 60.2 (8) |

Figure 1.

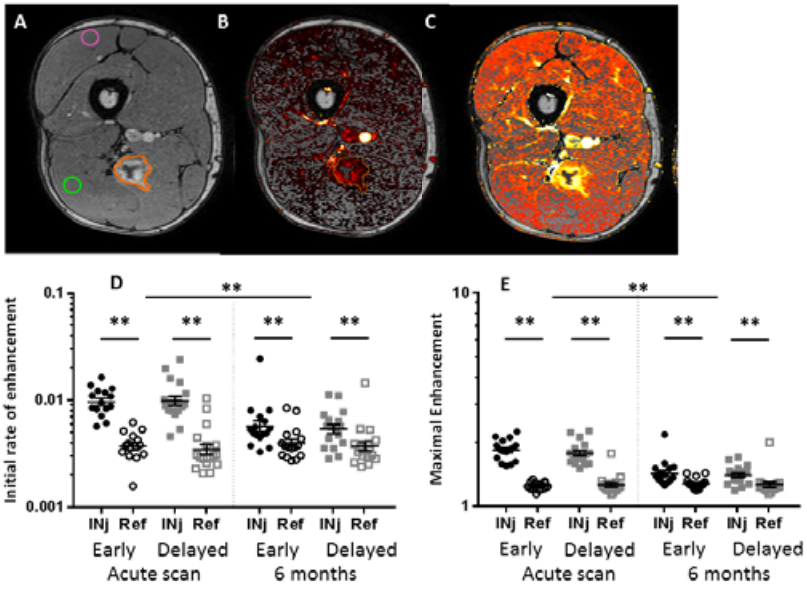

Figure 2.

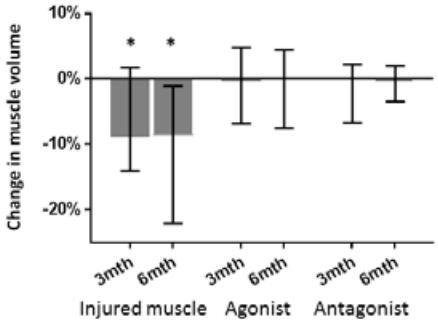

Figure 3.

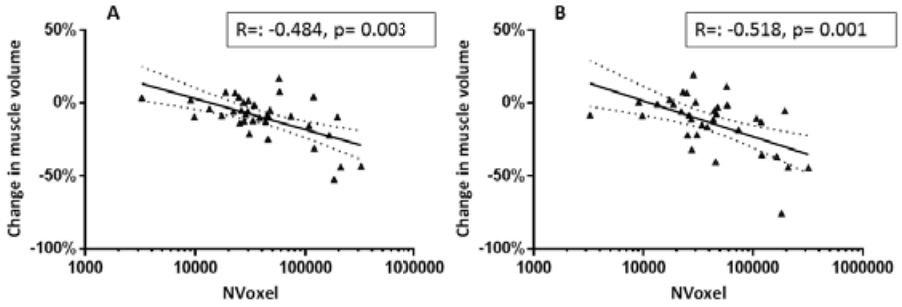

Figure 4.

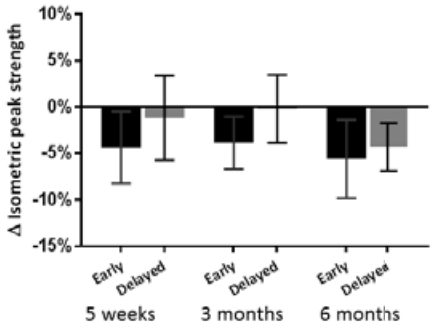

Figure 5