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Skeletal muscle adaptations to physical inactivity and subsequent retraining in young men.

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Abstract

Skeletal muscle structure and function are markedly affected by chronic disuse. With unloading, muscle mass is lost at rate of about 0.4%/day but little is known about the recovery of muscle mass and strength following disuse. Here we report an extensive data set describing in detail skeletal muscle adaptations in structure and function in response to both disuse and retraining.

Eight young men (23±2.2 yrs) underwent 3 weeks of unilateral lower limb suspension (ULLS) followed by a 3-week resistance training recovery program. Knee extensor (KE) isometric torque, voluntary activation (VA), quadriceps femoris (QF) muscle volume (QF_{vol}), fascicle length (Lf) and pennation angle (θ), physiological cross-sectional area (PCSA) of all four heads of the QF muscle, were measured before, after ULLS, and post-ULLS-resistance training. Needle biopsies were taken from the vastus lateralis muscle of a subgroup (n=6) of the same subjects and cross sectional area of individual muscle s and myosin content of muscle samples were determined.

Following 3 weeks of ULLS, isometric torque decreased by 26%, PCSA by 3%, QF_{vol} by 10%. Lf and θ of all four heads of QF significantly decreased ($P \leq 0.05$). Following the 3-week retraining period, isometric torque, PCSA, QF_{vol}, Lf and θ of all four heads of QF were all fully restored to pre ULLS values. CSA of individual muscle fibres and myosin content of muscle samples decreased by 26% and 35% respectively (post-ULLS) and recovered to almost pre-ULLS values following retraining. There were no significant changes in voluntary activation of the quadriceps muscles in response to either ULLS or subsequent retraining. These results indicate that, 1) the loss of muscle force with 3-week unloading in humans is mostly explained by muscle atrophy and by a decrease in myosin content and, 2) all the neuromuscular changes induced by this model of disuse can be fully restored after a resistance training intervention of equal duration.

Keywords
Disuse, Skeletal muscle, atrophy, recovery, resistance training

Introduction

Deficits in skeletal muscle mass and function are primary outcomes of chronic unloading, with antigravity muscles such as the knee extensors (KE) being particularly affected (Narici and De Boer 2011). An extensive knowledge of these adverse conditions is paramount, for they are inevitably linked to clinical situations such as prolonged best rest or the use of crutches due to bone fracture or musculoskeletal injury. Several models including bed rest, limb cast immobilization and unilateral lower limb suspension (ULLS) have been used to investigate the effects and recovery from disuse (Adams et al., 2003, de Boer et al. 2007). ULLS mimics the standard clinical practice of joint unloading commonly practiced following musculoskeletal injuries or surgery. In addition, this model offers the advantage of confining atrophy to the musculoskeletal system of the unloaded limb. Reported reductions in KE torque in response to ULLS range 15% to 21 % for durations of 14 to 28 days, 19% for mid-term, 15-28 days, and 21% for long-term, 28+ days, ULLS (Narici and De Boer, 2011).

Reported decreases in muscle size, represented by both anatomical cross sectional area (ACSA) and muscle volume of the quadriceps femoris muscle, average about 10% following 10 days to 5 weeks of ULLS, or approximately 2.6% per week (Narici and De Boer, 2011). The disuse-induced loss of muscle strength far exceeds that of muscle mass, indicating the contribution of other factors, such as neural drive, changes in muscle architecture (De Boer et al. 2007) and a decrease in single fibre specific tension (D'Antona et al., 2003).

Because muscle architecture is a main determinant of muscle function (Cutts, 1988, Gans and Bock, 1965, Lieber and Friden, 2001), changes in fascicle length (L_f) and pennation angle (θ) represent powerful indicators of alterations in functional deficit induced by disuse (Narici and Cerretelli, 1998). Pennation, a key-parameter of muscle architecture, is a natural strategy to pack more contractile elements in to a given muscle volume. A decrease in pennation angle (θ), due to muscle atrophy, is commonly interpreted as a decrease in the number of fascicles in parallel (Kawakami et al., 1993). Reductions in θ seen after only short durations of ULLS or bed rest (~8-13% in the vastus lateralis muscle after 23 to 35 days, (de Boer et al. 2007 & 2008) indicate that substantial muscle remodelling occurs with few days of muscle unloading.

In line with the decrease in pennation angle, a reduction in muscle fascicle length of ~8% was also found after 23 days ULLS (de Boer et al., 2007). Such a decrease implies a loss of sarcomeres in series and a consequential increase in sarcomere excursion during shortening of the whole muscle, shifting the operating muscle length towards a less favourable portion of its length-tension curve. Moreover, a reduction in the number of sarcomere in-sees also predicts a decrease in maximum shortening velocity. Hence, beyond gross atrophy, disused muscles are affected by subtle, yet substantial, architectural alterations, resulting in losses of force, velocity and power. Information regarding the reversibility of these changes during active recovery is currently lacking.

Neural drive and muscle activation capacity appear to be reduced by periods of prolonged disuse, as reflected by the decreased ability of muscles to fully activate during a maximal voluntary contraction. Bed rest studies have shown a decrease in the root mean square (RMS) of EMG of 6.5% after 20 days (Kawakami et al., 2001) and 19% after 42 days (Edgerton et al., 2001). Nevertheless, the contribution of reduced neural drive to the loss of muscle strength associated with disuse seems still an unresolved issue as several studies showed no change in maximal EMG activity in both vastus lateralis (de Boer et al., 2007) and plantar flexor muscles (Seynnes et al.) after ULLS, whilst others (Clark et al. 2006a) showed a 30% decrease in compound muscle action potential (CMAP).

Despite the importance of effective recovery strategies in clinical settings, little is known about the resilience of muscle functional properties following periods of disuse (Hanson et al., 2010). Disuse atrophy resulting from short periods of immobilization, ~10 days seems to be reversed rather quickly through passive recovery i.e. returning to normal weight bearing. However, without rehabilitation program full recovery from longer periods of disuse seems to require longer durations. For instance, (Berg and Tesch, 1996) reported a 13% decrease in KE torque following 10 days of

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ULLS, which returned to baseline levels after only 4 days of passive recovery. In a longer ULLS study which lasted 4 weeks and resulted in a 22% decrease in KE strength, muscle torque remained 11% below baseline levels after 4 days of passive recovery, eventually returning to normal 7 weeks following the ULLS period (Berg et al., 1991). Similarly, another study of 2-week immobilization showed that the KE torque could return to pre-ULLS levels within 4 weeks of active recovery (Suetta et al., 2009). The results of this study are noteworthy for they imply that early active recovery may not only improve patient functional status but is likely have a greater impact on quality of life, hereby reducing post-treatment hospitalisation times.

Here we report an extensive data set describing in detail skeletal muscle adaptations in structure and function in response to both disuse and active recovery. Knee extensor (KE) isometric and dynamic torque, voluntary activation (VA), contractile properties (Mwave and twitch response), volume (QF_{vol}), fascicle length (Lf) and pennation angle (θ) of all 4 heads of the quadriceps femoris were measured before and after 3 weeks ULLS and after 3 weeks active recovery. We hypothesised that substantial remodelling of skeletal muscle architecture would be induced by the 3 weeks of unloading and, unlike passive recovery, a rehabilitation program of equal duration would effectively restore all the muscle structural and functional parameters affected during disuse.

METHODS
Participants

Sixteen young men aged 18-25 yrs agreed to take part in a three-week ULLS program followed by a three-week resistance training program. Participants received supplements daily during the ULLS period and after every training session during recovery. Eight participants completed the three-week suspension period, followed by three weeks of active recovery. An additional 8 participants were recruited to act as ambulatory controls, with the same testing procedures three weeks apart, without ULLS. All participants signed a written informed consent and the study was approved by the Manchester Metropolitan Faculty of Science and Engineering Ethics Committee

Unilateral Lower Limb Suspension procedure. Participants underwent three weeks of disuse with the unilateral lower limb suspension (ULLS) model (Berg et al., 1991). Briefly, the left limb was fitted with a raised sole (8cm) to enable the right knee joint (suspended limb) to remain in a near extended position. The right limb was suspended above the ground by using a sling, maintaining the ankle joint at 0˚, with the foot at right angle to the tibia. Participants used the shoes, crutches and sling for the duration of the three week suspension period and were instructed to refrain from loading the right limb in any way, including driving vehicles.

Resistance training protocol. Following the ULLS, participants attended the laboratory three days per week for a period of three weeks to undergo a resistance training (RT) program. Quadriceps extensions were performed at 80% of participants' one repetition maximum (1RM). The 1RM was assessed weekly and the resistance level was adjusted accordingly. Participants first warmed up with a series of sub maximal contractions, after which the resistance was increased to find the maximum. The training load was set at 80% of 1RM, and three sets of ten contractions were performed during each training session.

Muscle biopsies. Using a procedure previously described (Bergstrom, 1979, Bottinelli et al., 1996), three needle biopsies were taken from vastus lateralis muscle of a subset of 6 subjects subjects : one pre suspension (pre ULLS), one post suspension (post ULLS), one post exercise recovery (post RT). Muscle biopsies were divided in several portions, and a tissue sharing approach among several laboratories collaborating in the MYOAGE consortium was used to optimize, for ethical and economic reasons, the burden on the volunteers. Two small (10 mg) portions were used in the present work. One

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was stored at -20 °C degrees in a skinning solution plus glycerol (50% v/v) and used for single fibre dissection and determination of CSA and myosin concentration. One was frozen in liquid nitrogen and stored at -80 °C for determination of myosin content.

Single fibre CSA measurement. From samples stored at -20°C degrees, 10 fibre per biopsy were dissected in a muscle chamber containing skinning solution (K-p 150 mM, KH2PO4 5mM, MgAc 5 mM, DTT 1 mM, EGTA 5 mM, Na2-ATP 3 mM, pH 7, Leupeptine Hydrochloride 20µg/ml, E64 10 µM). Each fibre was then mounted between two hooks on a stage of an inverted microscope. The width and the depth of every fibre was determined at 320x magnification on 10 different positions on the fibre, rotating the hooks. Assuming fibre section as an ellipse the CSA was calculated.

Myosin content. Muscle samples stored at -80 °C were pulverized with liquid nitrogen in order to obtain a powder that was immediately suspended in lysis buffer (50 mM Tris-HCl pH 7.6, 250 mM NaCl, 5mM EDTA, 1.5% v/v β-mercaptoethanol and 2% inhibitor-proteases cocktail by Sygma-Aldrich). A protein assay kit (RC DC Biorad) was used to determine protein concentration. For each subject group (pre ULLS, post ULLS, post RT) a sample mix was obtained putting together an equal quantity of protein taken from each sample. 15 µm of sample mix were loaded in triplicate into a precast gradient gel (AnykD, Biorad). The gel run 1 h at room temperature at 100 V and then it was stained with Coomassie Blue and acquired with a high resolution scanner (EPSON expression 1680 Pro). The brightness–area product (BAP) of myosin band of each sample was measured using the software Adobe Photoshop CS3 (Adobe).

Ultrasonography. Ultrasound scanning was used to assess muscle architecture at rest and during maximum voluntary contraction (MVC). With the subject lying in the supine position, images of muscle architecture (pennation angle θ and fascicle length Lf) at rest of all four heads of the quadriceps femoris were recorded. Scans were taken distally, at distance corresponding to 40% of femur length (rectus femoris scans were taken more proximally, at 50% of femur length). The final position and orientation of the probe at the scanning site were recorded on an acetate sheet using moles and angiomas as markers to ensure all subsequent scans were taken at the same anatomical location.
Muscles were scanned during MVC with the subjects sitting in the dynamometer (see position description below). Ultrasound video clips were recorded during slow (~4s) ramp contractions.
Images were analysed offline to assess muscle architecture. Muscle thickness was defined as the shortest distance between the superficial and deep aponeurosis. Pennation angle was measured as the angle between fascicles and the deep aponeurosis and fascicle length was measured by outlining the course fascicles between aponeuroses. For the latter, a small portion of the fascicles (10-25%) was not visible within the width of the ultrasound probe and was extrapolated as a straight line (Seynnes AP 2008). An average of three measurements was calculated for each architectural parameter and retained as the final value.

Muscle volume. Quadriceps muscle volume was measured using a 0.2 Tesla MRI scanner (E-Scan, Esaote Biomedica, Genova, Italy). Axial plane scans were acquired using a Turbo 3D T1 sequence with a slice thickness of 3.1mm. Oil-filled capsules were secured to the skin along the length of the thigh as external markers. Scans were performed while participants were lying in supine position with the knee fully extended.

Maximum Voluntary Contraction (MVC). Maximum knee extension and knee flexion torque were measured using an isokinetic dynamometer (Cybex Norm, Cybex International Inc., NY, USA) at three angles of knee flexion, 70˚, 80˚ and 90˚, with full knee extension corresponding to 0˚ flexion. Participants were seated in the dynamometer chair and secured into position using straps over the hip, so that this joint was maintained at an angle of 85˚ (full hip extension corresponding to 0˚). The centre rotation of the knee joint (right limb) was aligned to the centre of rotation of the dynamometer motor

using a laser pointer. Participants performed two maximal knee extension contractions and one knee flexion contraction at each angle, in a randomised order. Contractions lasted for 3-4 seconds. Particiants were provided with real-time visual feedback during MVC's by way of the trace produced by the dynamometers computer.

Electromyography. Surface EMG of the Vastus Lateralis muscle (VL) and biceps femoris (BF) were recorded throughout the functional muscle test performed on the dynamometer. Skin was shaved and cleaned with an abrasive gel and alcohol swabs to ensure inter-electrode impedance was below 5kΩ. Adhesive recording electrodes (REF) were placed between the innervation zones and the myotendinous junction and their position was recorded on acetate sheets for subsequent testing sessions. The reference electrode was placed over the lateral femoral condyle. The raw EMG signal was acquired with a sampling frequency of 2000 Hz and processed with a multi channel analogue-digital converter (Biopac EMG 100B Systems, USA). The raw signal was filtered using analogue high (10 Hz and) and low pass (500 Hz) filters, and was amplified with a gain of 2000, before being corrected for offset. Root mean square (RMS) was calculated over 500 ms around the peak isometric torque. Inter-individual and between testing variability in skin impedance and electrode positioning was accounted for by normalising VL RMS to the Mwave (see below). The BF co-activation level during isometric contraction of the knee flexors was estimated from the recorded RMS of this muscle when acting as an antagonist and as an agonist and was used to calculate net KE torque.

Maximal Voluntary Activation. Quadriceps activation was assessed with the twitch interpolation technique using electrical stimulation applied to the femoral nerve (model DS7, Digitimer stimulator, Welwyn, Garden City, UK). A hand-held monopolar cathode (0.5 cm diameter) was placed in the femoral triangle, where the femoral nerve is relatively superficial and easily accessible. The exact placing of the cathode electrode was determined by applying 50 mA stimulations. The anode (76 by 127 mm, Versa-Stim, Conmed) was placed on the gluteal fold. The position of the cathode electrode was chosen as the location where the highest M wave and twitch torque could be recorded in response to 50mA stimulations. Stimulation intensity was then increased until supramaximal level was reached, as evidenced by a plateau in twitch torque amplitude. Four maximal M waves were then recorded for analysis. Finally, twin-twitches (400 V) were administered during MVC and at rest following MVC and the ratio of these twitches was used as an index of activation capacity.

Statistics
The normal distribution of all data sets was verified with a Shapiro-Wilks test. Student's *t* tests for independent samples were applied to compare baseline values between the suspended and control groups. To determine the effect of the suspension period on the investigated variables, a one-way ANOVA for repeated measures was conducted with a Bonferroni adjustment for post hoc analysis. Statistical significance was set as α =0.05. Unless stated otherwise, results are means ± standard error of the mean.

Results
Statistical tests performed on baseline measurements showed no difference between the ULLS and control groups in any of the variables measured.

Muscle strength and activation.
Maximum voluntary contraction of MVC decreased by 26% following 3 weeks of ULLS (p<0.005), but increased during active recovery to give post-RT strength levels just 2.5% below baseline values (n.s. from baseline). Voluntary activation decreased by 5.1% during ULLS but this decline did not reach significance. However, a mirrored trend was observed after active recovery, when the voluntary

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activation capacity increased by 6.6% (p<0.05), (Table 1, Fig. 1). Control subjects showed no difference in MVC from baseline to post-ULLS testing.

Muscle volume.
Whole quadriceps muscle volume decreased by 10% during ULLS (p<0.05) but muscle mass was restored following 3 weeks of active recovery, with post-RT muscle volume being 2% higher than baseline measurements (Table 2, Figure 2). At single muscle level, atrophy of the rectus femoris did not reach significance in response to suspension, -5% (n.s.). However, RF volume increased by 12% from post ULLS to post-RT values (p<0.05) to increase post RT volume by 6% above baseline (n.s.). The volume of the 3 remaining heads of the quadriceps femoris all decreased during ULLS, VL and VI by 12% (p<0.05) and VM by 9% (p<0.005), and all muscles returning to pre ULLS values following active recovery (Figure 3). A one-way ANOVA of the percentage change in muscle volume of each of the four heads of the QF showed no significant differences in the individual responses of each heads during both ULLS and RT (Figure 3). Control subjects showed no difference in QF muscle volume from baseline to post-ULLS testing.

Resting twitch characteristics. Although there was some tendency for a change in the twitch characteristics at rest in response to both ULLS and retraining, none of the differences in twitch properties were significant (Table 3).

Muscle architecture.
Resting fascicle length decreased in all four heads of the quadriceps femoris following suspension, VL by 9% (p<0.005), VM by 6% (p<0.05), VI by 11% (p<0.005) and RF by 9 % (P<0.05). This parameter was fully restored to baseline values in all four muscles, following active recovery (Table 2, Figure 4). Similarly, pennation angle at rest decreased in all four heads, in VL by 10% (p<0.005), in VI and RF by 7% (P<0.05) and by 8.6% for VM (n.s.) (Table 2, Figure 7). There was no inter-muscle difference in the magnitude of changes in Lf or θ after ULLS or subsequent resistance training. Control subjects showed no difference in Lf or θ from baseline to post-ULLS testing.

Physiological cross sectional area and specific force.
Quadriceps PSCA did not change significantly across the study, despite a 3% reduction after ULLS, and a 5% increase above baseline values after re-training. Specific force did decrease significantly in response to suspension by 24% (p<0.05) and increased 10% above baseline values (n.s.). Control subjects showed no difference in PCSA or specific force from baseline to post-ULLS testing (Table 1, Figure 2).

Single fibre cross sectional area
Fig. 8 shows the mean values of single muscle fibre cross sectional area. Single muscle fibres went through significant atrophy (-26%) following ULLS and recovered almost pre-ULLS size following retraining (Table 4).

Myosin content
Fig. 9 shows the percentage values of myosin content. Myosin content was significantly lower in post-ULLS than in pre-ULLS (-35%). After active recovery, myosin content was significantly higher than post-ULLS, although it did not reach the levels observed before suspension (Table 5).

Discussion

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Data from the current study show that significant changes in skeletal muscle structure and function occur in response to unloading, but these changes can be reversed with active recovery with muscle function fully restored 3 weeks post disuse. A previous study showed that young men displaying muscle atrophy following 2 weeks of immobilisation regained both quadriceps MVC and muscle volume to reach baseline levels after 4 weeks of resistance training (Suetta et al., 2009). Our study shows that with resistive training performed in the early post-disuse phase recovery from a longer immobilisation period is possible in a shorter recovery timescale.

Muscle structure and function. Following 3 weeks of ULLS, MVC of the knee extensors decreased by 26%, corresponding to a daily rate of loss of 1.24%. With such a dramatic decrease in force one would expect a corresponding reduction in PCSA of the quadriceps femoris muscle, however PCSA in the present study only slightly decreased by 3%. This surprisingly small reduction in PCSA is simply due to the fact that PCSA is the ratio of muscle volume to fascicle length and since in this study QF_{vol} decreased by by 10% and Lf by 9%, this produced virtually no change in PCSA. For this reason, PCSA calculated in this way seems an unreliable indicator of muscle atrophy and a poor predictor of force loss with unloading. Instead, muscle volume seems to be a more reliable indicator of muscle atrophy, and our study suggests that the significant decrease in maximum isometric torque was largely accounted for by quadriceps atrophy. This contention is also supported by the results of the analysis of the CSA of individual muscle fibres which clearly indicates fibre atrophy (-26%) as a result of the 3 week ULLS.

Because of the paradoxically small reduction in PCSA, a large decrease in quadriceps specific force, -24% was found. Although, in principle a decrease in force per cross sectional area is consistent with previous observations on unloading (Berg et al., 1997, D'Antona et al., 2003), we believe that a 24% reduction is an overestimation due to the non-significant change in PCSA. Using a more conservative approach by which specific force is calculated using anatomical rather than physiological CSA, the resulting decrease in F/CSA is 19%. The muscular origin of this loss of specific force seems confirmed by the fact that in this experiment voluntary activation did not change during the ULLS period suggesting that single fibre changes are responsible for this phenomenon. Previous studies have shown that myosin content is a main determinant of single fibre specific tension (D'Antona et al., 2003). Hence the large decrease in myosin content (-35%) observed as a result of ULLS points to a decrease in single fibre specific tension, a likely contributing factor to the loss of specific force observed at whole quadriceps level.

Other candidates that could have contributed to the decrease in whole muscle F/CSA are changes in the structural integrity of the extracellular matrix as this is thought to largely contribute to the force transmitted by the contracting fibres to the tendon (Huijing et al. 1998) and, a decrease in fascicle length. No study has yet specifically investigated the role of changes in the extracellular matrix in the loss of muscle force/CSA. However studies examining the ECM components in immobilised human skeletal muscles have shown that within 48hrs of immobilisation mRNA for matrix metalloproteinases and ECM structural components such as collagen are down-regulated (Urso et al., 2006). As the ECM is important in regulating through cell behaviour via membrane permeability and interaction with growth factors and signal transduction pathways, any alterations in ECM composition is likely to affect normal cell function.

As for the contribution of a decrease in fascicle length to the loss of force/CSA, our study showed an average 9% decrease in Lf for all four heads of the QF. This reduced muscle fascicle length represents a loss of sarcomeres in series which predicts an increase in the sarcomere excursion required to achieve a given whole muscle shortening. Therefore, assuming tendon mechanical properties remain the same, just a small decrease in Lf may cause the fibres to operate at a less optimum portion of the length-tension curve. However, a previous similar study by our group observed a decrease in patellar tendon stiffness of -29% following 23 days ULLS (de Boer, *et. al.,* 2007). A more compliant tendon should

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theoretically cause a leftward shift of the length-tension curve and may to some extent compensate for the shift due to decreased fibre length (Narici and Maganaris 2007). Therefore decreased fibre length seems an unlikely cause of the decrease in F/CSA.

Preferential atrophy

Vastus lateralis is often used as a representative muscle of how the quadriceps muscles as a whole react to various alterations in loading conditions. In the present study changes in muscle volume, Lf and θ in response to unloading and active recovery were assessed individually in all four heads of the quadriceps. Whole quadriceps muscle volume as measured *in vivo* by MRI scanning showed a decrease of 10% following 3 weeks ULLS, equating to a daily rate of loss of 0.48%. This finding is of considerable clinical value since it highlights that disuse-atrophy is an extremely fast process requiring countermeasures from the very few days of unloading. VL, VI and VM showed similar extents of atrophy. However RF muscle volume decreased by only 5% and failed to reach significance, in line with previous observations of Capodaglio & Narici (1998). This is likely explained by the fact that the RF is a bi-articular muscle crossing both the knee and hip joints. In our model of immobilisation the knee joint was kept as fixed as possible (within limits of the model) however the hip joint was free to extend and flex during normal activity. Rectus femoris' role in stabilising the hip joint and aiding in hip flexion appears to be enough to preserve muscle mass and prevent atrophy to the extent displayed by the other QF muscles which act solely on the knee joint.

Effects of active recovery

The effect of active recovery on skeletal muscle after periods of immobilisation is not widely reported upon, however data exists to support our findings that full recovery from prolonged unloading is possible after 4 weeks retraining in young men (Suetta et al., 2009) (HortobÃ¡gyi et al., 2000). Our results show that resistance training in the very early stages of recovery following periods of disuse can reverse both the structural and functional changes resulting from skeletal muscle atrophy in as little as three weeks post-immobilization. Our data on muscle volume, architecture and strength following resistance training show that muscle recovery was complete after 3 weeks of active recovery immediately following ULLS. While PCSA and specific force resulted in post RT levels which were also not significantly different from baseline, PCSA and specific force attained values slightly higher than baseline. These data show that both increased PCSA and specific force contribute to this increase of MVC in disused muscle as it does in healthy individuals undergoing resistance training programs (Erskine *et al.* 2010).

Preferential hypertrophy.

Post RT muscle volume changes with respect to baseline a show RF to appear to be more responsive to resistance training. However on closer inspection it can be seen that all 4 heads have a similar response to active recovery following disuse, however due to the fact that RF displayed less atrophy during disuse, the similar increases in muscle mass result in a higher than baseline muscle mass for RF after 3 weeks active recovery. It could be hypothesised that if training were to continue the remaining 3 muscles, VL, VI and VM would eventually approach similar post-RT to baseline increases in muscle mass as RF. The 3 week suspension period also resulted in changes in Lf and θ in line with whole QF muscle volume decrease. The difference in RF muscle atrophy was not accompanied by similar preservation on RF individual architectural parameters. In fact all four heads of the QF displayed similar adaptations in Lf and θ with no statistically differences between responses.

Voluntary activation and neural drive.

The observed changes in net KE torque could not be accounted for by changes in maximum voluntary activation or neural drive since neither VA or VL activation (EMG$_{rms/M-wave}$)changed with ULLS or subsequent retraining. These findings are in line with previous findings (de Boer et. al. 2007 & Seynnes at.al., 2010) but at odds with others (Berg. Et.al., 1997; Koryak, 2001; Reugg et.al., 2003). As

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EMG data was only recorded for VL as a representative of quadriceps femoris activation, comment cannot currently be made on the individual responses in VM, VI and RF neural activation. Similar results would have been expected for the VM and VI too, perhaps with the exception of RF which, being a bi-articular muscle, is likely to have been more activated that the other three heads of the quadriceps during the ULLS period. This hypothesis seems supported by the lesser atrophy displayed by this muscle in response to ULLS.

Muscle twitch characteristics.
Previous studies have shown a decrease in human triceps surae muscle TPT (7%) and 1/2RT (2%, n.s.), in response to plaster cast immobilisation (Davies. et.al,. 2008). However these authors found an increase in PT with immobilisation, contrary to our finding of a decrease in PT in the quadriceps muscle. An increase in PT has been attributed to a decreased reuptake of calcium by the sarcoplasmic reticulum during immobilisation leading to increased levels of Ca2+ and therefore to twitch potentiation (Thom et. al., 2001). However, significant decreases, -22% in PT of quadriceps of young individuals in response to disuse have been observed (Suetta, 2009) whilst in the same study TPT was not affected by immobilisation or retraining. The results of Suetta's study appear to be more in accordance with the results of the present study than with those of the previous two group of authors (Davies. et.al,. 2008). Whilst in our study no significant changes were observed in resting twitch characteristics, there was a trend in the decrease of the PT in response to ULLS, -12%, with PT subsequently increasing at 6 weeks post ULLS to 4.5% below baseline following resistance training protocol. Also, no significant changes in TPT were found in the present study.
The above conflicting results seem to indicate that different muscle groups respond differently to immobilisation. The lack of change in quadriceps twitch characteristics may also be due to the fact that ULLS is a less drastic method of disuse than immobilisation. For Instance this difference is reflected by more pronounced spinal and supra-spinal neural adaptations induced by immobilisation when compared to ULLS (Clark, 2009).
The lack of significant changes in muscle twitch characteristics found in the present study seems consistent with the absence of changes in VL myosin heavy chain composition in the participants of this ULLS study (Bottinelli, personal communication 2012).

Implications of the findings for the recovery of muscle in young and older individuals following period of disuse
The results of this work strongly suggest that early rehabilitation is an effective strategy for recovering skeletal muscle mass and function following a period of immobilisation in young individuals. The fast recuperation of muscle mass observed with RT suggests that overloading of skeletal muscle of young individuals during the recovery period, quickly overcomes the anabolic blunting of protein synthesis caused by inactivity (Glover *et al*. 2008). This fast recovery of muscle mass seems also due to the 'muscle memory' phenomenon, described by Brudsgaard et al (2010), which shows that myonuclei of muscle fibres are preserved during a period of inactivity and are thus readily available for supporting fibre hypertrophy when muscle is reloaded again (Bruusgaard *et al*. 2010). However the same does not appear to hold true for older individuals. A previous study has shown that after 2 weeks ULLS young individuals restored MVC, muscle volume and architecture to baseline levels by following a resistance training protocol for 4 weeks. Older individuals following the same unloading procedures and training protocol did regain pre-immobilization MVC levels however muscle volume and architecture did not fully recover (Suetta *et. al.,* 2009). The reduced capacity for skeletal muscle recovery from disuse atrophy observed in older individuals may be attributed to a combination of age related changes in skeletal muscle physiology. In particular, a decrease in the anabolic response of aged skeletal muscle to both resistance exercise and protein availability (Drummond *et. al*., 2008) could explain the slower recovery of older individuals from disuse atrophy. In addition, it is thought that older individuals not only have a decrease number of satellite cells, but that the remaining pools of satellite cells exhibit reduced activation capacity (Gallegly *et. al.,* 2004).

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With periods of immobilisation more common in the elderly population due to illness and injury, further research is necessary to fully understand the observed reduced plasticity of aged skeletal muscle and the exploration of potential countermeasures.

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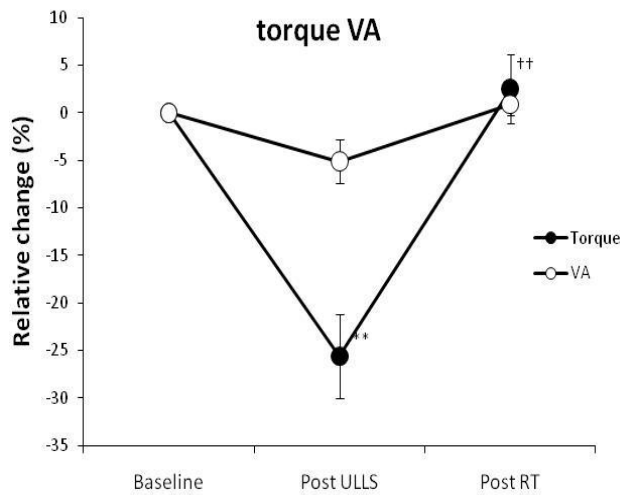
Figures

Figure 1. Percentage change in MVC torque and voluntary activation (VA) capacity of the quadriceps femoris at baseline, following 3 weeks suspension and after 3 weeks subsequent active recovery. Data are means ± SEM. No changes in VA were statistically significant. ** Statistically significant from baseline $P<0.005$, †† significantly different from post-ULLS values $P<0.005$
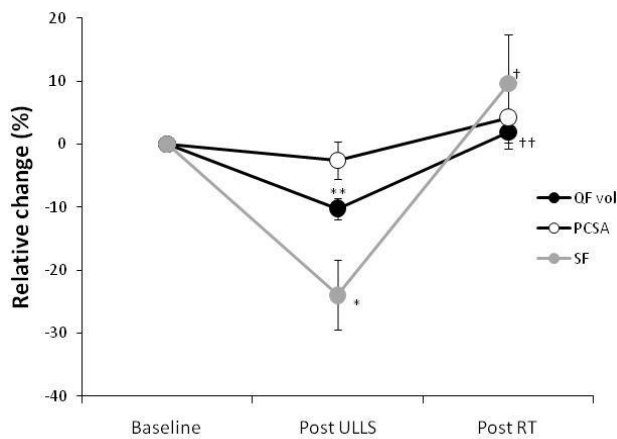

Figure 2. Relative change from baseline of whole QF volume, PCSA and specific force following 3 weeks suspension and after 3 weeks subsequent resistance training. Data are means ± SEM. No changes in PCSA were statistically significant. *Statistically significant from baseline $P<0.05$, ** statistically significant from baseline $P<0.005$, †statistically significant from post-ULLS $P<0.05$, ††statistically significant from post-ULLS $P<0.005$.
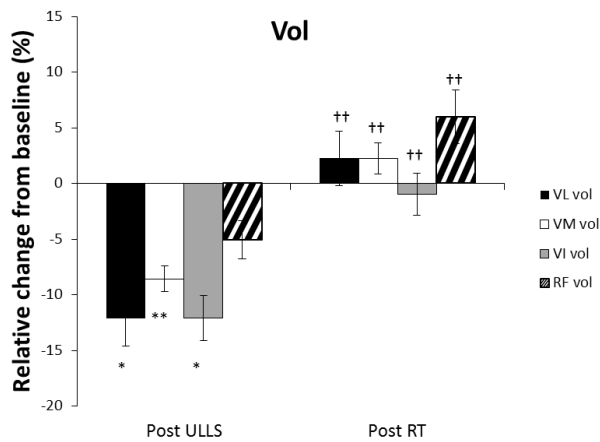
Figure 3. Relative change from baseline of muscle volume of the all 4 heads of quadriceps following 3 weeks suspension and after 3 weeks subsequent active recovery. Data are means ± SEM. *Statistically significant from baseline P<0.05, ** statistically significant from baseline P<0.005, † statistically significant from post-ULLS P<0.05, †† statistically significant from post-ULLS P<0.005.
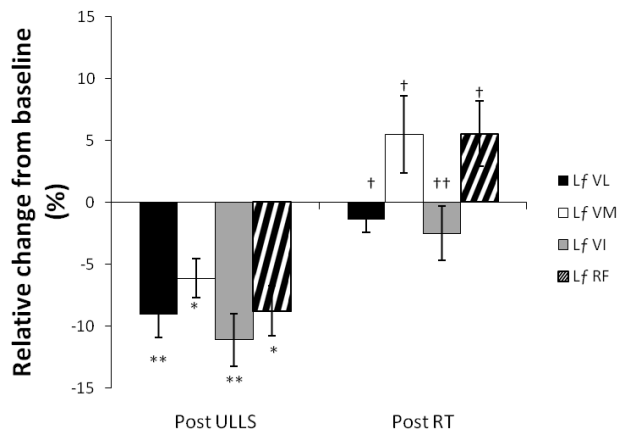

Figure 4. Relative change from baseline of fascicle length of the all 4 heads of quadriceps following 3 weeks suspension and after 3 weeks subsequent active recovery. Data are means ± SEM. *Statistically significant from baseline P<0.05, ** statistically significant from baseline P<0.005, † statistically significant from post-ULLS P<0.05, †† statistically significant from post-ULLS P<0.005
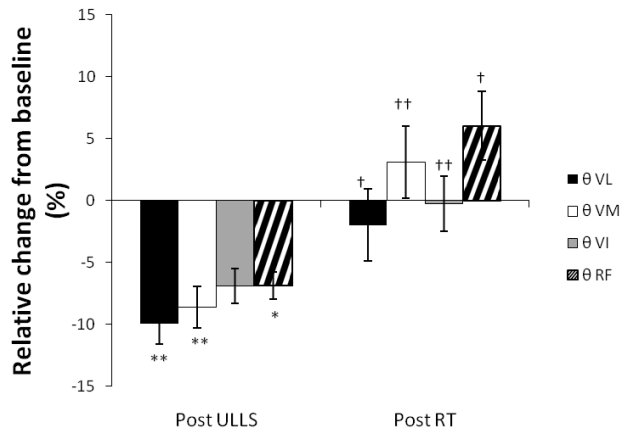
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Figure 7. Relative change from baseline of fascicle pennation angle of the all 4 heads of quadriceps following 3 weeks suspension and after 3 weeks subsequent active recovery. Data are means ± SEM. *Statistically significant from baseline P<0.05, ** statistically significant from baseline P<0.005, † statistically significant from post-ULLS P<0.05, †† statistically significant from post-ULLS P<0.005
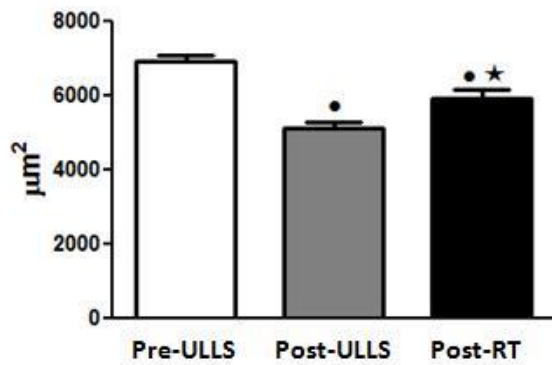

Figure 8. Single fibre CSA of vastus lateralis fibres following 3 weeks suspension (Post ULLS) and after 3 weeks subsequent resistance training (ULLS Ex). Data are means ± SEM. ● Statistically significant from Pre ULLS P<0.05, ★ statistically significant from Post ULLS P<0.05.
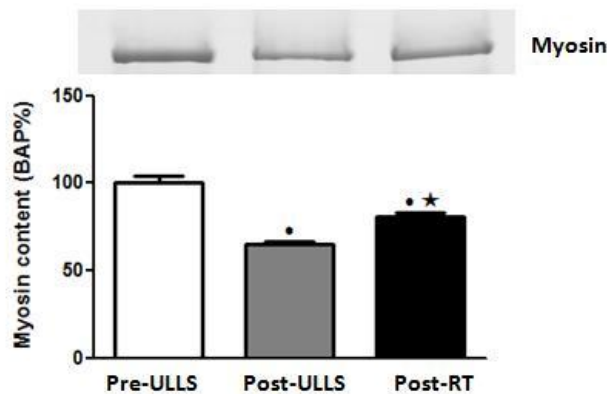

Figure 9. Percentage values of myosin content following 3 weeks suspension and after 3 weeks subsequent resistance training. Data are means ± SEM. ● Statistically significant from Pre ULLS P<0.05, ★ statistically significant from Post ULLS P<0.05.

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Tables

Table 1 Quadriceps MVC, voluntary activation capacity, PCSA and specific force and baseline, post-ULLS and post-RT. Data are means±SEM *significantly different from baseline p<0.05 **significantly different from baseline p<0.005 †significantly different from post-ULLS p<0.05 ††significantly different from post-ULLS P<0.005

	ULLS (n=8)			Control (n=8)	
	Baseline	3 wks Post-ULLS	6 wks Post-RT	Pre	Post
MVC	299±14	221±14**	291±14††	311±21	294±26
VA	87±3.5	83±3.5	88±3	--	--
EMG$_{RMS/M-wave}$	0.073±0.02	0.0614±0.02	0.073±0.02	0.070±0.01	0.070±0.01
PCSA	208±6	203±8	218±14	199.98±18.0	202.66±21.7
SF	32±2	24±1*	35±3†	38.0±4.4	37.1±6.3

Table 2 Quadriceps muscle volume at baseline, post-ULLS and post-RT. Data are means±SEM *significantly different from baseline p<0.05 **significantly different from baseline p<0.005 †significantly different from post-ULLS p<0.05 ††significantly different from post-RT P<0.005

	ULLS (n=8)			Control (n=8)	
	Baseline	3 wks Post-ULLS	6wks Post-RT	Pre	Post
Volume (cm3)					
QF	1983±116	1771±79**	2021±122††	1835.2±186.4	1870.5±192.3
VL	663±32	585±16*	675±29†	633.8±68.7	643.2±73.3
VM	493±10	449±23**	503±30††	431.4±40.1	443.7±45.7
VI	533±50	466±40**	531±53†	495.8±49.8	508.2±42.1
Lf					
VL	8.4±0.2	7.7±0.3**	8.3±0.5†	7.2±0.5	7.8±0.4
VM	9.3±0.5	8.6±0.4*	9.7±0.4†	9.4±0.7	9.4±0.6
VI	11.0±0.5	9.8±0.4**	10.7±0.4††	7.4±0.2	7.9±0.5
RF	8.1±0.3	7.4±0.3*	8.6±0.4†	8.5±0.5	8.7±0.6
θ					
VL	12.8±0.6	11.5±0.5**	12.5±0.6†	17.1±0.5	16.5±1.0
VM	15±1.0	13.7±0.6*	15.4±0.9†	16.3±1.8	16.3±0.6
VI	11.3±0.8	10.5±0.8**	11.2±0.8††	8.2±1.0	7.8±1.0
RF	15.1±1.4	14.1±1.3	15.9±1.3†	8.2±1.1	7.8±1.1

Table 3 Resting twitch characteristics at baseline, post-ULLS and post-RT. Data are means±SEM all data are not statistically significant.

	Baseline	3 wks Post-ULLS	6 wksPost-RT
PTw	66±4	58±6	63±4
TPT	58±2	62±2	60±1
1/2RT	71±6	78±9	72±4

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dT/dt	1.15±0.1	0.96±0.1	1.06±0.1
Tw/MVC	0.23±0.02	0.27±0.03	0.22±0.02

Table 4 Vastus lateralis single fibre CSA, Pre-ULLS, Post-ULLS and Post-RT.

	ULLS (n=6)		
CSA (µm2)	Mean	SD	n
Pre-ULLS	6924.493	1788.739	115
Post-ULLS	5145.383	1441.211	118
Post-RT	5919.379	2461.265	113

Table 5 Percentage values of Myosin content of vastus lateralis fibres, Pre-ULLS, Post-ULLS and Post-RT.

	ULLS (n=6)		
	Mean	DS	n
Pre-ULLS	100	7.39053	3
Post-ULLS	65.088760	2.711583	3
Post-RT	80.473370	4.467358	3