Effects of aging on human skeletal muscle after immobilization and retraining


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Suetta C, Hvid LG, Justesen L, Christensen U, Neergaard K, Simonsen L, Ortenblad N, Magnusson SP, Kjaer M, Aagaard P. Effects of aging on human skeletal muscle after immobilization and retraining. J Appl Physiol 107: 1172–1180, 2009. First published August 6, 2009; doi:10.1152/japplphysiol.00290.2009.—Inactivity is a recognized compounding factor in sarcopenia and muscle weakness in old age. However, while the negative effects of unloading on skeletal muscle in young individuals are well elucidated, little is known about the consequence of immobilization and the regenerative capacity in elderly individuals. Thus the aim of this study was to examine the effect of aging on changes in muscle contractile properties, specific force, and muscle mass characteristics in 9 old (61–74 yr) and 11 young men (21–27 yr) after 2 wk of immobilization and 4 wk of retraining. Both young and old experienced decreases in maximal muscle strength, resting twitch peak torque and twitch rate of force development, quadriceps muscle volume, pennation angle, and specific force after 2 wk of immobilization (P < 0.05). The decline in quadriceps volume and pennation angle was smaller in old compared with young (P < 0.05). In contrast, only old men experienced a decrease in quadriceps activation. After retraining, both young and old regained their initial muscle strength, but old had smaller gains in quadriceps volume compared with young, and pennation angle increased in young only (P < 0.05). The present study is the first to demonstrate that aging alters the neuromuscular response to short-term disuse and recovery in humans. Notably, immobilization had a greater impact on neuronal motor function in old individuals, while young individuals were more affected at the muscle level. In addition, old individuals showed an attenuated response to retraining after immobilization compared with young individuals.

unloading; disuse; recovery; sarcopenia

THE LOSS OF MUSCLE MASS WITH aging, i.e., sarcopenia, and the concomitant decline in muscle strength are associated with increased disability and mortality (36, 50). In addition, elderly individuals are more prone to periods of bed rest due to a higher degree of comorbidity and hospitalization (47), which, per se, result in a rapid and accelerated loss of skeletal muscle mass (34, 55). Despite this, very little is known about the physiological consequences of unloading on muscle mass and neuromuscular function in the elderly, while even less is known about the regenerative capacity of skeletal muscle in the elderly human being.

The negative effects of unloading on skeletal muscle in young individuals are well elucidated (8, 18, 45). Furthermore, chronic disuse in old individuals seems to accelerate the age-related decrease in the contractile capacity of the quadriceps muscle (69), as well as in single muscle fibers (17). However, only very few studies have investigated the effects of immobilization in old compared with young humans (21, 72), and, so far, none have addressed the atrophy response to unloading of weight-bearing muscles in aging individuals. Thus the present knowledge is primarily based on animal data, where hindlimb suspension (HS) has been used as a model of muscle unloading to investigate the underlying mechanisms associated with disuse muscle atrophy in aging (3, 4, 9–11, 13, 19, 20, 22). However, the data obtained by HS in young vs. old animals are somewhat inconsistent. The majority of studies have reported young animals to be more affected by HS (13, 59), while others find a similar degree of muscle atrophy between young and old animals (67) or even greater magnitude of muscle atrophy in old animals following HS (20). Further, there are substantial indications that the muscle tissue of old animals demonstrates an attenuated recovery response after immobilization and injury (12, 19, 66). Although it is evident that aging leads to a multitude of changes in the neuromuscular system that are similar to those evoked by unloading (73), the lack of research into the effect of unloading in elderly humans makes it difficult to ascertain what effects can be attributed to a decreased physical activity per se and which to the aging process, as such.

The purpose of the present study was to investigate the effects of unilateral lower limb immobilization and subsequent retraining on muscle mass, muscle architecture, neuromuscular activation, and resting twitch characteristics in young and aged human individuals. By assessing these changes, we also aimed to study the potential interaction between changes in muscle contractile properties, specific force (Spforce), and muscle mass characteristics after immobilization, and also to examine the regenerative capacity of old compared with young individuals. Based on the literature, it was difficult to hypothesize if aging would affect the response to disuse muscle atrophy; however, due to the more consistent data regarding the capacity for regrowth in aging muscle, we hypothesized that old individuals would display an attenuated response to subsequent retraining.

METHODS

Subjects and Study Design

Twenty healthy men, 9 old (OM: 67.3 yr, range 61–74 yr) and 11 young (YM: 24.4 yr, range 21–27 yr), volunteered to participate in the study. Before inclusion, subjects were screened by a physician to exclude subjects with cardiovascular disease, diabetes, neural- or musculoskeletal disease, inflammatory or pulmonary disorders, and any known predisposition to deep venous thrombosis. Only healthy, nonmedicated individuals were included in the study. Physical activity during work and leisure time was graded in four levels based on...
questionnaire assessment (65). All subjects were moderately active (OM: 5.2 ± 1.4 h/wk, YM: 5.0 ± 0.9 h/wk) with no difference between groups, and none of the subjects had previously participated in systematic strength training. The local Ethics Committee approved the conditions of the study (KF01–322606), and all experimental procedures were performed in accordance with the Declaration of Helsinki. Written, informed consent was obtained from all participants before inclusion in the study. After separate familiarization trials and baseline test procedures, all subjects were subjected to unilateral (randomly selected limb) lower limb casting from the hip to the ankle for 2 wk. All measurements were conducted at baseline previous to the immobilization procedure (Pre), after 2 wk of immobilization, and again after 4 wk of heavy resistance training (6 wk). All measurements were performed on both sides, with the nonimmobilized side serving as within-subject control.

Immobilization Protocol

Immobilization was accomplished by 2 wk of randomized, unilateral, whole leg casting using a lightweight fibercast applied from just above the malleoli to just below the groin, which previously has proven to induce substantial muscle atrophy in short-term immobilization studies in young individuals (33, 35, 37). The cast was positioned in 30° of knee joint flexion to circumvent walking ability of the casted limb, and the subjects were carefully instructed to perform all ambulatory activities on crutches and abstain from ground contact, as well as performing isometric contractions of quadriceps of the immobilized leg. During the 2-wk immobilization period, the subjects were contacted on a regular basis and carefully instructed to contract the muscles around the ankle joint (venous pump exercises) several times a day to prevent potential formation of deep venous thrombosis.

Retraining Procedure

After removal of the whole leg cast, the subjects received manual mobilization of their immobilized leg by a trained physiotherapist. This was carried out to ensure that minimal pain was present and that normal range of motion could be obtained at the knee joint. The retraining protocol was accomplished by 4 wk of supervised unilateral strength training on the immobilized leg, with three sessions each week, and has previously proven to elicit increases in muscle size and maximal muscle strength in elderly individuals (27, 71). After a 5-min warm-up on a stationary bike, the subjects performed knee extension, leg press, and knee flexion, with all of the machines being adjustable (Technogym International). To induce a sufficient response in the thigh musculature, the training intensity was 3–4 sets × 10 repetitions (15 repetitions maximum (RM)) in week 1, 5 sets × 10 repetitions (12 RM) in weeks 2 and 3, and 4 × 10 repetitions (12 RM) in week 4. Training load was adjusted on a weekly basis by the use of 5-RM tests.

Body Composition

Dual-energy X-ray absorptiometry (Lunar DPX, version 3.6Z software) was used to estimate whole body composition and percent body fat.

Maximal Muscle Strength and Neuromuscular Activation

Maximal voluntary and evoked muscle force was measured in a custom-made setup, where the subjects were seated in an upright position with back support and the hip and knee joint were flexed at 90° (69). A steel cuff was strapped around the lower leg, ~2 cm above the medial malleoli, and was connected via a rigid steel bar to a strain-gauge load cell (Bofors KRG-4, Bofors, Sweden), which was connected to an instrumentation amplifier (Gould 5900, Gould, Valley View, OH).

Resting muscle twitches. Each test procedure began with the determination of the maximal twitch response in the resting muscle (Fig. 1A). Percutaneous surface stimulation electrodes (Bioflex, model PE3590) were placed over the distal and proximal parts of the quadriceps femoris muscle, ~10 cm above the patella and 15 cm below the anterior superior iliac spine, respectively. The exact electrode position, including interelectrode distance, was registered in relation to anatomic landmarks by use of ink markings, transparent sheet, and vertical height measurements during upright standing to ensure identical electrode positioning throughout the study period. The stimulation protocol and data sampling were controlled from a personal computer by predesigned algorithms written in Spike 2 (version 6.02, Cambridge Electronic Design, Cambridge, UK) using an external analog-to-digital converter (CED Micron 1401 II, 16 bit, Cambridge, CED), and all force signals were sampled and subsequently filtered with a 20-Hz Butterworth low-pass filter (~3-dB gain) (52). Twitch contractions were evoked in the passive muscle using electrical stimulation consisting of single square-wave pulses of 0.1-ms duration delivered by a direct current stimulator (Digitimer
Electronics, model DS7). Stepwise increments in the current were delivered until no further increase in twitch amplitude was seen (32). The following twitch characteristics were determined: 1) peak torque (PT); 2) twitch time to peak torque (TPT), defined as the time elapsed from onset (2% of twitch amplitude) to PT; and 3) rate of force (torque) development (RFD) in time intervals of 0–30 ms and 0–50 ms, which was determined as the mean slope of the torque-time curve in time intervals of 0–30 ms and 0–50 ms, respectively (time = 0 denotes onset of twitch force).

Superimposed twitches. To evaluate the ability to activate the quadriceps muscle, i.e., to assess the magnitude of central activation (neuromuscular activation), electrically evoked muscle doublet twitches were superimposed onto maximal voluntary muscle contraction (MVC) (49, 68). Contractions were evoked using doublet square-wave pulses of 0.1-ms duration, and a minimum of two trials were performed with a requirement to reach within 5% of the peak MVC force measured in preceding trials. Supramaximal doublet stimulation (100-ms pulse duration, 10-ms interpulse interval) was manually delivered 5 s before (nonpotentiated doublet), at the highest attained force plateau (superimposed doublet), and 2 s after (potentiated resting doublet), with the latter being used as the resting reference twitch (Fig. 1B). The force recording of each contraction was displayed online on a computer screen, which enabled stimuli to be triggered manually on top of a MVC. The height of the superimposed and resting doublet twitches were measured, and central activation (CA) (i.e., neuromuscular activation) was calculated as (49, 68):

\[ CA(\%) = 100 - \left( \frac{D\times Tb/T_{max}}{T_{DTW}} \right) \times 100 \]

where D is the difference between T5 and the maximum torque attained during the superimposed doublet stimulation; T5 is the torque recorded just before the instant of doublet stimulation; T_{max} is the maximal attained torque measured during the preceding MVC trials; and T_{DTW} is the torque recorded during the potentiated resting doublet twitch. D is indicative of additional activity from motor units not fully activated at the time of stimulus. Correction of D (~T5/T_{max}) was included in the equation, since the manually controlled doublet twitch stimulation was not always perfectly timed at T_{max} (49, 68).

Quadriceps Muscle Volume

Muscle volume of the quadriceps muscle (Qvol) was obtained by use of axial magnetic resonance imaging measurements (1). Imaging was performed in a body array coil with the subject in a supine position at all time points. Dependent on the femur length of the subject, the joint was included in the field (field of view 48). The following first scan was centered just below the femur condyles to ensure the same scan position at all time points. Dependent on the femur length of the subject, seven to eight transverse scans were carried out with a slice thickness of 10 mm and an interslice gap of 50 mm. The scans were T1-weighted with a field of view 42 and matrix 512 x 512. The anatomic cross-sectional area of each scan was measured three times by a blinded trained person using a Web1000 imaging software. The mean value of the three measurements was recorded as the result, and the coefficient of variation between consecutive measurements was <5%. Qvol was calculated by the summation of six successive anatomic cross-sectional area values (scans 2–7), each multiplied by the sum of the slice thickness and interslice gap.

Muscle Architecture

Sagittal ultrasound images of the quadriceps femoris muscle were recorded with the use of a Siemens real-time scanner with a 7.5-MHz linear array transducer. Images were obtained with the subject in a seated position (90° flexion in the hip and knee joint) at 50% of femur length over the midbelly of the vastus lateralis (VL) muscle, according to the procedures described previously (70). To ensure identical scan position at each time point, the specific scan position was marked with traces drawn on acetate paper, which was aligned relative to individual skin marks and anatomic landmarks. The pennation angle (θp) of the VL fascicles was measured as the angle between the VL muscle fascicles and the deep aponeurosis of the insertion, i.e., the fascia separating VL and the vastus intermedius muscle (70). Two images from each limb were obtained from each subject. Each image was evaluated three times, and the mean value was recorded as the average fiber θp. The coefficient of variation between consecutive measurements was <5%.

\[ S_{\text{force}} = Ff/PCSA \]

To quantify the relative contributions to changes in muscle mass and muscle architecture with immobilization in old and young individuals, S_{force} of the quadriceps muscle was calculated by dividing fascicle force (Ff) by the physiological cross-sectional area (PCSA) of the quadriceps muscle (46, 63):

\[ \text{PCSA} = Q_{\text{vol}} \times \cos(\theta_p)/F1 \]

As previously described (1), quadriceps contraction force (FQ) was estimated from measurements of the maximal voluntary isometric knee extension torque (MVC), assuming that patella tendon moment arm length (M_{pa}) was 4.0 cm (53, 54), and that the ratio of patella tendon force to quadriceps tendon force was 0.7 (53, 54).

\[ F_Q = (\text{MVC}/M_{pa})/0.7 \]

Ff was then calculated as the FQ divided by cosine to the θp of the VL fascicles:

\[ Ff = F_Q/\cos(\theta_p) \]

Correlation Analyses

Correlation analysis between premuscle volume and the relative decrease after immobilization was performed to investigate the importance of habitual muscle mass on the individual responses to immobilization. Furthermore, to investigate the association between the individual responsiveness to immobilization and subsequent retraining, correlation analysis was performed on the individual (relative) decreases in muscle size after immobilization and the subsequent individual (relative) increase after retraining.

Statistics

Changes in muscle strength, neuromuscular activation, muscle volume, θp of the VL fascicles, and S_{force} were evaluated by using the Friedman two-way analysis of variance by ranks of related samples with subsequent analysis using the Wilcoxon signed rank test for paired samples and presented as group means ± SE. Intergroup differences were evaluated using Kruskal-Wallis rank test. Spearman's rho (r_s) was used to determine the presence of any rank-order association. A 0.05 level of statistical significance (two-tailed) was used.

RESULTS

Subjects

At baseline, there was no difference in body mass (OM: 84.8 ± 3.4 kg, YM: 72.2 ± 2.3 kg) or height (OM: 178.7 ± 2.6 cm, YM: 181.4 ± 1.8 cm), whereas OM had a larger percentage of body fat (OM: 26.0 ± 3.9%, YM: 14.7 ± 5.7%) and a
higher body mass index (OM: 26.3 ± 0.5 kg/m², YM: 22.1 ± 0.5 kg/m²) than their young counterparts.

**Maximal Muscle Strength and Central Activation**

At baseline, maximal quadriceps strength was 31% (P < 0.05) lower in OM compared with YM (Table 1). After 2 wk of immobilization, OM and YM lost 15.7% and 19.8% of the maximal quadriceps strength, respectively (P < 0.05); however, after 4 wk of retraining, both OM and YM had regained their baseline MVC (Table 1). No changes were observed in the control leg (Con) in either OM or YM. Before immobilization, OM and YM showed similar levels of central activation in either OM or YM. However, while the activation level of YM remained unchanged, OM experienced a 9.9% (P < 0.05) decline after immobilization. After 4 wk of subsequent strength training, OM returned to the initial activation level, whereas YM reached values above the baseline activation level (P < 0.05). There was no change in the activation level of the Con leg in any of the two groups.

**Resting Twitch Characteristics**

Before intervention, peak twitch torque (PTT: -42.6%, P < 0.05) and twitch RFD at 0–30 ms (-46.3%, P < 0.05) and 0–50 ms (-44.9%, P < 0.05) were reduced in OM compared with YM (Fig. 1 and Table 2). In contrast, there was no difference between young and old in the TPT. After immobilization, peak twitch torque decreased in both young and old (OM: -27.7%, YM: -22.2%, P < 0.05) with subsequent increases after retraining (OM: +30.7%, YM: +21.5%, P < 0.05). Furthermore, twitch RFD decreased after immobilization in time intervals of 0–30 ms (OM: -30.7%, YM: -21.7%, P < 0.05) and 0–50 ms (OM: -30.4%, YM: -21.5%, P < 0.05), while subsequently increasing after retraining at 0–30 ms (OM: +38.3%, YM: +16.8%, P < 0.05) and 0–50 ms (OM: +36.5%, YM: +17.8%, P < 0.05), respectively. Moreover, there was no change in TPT in either old or young individuals in response to immobilization or retraining (Table 2). No changes were observed in the Con leg. All relative changes reported above did not differ between OM and YM.

**Quadriceps Muscle Volume**

Before intervention, quadriceps muscle volume (Qvol) was 11% reduced in OM compared with YM (P < 0.05, Table 1). After immobilization, muscle volume decreased more in young than old (YM: -8.9%, OM: -5.2%, P < 0.05). Moreover, YM showed greater increases in Qvol in response to retraining (YM: +8.2%, OM: +3.8%, P < 0.05) and reached the initial baseline level (Fig. 2). In contrast, OM did not fully recover their Qvol after retraining (P < 0.05) (Fig. 2). No change was observed in the Con leg. Moreover, correlations emerged

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**Table 1. Effects of immobilization and retraining on muscle contractile properties, specific force, and muscle mass characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Immobilized</th>
<th>Control</th>
<th>Immobilized</th>
<th>Control</th>
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</thead>
<tbody>
<tr>
<td>MVC, N·m</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>214±27</td>
<td>212±44</td>
<td>139±21†</td>
<td>142±30‡</td>
</tr>
<tr>
<td>2 wk immobilization</td>
<td>171±23†</td>
<td>218±39</td>
<td>118±24‡</td>
<td>135±11‡</td>
</tr>
<tr>
<td>4 wk Retraining</td>
<td>226±30*</td>
<td>229±30</td>
<td>145±31*‡</td>
<td>152±30‡</td>
</tr>
<tr>
<td>Muscle activation, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>91.6±1.6</td>
<td>92.3±1.5</td>
<td>88.6±1.6</td>
<td>86.9±3.2</td>
</tr>
<tr>
<td>2 wk immobilization</td>
<td>90.6±2.8</td>
<td>89.6±2.9</td>
<td>80.2±2.8†</td>
<td>83.2±2.5</td>
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<tr>
<td>4 wk Retraining</td>
<td>95.2±1.5†‡</td>
<td>92.4±1.7</td>
<td>90.6±2.8‡</td>
<td>90.8±2.8</td>
</tr>
<tr>
<td>Quadriiceps volume, cm³</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pre</td>
<td>1,841.3±62.2</td>
<td>1,829.5±72.4</td>
<td>1,633.1±46.3‡</td>
<td>1,580.1±61.3‡</td>
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<tr>
<td>2 wk immobilization</td>
<td>1,676.9±47.3†</td>
<td>1,824.3±78.5</td>
<td>11,545.0±39.7†</td>
<td>1,577.1±65.1‡</td>
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<tr>
<td>4 wk Retraining</td>
<td>1,813.7±61.6*</td>
<td>1,814.7±73.0</td>
<td>1,605.5±45.3*‡</td>
<td>1,558.7±61.1‡</td>
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<tr>
<td>PCSA, cm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>164.4±7.1</td>
<td>160.3±7.2</td>
<td>135.8±6.8‡</td>
<td>145.5±7.8‡</td>
</tr>
<tr>
<td>2 wk immobilization</td>
<td>157.5±7.0</td>
<td>158.1±8.5</td>
<td>139.7±8.6‡</td>
<td>149.4±9.6‡</td>
</tr>
<tr>
<td>4 wk Retraining</td>
<td>173.1±7.1*</td>
<td>161.2±6.8</td>
<td>143.0±8.3</td>
<td>146.5±8.9‡</td>
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<tr>
<td>Pennation angle, °</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>10.4±0.4</td>
<td>9.8±0.3</td>
<td>9.0±0.5‡</td>
<td>9.0±0.5‡</td>
</tr>
<tr>
<td>2 wk immobilization</td>
<td>9.4±0.4†‡</td>
<td>10.0±0.2</td>
<td>8.4±0.5‡†</td>
<td>8.7±0.4‡</td>
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<tr>
<td>4 wk Retraining</td>
<td>10.5±1.5*†</td>
<td>10.1±0.4</td>
<td>8.6±0.4‡</td>
<td>8.9±0.5‡</td>
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<tr>
<td>Fascicle length, mm</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Pre</td>
<td>11.7±0.3</td>
<td>11.9±0.3</td>
<td>11.6±0.5</td>
<td>11.7±0.5</td>
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<tr>
<td>2 wk immobilization</td>
<td>10.5±0.4†‡</td>
<td>11.6±0.5</td>
<td>11.1±0.5</td>
<td>11.4±0.7</td>
</tr>
<tr>
<td>4 wk Retraining</td>
<td>11.0±0.3</td>
<td>11.7±0.5</td>
<td>11.4±0.5</td>
<td>11.3±0.5</td>
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<tr>
<td>Specific force, N/cm²</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>47.9±1.8</td>
<td>48.7±3.5</td>
<td>33.0±1.9‡</td>
<td>34.5±2.0‡</td>
</tr>
<tr>
<td>2 wk immobilization</td>
<td>40.1±2.4†‡</td>
<td>51.3±3.9</td>
<td>25.3±2.0†‡</td>
<td>32.4±1.2‡</td>
</tr>
<tr>
<td>4 wk Retraining</td>
<td>48.4±2.64*</td>
<td>51.4±2.4</td>
<td>32.2±3.3‡</td>
<td>34.0±1.4‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. Changes are shown for maximal muscle strength, quadriceps muscle volume, quadriceps activation, muscle architecture, and specific force with unloading and retraining in old and young men. Measurements were conducted at baseline (Pre), after 2 wk of unilateral immobilization, and following 4 wk of retraining on both limbs, immobilized and control. MVC, maximal voluntary contraction; PCSA, physiological cross-sectional area. †Significant different from Pre, *significant different from 2-wk immobilization, ‡old men significant different from young men: P < 0.05.
values in 0–50 ms (RFD 0–50) with unloading and retraining in old and young men. Measurements were conducted at Pre, after 2 wk of unilateral immobilization, and following 4 wk of retraining on both limbs, immobilized and control. †Significant different from Pre, *significant different from 2-wk immobilization, ‡old men significant different from young men:

Physiological CSA and Specific Force

Physiological CSA (PCSA) and specific force (Spforce) were reduced by 17 and 31% in OM compared with YM (P < 0.05), respectively, before immobilization. PCSA remained unaltered in both OM and YM after immobilization; however, the retraining regime led to a 10.6% increase in PCSA in YM (P < 0.05), whereas no change was observed in OM. Moreover, there was a marked decrease in Spforce after immobilization in both groups (OM: −23.1%, YM: −16.6%, P < 0.05), where YM tended to decrease more than OM (P = 0.09). After 4 wk of retraining, Spforce returned to baseline level in both old and young individuals (OM: +34.4%, YM: +23.7%, P < 0.05). There were no changes in PCSA or Spforce in the Con leg in either of the two groups.

Table 2. Effects of immobilization and retraining on resting twitch characteristics

<table>
<thead>
<tr>
<th></th>
<th>Young Men Immobilized</th>
<th>Young Men Control</th>
<th>Old Men Immobilized</th>
<th>Old Men Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak torque, N·m</td>
<td>47.72 ± 3.81</td>
<td>44.99 ± 3.77</td>
<td>27.40 ± 1.95</td>
<td>26.42 ± 1.54</td>
</tr>
<tr>
<td></td>
<td>35.79 ± 2.07†</td>
<td>45.38 ± 3.70</td>
<td>19.80 ± 3.65†‡</td>
<td>22.84 ± 2.56‡</td>
</tr>
<tr>
<td>4-wk Retraining</td>
<td>43.32 ± 3.11*</td>
<td>45.24 ± 3.03</td>
<td>25.87 ± 1.64†‡</td>
<td>26.29 ± 1.80‡</td>
</tr>
<tr>
<td>Time to peak tension, ms</td>
<td>88 ± 2</td>
<td>88 ± 2</td>
<td>89 ± 2</td>
<td>89 ± 3</td>
</tr>
<tr>
<td></td>
<td>88 ± 2</td>
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<td></td>
<td>89 ± 2</td>
<td>88 ± 2</td>
<td>87 ± 2</td>
<td>86 ± 1</td>
</tr>
<tr>
<td>RFD 0–30 ms, N·m·s⁻¹</td>
<td>1.375 ± 0.99</td>
<td>1.286 ± 1.02</td>
<td>738 ± 48‡</td>
<td>784 ± 68‡</td>
</tr>
<tr>
<td></td>
<td>1.053 ± 0.72†</td>
<td>1.301 ± 1.05</td>
<td>546 ± 97‡‡</td>
<td>678 ± 103‡‡</td>
</tr>
<tr>
<td>4-wk Retraining</td>
<td>1.226 ± 0.86*</td>
<td>1.328 ± 0.96</td>
<td>756 ± 65‡‡</td>
<td>788 ± 70‡‡</td>
</tr>
<tr>
<td>RFD 0–50 ms, N·m·s⁻¹</td>
<td>1.778 ± 126</td>
<td>1.673 ± 132</td>
<td>980 ± 66‡</td>
<td>1,033 ± 87‡‡</td>
</tr>
<tr>
<td></td>
<td>1.361 ± 0.90†</td>
<td>1.692 ± 136</td>
<td>734 ± 131‡‡</td>
<td>896 ± 136‡‡</td>
</tr>
<tr>
<td>4-wk Retraining</td>
<td>1.598 ± 110*</td>
<td>1.705 ± 121</td>
<td>1,001 ± 86‡‡</td>
<td>1,047 ± 93‡‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. Changes are shown for peak twitch torque, time to peak tension, and twitch rate of force development in 0–30 ms (RFD 0–30) and in 0–50 ms (RFD 0–50) with unloading and retraining in old and young men. Measurements were conducted at Pre, after 2 wk of unilateral immobilization, and following 4 wk of retraining on both limbs, immobilized and control. †Significant different from Pre, *significant different from 2-wk immobilization, ‡old men significant different from young men: P < 0.05.

Muscle Architecture

Before immobilization, OM had smaller θf than YM (Table 1) and a tendency toward smaller fascicle lengths (P = 0.08). However, after immobilization, the θf decreased to a larger extend in YM than in OM (OM: −6.5%, YM: −9.3%, P < 0.05). In both young and old, there was a tendency toward a decrease in fascicle length after immobilization (OM: −6.1%, P = 0.08; YM: −9.7%, P = 0.06, both groups collapsed: P < 0.05). After retraining, θf increased in YM by 12.0% (P < 0.05), whereas the 4.5% increase observed in OM did not reach statistical significance. No change was observed for the Con leg in YM or OM.

Fig. 2. Relative changes in quadriceps muscle volume after 2 wk of immobilization (Immob) and subsequent 4 wk of retraining. Qvol, quadriceps muscle volume.

Fig. 3. Correlation analysis between premuscle volume and the relative decrease after immobilization. Qvol, quadriceps muscle volume; ns, nonsignificant.

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of 7 days of limb immobilization in young and old men (21), and by Urso et al. (72), who examined the effects of 2-wk immobilization of the adductor pollicis muscle in young and old human individuals, respectively. Furthermore, immobilization led to significant reductions in Qvol, comparable to previous data obtained in young individuals after 2 wk of immobilization (18, 33, 37). Interestingly, the observed decrease in muscle volume in old subjects was significantly smaller than the decrease observed in young subjects, in contrast to earlier findings obtained in the human adductor pollicis muscle (72). This discrepancy could, however, be due to the different muscle groups investigated, as different adaptation strategies have been suggested for small muscle groups compared with that of bigger muscle groups (29). Animal data are somewhat inconsistent as well. Some studies report old animals to be more affected by hindlimb suspension (20), some find a similar degree of muscle atrophy between young and old animals (67), while others, in line with findings from the present study, find a higher magnitude of muscle atrophy in young compared with old animals (13, 59). The attenuated decline in muscle size in OM following immobilization could hypothetically be related to a reduced muscle protein breakdown rate with aging concurrent with the observed decrease in muscle protein synthesis rate (6, 74). In some support of this notion, MuRFbx and atrogen-1 that drive ubiquitin-proteasome-mediated myofibrillar proteolysis were downregulated in skeletal muscle of old rats (26), although not consistently shown to differ between young and old humans, comparable to the age of the present subjects (76). Furthermore, the observed correlation between baseline muscle volume and relative decrease after immobilization in young but not old subjects in the present study suggests that the habitual muscle volume predicts the individual response to muscle disuse in young but not aged individuals. This finding indicates qualitative differences in the myogenic response to immobilization between young and old individuals, with old subjects being more affected on the efferent neuronal function, while young individuals were more affected at the muscle protein level.

In accordance with the changes in muscle volume, the present decrease in pennation angle of the VL fascicles observed in young subjects after immobilization was larger than that observed in old subjects, underlining the importance of muscle architecture to explain part of the discrepancy between the average relative decrease in muscle strength (OM: −15.7%, YM: −19.8%, P < 0.05), being about twice as large compared with the average relative decrease in muscle mass (YM: −8.9%, OM: −5.2%, P < 0.05).

Although Spforce of the old individuals was markedly reduced compared with young subjects before immobilization, the relative decrease in Spforce was similar in old and young subjects following immobilization. Similarly, the present study demonstrated comparable decreases in resting twitch PT and twitch rate of force development between young and old individuals after immobilization, which indicates that the change in intrinsic (“qualitative”) mechanical muscle function (including muscle phenotype expression and/or tendon stiffness) did not differ between old and young individuals. The age-related differences in twitch PT and twitch RFD observed before immobilization may be due to potential changes in muscle fiber composition (5) and/or tendon stiffness (51) with aging, whereas the effect of immobilization in both young and

**Fig. 4.** Correlation analysis between the individual (relative) decreases in muscle size after immobilization and the subsequent individual (relative) increase after retraining.

**DISCUSSION**

In the present study, concurrent data were obtained on the change in muscle contractile function, muscle size, central activation, and muscle architecture induced by unloading and retraining in old vs. young human individuals, respectively. It was the purpose to address the effect of aging on 1) the magnitude of acute muscle disuse atrophy, and 2) the adaptive plasticity of subsequent exercise rehabilitation. The main and novel findings were 1) that young subjects showed a greater magnitude of muscle atrophy and more marked changes in VL fascicle pennation angle after immobilization compared with old subjects; 2) old subjects demonstrated a diminished capacity to restore muscle size and muscle architecture during subsequent retraining; and 3) immobilization led to reduced muscle activation in old but not young subjects. Thus the present data suggest that the adaptive plasticity in skeletal muscle mass and central nervous system function associated with unloading and subsequent remobilization, respectively, may differ substantially between old and young individuals.

Multiple interrelated factors appear to contribute to the deterioration of muscle mechanical function with aging as a result of changes in both quantitative and qualitative factors (23, 25, 73, 75). In addition to these changes in intrinsic factors, the level of physical activity has been shown to modify the age-related loss in muscle size and function (2, 43, 57, 61). However, as there was no difference in the activity level between old and young individuals examined in the present study, we believe the observed differences were mainly attributable to the effect of aging per se.

**Effects of Immobilization**

It is evident that aging and disuse bring about analogous impairments in the neuromuscular system, and, although there are indications of different strategies to muscle disuse in young and old animals, these aspects remain to be elucidated in humans. In the present study, the average decrease in maximal muscle strength after 2 wk of immobilization did not differ between the two age groups, which is in line with previous results reported by Deschenes et al., who examined the effects...
old individuals may have included changes in sarcoplasmic Ca\(^{2+}\) kinetics (44).

Importantly, old subjects demonstrated an impaired ability to activate the quadriceps muscle after immobilization (OM: \(-9.9\%, P < 0.05\)), whereas young subjects, latter in line with recent findings, remained unaffected (18). This finding could partly be explained by a potential age-related decline in somatosensory afferent inflow on motor unit activation (30) and a reduced maximal motor unit firing rate at MVC (39, 41, 56) that occasionally is accompanied by reduced levels of muscle activation (42). It is possible that immobilization leads to an amplified age-related gap in these parameters that could, at least in part, explain the present findings, which should be examined in future experiments.

**Effects of Retraining**

The capacity for muscle regrowth in elderly human individuals after immobilization has not previously been investigated, despite the obvious clinical importance of such knowledge. Importantly, the present data demonstrate that old subjects, in contrast to young, did not fully recover from the decrease in muscle volume and pennation angle, despite 4 wk of intensive resistive exercises. These findings corresponds well with previous animal data, indicating an attenuated response to reloading in old animals (12, 48, 77), and, although the knowledge about the molecular processes involved in muscle regrowth is limited, there are indications that aging impairs the activation of satellite cells (28). Furthermore, animal data from the Conboy group indicate that the impaired regenerative capacity in old skeletal muscle is due to a diminished activation of the Notch signaling pathway with aging (16). Moreover, it has been put forward that the satellite cell pool, and thereby the myogenic potential, is reduced with aging (31, 38), although this is not a universal finding (15, 64). The attenuated rate of muscle size gain in old compared with young during the period of subsequent retraining could also be due to a lesser or delayed rise in protein synthesis rate, and/or an increased protein breakdown rate during the acute training sessions in OM. Furthermore, attenuated exercise-induced changes in myogenic regulatory factor expression may have been involved in the smaller gain in muscle size in OM observed after the period of retraining (40, 48).

Even though the present data suggest that the rate and magnitude of change in muscle mass is attenuated in old compared with that of young individuals after 4 wk of resistance training, more prolonged regimes of resistance training have revealed marked increases in muscle size and muscle architecture in old individuals (62, 70), comparable to that of young individuals (1). These data are further supported by recent data from Carey and colleagues (14), who have elegantly demonstrated that the expression of Notch genes is reduced in aged human skeletal muscle compared with that of the young; however, after 12 wk of resistance training, there was no difference in basal Notch gene expression between young and old subjects.

While long-term resistance training may lead to changes in resting twitch TPT (7, 24), no longitudinal changes were observed in the present study, suggesting that the present 2 wk of immobilization followed by 4 wk of retraining were too short in duration to elicit any major change in muscle fiber-type composition and/or tendon stiffness properties. Consequently, the differential age-related changes in muscle size and muscle activation likely were the major factors to explain the present changes in mechanical muscle function induced by immobilization and subsequent retraining. On the other hand, the correlations observed in the present study between the individual relative decrease in muscle volume after immobilization and the subsequent individual relative gain after retraining (YM: \(r = -0.702, OM: r = -0.778, P < 0.05\)) indicate that, besides aging per se, genetic factors are also likely to play an important role for the myogenic potential, in line with recent findings by Petrella et al. (58).

In conclusion, the present data shows that aging is accompanied by an attenuated rate of muscle atrophy in response to immobilization compared with that of young individuals, and importantly that old subjects demonstrate a diminished capacity to restore muscle size and muscle architecture during subsequent retraining. Moreover, immobilization led to reduced muscle activation in old but not young subjects. Thus the present data suggest that the adaptive plasticity in skeletal muscle mass and central nervous system function associated with unloading and subsequent remobilization, respectively, may differ between old and young individuals. Collectively, these findings suggest that old individuals may be more affected with respect to neural function, and young individuals more affected in terms of muscle size, in response to short-term immobilization. Furthermore, the present data indicate that aging is accompanied by an impaired ability to recover from disuse muscle atrophy, and, consequently, old individuals may need a longer time to recover from periods of disuse compared with young individuals.

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EFFECTS OF IMMobilIZATION AND RETRAINING IN OLD AGE


