Plasticity and function of human skeletal muscle in relation to disuse and rehabilitation: Influence of ageing and surgery

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THE PRESENT THESIS IS BASED ON THE FOLLOWING PAPERS:


INTRODUCTION

In humans skeletal muscle tissue accounts for about 40% of the total body mass and, in addition to being a crucial factor for locomotion, skeletal muscle represent a key element in maintaining metabolic function and as an energy reservoir in catabolic conditions. Thus, deteriorations in the contractile and metabolic properties of skeletal muscle have significant negative effects on human health and even short periods of muscle disuse rapidly leads to a number of negative consequences, such as skeletal muscle atrophy [260] reduced muscle strength [59, 114, 260] and a decline in basal metabolic rate [102] in otherwise healthy individuals.

In parallel, the loss of muscle mass observed with ageing i.e. sarcopenia and the concomitant decline in muscle strength and power have extensive consequences for the elderly since associated with an impaired ability to perform tasks of daily living, along with an increased risk of disability and mortality [172, 199]. Moreover, periods of skeletal muscle...
disuse due to a higher degree of comorbidity and hospitalisation per se results in a rapid and accelerated loss of skeletal muscle mass [118]. In fact, immobilisation due to major surgery and hospitalisation markedly increases the risk of deterioration in muscle function, often leading to onset of disability in frail elderly individuals [49, 135]. In addition, the recovery of losses in muscle mass and muscle strength is often very slow [203, 249, 259] and many elderly patients fail to regain the level of function that was present prior to hospital admission [49, 108, 217, 259]. Thus attempts to counteract the muscle atrophy associated with surgery and hospitalisation in the elderly seem highly relevant. We therefore set to investigate the mode and magnitude of muscle activity required to effectively counteract the decline in muscle mass and function associated with surgery and hospitalisation (Study I, II, IV and V).

The observation that skeletal muscle disuse leads to substantial atrophy is far from new and the negative effects of unloading on skeletal muscle are relatively well described in young individuals [23, 24, 53, 106, 148]. In contrast, very little is known about how immobilisation and skeletal muscle disuse affects skeletal muscle size and function in old adults [59, 137, 251]. Thus, our present knowledge is primarily based on animal data where hind-limb suspension (HS) has been used as a model of muscle un-loading to investigate the underlying mechanisms associated with disuse muscle atrophy in aging [4, 12, 13, 30, 33, 34, 37, 60, 61, 253]. Although it is evident that aging leads to a multitude of changes in the neuromuscular system that are similar to those evoked by unloading [4, 253], 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. An important question is therefore whether processes responsible for the loss of muscle mass due to acute or chronic disuse are similar to those underlying sarcopenia and additionally, whether skeletal muscle disuse leads to similar effects in old and young individuals. On this background, studies III and VI were carried out, in order to investigate the effects of skeletal muscle disuse in aged individuals, initially in individuals exposed to chronic disuse (Study III) while in Study VI, we intended to discriminate between the differential effects of a defined period of muscle disuse and aging per se.

Moreover, based on previous animal data demonstrating an attenuated recovery response after immobilisation and injury in old compared to young muscle tissue [36, 44, 85, 269] we investigated the ability of young and older individuals to recover muscle mass and mechanical muscle function after 14 days and 4 days of immobilisation, respectively (Study VI and IX).

At the molecular level effective cellular communication is known to play an essential role in skeletal muscle plasticity and in adult skeletal muscle tissue the size of the muscle is, in essence, determined by the relative rates of protein synthesis and protein degradation [78]. Thus, skeletal muscle atrophy is a consequence of a reduction in muscle protein synthesis and/or an increase in protein degradation. However, despite the existence of several robust candidate pathways [211, 218], the molecular mechanisms responsible for the regulation of skeletal muscle atrophy and the subsequent restoration in skeletal muscle mass in response to exercise based rehabilitation are relatively unknown, particularly in humans. A better understanding of the pathways regulating myofibrillar protein synthesis and protein degradation in humans and their temporal relationship to changes in muscle function and lean mass is hence of considerable clinical importance and has far-reaching implications to counteract muscle wasting during periods of skeletal muscle disuse. In animal models, loss of muscle mass with immobilisation or unloading has been shown primarily to occur through an accelerated degradation of myofibrillar proteins via the ubiquitin-proteasome path-way [29, 82]. Somewhat in contrast, studies in young human individuals have suggested that a decline in protein synthesis rather than accelerated protein breakdown is responsible for the atrophy related muscle loss [54, 76, 78]. With aging, loss of muscle has been associated with increased inflammation [38] and decreased anabolic signalling [50], increased apoptosis [62; 66], impaired myogenic responsiveness [43, 47, 85] as well as de-creased mitochondrial function [165]. Moreover, aging has been found to affect signalling pathways that regulate myogenic growth factors and myofibrillar protein turnover in skeletal muscle of rodents [13]. In order to investigate some of these cellular and molecular mechanisms suggested being responsible for the age-related changes in skeletal muscle with disuse and recovery, including the differential involvement and time course of such signalling pathways, Study VII and VIII was carried out.

The present thesis provides an overview of the information gathered from Study I-IX and the current knowledge about the plasticity of aging muscle in relation to disuse and retraining.

MATERIAL AND METHODS

While relevant details related to Material and Methods are described below, more detailed information can be found in the respective articles.

SUBJECTS

The patient population recruited for Study I-V consisted of patients scheduled for a primary unilateral hip-replacement operation at Bispebjerg University Hospital, Copenhagen, Denmark from May 2000 to May 2002. Eligibility criteria included; age 60 years or older, unilateral primary hip replacement due to primary hip osteoarthritis in patients without cardiopulmonary, neurological or cognitive problems. In the two immobilisation studies (Study VI – IX) comparable groups of healthy young (20-30 years) and elderly (60-75 years) individuals were recruited. Prior to inclusion, all subjects were screened by a physician to exclude individuals with cardiovascular disease, diabetes, neural- or musculoskeletal diseases, inflammatory or pulmonary disorders or any known predisposition to deep venous thrombosis.
<table>
<thead>
<tr>
<th>Study</th>
<th>N=</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Body weight (kg)</th>
<th>Height (cm)</th>
<th>BMI (kg/m²)</th>
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</thead>
<tbody>
<tr>
<td>Retraining after Hip-replacement Study I, II, IV, V</td>
<td>N=</td>
<td>Age (years)</td>
<td>Gender</td>
<td>Body weight (kg)</td>
<td>Height (cm)</td>
<td>BMI (kg/m²)</td>
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<tr>
<td>Conventional rehab</td>
<td>12</td>
<td>68 (62-78)</td>
<td>5M/7W</td>
<td>81.3 ± 5.8</td>
<td>169.8 ± 2.1</td>
<td>28.2 ± 1.7</td>
</tr>
<tr>
<td>NMES</td>
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<td>69 (60-75)</td>
<td>5M/6W</td>
<td>79.0 ± 4.2</td>
<td>167.7 ± 2.8</td>
<td>27.9 ± 0.9</td>
</tr>
<tr>
<td>Resistance training</td>
<td>13</td>
<td>69 (60-86)</td>
<td>7M/6W</td>
<td>77.8 ± 4.5</td>
<td>168.0 ± 2.0</td>
<td>27.4 ± 1.4</td>
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<td>Chronic disuse Study III</td>
<td>Men</td>
<td>19</td>
<td>69 (60-79)</td>
<td>M</td>
<td>85.8 ± 3.6</td>
<td>173 ± 2.0</td>
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<td></td>
<td>Women</td>
<td>20</td>
<td>70 (60-86)</td>
<td>W</td>
<td>71.5 ± 3.6</td>
<td>163 ± 1.0</td>
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<td>74.3 ± 2.4</td>
<td>180.4 ± 2.7</td>
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<td>Old</td>
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<td>67.2 (60-72)</td>
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<td>87.7 ± 3.0</td>
<td>178.8 ± 1.7</td>
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<td>24.4 (21-27)</td>
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<td>72.2 ± 2.3</td>
<td>181.4 ± 1.8</td>
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<tr>
<td></td>
<td>Old</td>
<td>9</td>
<td>67.3 (61-74)</td>
<td>M</td>
<td>84.8 ± 3.4</td>
<td>178.7 ± 2.6</td>
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</tbody>
</table>

Table 1. Anthropometrical data of the study participants

IMMOBILISATION PROTOCOLS
Two different immobilisation studies were conducted. In the first experiments (Study VI, VII & VIII), subjects had one lower limb immobilised for 14 days by unilateral whole-leg casting using a lightweight fibre cast applied from just above the malleoli to just below the groin. The cast was positioned in 30 degrees 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 immobilised leg.

In the following short-term study (Study VII & IX), subjects had a randomly assigned leg immobilised for 4 days using a knee brace (DonJoy, Orthopedics, Sunny Vista, CA, US) fixated at a knee angle of 30 degrees (similar to the cast). Using a knee-brace instead of a whole-leg cast enabled us to obtain muscle biopsies during the immobilisation period without removing the brace. Both methods have previously been shown equally effective of inducing muscle atrophy in young individuals [53, 59, 106]. Similarly to the 14 days immobilisation intervention, subjects were provided with crutches during the immobilisation period.

RE-TRAINING PROCEDURES
Hip-replacement patients
Patients were stratified by age and sex and randomly allocated to one of three groups: home-based standard rehabilitation (SR), SR plus unilateral lower-limb resistance training (RT), or SR plus unilateral neuromuscular electrical stimulation (ES). The RT and electrical stimulation (ES) groups performed the additional training or received ES on the operated leg, so the non-operated side could serve as a within-subject control.

Home-Based Standard Rehabilitation
All three intervention groups were provided the same standard rehabilitation (SR) procedure for hip-replacement patients at Bispebjerg Hospital. The standard rehabilitation program consisted of 15 functional exercises with no use of external loads. The SR group was instructed to perform the exercises twice a day and attend weekly control sessions in the Physical Therapy department, during which an experienced physical therapist guided them through all the exercises to ensure they were performed correctly. Identical instructions were given to the two other treatment groups.

Neuromuscular Electrical Stimulation
The ES group began the stimulation program on the operated leg the day after hip surgery. Patients were carefully instructed in the use of the stimulator and the placement of the electrodes. The stimulator was a pocket-sized battery-operated unit (Elpha 2000, Biofina, Denmark) that delivered a constant biphasic current (0–60 mA). After careful preparation of the skin, two stimulation electrodes (Bio-Flex, 50 x 89 mm, Biofina A/S; Odense, Denmark) were placed over the quadriceps muscle belly 5 cm below the inguinal ligament and 5 cm above the patella. The pulse rate was 40 Hz, with a pulse width of 250 µs, and stimulation time of 10 s, followed by 20 s of rest [101]. The amplitude increased and decreased gradually during the first and last 2 s. The intensity of the stimulation was adjusted according to patient tolerance, at maximal tolerable stimulus intensity. The total stimulation time was 1 h/d for 12 weeks, and all patients registered total stimulation time and intensity. After discharge from the hospital, the stimulator was used at home, and weekly controls were conducted.

Resistance exercise
Resistance training was performed as unilateral progressive exercise for the operated lower limb. The post-operative training was initialized the first day after surgery and consisted of daily knee extension exercises (3 x 10 reps) in a
seated position with sandbags strapped around the ankle. As soon as possible (day 5-7 post surgery) training was performed by use of adjustable leg-press and knee-extension machines (Techno gym international) three times per week. Following a brief 10 min warm-up on a stationary bicycle, knee-extension and leg-press exercises were performed. A trained physical therapist carefully supervised all training sessions. Training intensity was progressively increased in intensity from 20 RM (~50% of 1-RM) the first week, 15 RM (~65% of 1-RM) during week 2-4, 12 RM (~70% of 1-RM) during week 5-6 and 8 RM (~80% of 1-RM) the last six weeks. Training loads were carefully adjusted on a weekly basis, measured by a multiple-RM testing based on goal repetitions, to ensure that all patients exercised at the intended intensity.

**Re-training subsequent to immobilisation**

The re-training protocol for the 2 weeks immobilisation protocol consisted of a 4 weeks supervised resistance exercise for the intervention leg that was fairly similar to the above program (cf. 5.3.1c) during week’s two to six. The program was previously shown to elicit increases in muscle size and maximal muscle strength in elderly individuals [68]. Training sessions were carried out three times per week and after a 10 min warm-up on a stationary bike, subjects performed knee extension, leg press, and knee flexion, with all the machines being adjustable (Technogym International). The subjects were instructed to use moderate (~1-2 s) and slow speed (~3-4s) in the concentric and eccentric contraction phases, respectively. Load intensity was 3-4 sets x 12 reps (at 15 repetition maximum (RM)) in week 1, followed by 5 sets x 10 reps (at 12 RM) in weeks 2 and 3, and 4 sets x 10 reps (at 12 RM) in week 4. Training loads were determined and progressively adjusted on a weekly basis by use of 5-RM testing.

The 7 days re-training protocol following 4 days of immobilisation was designed in a similar way as the first week of the 4 week re-training period with a load intensity of 3-4 sets x 12 reps (at 15 RM) determined by use of 5-RM testing.

**FUNCTIONAL CAPACITY**

To evaluate changes in functional performance in the group of hip-replacement patients (Study I) a number of functional parameters were obtained that have previously been shown to correlate significantly with risk of physical disability, dependency and falls [52, 118]. Maximal gait speed over a 10-meter course was measured to the nearest 0.1 s and stair-climbing performance was measured as the time to ascend 10 steps (height 20 cm). Both tests were started from a standing position and stopped when both feet were at the determined ending position. The ability to rise from a chair (Sit-to-stand test) was measured on a standardised chair, as the 5-repetition time to the nearest 0.1 s. Furthermore, maximum stair walking power per kg body mass (watt/kg) was calculated as the distance of vertical dis-placement of the body centre mass times g (9.81 m/s²), i.e. the change in potential energy, divided by the fastest time of stair ascent (Study III). Each subject performed three trials, and the stairs consisted of 10 steps each with a height of 16.5 cm leading to a total vertical displacement of 1.65 m.

All three tests are highly validated showing high test-retest reliability and with strong relationships to muscle strength, frailty and mortality [88, 199, 200]. Moreover, the tests are easy to carry out, cheap and not very time-consuming. There are, however, also certain limitations of these tests. The most important being a relatively low sensitivity and an early ceiling effect, which makes them best suitable for frail populations.

**MECHANICAL MUSCLE FUNCTION**

**Isokinetic muscle strength**

To assess mechanical muscle function, isokinetic dynamometry (Kinetic Communicator, Chattanooga, TN, KinCom) was employed in Study I-III, V-VII and IX. Dynamic muscle strength was measured as the maximal voluntary isokinetic knee extensor moment [peak moment, Nm] during concentric quadriceps con-traction performed at slow (60°/s) and fast (180°/s) knee joint angular velocity. Maximal isometric quadriceps and hamstring muscle strength were assessed at a 60° knee joint angle (0° = full knee extension) and the trial with the highest maximal voluntary contraction moment (MVC) was selected for further analyses of rate of force development (RFD) and contractile impulse [3]. Individual settings of the seat, backrest, dynamometer head and lever arm length was registered, so identical positioning was ensured for each subject at all time-points. All measurements were performed on both thighs, and were preceded by a familiarisation trial conducted on a separate day. Verbal encouragement was given and visual feedback was provided as a real-time display of the force output [130]. Successive trials were performed until peak moment could not be improved any further [3], which typically included 7-9 attempts at each velocity. Reliability and validity of the KinCom dynamometer has been verified in detail by Farrell & Richards and is characterized by a high validity and reliability [69].

### Table 2: Overview of methods used in Study I-IX

<table>
<thead>
<tr>
<th>Study</th>
<th>I</th>
<th>II</th>
<th>III</th>
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<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
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<tr>
<td>Functional performance</td>
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<td>Dynamometry</td>
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<tr>
<td>Interpolated twitch</td>
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<td>Surface EMG</td>
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<td>MR Imaging</td>
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<td>Ultrasound Imaging</td>
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<td>Dexe Imaging</td>
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<td>Western Blotting analyses</td>
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<td>Satellite cell analyses</td>
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Furthermore, strong test-retest robustness has previously been demonstrated for the use of isokinetic dynamometry to assess maximal strength of the knee extensors, knee flexors and plantar flexors in both young [235, 264] and old adult [109].

**Interpolated twitch technique (ITT)**

In Study V and VI maximal evoked muscle force was measured using a custom-made set-up [100] with the subjects seated in an upright position with a rigid back support and the hip and knee joint flexed at 90° [100]. A steel cuff was strapped around the lower leg, approximately 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 a linear instrumentation-amplifier (Gould 5900, Gould Inc. Valley View, OH USA).

**Resting muscle twitches**

Each test session was initiated by determination of the maximal twitch response in the resting muscle. 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 [98].

**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 [171, 239]. Contractions were evoked using doublet square-wave pulses of 0.1 ms duration and a minimum of two trials was performed with a requirement to reach within ±5% of the peak MVC force measured in preceding trials. Supra-maximal doublet stimulation (100 ms pulse duration, 10 ms interpulse interval) was manually delivered 5 s before (non-potentiated rest doublet), at the highest attained force plateau (superimposed doublet), and 2 s after (potentiated rest doublet), with the latter response being used as the resting reference twitch.

**MUSCLE SIZE AND ARCHITECTURE**

**Computed tomography (CT)**

Computed tomography (CT, Picker 5000, Ohio, US) was used to obtain anatomical cross-sectional area of the quadriceps muscle in the population of patients undergoing hip-replacement surgery (Study I, II and VI). A slice thickness 8 mm was used and a scanning time of 55s with an image matrix of 512 x 512 pixels. Axial scans of the quadriceps muscle were obtained at the mid-point between the great trochanter and lateral joint line of the knee. A trained radiologist measured cross sectional areas using the Picker VOXEL-Q CT/MR Software Package for real-time Analysis after each scan was blinded for both subject and time point. Each scan was evaluated three times and the mean value was recorded as the result. The coefficient of variation between two consecutive measurements was < 2%.

**Magnetic resonance imaging (MRI)**

In Study VI muscle cross sectional area and muscle volume of the quadriceps muscle was measured by use of axial Magnetic Resonance Imaging (MRI) [1]. Imaging was performed in a body array coil with the subject in a supine position with both limbs extended and relaxed. Prior to the first scan a localising scan centred mid femur was conducted to ensure the knee joint was included in the field (Field of View, FOV 48). The subsequent scan was centred just below the femur condyles to ensure the same scan position at all time-points. Dependent on the femur length of the subject 7-8 T1-weighted transverse scans with a FOV 42 and matrix 512 x 512, pixel matrix were obtained with a slice thickness of 10 mm and an inter-slice gap of 50 mm. After blinding of each scan the Anatomical Cross Sectional Area (ACSA) of each scan was measured three times using Web1000 imaging soft-ware. The mean value of the three measurements was recorded as the result and the coefficient of variation between consecutive measurements was < 5 %. Subsequently, Quadriceps muscle volume (Qvol) was calculated by the summation of 6 successive ACSA values (scan 2-7), each multiplied by the sum of the slice thickness and inter-slice gap. Based on cadaver analysis, a high validity has been reported for the non-invasive assessment of human skeletal muscle CSA and volume by means of MRI [20, 175]. Furthermore, high test-retest reliability is reported for the repeated recording of transversal anatomical CSA of the human quadriceps muscle by means of MRI, demonstrating excellent test-retest reliability [205].

**Ultrasound (UL)**

To assess changes in muscle architecture measurements of muscle fibre pennation angle and muscle thickness ultrasonography was performed in Study III and VI. Sagittal ultrasound images of the quadriceps muscle were recorded with the using a Siemens real-time scanner with a 7.5 MHz linear array transducer. Images were obtained with the subject in a seated position (90 deg. flexion in the hip and knee joint) at 50 % of femur length over the mid-belly of VL muscle [1]. Vastus lateralis (VL) fibre pennation angle (qp) was measured as the angle between VL muscle fibre fascicles and the deep aponeurosis of the insertion, i.e. the fascia separating VL and the vastus intermedius muscle [104, 208, 215]. Two images from each limb were obtained from all subjects. Each image was evaluated three times and the mean value was recorded as the average fibre pennation angle. The coefficient of variation between two consecutive measurements was < 5%. In order to obtain ultrasound images from an identical position at the thigh during longitudinal sessions, anatomical landmarks were carefully drawn on a transparent sheet. Moderate-to-excellent test-retest reliability has been reported for the measurement of qp in the human quadriceps muscle in vivo [27, 198, 206] as well as in other lower limb muscles [10, 198].

**Dual-energy X-ray absorptiometry (DEXA)**

In Study VI, Dual-energy X-ray absorptiometry (DEXA) (Lunar DPX, version 3.6Z software) was used to assess whole body composition and percent body fat. Subjects were measured in supine position on the same time of the day on consecutive measurements. Good-to-excellent test-retest
reliability has been reported for the measurement of body composition, body fat and lean muscle mass in young and old women [161].

**NEURAL ACTIVATION**

**Electromyographic recordings (EMG)**

In Study II and V surface EMG-recordings were carried out to measure neuromuscular activity in the quadriceps and hamstring muscles. After careful preparation of the skin, pairs of surface electrodes (Medicostest Q-10-A, 20 mm inter electrode distance) were placed over the belly of vastus lateralis (VL), vastus medialis (VM) and rectus femoris (RF). All electrode positions were carefully measured for each subject to ensure identical recording sites throughout all tests. EMG and dynamometer strain-gauge signals were synchronously sampled with a 1000-Hz analogue-to-digital conversion rate using an external analogue-to-digital converter (dt 2801-A, Data Translation, Marlboro, MA). Subsequent, during later off-line analysis, EMG signals were digitally high-pass filtered with a fourth-order, zero-lag Butterworth filter with a 5-Hz cut-off frequency, followed by a moving root-mean-square filter with a time constant of 50 ms. Maximum EMG amplitude of the RMS-filtered signal was identified for the entire contraction phase and to reflect neural adaptations in the early phase of contraction, integrated EMG (iEMG) and mean average voltage EMG (MAV=iEMG/integration time) were calculated in time intervals of 0-30 ms, 50 ms, 100 ms and 200 ms relative to the onset of EMG integration, which was initiated 70 ms before force onset to account for electromechanical delay [3]. The degree of antagonist co-contraction was calculated by dividing maximum antagonist hamstring EMG by maximum agonist hamstring EMG measured during maximal isometric knee flexion. For the quadriceps muscle acceptable reproducibility has been observed for the surface EMG recorded during static as well as dynamic contraction conditions, including isokinetic knee extension [176, 258].

**CELLULAR AND MOLECULAR ANALYSES**

In Study III, IV and VII-IX muscle samples were obtained from the middle portion of m. vastus lateralis utilising the percutaneous needle biopsy technique of Bergström [25] in order to perform cellular and molecular analyses.

**Muscle fibre CSA and fibre type composition**

After dissecting the muscle samples of all visible blood, adipose and connective tissue, the muscle samples were oriented in embedding medium (Tissue Tec) frozen in isopentane cooled with liquid nitrogen and stored at -80° C. Subsequently serial transverse sections (10 mm) were cut in a cryostate at -20° C and stained for myofibrillar ATPase at pH 9.4 after both alkaline (pH 10.3) and acid (pH 4.3 and 4.6) pre-incubations [35]. All samples of each individual person were stained in the same batch to avoid inter-assay variation. Fibre type distribution (fibre number percentage, fibre area percentage) and fibre cross-sectional area for each of the three major fibre types (I, IIA, IIX) were analysed in a blinded fashion using computerized digital image analysis techniques. Since myofibre area is known to vary in a systematic way along the length and depth of the human VL muscle [152], all biopsies were obtained by the same investigator, and careful efforts were made to extract tissue from the same depth and with ~2 cm distance between each biopsy, which has been shown to be sufficient to avoid the influence of muscle damage from repeated biopsies [87].

Using comparable procedures, relatively high test-retest reliability has been reported for the assessment of fibre type composition (ICC of 0.88, 0.82, 0.56 for type I, IIA and IIX fibres) and fibre-type specific area (ICC of 0.74-0.82) [227]. Moreover, a 4-7% variation in fibre type composition has been reported between duplicate biopsy samplings, while the variation in fibre type distribution within a single biopsy was small (2-3%) when 200 fibres were analysed [28]. However, it should be recognized, that greater variations (12-19%) in fibre type composition and area have been reported with duplicate biopsy sampling procedures in the human VL muscle [95]. Yet, despite the needle biopsy sampling technique in the human VL muscle may show substantial with-in subject variability [152, 153], this remains the only known method for the evaluating of cellular morphology and phenotype composition in human skeletal muscle in vivo.

**Myogenic stem cells and myonuclei**

In Study VIII myogenic stem cells, also referred to as satellite cells (SCs), were identified by using antibody staining of mononuclear cells located between the basal lamina and the sarcolemma, to mark SCs expressing Pax-7 [123, 159]. In contrast to the membrane localization of membrane-bound neural cell adhesion molecule (N-CAM/CD56), the paired-box transcription factor Pax-7 is confined to the satellite cell nucleus [159], resulting in a lower density of Pax-7+ compared to the number of CD56+ cells typically observed in resting human skeletal muscle [159, 174], although fairly similar SC numbers also have been reported [156].

**Gene expression analyses**

In Study IV, VII and VIII total RNA was isolated [125] in order to study the molecular regulation in muscle size during disuse and subsequent re-training. Total RNA (500 ng) was converted into cDNA in 20 µl using the OmniScript reverse transcriptase (Qi-agen, CA, USA) according to the manufacturer’s protocol. The mRNA expression of FoxO1, FoxO3, FoxO4, PGC-1α, PGC-1β, IL-6, MGF, IGF-1Ea, GAPDH and RPLP0 were analysed by quantitative real-time RT-PCR. The amplification was monitored real-time using the real-time PCR analysis (MX3000P; Stratagene, CA, USA). The threshold cycle (Ct) values were related to a standard curve made with the cloned PCR products and specificity ensured by melting curves analysis and the quantities were normalized to RPLP0. Furthermore, TaqMan based quantitative real-time RT-PCR of MuRF-1, Atrogin-1, NF-κB, Bax, BCL2L1, p53, TNF-α, AGABARAPL1, and RPLP0 mRNA were performed in the ABI Prism 7900HT Sequence Detection System (Applied Biosys-tems) using ABI TaqMan Low Density Arrays (Applied Biosys-tems). Each sample was run in triplicates with 4 samples per card. Raw data were extracted and analysed using the SDS 2.1 software (Applied Biosystems), while qBasePlus (Biogazelle) was used to quality-check Ct-values, asses triplicates, exclude runs for difference among triplicates >0.5 Ct and finally to normalize data to RPLP0 using the 2-ΔΔCt method [157].

**Protein quantification**

In Study VII and IX mRNA expression data were
supplemented by protein quantification using Western Blotting analysis. From each muscle biopsy 150 cryosections (10 µm) were homogenized in a micro vial containing 1 silicium carbide crystal, 5 steel beads (2.3 mm) and 250 µl ice-cold homogenization buffer (Complete, Roche, Basel, Switzerland), using a FastPrep-24 (MP Biomedicals, Solon, OH, USA) homogenizer. Laemmli buffer was added and protein concentrations were determined with the EZQ Protein Quantitation Kit according to the manufacturer’s protocol (Molecular Probes, Eugene, OR, USA). After heating samples were separated by SDS-PAGE and gels were blotted (Trans-blot cell, Bio-Rad, 400 mA, 2 h) to polyvinylidene difluoride mem-branes (Amersham Hybond LFP, GE Healthcare, Buckinghamshire, UK). Total and phosphorylated protein pairs were detected simultaneously on the same membrane. Band intensities were quantified using densiometry analysis (Image); National Institutes of Health, Bethesda, MD, USA).

RESULTS AND DISCUSSION

EFFECTS OF SKELETAL MUSCLE DISUSE

Skeletal muscle mass and muscle strength are both known to decline in response to disuse, and the effects of inactivity on human skeletal muscle have been studied in a variety of different modes including bed-rest, unilateral lower limb suspension, immobilisation as well as actual and simulated microgravity [15, 33, 112] in order to gain knowledge about how muscle disuse affects the human locomotor system. Yet, the effect of ageing on human skeletal muscle disuse has not previously been given much attention, although it seems important to enhance our understanding of disuse-atrophy in bed-ridden patients and to shed light on its contribution to sarcopenia in older individuals. Based on such recent experiments, we here present and discuss data from three different human muscle disuse interventions to focus on the effects of ageing on skeletal muscle disuse-atrophy. At the outset, our population of elderly patients with hip-osteoarthritis was examined in order to enhance our knowledge about the effects of chronic muscle disuse on aging skeletal muscle (Study III). Further, in order to investigate potential age-related differences to human skeletal muscle disuse, we studied two well-defined periods of immobilisation in able-bodied elderly individuals, compared to young individuals with a comparable activity level (Study VI-VIII).

The impact of chronic disuse on aged human skeletal muscle

Chronic joint pain is a surprisingly frequent condition, which is estimated to affect more than 33% of individuals above the age of 45 years of age [70]. Furthermore, osteoarthritis (OA) has been shown to be the most common cause of inactivity and long-term disability in persons above the age of 65 years [70]. The impact of osteoarthritis on disability is therefore substantial and the risk of disability due to osteoarthritis is greater than any other medical condition in elderly persons [86]. Moreover, the presence of concurrent chronic conditions further increases the likelihood of subsequent disability [70]. Based on this knowledge, we studied a mixed population of men and women with osteoarthritis of the hip on the waiting list for a hip-replacement operation, to get deeper insights into how long-term (months to years) inactivity affects muscle function in elderly individuals (Study III).

Figure 1. Overall study scope and experimental methods

Skeletal muscle size and architecture

Decreased loading in terms of immobilisation, bed-rest or spinal-cord injury is known to induce marked muscle atrophy in younger human individuals [53, 106, 110, 119, 147, 260]. Notably, the relative loss in muscle mass over time is not linear but tends to plateau over time, with the highest atrophy rate observed within the first 7-10 days [53, 184, 242]. Even though chronic disuse due to joint pain may not be directly comparable to a standardised period of immobilisation, patients with chronic hip osteoarthritis in Study III showed a ~10% reduced anatomical quadriceps muscle cross sectional area (ACSA) on the affected side compared to the unaffected side (Study III). Additional contributing factors to the decrement in muscle strength with ageing comprise changes in structural components, such as increased intramuscular fat and connective tissue [154]. Therefore, in addition to ACSA, lean tissue cross-sectional area (LCSA) as well as inter- and intramuscular fat CSAs was measured using known CT density limits for fat and lean tissue to discriminate between contractile and non-contractile tissue within the muscle compartment area in Study V [229]. Both men and women showed an 8-10 % reduced lean quadriceps muscle CSA (LCSA) on the affected compared to the unaffected side (Study IV), in line with findings by Rasch et al that observed marked atrophy in all muscles around the hip and knee in a similar group of patients [201]. These results were further underlined by our findings of a reduced myofibre area (type I and type IIA fibres) on the affected compared to the non-affected side (Study IV).

In addition to a reduction in muscle size, changes in muscle architecture also contribute to the decrease in muscle force production with aging and/or disuse [127; 183].

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Consequently, in sarcopenia as well as skeletal muscle disuse-atrophy, it has been demonstrated that muscle fibre fascicles have a reduced pennation angle compared to healthy young individuals [177, 185]. In agreement with these findings, muscle fibre pennation angles on the osteoarthritic side in Study III were significantly smaller (-15%) compared to the healthy side, indicating that chronic disuse leads to significant changes not only in muscle size but also muscle architecture.

**Neuromuscular function**

Maximal contractile muscle strength and rapid force characteristics (RFD) are known to decrease with aging as well as in response to disuse [4, 253]. Notably, isokinetic strength has been demonstrated to be an important predictor of pain and disability in patients with gonarthrosis [160]. Somewhat surprising, maximal isometric quadriceps strength (MVC) of the unaffected leg was similar to that of healthy age-matched subjects in our cohort of patients with unilateral hip-osteoarthritis, [68, 146, 240]. In contrast, MVC on the affected side was markedly reduced (~20 %), along with a decline in muscle quality reflected by decreased specific strength (MVC/moment/CSA), underlining the severe consequences of chronic pain/disuse on mechanical muscle function (Study V). Notably, the clinical consequences of muscle strength asymmetry in the lower limbs are significant since a close relation to postural balance problems, decreased walking speed, as well as increased risk of falling has been shown [195, 196].

In general, women demonstrate lower muscle mass and maximal muscle strength than men throughout the adult life span, and therefore the risk of frailty is increased with ageing in women in particular [56]. Accordingly, women span, and therefore the risk of frailty is increased with maximal muscle strength than men throughout the adult life has been shown [195, 196].

Maximal contractile muscle strength and rapid force characteristics (RFD) is substantially reduced in healthy elderly compared to young individuals of both genders [46, 115, 194] although not all agree [58, 231]. In contrast, MVC on the affected side was markedly reduced (~20 %), along with a decline in muscle quality reflected by decreased specific strength (MVC/moment/CSA), underlining the severe consequences of chronic pain/disuse on mechanical muscle function (Study V). Notably, the clinical consequences of muscle strength asymmetry in the lower limbs are significant since a close relation to postural balance problems, decreased walking speed, as well as increased risk of falling has been shown [195, 196].

In summary, the present data demonstrate that chronic muscle disuse in the elderly is associated with marked quantitative as well as qualitative neuromuscular impairments. More specifically, chronic muscle disuse leads to decreases in muscle strength, muscle size (ACSA, LCSA, and myofibre area of type I and II A fibres), accompanied by impairments in muscle architecture (muscle fibre pennation angle), contractile properties (rapid muscle force characteristics) and neuromuscular activation (maximal EMG amplitudes). Furthermore, large side-to-side deficits were observed for specific strength (MVC/LCSA) and normalised RFD (RFD/CSA), indicating that a major part of the observed changes with disuse are explained by decreases in muscle quality.

**The effect of ageing on skeletal muscle disuse**

The plasticity in skeletal muscle mass homeostasis in response to decreased activity is fairly well described in young adults [8, 15, 22]. On the other hand, almost no attention has been given to the combined effects of ageing and skeletal muscle disuse, although muscle contractile function is known to be a crucial factor to maintain an independent lifestyle with ageing. Notably, the deterioration of mechanical muscle function with aging seems to be a result of changes in both quantitative and qualitative factors [5, 64, 254]. Moreover, in addition to changes in intrinsic factors, the level of physical activity is known to modify the age-related loss in muscle size and function [2, 134, 189, 199]. Hence, the fact that that our young and elderly able-bodied participants reported comparable levels of activity in the two present immobilisation experiments (Study VI-IX) lead us to believe that the observed differences between young and old prior to the intervention were mainly attributable to the effect of aging per se.

**Changes in muscle size and muscle architecture**

Muscle disuse in terms of immobilisation and bed-rest is known to induce significant reductions in anatomical muscle cross sectional area, muscle fibre area and muscle fibre pennation angle in young individuals [55, 106, 119, 183]. Data from older human individuals are limited, but in the animal model the majority of studies, find a higher magnitude of muscle atrophy in young compared to old animals [13, 37, 40, 194] although not all agree [58, 231]. In agreement with earlier findings in young subjects, we found significant reductions in muscle size (anatomical muscle cross sectional area and quadriceps muscle volume) in young individuals after 2 weeks of immobilisation [54, 106, 119],

**Changes in muscle size and muscle architecture**

Muscle disuse in terms of immobilisation and bed-rest is known to induce significant reductions in anatomical muscle cross sectional area, muscle fibre area and muscle fibre pennation angle in young individuals [55, 106, 119, 183]. Data from older human individuals are limited, but in the animal model the majority of studies, find a higher magnitude of muscle atrophy in young compared to old animals [13, 37, 40, 194] although not all agree [58, 231]. In agreement with earlier findings in young subjects, we found significant reductions in muscle size (anatomical muscle cross sectional area and quadriceps muscle volume) in young individuals after 2 weeks of immobilisation [54, 106, 119],
Study VI), and recently also demonstrated after 5 days of immobilisation [260]. However, in agreement with the majority of various animal models [13, 37, 40, 194], the atrophy response was significantly smaller in old compared to young individuals (Study VI).

In contrast, a larger muscle atrophy response has been reported in old compared to young adults, after 14 days of immobilisation of the human adductor pollicis muscle [251]. Part of the explanation may be due to the different muscle groups investigated, as smaller muscle groups have been suggested to respond differently to changes in loading [75]. Yet, a significantly larger muscle loss has also been observed after 10 days bed-rest in able-bodied older individuals [138].

In general, immobilisation and bed-rest induce similar atrophy responses of the lower limbs [8, 48] which makes the difference between the marked muscle loss (950 g lean lower limb mass) shown by Kortebein et al. [138] and the more moderate atrophy response observed in our study (Study VI) somewhat puzzling. The difference may, however, be explained by the observation of a negative nitrogen balance before the intervention with a further decline during bed rest in the bedridden participants [138], since a negative nitrogen balance is known to aggravate the magnitude of skeletal muscle loss with immobilisation [188].

In addition, changes in muscle size were assessed by histological analyses of muscle fibre cross sectional area after 4 and 14 days of immobility. Prior to the immobilisation interventions, myofibre CSA of type II fibres were smaller (~25-30%) in old compared to young muscle in both intervention studies, whereas no difference was observed in type I fibre CSA. Notably, decreases in mean myofibre area of approximately 10% were detected in both age groups, despite the brief period of muscle disuse (4 days) (Study VII). However, no difference was observed between the decline in type I and type II fibres at the 4 day time-point (Study IX), despite that the decline in type II fibres was significantly larger than in type I fibres in both young and old following 14 days of immobilisation [112]. Yet, in line with our whole muscle assessments, the decrease in muscle fibre CSA following 14 days of immobilisation was larger in young compared to older individuals ([112], Study VII) and consequently elderly individuals demonstrated less overall muscle loss with disuse than their young counterparts after 14 days of immobility (Study VI & VII).

Along with the reduction in muscle size, marked changes in muscle architecture were observed following 14 days of immobilisation (Study VI). Accordingly, more marked decreases in muscle fibre pennation angles of the VL fascicles were observed in young subjects compared to aged individuals (Study VI), underpinning the importance of muscle architecture to explain part of the discrepancy between the average relative decrease in muscle strength, which was about twice as large compared with the average relative decrease in muscle mass [1, 182, 215]. Collectively our findings in Study VI, VII and IX, are in line with previous findings obtained using various animal models, indicating that skeletal muscle disuse leads to larger loss of muscle mass (quadriceps volume, anatomical CSA, muscle fibre CSA and muscle fibre pennation angles) in young compared to older individuals, at least with more prolonged immobilisation (14 days). Interestingly, however, the muscle atrophy response observed within the first 4 days of immobilisation did not seem to be affected by age, since similar reductions in myofibre area were observed in young and old muscle.

Changes in neuromuscular function

It is evident, that ageing as well as muscle disuse bring about negative effects on the neuromuscular system, and although there are indications of differential effects of muscle disuse in young and old animals, data from human individuals are still limited.

In Study VI and IX, various parameters of mechanical muscle function (dynamic & isometric knee extensor muscle strength, specific force (MVC/CSA) and contractile rate of force development (RFD)) were assessed prior to immobilisation. In agreement with previous data [1, 54, 59, 106, 110, 119] marked reductions in contractile capacity were demonstrated following 4 days as well as 14 days of lower limb disuse, independently of age (Study VI & IX). Notably, the magnitude and time-course of changes were similar to earlier findings in young [17, 24, 54, 145] as well as old individuals [59, 139]. Of notice, maximal leg extension power and rapid muscle force capacity (RFD) have been shown to decline to a greater relative extent than maximal muscle strength with aging [133, 189, 232] and more importantly, has been advocated to be of greater importance than maximal muscle strength for the observed decline in functional status and the ability to counteract a fall [196, 233, 234]. Notably, our data demonstrated rapid force capacity (RFD, impulse) was affected to a greater extent in older individuals compared to young after 4 days and 14 days of disuse, especially during the very initial phase of muscle contraction (0–50 ms) (Study IX, [112]). In support hereof, maximal dynamic muscle strength during fast contractions (120°/s) has been shown to decline to a greater extent in old compared to young individuals following 7 days of immobilisation [59].

In addition to mechanical muscle strength parameters, the magnitude of central activation (neuromuscular activation) [171, 239] and resting twitch characteristics were assessed in the present line of experiments. Indicating changes in intrinsic (“qualitative”) mechanical muscle function with ageing, older subjects demonstrated lower resting twitch peak torque and resting twitch RFD prior to intervention compared to young individuals, followed by comparable decreases in activation as a result of muscle disuse (Study VII). In contrast, both young and old adults showed similar levels of central activation prior to immobilisation, in line with previous findings [94, 131, 212] and further supporting the observation of comparable habitual activity levels between our two age-groups. However, following immobilisation (14 days) older individuals experienced a decline (Study VII), whereas young subjects in agreement with previous findings remained unaffected [53]. Collectively, these observations suggest that disuse may enhance the age-related gap in neuromuscular function, observed with natural ageing.
Taken together, our neuromuscular data demonstrate that young and aged men experience similar declines in muscle contractile function following a brief period of muscle unloading at isometric and slow velocities of muscle contraction, whereas at faster contraction speeds as well as in terms of rapid force characteristics (RFD and Impulse), older individuals may experience more marked declines than young. Moreover, the present findings point toward an age-related difference in the susceptibility of central activation parameters to short-term disuse.

Molecular regulation of muscle disuse-atrophy

Although, important insights have been made concerning the molecular and genetic bases of skeletal muscle atrophy and aging in cell culture and animal models, only little is known about the underlying molecular mechanisms of skeletal muscle atrophy with aging and disuse in humans. Certain controversy exists in the literature regarding whether muscle atrophy in human skeletal muscle is regulated primarily by increases in myofibrillar protein degradation or a decrease in protein synthesis (Figure 2). In sup-port of the latter, solid evidence in the murine model, has pointed at protein degradation as the driving factor for skeletal muscle atrophy, with the ubiquitin-dependant proteolytic system being rapidly activated [29, 82, 149] in relation to unloading and various disease states [29, 149, 216]. In contrast, data from human in vivo studies have been less consistent [6, 45, 54, 119, 150]. Our data revealed a 1-2 fold up-regulation in MuRF-1 and Atrogin-1 mRNA expression in both young and old muscle during the initial days of immobility (~2-4 days), supporting a role for the ubiquitin-proteasome pathway in the initiation of human skeletal muscle atrophy. The fact that we observed more modest changes compared to previous animal reports may reflect that more drastic and hence more systemic wasting models were used in these animal studies [29, 82, 149] compared to human immobilisation models. Notably, the present data revealed that the expression levels of both Atrogin-1 and MuRF-1 returned to basal levels after 14 days of immobility, indicating that in human skeletal muscle the ubiquitin-proteasome pathway may not be important to maintain a more chronic atrophy response but rather plays a role in the very initial atrophy response (~days). In support of this notion, a biphasic time-course has previously been shown to exist for the mRNA expression profiles of selected atrogenes in the rodent model [216], which may explain that early transcriptional changes have been overlooked in previous human studies, which mainly have studied later time points of disuse. A coordinated regulation of the ubiquitin-proteasome and the autophagy-lysosome pathways has been shown to exist in the murine model [164, 218, 270], but somewhat surprisingly we did not observe any change in the mRNA expression profiles of ATG4, GABARAPL or FoxO3 (Study VII). However, we did see a trend towards an increase in LC3B II/I protein ratio selectively in young muscle after 1 and 4 days of immobility, indicating that the autophagic process (lipidation) was initiated at least in the young myofibres and thus, crosstalk between the ubiquitin-proteasome and the autophagy-lysosome pathways may also exist in the human model. The limited activation of the autophagy pathway could indicate that cross-talk between ubiquitin-proteasome and autophagy-lysosome pathways mainly occurs in more systemic atrophy models, although species-specific differences between the rodent and human model have been suggested to exist as well [262].

In addition to being a central regulator of muscle protein synthesis and muscle hypertrophy the IGF-1/Akt signalling pathway has been proposed to be a potent suppressor of myofibrillar proteolysis and atrophy related ubiquitin ligases, respectively [30]. Importantly, our finding of an age-specific decrease in P-Akt protein content indicates that immobility leads to a rapid (2-4 days) as well as more sustained (14 days) decrease in myofibrillar protein synthesis exclusively in young muscle, which at least in part explains the observation of a larger muscle loss in young compared old individuals (Study VII). In support of this notion, a diminished phosphorylation of Akt pathway components has been reported following 48h of immobility in young human subjects [6]. In contrast, the present data point toward an up-regulation of the muscle specific IGF1-pathway exclusively in old subjects (Study V), which in combination with a lack of change in the Akt pathway may explain the attenuated atrophy response in old muscle. However, our current knowledge regarding the age-related differences in the regulation of this pathway remain highly limited, and more studies clearly are needed to uncover the mechanisms underlying the apparent age-specific influence on disuse induced muscle loss that was observed in the present line of experiments.

Figure 2. Schematic overview of selected molecular signalling pathways regulating skeletal muscle homeostasis (see further details in the text). Dashed lines indicate pathways that still have to be completely defined.

Further, a marked down-regulation in genes involved in mitochondrial metabolism was observed (Study VII), consistent with recent human gene array studies [6, 45]. A rapid decrease in PGC-1α mRNA and PGC-1β mRNA gene expression was observed in young but not old muscle in the
most initial phase of immobilisation (1 day), where the response in old muscle was slightly slower (Study VII), although reaching statistical significance within the first 4 days of immobilisation (Study VII). However, there was no difference between age groups at the 14 days’ time-point, in accordance with recent data from Gram et al. [84]. These findings support the hypothesis that down-regulation in accordance with recent data from Gram et al. [84]. These findings support the hypothesis that down-regulation

In summary, our data point toward a number of intracellular signalling pathways for both muscle atrophy and hypertrophy being activated in the very initial phase of immobility, in turn leading to a rapid initial atrophy response in both young and aged muscle, followed by a decaying atrophy response at later time-points. Notably, our data showed a parallel activation of the ubiquitin-proteasome pathway along with the IGF/Akt indicating that proteolyses may be an important component in the initiation of human disuse atrophy in both young and old muscle, whereas the myocellular regulation in protein synthesis and IGF-1 and PGC1α are important determinants for the initiation of human skeletal muscle atrophy, as also observed in rodents [219], although no indications of FoxO3-dependant transcriptional changes were noted in the present study in contrast to previous animal data obtained using systemic muscle wasting [219, 220]. It can be speculated, that one reason for the slower and/or attenuated atrophy response to immobility in aged compared to young human muscle could be a consequence of the general decrease in oxidative metabolism observed with aging. However, recent findings from Gram et al elegantly have demonstrated that 14 days of inactivity and subsequent re-training alter mitochondrial biogenesis to a similar extend in young and elderly males [84].

Another topic of debate has been the role of the apoptotic pathway in human skeletal muscle atrophy and sarcopenia. Using animal models there are a significant amount of data indicating an important role for apoptosis in the development of muscle atrophy observed with aging [62, 166, 167, 192, 193, 230], whereas human data have been more inconsistent [163, 238, 265]. In essence, our data showed a marked and rapid increase in the expression of apoptotic markers with immobilisation, with indications of a more pronounced response in old muscle cells (Study VII). Notably, despite that a general (i.e. non-specific) increase in TUNEL-positive nuclei was observed primarily in muscle biopsies from old individuals after immobilisation, specific myocellular TUNEL-positive myonuclei did not appear to increase in neither young nor old adults, in contrast to previous findings in the murine model [67]. Thus, in the present experiments an up-regulated number of TUNEL-positive nuclei mainly were localized in the interstitiel space, indicating that intrinsic myofibre apoptosis may not play a key role for the mediation of human disuse.

In summary, our data point toward a number of intracellular signalling pathways for both muscle atrophy and hypertrophy being activated in the very initial phase of immobility, in turn leading to a rapid initial atrophy response in both young and aged muscle, followed by a decaying atrophy response at later time-points. Notably, our data showed a parallel activation of the ubiquitin-proteasome pathway along with the IGF/Akt indicating that proteolyses may be an important component in the initiation of human disuse atrophy in both young and old muscle, whereas the myocellular regulation in protein synthesis and mitochondrial function seems more age-dependent.

Although fundamental mechanistic questions still remain to be answered, our data indicate that the orchestrating of cellular and molecular mechanisms that contribute to the ability of skeletal muscle to repair and re-growth are largely unknown and the cellular and molecular mechanisms that contribute to the

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<th>Measured variable from signalling pathways</th>
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Table 3: Skeletal muscle signalling responses during immobilisation in young and old individuals.

RE-GROWTH OF HUMAN SKELETAL MUSCLE

Human skeletal muscle is a highly plastic tissue, which is reflected in its rapid ability to adapt to changes in loading intensity, at least in young individuals [112, 260]. In contrast, the ability of skeletal muscle to repair and re-growth is known to diminish with aging [36, 44, 85, 269]. Yet, the mechanisms responsible for the diminished ability of aged skeletal muscle to re-growth are largely unknown and the cellular and molecular mechanisms that contribute to the
recovery in muscle mass after reduced mechanical loading are just beginning to become unravelled. However, as muscle atrophy not only compromises physical functioning but also is associated with increased frailty and mortality, it seems important to expand our understanding of the mechanisms involved in muscle re-growth to develop methods to maintain or improve muscle mass during or following periods of disuse. Thus, in order to expand our knowledge regarding the ability of aged human skeletal muscle to recover from longer or shorter periods of disuse, we investigated the capacity for muscle re-growth and restoration of mechanical muscle function in the three groups of patients/participants described above who underwent skeletal muscle disuse of various lengths (hip-replacement patients, as well as two groups of healthy elderly and young individuals, respectively).

**Effects of re-training in elderly post-operative patients**

An infinite number of medical and surgical illness states lead to the development of hospital associated deconditioning [136]. Several common etiologic factors contribute to this effect, including the specific medical or surgical illness necessitating hospitalisation, the adverse effects of treatment (including surgical interventions), bed rest inactivity, as well as the detrimental effects of aging per se [14, 107, 138, 254].

Although much effort is done to minimize surgical intervention, effects of major surgery are still associated with an increased risk of morbidity, convalescence and disability [49, 73, 126, 128]. Major surgery is furthermore known to elicit a catabolic stress response that leads to a reduced protein synthesis and a reduction of lean tissue mass [48, 129]. Consequently, a significant number of elderly patients experience a decline in functional performance after surgery, and more importantly a large proportion of these patients do not regain their functional level without specific rehabilitation programmes [162, 203, 221, 225, 226, 249, 259, 261]. To minimize deconditioning and enhance recovery, the concept of “fast-track surgery” has been introduced as a coordinated effort to combine uni-modal evidence-based principles of care into a multi-modal effort, which has evolved as a valid concept to improve post-operative outcome [128, 245]. Yet, loss of muscle mass is not completely counteracted by the implementation of “fast-track regimes” [129] and growing evidence indicates that rehabilitation programmes have to be highly specific and of sufficient intensity to counteract decreases in muscle strength and muscle mass in postoperative elderly patients [103, 221, 240, 241, 250]. Notably, the use of resistance training is known to improve muscle strength and functional performance both when initiated in the early post-operative phase (weeks to months) [103] as well as in the late post-operative phase (months to years) [221, 224, 250]. Yet, knowing that the detrimental effects on muscle tissue properties are most dramatic during the first weeks of immobilisation [15, 48, 240], Study VII it seems rational to initiate specific training intervention as soon as possible after surgery. We therefore set out to investigate the effect of various rehabilitation regimes initiated in the very early postoperative phase (1-2 days post-surgery) in elderly individuals undergoing elective hip-replacement surgery [241, 244]. Based on the findings from earlier studies [221, 224, 250] voluntary resistance training was compared to peripheral muscle stimulation (NMES) and conventional rehabilitation activities in order to evaluate the neuromuscular adaptations elicited by these three different exercise modalities (Study I-IV).

**Skeletal muscle size and architecture**

Despite resistance training is known to induce marked increases in anatomical muscle cross sectional area and muscle fibre area, in aged [90, 92, 179, 213, 248, 263] as well as in very old individuals [71, 142] the use of resistance training is still not widely used to rehabilitate elderly patients following major surgery. In fact, only few studies have tried to apply intensive strengthening exercises in the acute post-operative phase (Study I, II) [111, 116]. In the light hereof, one of the most important findings of Study I and II was the feasibility of applying resistance training and electrical stimulation in the acute post-operative phase (1 day after surgery). Moreover, there was marked differences between the three different rehabilitation regimes investigated (resistance training, electrical muscle stimulation and functional exercises) on quadriceps muscle size and quadriceps muscle architecture. Despite successful surgical outcome and the use of early mobilisation strategies during hospitalisation, we observed a further decrease in CSA at five as well as twelve weeks post-surgery in the patients who received the conventional rehabilitation program based on functional exercises with no external loads applied. In contrast, 1 hour/day of neuromuscular electrical stimulation (NMES) of the quadriceps muscle, nearly counteracted a decline in muscle size (Study I & II), in accordance with previous results from young patients [16, 77], and recently also in intensive care unit patients [63]. Amplifying this effect, resistive exercises not only prevented the surgery associated muscle atrophy at five weeks, but further increased CSA after twelve weeks. As a result, a significant difference in treatment outcome was observed between the resistance-training group and the conventional rehabilitation group after 12 weeks of rehabilitation. Further, muscle fibre area increased by 32% following 12 weeks of resistance training, and in line with previous studies in young [1] and old individuals [142] more marked gains were observed for the type Ila and Ix fibres compared to the type I fibres (Study III). Consequently, side-to-side deficits in anatomical CSA and muscle fibre CSA (type 1 and type 2a) were fully eliminated after 12 weeks of resistance training, while still persistent following NMES and conventional rehabilitation (Study III).

In addition to the muscular changes relating to muscle fibre size, ageing also leads to marked alterations in muscle architecture that potentially contribute to the loss in muscle strength [185]. A reduction in muscle fibre pennation angle in old com-pared to young individuals has previously been observed, suggesting that a significant part of the decrease in muscle function with aging may be related to changes in muscle architecture [185]. Both in sarcopenia and disuse atrophy, muscle fibre fascicles seem to have a reduced pennation angle compared to healthy young individuals, likely due to decreased amounts of contractile tissue [177, 183]. In agreement with these findings, muscle fibre...
penetration angles on the osteoarthritic side were substantially reduced compared to the unaffected side (Study IV). However, after 12 weeks of RT there was a significant increase in VL muscle fibre penetration angle, comparable to the changes typically reported following resistance training in able-bodied young and old individuals after comparable periods of resistance training [1, 178]. In contrast, no improvements in muscle fibre penetration angle were observed for patients subjected to electrical stimulation or functional exercises.

Neuromuscular function and functional capacity
Alongside the above morphological adaptations, marked changes in mechanical muscle strength parameters and functional capacity were observed after 12 weeks of rehabilitation, however, the observed changes were highly dependent on the type of rehabilitation regime (Study I & II). Notably, marked increases was observed maximal muscle strength (dynamic and isometric) in response to 12 weeks of resistance training, despite patients being rather frail during the initial 4-6 weeks after surgery. Comparable gains in maximal muscle strength have been demonstrated following resistance training in healthy elderly individuals [93, 151, 207], as well as in frail elderly [26, 71, 99, 100]. In contrast, no gains in maximal muscle strength parameters could be observed with electrical stimulation (Study II), which is in agreement with earlier findings in young patients after ACL-reconstruction and conventional rehabilitation [16], while also lacking to be demonstrated in studies evaluating the effect of physiotherapy exercises (with no external loads) after hip-surgery [226, 249]. Consequently, the muscle strength asymmetry observed in all patients prior to the operation was fully reversed following twelve weeks of resistance training, while not affected by electrical stimulation or conventional rehabilitation (Study I). This finding is potentially of high importance since asymmetry in lower limb muscle strength is related to fall prevalence in elderly adults [196, 234]. Moreover, the elimination of muscle asymmetry is noteworthy, since the non-operated side was equally strong compared to that of able-bodied age-matched individuals [68], resulting in a normalisation of neuromuscular performance after only 12 weeks of resistance training in patients subjected to many years of chronic disuse and subsequent hip-replacement surgery. Of note, is also the fact that no training related complications were observed in any of the three intervention groups, despite all three training regimes were commenced in the very early post-operative phase (1-2 days after surgery).

The ability to develop force rapidly (i.e., demonstrating high contractile RFD) is an important performance characteristic, especially in older people, contributing to several tasks of daily life such as climbing stairs, walking, and attempting to avoid a fall [19, 72]. However, significant increases in RFD and elevated neuromuscular (EMG) activity have been demonstrated in healthy elderly individuals following 3-6 months of resistance training with special focus on increasing muscle power [89, 91]. In order to avoid postoperative injuries while still ensuring a sufficiently high loading intensity to induce measurable gains in muscle size [57, 71] and neuromuscular performance characteristics, as previously demonstrated in young individuals [3] we used a progressively adjusted exercise program known to be effective of inducing adaptive muscular changes in able-bodied elderly individuals [68]. To our best knowledge, potential changes in RFD characteristics have not previously been evaluated after NMES or conventional rehabilitation.

In accordance with earlier findings in young individuals [3, 93, 141], resistance training lead to marked increases in rapid force production, in the very initial phase (30–50 ms) as well as the later part (100–200 ms) of the isometric force-time curve, with similar gains in contractile impulse. Notably, the increase in RFD was still present after normalising for muscle size (RFD/CSA), indicating qualitative changes in muscle contraction characteristics may have occurred, such as increased maximal motor unit firing frequency [252] and/or changes in myosin heavy chain isoform composition toward an increase in type II fibre area percentage [1]. The importance of these positive adaptations in rapid muscle force characteristics are underlined by the fact, that a positive correlation was observed between the increase in maximal gait speed and the increase in absolute RFD following 12 weeks of resistance training, which was even stronger when related to normalized RFD (RFD/CSA) in the very initial phase of muscle contraction (0–30 ms). In contrast, no relationship could be observed between the change in maximal walking speed and changes in maximal muscle strength characteristics and/or muscle size (Study II).

Apart from gains in muscle size and neuromuscular characteristics, resistance training and NMES produced marked increases in functional performance parameters (walking speed, chair rise performance and stair climbing), although most present following resistance training (Study I). The enhancement in horizontal walking speed was particularly noticeable, since maximal and habitual walking speed are powerful predictors of future disability and dependency [88, 236]. Moreover, it is thoughtful that despite all resistance training exercise were performed unilaterally the marked changes in unilateral muscle strength and muscle size were translated (nearly 1:1) into increases in functional tasks that comprise two-legged coordinated movement. This finding underlines the importance of specifically focusing on reducing observable deficits (muscle size and strength) between sides before both legs are trained simultaneously. Another puzzling finding was the observed increase in functional performance with NMES that, although being more modest than the changes observed with resistance training, clearly were superior to those achieved by conventional rehabilitation (Study I). However, despite there was no measurable gains in neither neuromuscular function nor muscle size with NMES, a majority of the decline observed in the assessed parameters at 5 weeks post-operatively with conventional rehabilitation was largely prevented with NMES (Study I), underlining the importance of focused intervention to counteract the decline in muscle mass and function during hospitalisation.

In summary, the marked increases in neuromuscular performance observed with resistance training were translated into significant gains in activities of daily life (ADL) function, manifested by increased walking speed, enhanced ability to rise from a chair and improved stair climbing performance (Study I-II). In contrast, despite producing no increases in neuromuscular performance characteristics (dynamic and
The effect of post-operative re-training on the IGF-I pathway

The IGF-I pathway is known to be an important stimulator of anabolic signalling and one of the key factors responsible for increasing rate of protein synthesis in skeletal muscle [7]. How-ever, based on the findings that senescent muscle in the animal model demonstrates an attenuated recovery response after immobilisation and injury [36, 44, 85, 269], it has been hypothesized that sarcopenia may in part be due to a failure to generate IGF-I isoforms necessary to initiate the remodelling of muscle by stimulating satellite cell activation and proliferation [80]. In order to describe the potential interaction between changes in muscle morphology and IGF-I splice variants in elderly frail patients, changes in muscle fibre area and mRNA expression levels of IGF-Ia and the mechano sensitive IGF isoform IGF-Ic (MGF) were assessed in our group of hip replacement patients prior to surgery and subsequent to our three intervention regimes (Study IV). In human exercise studies, the expression of IGF-I mRNA has been found to increase acutely after a single bout of resistance exercise [18], although no total agreement exists [97, 197]. However, more consistent increases in IGF-I have been demonstrated at both the mRNA level [96, 144, 190] and the protein level [228] following prolonged periods of resistance training.

The activation of myogenic stem cells (satellite cells) and their donation of new nuclei to the exercised myofibres seem required for hypertrophied fibres to maintain an optimal DNA to protein ratio [121]. Since MGF is suggested to play an important role in the activation of satellite cells [268], the possibility exists that IGF isoforms are involved in the promotion of muscle growth and repair during the process of reloading subsequent to periods of disuse. Known to be involved in muscle repair [81] it does not seem surprising that MGF mRNA levels were elevated in all three intervention groups compared to the non-operated-side at 48 h post-surgery (Study IV). However, in contrast to NMES and conventional rehabilitation, MGF mRNA expression levels did not decrease in the RT group, which could support the hypothesis that MGF could be involved in both muscle repair (1-2 days post-surgery) as well as in the robust hypertrophy response observed with resistance training (5 weeks and 12 weeks). Moreover, increases in absolute levels of IGF-Ia mRNA and MGF mRNA were only observed in response to resistance training intervention, with no changes detected following NMES or conventional rehabilitation (Study IV).

Importantly, albeit the response may be attenuated in old human muscle as well as in old muscles of various animals, older muscles appear capable of up-regulating the expression of both IGF-Ia and MGF mRNAs in response to a period of prolonged resistance training even after surgery and/or immobilisation.

Collectively, Study IV-V, demonstrates that elderly patients who undergo elective hip-replacement surgery achieve significant muscular, neuronal and functional benefits from intensive physical training initiated in the very early the postoperative phase. Specifically, resistance training more effectively increased muscle morphology (size and architecture), neuromuscular characteristics (muscle strength, RFD, impulse, EMG, central activation) and functional performance (walking speed, chair rise and stair climbing) compared to NMES or conventional rehabilitation.

The effect of aging on skeletal muscle re-growth

It is well known that the ability to produce muscle re-growth after injury and immobilisation is impaired in animal senescent muscle [44, 85, 269]. In human individuals however, it still re-mains largely unknown, which factors and mechanisms promote or hinder the recovery of muscle mass following short or longer periods of disuse. Based on the findings of an impaired ability for muscle re-growth in animal models and our findings of an age-specific regulation and time-course of skeletal muscle disuse-atrophy, we hypothesized that a similar age-specific regulation might exist in response to human skeletal muscle recovery after disuse atrophy. In the light of a steadily increasing aging population leading to an increasing number of persons recovering from shorter or longer periods of muscle disuse, it seems rational to expand our current understanding of the mechanisms involved in human muscle re-growth in order to facilitate the development of approaches to maintain and regain muscle mass during periods of skeletal muscle disuse. We therefore assessed the effect of recovery following 4 days and 14 days of immobilisation, respectively. To optimize the conditions for complete muscle recovery, supervised resistance training was applied in both age groups. Following the 4 days disuse intervention participants were admitted to a 7 days recovery regime (Study IX), whereas participants exposed to 14 days disuse intervention were re-trained for 4 weeks (Study VIII).

Skeletal muscle size and muscle architecture

Reloading of disuse-induced skeletal muscle atrophy, by means of resistive types of exercise, is known to fully restore muscle mass through hypertrophy in young human individuals [22, 32, 106, 110, 119], however, only few data exists on the changes in skeletal muscle size following brief periods (less than a week) of unload-ing followed by active exercise-based recovery (Study IX). Thus, our somewhat limited assessments of changes in muscle size and architecture following 7 days of recovery subsequent to 4 days of disuse (Study IX) reflected that we did not expect major changes following such a brief intervention period. Yet, 4 days of unloading induced substantial atrophy in both type I and type II myofibres of young and old individuals alike (Study VII & IX), which was reversed to pre-disuse values in both age-groups following 7 days of recovery, except for type I myofibre area in old individuals that remained suppressed compared to pre-levels (Study IX).

In comparison, despite a larger atrophy response following 14 days of immobilisation young individuals demonstrated a greater increase in quadriceps muscle size (Qvol, ACSA and PCSA) in response to 4 weeks of retraining leading to a full restoration in muscle mass. In contrast, despite a smaller atrophy response, old individuals did not fully recover quadriceps muscle size after retraining. A similar pattern was observed for the changes in muscle architecture, with a more modest decrease in muscle fibre pennation angle seen.
in old compared to young with no full reversal in old participants despite 4 weeks of intensive re-training (Study VI). This finding was further supported by our analyses of muscle fibre cross sectional area which showed robust increases in type I and II myofibre area in young subjects during the recovery phase, whereas aged individuals showed no changes in neither type I nor type II myofibre area, leading to an overall difference in the recovery response to re-training between young and old (Study VIII). Notably however, apart from reflecting a significant age-related difference to re-loading, the observed differences between young and old adults may also reflect that a limited period of re-training was used in the present experiments, as substantially longer intervention periods have demonstrated significant gains in quadriceps size ([31], Study I-II), muscle architecture (Study III) and muscle fibre area (Study III) in older individuals recovering from muscle disuse and/or surgery.

Altogether, the present data indicate that despite intensive rehabilitation efforts aging is accompanied by an impaired ability to recover from short-term disuse muscle atrophy, and consequently old individuals may need a longer time to recover from periods of disuse compared to young individuals.

**Neuromuscular function**

Parameters of mechanical muscle output (contractile capacity) and neuromuscular function are both strong predictors of general functional capacity, quality of life, and risk of mortality in aged individuals [39, 186, 267], which underlines the importance of gaining more insight to the time-course and potential age-related differences in the recovery of neuromuscular function in human individuals following periods of disuse. In young adults, mechanical muscle function (isometric and dynamic MVC) has been demonstrated to be fully reversed following 3-6 weeks of resistance training subsequent to 2 weeks of unloading / immobilisation [32, 106, 119, 145] in agreement with the present findings (Study VI, [112]). Moreover, it is noteworthy that decreases in dynamic muscle strength observed following 4 weeks of unloading in young individuals were fully reversed after 7 weeks, despite no training intervention [22]. However, almost no previous studies have focused on disuse lasting less than 1 week, and the subsequent recovery phase. Based on the present experiments, 7 days recovery (subsequent to 4 days immobilisation) appeared effective of restoring knee extensor mechanical muscle function in young subjects, while in contrast maximal muscle strength characteristics remained suppressed in our old subjects (Study IX). Similar trends were observed for rapid force capacity (RFD, impulse) at the very initial phase of contraction (0–50 ms) that tended to remain reduced relative to baseline levels after 4 weeks of re-training subsequent to 14 days of disuse [112]. The observed impairment in restoring lower limb mechanical muscle function following short-term disuse in old adults may in part reside from alterations in qualitative muscle factors, as contractile rate of force development tended to remain reduced in old individuals following 7 days of recovery (Study IX), as well as following 4 weeks of re-training [112], whereas maximal isometric strength capacity recovered to a more full extent. Yet, our older individuals were able to reverse the deficit in central activation with 4 weeks of resistance training (following 14 days of immobility), whereas young individuals reached values above the baseline activation level (Study VI).

In summary, the findings of the present data suggest that the magnitude and time-course of changes in mechanical muscle function during the recovery phase following short-term disuse are compromised in old compared to young individuals.

**Myogenic stem cells**

The regulation in muscle growth and maintenance of muscle mass is known to be influenced by a unique population of muscle resident stem cells referred to as myogenic progenitor cells or satellite cells (SCs) [168]. Satellite cells represent a heterogeneous population of adult muscle stem cells that are normally quiescent and were identified more than 50 years ago as nuclei located in a niche between the sarcolemma and the basement membrane of the muscle fibre, and known to play a key role in the maintenance, growth and repair of myofibres [105, 168, 180, 181].

In humans, the pool of SCs seems to be maintained into the sixth-to-seventh decade of life [190, 214], with a decline in con-tent and activation capabilities with progressive ageing [122, 256] leading to a reduced muscle regeneration capacity in response to myofibre injury and disuse [41, 42]. However, despite the group of aged individuals in the present experiments our old participants (~67 years) demonstrated impaired SC proliferation capacity compared to young individuals (~24 years), indicating that the SC response to re-loading and exercise might be attenuated. Interestingly, age-related differences in SC proliferative capacity were detectable in the acute (+ 3 days) as well as the prolonged (+ 4 weeks) phase of re-training, in line with reports by Dreyer et al. [65] who reported a greater increase in SC content in young compared to aged human skeletal muscle within 24h following 92 eccentric contractions [65].

The importance of SC number in relation to muscle size has previously been reported by Petrella et al [191] that found a positive association between SC number at baseline and gains in muscle fibre area following 16 weeks of resistance training in young and older human individuals [191], suggesting that the individual myogenic potential may at least partially be pre-determined by the availability of satellite cells prior to training. Expanding those observations, we observed for the first time in human individuals a positive relationship exists between SC number and mean muscle fibre area (MFA) following disuse-atrophy (2 weeks) as well as moderate-to-strong associations the magnitude of muscle hypertrophy and gains in SC number in response to exercise-based reloading (4 weeks) (Study VIII).

As aging is associated with a preferential reduction in muscle fibre type II size it may be speculated that SC content would decrease more in type II fibres compared to type I fibres. In young human individuals SC content is similar between type I and II muscle fibres [124, 256]. In contrast, age-related type II muscle fibre atrophy is accompanied by a type II muscle fibre specific reduction in SC content [256, 257]. Interestingly, in the present line of experiments positive associations were observed between the number of Pax7+
In summary, our data demonstrated substantial age-specific differences in the re-growth capacity of human skeletal muscle following immobility-induced muscle atrophy. Specifically, aged individuals showed a reduced responsiveness to the re-loading exercise paradigm, reflected by reduced gains in myofibre area that were accompanied by an attenuated capacity for SC proliferation.

Molecular regulation of skeletal muscle re-growth

The reduced capacity of aging skeletal muscle to recover after disuse indicates that molecular signalling pathways regulating muscle hypertrophy/re-growth are altered at increasing age and/or that negative regulators of muscle mass become progressively more active with aging. We therefore set out to profile a range of positive and negative growth factors associated with local skeletal muscle milieu that are known to stimulate the proliferation of SCs [83]. Among these factors, Insulin-like growth factor (IGF-1Ea) and mechano-growth factor (MGF) mRNA expression levels and protein content have been shown to correlate with the increase in whole muscle DNA content in response to compensatory muscle overloading [9]. Further, MyoD and myogenin mRNA expression levels were also assessed in the present experiments since these factors are part of the family of myogenic regulatory factors (Myf5, MyoD, Mrf4 and myogenin) that play a key factor in the myogenic specification and differentiation of SCs in mature skeletal muscle [79, 187]. MyoD is primarily related to SC activation and proliferation, whereas myogenin reflects the phase of terminal myoblast differentiation [143, 187]. Among the multiple growth factors associated with the local skeletal muscle milieu that stimulate the proliferation of SCs, hepatocyte-growth factor (HGF) is considered one of the most important parameters [83]. Together with its trans-membrane receptor (c-met) HGF is a vital link in the cascade of signalling events that lead to activation of skeletal muscle SCs when myofibres are exposed to strain or injury [266]. Furthermore, a number of studies have identified various fibroblast growth factors (FGFs) and their receptors (FGFRs) to be key regulators of both senescence and self-renewal capacity in a variety of stem cell types [222]. Among those, fibroblast growth factor 2 (FGF2) and its receptor FGFR1 are known to stimulate SC proliferation [11, 173]. In addition to these factors, we also assessed the expression levels of myostatin mRNA, a member of the transforming growth factor-β family, which exerts a strong negative regulation on skeletal muscle mass [170] partly by inducing a sustained satellite cell quiescence [120, 169].

Together, our experiments showed age-independent differences in the time-course of IGF-1Ea and MGF regulation, respectively, with an acute and sustained up-regulation of MGF mRNA expression in response re-training, whereas IGF-1Ea expression was up-regulated only in the later phase of re-loading (Study VIII). Notably, changes in myofibre area were positively related to the corresponding changes in IGF-1Ea and MGF expression levels after 4 weeks of resistance training, strongly suggesting an essential role of these IGF isoforms for the induction of human muscle hypertrophy with training/re-loading. Moreover, MyoD and myogenin demonstrated markedly up-regulated expression levels with immobilisation in both age groups, whereas the sub-sequent recovery phase led to acute and sustained decreases in both MyoD and myogenin mRNA in young as well as aged skeletal muscle. While elevated mRNA expression levels of myogenic regulatory factors have been reported following prolonged (months) resistance exercise in both young and older adults [132, 140, 202], only a modest up-regulation in myogenin expression was observed following 4 weeks of resistance training in the present experiments (Study VIII), which may indicate that short-term (days-weeks) re-training after disuse-atrophy generate a different molecular signalling stimuli compared to that evoked by more prolonged exercise intervention.

Moreover, marked increases in HGF mRNA expression were observed in response to immobilisation as well as early and sustained re-training (+3d and +4wks) in line with the overall increase in SCs at these time-points (Study VIII). However, de-spite the age-dependent differences in SC proliferation no age-specific difference was found in the expression profiles of HGF. These seemingly conflicting observations may be due to a somewhat low number of subjects examined and/or could be caused by the relative large variation seen especially in the elderly sub-jects. Further, there was no significant change in the expression levels of FGF2 at any time-points, indicating that FGF may be of minor importance for SC proliferation in human skeletal muscle irrespectively of age.

Notably, we observed an age-specific influence on the pattern of myostatin regulation, with a larger increase seen in response to immobilisation followed by a smaller reduction with re-loading in our aged individuals (Study VIII). In turn, these observations may partly explain the impaired ability of SC proliferation and myofibre re-growth observed with re-training in aged skeletal muscle (Study VIII). In line with these findings, myostatin mRNA expression has previously been shown to increase following dis-use in young individuals, where the elevation in myostatin expression was related to the magnitude of myofibre atrophy [204]. Notably, our data revealed that the change in myostatin expression within the first days of re-loading (+3d) was negatively related to the change in Pax7+ cells, underlining the negative effect of myostatin on SC proliferation [120, 169].

Collectively, our data demonstrate that important age-specific differences exist for the capacity of myofibrillar re-growth of human skeletal muscle following immobility-induced muscle atrophy. Specifically, aged individuals seemed to respond less sensitively to the re-loading program, as reflected by significantly smaller gains in myofibre area, in parallel with a smaller increase in SC
number despite that no age-related differences were observed in local growth factors known to promote skeletal muscle hypertrophy and satellite cell proliferation (IGF-Ea, MGF, MyoD, myogenin, HGF). This attenuated responsiveness of old adults to the re-loading stimulus may partly be explained by a reduced sensitivity to the above growth factors since basal MRF mRNA expression appears to be generally up-regulated in senescent muscle [97, 132, 140, 202]. In addition, the present experiments also indicate that myostatin may play an important role for the impaired re-growth response of aged human skeletal muscle, as the regulation in myostatin mRNA expression was influenced by age, with old adults demonstrating greater increases with immobilisation followed by a less degree of down-regulation in response to subsequent re-loading. Notably, an association between the down-regulation in myostatin mRNA expression and SC proliferation was observed in the acute phase of re-loading, indicating a strong influence of myostatin signalling on the myogenic potential of human skeletal muscle in vivo.

**MAJOR CONCLUSIONS**

In summary, chronic muscle disuse in the elderly was associated with marked quantitative as well as qualitative neuromuscular impairments. More specifically, decreases were observed in muscle strength, quadriceps muscle size and myofibre area, muscle architecture, contractile properties and neuromuscular activation. Furthermore, substantial side-to-side differences in specific strength (MVC/LCSA) and normalised rapid muscle force capacity (RFD/CSA) were observed, indicating that a significant part of the observed changes in mechanical muscle function with disuse were explained by impairments in muscle quality. Importantly, within the first 4 days of immobility the observed atrophy responses did not seem affected by age, as manifested by comparable reductions in myofibre area in young and old individuals. However, in line with previous observations using various animal models, we observed a larger loss in muscle mass in young compared to older individuals after more prolonged immobilisation (14 days). Conversely, old individuals were more negatively affected with respect to neural function and rapid force characteristics than their young counterparts. Moreover, we showed that the initiation and regulation of human skeletal muscle atrophy with short-term disuse is age-dependant. Based on the present experiments it can be concluded that a multitude of signalling pathways related to both muscle atrophy and protein synthesis are activated in the initial phase of disuse, which in turn lead to a rapid initial atrophy response (~1-4 days) in both young and old individuals followed by a gradually attenuated atrophy response at later time-points (~2 weeks). Notably, during the first 1-2 days of immobility a parallel activation of the ubiquitin-proteasome pathway and the IGFI/Akt pathway seem to occur along with a deactivation of PGC-1α and PGC-1β, suggesting that cellular proteolysis plays an important role in the initiation of human disuse atrophy in both young and old muscle, whereas the concurrent regulation in protein synthesis signalling and proteolysis inhibition appears to affect young adults more pronouncedly compared to older adults. Gaining a better understanding of the ability of human skeletal muscle to recover from disuse-induced atrophy has important implications for the development and implementation of effective counter-measures against physical frailty in the increasing population of elderly. Importantly therefore, the present experiments demonstrate that resistance training is highly effective of increasing maximal muscle strength and neuromuscular function in elderly post-operative patients. Importantly, these increases in mechanical muscle function were accompanied by gains in muscle size, architecture and in the expression of IGF-I mRNA splice variants, resembling that typically seen in young healthy individuals when exposed to resistance training. In contrast, these positive adaptations could not be achieved with the use of neuromuscular electrical stimulation or conventional rehabilitation efforts alone. Collectively, these findings strongly underline the importance of implementing resistive exercises in future rehabilitation programs for elderly individuals.
myostatin play an important role in the impaired ability of aged human skeletal muscle to recover from immobility-induced muscle atrophy.

**PERSPECTIVES**

The present line of experiments revealed that the adaptive plasticity in skeletal muscle mass and central nervous system function associated with unloading and subsequent re-mobilisation, differ between young and older individuals (Study VI-IX). Notably, this influence of aging on muscle mass homeostasis is well documented in various animal models [36, 44, 85, 269]. However, in human research there has been a tendency to overlook the importance of investigating the very early phase of disuse/unloading (1-5 days) where the atrophy response is most strongly manifested and important information regarding the regulation of human disuse atrophy has therefore been left unnoticed. Yet, our findings (Study VII) as well as others [6, 54, 243, 246] show evidence of an early rise in atrogens during human disuse with time-course patterns similar to what have previously been demonstrated in the murine model [216]. Collectively, these findings indicate that the regulation of human muscle disuse is more complex and not merely driven by a decrease in myofibrillar protein synthesis, as previously suggested [78, 210].

Despite, many links still remain to be elucidated in the puzzle of human muscle plasticity, the observation that the initiation and regulation of human skeletal muscle atrophy is age dependent may be important for the identification of biomarkers and future therapeutic intervention paradigms, which can be used to counteract human skeletal muscle atrophy in relation to aging and disuse.

Moreover, our finding that aging is accompanied by an impaired ability to recover from disuse muscle atrophy despite intensive re-training efforts, and, consequently, need a longer time to recover from periods of disuse may also explain the somewhat disappointing results from shorter rehabilitation studies [117]. Importantly, however, the findings from study I-V clearly demonstrate, in line with previous data [5, 71, 142] that elderly skeletal muscles respond very well to prolonged intensive resistance training. Consequently, this intervention modality should be more clearly recognized as one of the key tools in the rehabilitation of elderly individuals, including very old and frail patients. The findings from Study I-V showed that resistance training can be successfully initiated during a hospital stay, including the acute post-operative phase and in the initial days after discharge in order to counteract the decline in muscle function and loss of muscle mass normally associated with hospitalisation in elderly patients [136].

Additionally, the observation from Study II & IV that rapid muscle force capacity (RFD) and neuromuscular function remain trainable in elderly recovering from surgery has important implications for the design of future rehabilitation programs, especially when considering the importance of rapid muscle force capacity on postural balance control, maximal walking speed and other tasks of daily living [5].

**SUMMARY**

In order to study the influence of disuse and aging on skeletal muscle homeostasis, different human models were employed. Effects of chronic disuse were investigated in elderly patients suffering from uni-lateral hip-osteoarthritis, whereas the effect of short-term disuse (4 and 14 days of unilateral lower limb immobilisation) was assessed in healthy young and old individuals.

In summary, chronic muscle disuse in the elderly was associated with marked quantitative as well as qualitative neuromuscular impairments. More specifically, decreases were observed in muscle strength, quadriceps muscle size and myofibre area, muscle architecture, contractile properties and neuromuscular activation. Furthermore, substantial side-to-side differences in specific strength (MVC/LCSA) and normalised rapid muscle force capacity (RFD/CSA) were observed, indicating that a significant part of the observed changes in mechanical muscle function with disuse were explained by impairments in muscle quality.

Importantly, within the first 4 days of immobility the observed atrophy responses did not seem affected by age, as manifested by comparable reductions in myofibre area in young and old individuals. However, in line with previous observations using various animal models, we observed a larger loss in muscle mass in young compared to older individuals after more prolonged immobilisation (14 days). Conversely, old individuals were more negatively affected with respect to neural function and rapid force characteristics than their young counterparts.

Moreover, we showed that the initiation and regulation of human skeletal muscle atrophy with short-term disuse is age-dependant. Based on the present experiments it can be concluded that a multitude of signalling pathways related to both muscle atrophy and protein synthesis are activated in the initial phase of disuse, which in turn lead to a rapid initial atrophy response (~1-4 days) in both young and old individuals followed by a gradually attenuated atrophy response at later time-points (~2 weeks). Notably, during the first 1-2 days of immobility a parallel activation of the ubiquitin-proteasome pathway and the IGF-1/Akt pathway seem to occur along with a deactivation of PGC-1α and PGC-1β, suggesting that cellular proteolysis plays an important role in the initiation of human disuse atrophy in both young and old muscle, whereas the concurrent regulation in protein synthesis signalling and proteolysis inhibition appears to affect young adults more pronouncedly compared to older adults.

Gaining a better understanding of the ability of human skeletal muscle to recover from disuse-induced atrophy has important implications for the development and implementation of effective countermeasures against physical frailty in the increasing population of elderly. Importantly therefore, the present experiments demonstrate that resistance training is highly effective of increasing maximal muscle strength and neuromuscular function in elderly post-operative patients. Importantly, these increases in mechanical muscle function were accompanied by gains in muscle size, architecture and in the expression of IGF-I mRNA splice variants, resembling that typically seen in young healthy individuals when exposed to resistance training. In contrast, these positive adaptations could not be achieved with the use of neuromuscular...
recovery phase following short-term disuse were course of changes in mechanical muscle function during the recovery phase following short-term disuse were compromised in old compared to young individuals. Likewise, aged individuals demonstrated an impaired response to re-loading reflected by attenuated gains in myofibre area, in parallel with smaller increases in satellite cell number despite no age-related differences were observed in factors known to promote skeletal muscle hypertrophy and myogenic stem cell proliferation (IGF-Ea, MGF, MyoD, myogenin, HGF). Moreover, an age-specific regulation in myostatin mRNA expression was observed, characterized by an amplified increase in aging skeletal muscle with immobilisation that was followed by less down-regulation during the subsequent phase of re-loading. In combination with an association observed between the changes in myostatin expression and satellite cell proliferation in the acute phase of re-loading, these data indicate that myostatin play an important role in the impaired ability of aged human skeletal muscle to recover from immobility-induced muscle atrophy.

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