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

Charlotte Suetta

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Official opponents: Marco Narici and Jan Lexell

Correspondence: Department of Clinical Physiology, Nuclear Medicine & PET, Rigshospitalet Glostrup, Ndr. Ringvej 57, 2600 Glostrup, Denmark

E-mail: charlotte.suetta@regionh.dk

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THE PRESENT THESIS IS BASED ON THE FOLLOWING PAPERS:

I. Suetta C, Magnusson SP, Rosted A, Aagaard P, Jakobsen AK, Larsen LH, Duus B, Kjaer M. Resistance training in the early post-operative phase reduces hospitalization and leads to muscle hypertrophy in elderly hip surgery patients – a controlled random-ized Study. J Am Geriatr Soc. Dec;52(12):2016-22, 2004

II. Suetta C, Aagaard P, Rosted A, Jakobsen AK, Duus B, Kjaer M, Magnusson SP. Training-induced changes in muscle CSA, muscle strength, EMG and rate of force development in elderly subjects after long-term unilateral disuse. J Appl Physiol. 97, 1954-1961, 2004

III. Suetta C, Andersen JL, Dalgas U, Berget J, Koskinen SO, Aa-gaard P, Magnusson SP, Kjaer M: Resistance training induces qualitative changes in muscle morphology, muscle architecture and muscle function in elderly postoperative patients. J Appl Physiol. 2008 Jul;105(1):180-6.

IV. Suetta C, Clemmensen C, Andersen JL, Magnusson SP, Schjerling P, Kjaer M. Coordinated increase in skeletal muscle fiber area and expression of IGF-1 with resistance exercise in elderly post-operative patients. Growth Horm IGF Res. 2010 Apr;20(2):134-40.

V. Suetta C, Aagaard P, Magnusson SP, Andersen LL, Sipilä S, Rosted A, Jakobsen AK, Duus B, Kjaer M: Muscle size, neural activation and rapid force characteristics in elderly men and women - effects of unilateral long-term disuse due to hip-osteoarthritis. J Appl Physiol. 2007 Mar;102(3):942-8.

VI. Suetta C, Hvid LG, Justesen L, Christensen U, Neergaard K, Simonsen L, Ortenblad N, Magnusson SP, Kjaer M, Aagaard P. Effects of ageing on human skeletal muscle after immobilisation and re-training. J Appl Physiol. 2009 Oct;107(4):1172-80

VII. Suetta C, Frandsen U, Jensen L, Munk Jensen M, Jespersen JG, Hvid LG, Bayer M, Petersson SJ, Schrøder HD, Andersen JL, Heinemeier KM, Aagaard P, Schjerling P, Kjaer M. Aging affects the transcriptional regulation of human skeletal muscle atrophy. PLoS One 2012;7(12):e51238

VIII. Suetta C, Frandsen U, Mackey A, Jensen L, Hvid LG, Bayer M, Petersson SJ, Schrøder HD, Andersen JL, Aagaard P, Schjerling P, Kjaer M. Aging is associated with diminished muscle re-growth and myogenic precursor cell expansion in the early phase after immobility-induced atrophy in human skeletal muscle. J Physiol. 2013 Aug 1;591(Pt 15):3789-804.

IX. Hvid LG, Suetta C, Nielsen JH, Jensen MM, Frandsen U, Ør-tenblad N, Kjaer M, Aagaard P. Aging impairs the recovery in mechanical muscle function following 4 days of disuse. Exp Ge-rontol. 2014 Apr;52:1-8.

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 re-training.

MATERIAL AND METHODS

While relevant details related to Material and Methods are de-scribed 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.

Study	N=	Age (years)	Gender	Body weight (kg)	Height (cm)	BMI (kg/m²)
Retraining after Hip-replacement						
Study I,II,IV,V						
Conventional rehab	12	68 (62-78)	5M/7W	81.3 ± 5.8	169.8 ± 2.1	28.2 ± 1.7
NMES	11	69 (60-75)	5M/6W	79.0 ± 4.2	167.7 ± 2.8	27.9 ± 0.9
Resistance training	13	69 (60-86)	7M/6W	77.8 ± 4.5	168.0 ± 2.0	27.4 ± 1.4
Chronic disuse						
Study III						
Men	19	69 (60-79)	M	85.8 ± 3.6	173 ± 2.0	29 ± 1.0
Women	20	70 (60-86)	W	71.5 ± 3.6	163 ± 1.0	27 ± 1.0
4 days immobilisation study						
Study VII, IX						
Young	11	24.3 (21-30)	M	74.3 ± 2.4	180.4 ± 2,7	22.9 ± 0.5
Old	11	67.2 (60-72)	M	87.7 ± 3.0	178.8 ± 1.7	27.5 ± 0.6
14 days immobilisation study						
Study VII, VIII						
Young	11	24.4 (21-27)	M	72.2 ± 2.3	181.4 ± 1.8	22.1 ± 0.5
Old	9	67.3 (61-74)	M	84.8 ± 3.4	178.7 ± 2.6	26.3 ± 0.5

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 immobilization

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.

Study	I	II	III	IV	V	VI	VII	VIII	IX
Functional performance	X		X						
Dynamometry	X	X	X		X	X	X		X
Interpolated twitch					X	X			
Surface EMG		X			X				
CT Imaging	X	X			X				
MR Imaging						X			
Ultrasound Imaging			X			X			
Dexa Imaging						X			
Muscle biopsy analyses			X	X			X	X	X
Gene expression analyses				X			X	X	
Western Blotting analyses							X		X
Satellite cell analyses								X	

Table 2. Overview of methods used in Study I-IX

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, Chattecx, 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 dis-play 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].

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 fur-ther 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 super-imposed 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 ≥95% 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 resting doublet), at the highest attained force plateau (superimposed doublet), and 2 s after (potentiated resting 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 V). A slice thickness 8 mm was used and a scanning time of 5s 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 + 512 pixel matrix were obtained with a slice thick-ness 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 (Medicotest 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 cryotome 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, ATG4B, GABARAPL1, 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, Schwitzerland), using a FastPrep-24 (MP Biomedi-cals, 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, Buckingham-shire,UK). Total and phosphorylated protein pairs were detected simultaneously on the same membrane. Band intensities were quantified using densiometry analysis (ImageJ; 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 giving 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 stud-ied 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).

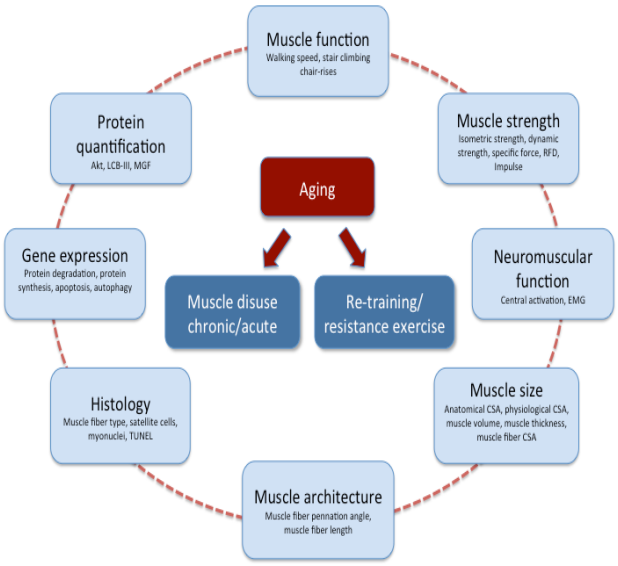

Figure 1. *Overall study scope and experimental methods*

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 V), 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].

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, maxi-mal 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 showed markedly lower MVC on both sides (~40%) compared to male subjects (Study IV). Notably, no difference between genders was detected when maximal muscle strength was normalised to lean cross sectional area (MVC/LCSA) (Study IV), in agreement with earlier investigations [155, 248, 255]. Importantly, however, specific strength on the affected side was reduced compared to the unaffected side in both genders of about 12-14%, in line with earlier results demonstrating a lower specific strength in sedentary elderly subjects compared to young individuals, whereas elderly subjects with a long-term history of strength or endurance training typically show similar specific strength compared to young individuals [134]. Furthermore, immobilisation leads to decreased specific force capacity in single muscle fibres of the quadriceps muscle after immobilisation in both young and old individuals [51, 113], indicating a deterioration in cellular muscle quality with skeletal muscle disuse.

In parallel with the decrease in maximal contractile muscle strength, the ability to develop force rapidly (i.e. contractile RFD) is substantially reduced in healthy elderly compared to young individuals of both genders [46, 115, 247, 255], although not a universal finding [46, 247]. However, we found absolute contractile RFD significantly to be reduced on the affected side com-pared to the unaffected side in both men and women (Study III). Notably, the affected side remained reduced when RFD was normalized to CSA in both genders, supporting the finding of a decrease in muscle quality with prolonged disuse.
Along with changes in mechanical muscle function, marked reductions in maximal EMG signal amplitude were observed during MVC testing on the affected side compared to the unaffected side in both genders (Study III), in agreement with earlier findings in young individuals after 4 weeks of unloading [21], suggesting that the decreases in contractile RFD on the affected limb at least partly was explained by changes in neuromuscular activation. In further support hereof, using interpolated twitch analysis we observed a significant muscle activation deficit on the affected compared to the non-affected side (Study III), in line with that observed by Stevens et al. after 7 weeks of cast immo-bilization in young subjects [237].

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 IIA 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 dete-rioration 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 elder 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],

Study VI), and recently also demonstrated after 5 days of immobility [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 be-fore the intervention with a further decline during bed rest in the bed-ridden 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 1 and type 2 fibres at the 4 day time-point (Study IX), despite that the decline in type 2 fibres was significantly larger than in type 1 fibres in both young and old following 14 days of immobilisation [112]. Yet, in line with our whole mus-cle 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 immobility 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 VI). 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 VI), 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 points 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 myofibril-lar 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.

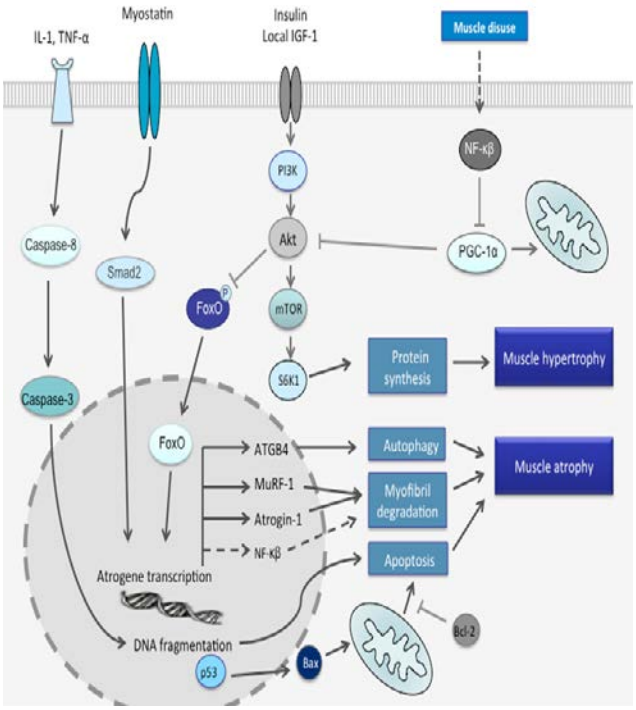

Figure 2. Schematic overview of selected molecular signalling pathways regulating skeletal muscle homeostasis (see further details in the text). Dashed lines indicate pathways that still have to be completely defined.

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

Measured variable from signalling pathways			Young	Old
Protein degradation	Atrogin-1	0-4 d imm	↑	↑
		0-14 d imm	↔	↓
	MuRF-1	0-4 d imm	↑	↑
		0-14 d imm	↔	↓
Protein synthesis	IGF-1Ea	0-4 d imm	↔	↑
		0-14 d imm	↑	↑
	MGF	0-4 d imm	↔	↑
		0-14 d imm	↑	↑
Apoptosis	Bax	0-4 d imm	↑	↑
		0-14 d imm	↑	↑
	P53	0-4 d imm	↑	↑
		0-14 d imm	↑	↑
	BCL2L1	0-4 d imm	↔	↔
		0-14 d imm	↔	↔
Autophagy	GABARAPL	0-4 d imm	↔	↔
		0-14 d imm	↔	↔
	ATG4B	0-4 d imm	↔	↔
		0-14 d imm	↑	↑
	LC3B-I/II	0-4 d imm	↑	↔
Mitochondrial biogenesis	PGC-1α	0-4 d imm	↓	↓
		0-14 d imm	↓	↓
	PGC-1β	0-4 d imm	↓	↓
		0-14 d imm	↔	↔

Table 3. Skeletal muscle signalling responses during mmobilisation in young and old individuals

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 PGC-1α and PGC-1β 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 human skeletal muscle atrophy is age-dependent, with a number of cellular signalling pathways being modified interdependently of each other.

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 see [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 intervention programs [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], 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 IIa and IIx) 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

pennation 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 pennation 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 pennation 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. Com-parable 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, de-spite 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

isometric MVC, RFD, EMG and contractile impulse) NMES led to moderate albeit statistically significant gains in functional performance, while conventional rehabilitation did not result in any increases in neuromuscular or functional performance (Study I & II).

The effect of post-operative re-training on the IGF-1 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-IEa and the mechano sensitive IGF isoform IGF-IEc (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-IEa 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-IEa and MGF mRNAs in response to a period of prolonged resistance training even after surgery and/or immobilisation.
Collectively, Study I-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 unload-ing in young individuals were fully reversed after 7 weeks, despite no training intervention [22]. However, almost no previous stud-ies 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 after 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 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+

cells and the size of both type I and II fibres, respectively. In further support of the importance of SC proliferation for muscle re-growth, a relationship was observed between the relative change in MFA and total number of Pax7+ cells, as well as the relative change in fibre type I area and the change in number of type I associated Pax7+ cells subsequent to 4 weeks of re-training (Study VIII). However, although muscle regenerative capacity appears to decline at a more advanced age reflected by a decline in SC number and/or activation [74, 209, 223], a re-duced SC pool does not abolish the myogenic potential for adaptive muscle hypertrophy if the intervention period is sufficiently long (≥12 weeks), even at old age [158, 223].
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 those 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 IGF1/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 patientImportantly, 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.

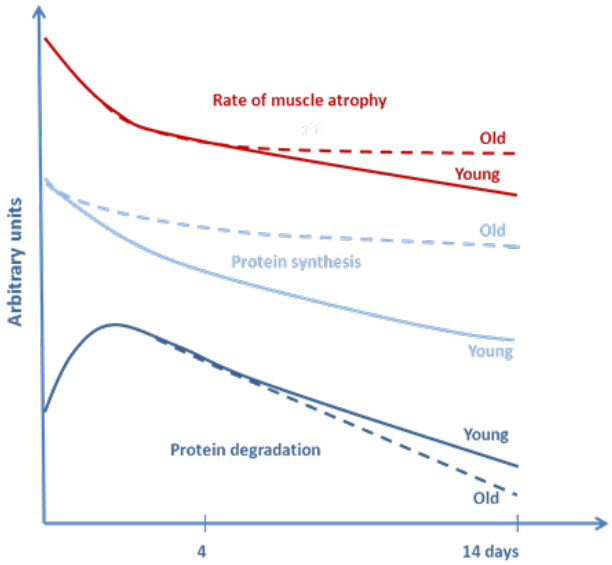

Figure 3. Schematic figure of muscle atrophy rates as well as changes in protein synthesis and protein degradation in response to 4 and 14 days of immobilisation in young (full line) and old individuals (dotted line), respectively. The schematic changes in protein synthesis and protein degradation rates are based on our gene expression data (Study VII).

In addition, comparing young and old able-bodied individuals, we observed that the magnitude and time-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 SC 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 indicates that

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 atrogenes 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 depend-ant 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

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.

In addition, comparing young and old able-bodied individuals, we observed that the magnitude and time-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 indicates that myostatin play an important role in the impaired ability of aged human skeletal muscle to recover from immobility-induced muscle atrophy.

REFERENCE LIST

1. Aagaard P, Andersen JL, Dyhre-Poulsen P, et al. A mechanism for increased contractile strength of human pennate muscle in response to strength training: changes in muscle architecture. Journal of Physiology-London 2001;534: 613-623.
2. Aagaard P, Magnusson PS, Larsson B, et al. Mechanical muscle function, morphology, and fiber type in lifelong trained elderly. Med Sci Sports Exerc 2007:39: 1989-1996.
3. Aagaard P, Simonsen EB, Andersen JL, et al. Increased rate of force development and neural drive of human skeletal muscle following resistance training. Journal of Applied Physiology 2002;93: 1318-1326.
4. Aagaard P, Suetta C, Caserotti P, et al. Role of the nerv-ous system in sarcopenia and muscle atrophy with ag-ing: strength training as a countermeasure. Scand J Med Sci Sports 2010;20: 49-64.
5. Aagaard P, Suetta C, Caserotti P, et al. Role of the nerv-ous system in sarcopenia and muscle atrophy with ag-ing: strength training as a countermeasure. Scand J Med Sci Sports 2010;20: 49-64.
6. Abadi A, Glover EI, Isfort RJ, et al. Limb immobilization induces a coordinate down-regulation of mitochondrial and other metabolic pathways in men and women. PLoS ONE 2009;4: e6518.
7. Adams GR. Role of insulin-like growth factor-I in the regulation of skeletal muscle adaptation to increased loading. Exerc Sport Sci Rev 1998;26: 31-60.
8. Adams GR, Caiozzo VJ and Baldwin KM. Skeletal muscle unweighting: spaceflight and ground-based models. J Appl Physiol 2003;95: 2185-2201.
9. Adams GR and Haddad F. The relationships among IGF-1, DNA content, and protein accumulation during skele-tal muscle hypertrophy. J Appl Physiol 1996;81: 2509-2516.
10. Aggeloussis N, Giannakou E, Albracht K et al. Reproduc-ibility of fascicle length and pennation angle of gas-trocnemius medialis in human gait in vivo. Gait Posture 2010;31: 73-77.
11. Allen RE and Boxhorn LK. Regulation of skeletal muscle satellite cell proliferation and differentiation by trans-forming growth factor-beta, insulin-like growth factor I, and fibroblast growth factor. J Cell Physiol 1989;138: 311-315.
12. Alway SE, Degens H, Krishnamurthy G and Chaudhrai A. Denervation stimulates apoptosis but not Id2 expression in hindlimb muscles of aged rats. J Gerontol A Biol Sci Med Sci 2003;58: 687-697.
13. Alway SE, Lowe DA and Chen KD. The effects of age and hindlimb supension on the levels of expression of the myogenic regulatory factors MyoD and myogenin in rat fast and slow skeletal muscles. Exp Physiol 2001;86: 509-517,.
14. Appell HJ. Skeletal muscle atrophy during immobiliza-tion. Int J Sports Med 1986;7: 1-5.
15. Appell HJ. Muscular atrophy following immobilisation. A review. Sports Med 1990;10: 42-58.
16. Arvidsson I, Arvidsson H, Eriksson E et al. Prevention of quadriceps wasting after immobilization: an evaluation of the effect of electrical stimulation. Orthopedics 1986;9: 1519-1528.
17. Bamman MM, Clarke MS, Feeback DL, et al. Impact of resistance exercise during bed rest on skeletal muscle sarcopenia and myosin isoform distribution. J Appl Physiol 1998;84: 157-163.
18. Bamman MM, Shipp JR, Jiang J, et al. Mechanical load increases muscle IGF-I and androgen receptor mRNA concentrations in humans. Am J Physiol Endocrinol Metab 2001;280: E383-E390,.
19. Bassey EJ, Fiatarone MA, O'Neill EF, et al. Leg extensor power and functional performance in very old men and women. Clin Sci (Colch) 1992;82: 321-327,.
20. Beneke R, Neuerburg J and Bohndorf K. Muscle cross-section measurement by magnetic resonance imaging. Eur J Appl Physiol Occup Physiol 1991;63: 424-429.
21. Berg HE, Dudley GA, Haggmark T, et al. Effects of lower limb unloading on skeletal muscle mass and function in humans. J Appl Physiol 1991;70: 1882-1885.
22. Berg HE, Dudley GA, Haggmark T, et al. Effects of lower limb unloading on skeletal muscle mass and function in humans. J Appl Physiol 1991;70: 1882-1885.

23. Berg HE, Larsson L and Tesch PA. Lower limb skeletal muscle function after 6 wk of bed rest. J Appl Physiol 1997;82: 182-188.
24. Berg HE and Tesch PA. Changes in muscle function in response to 10 days of lower limb unloading in humans. Acta Physiol Scand 1996;157: 63-70.
25. Bergström J. Muscle electrolytes in man. Scand J Clin Lab Invest 1962;68(suppl): 1-110.
26. Beyer, N, Simonsen, L, Bülow J, et al. Old women with a recent fall history show improved muscle strength and function sustained for six months after finishing training. Aging Clin Exp Res. 2007;19(4):300-9.
27. Blazevich AJ, Gill ND and Zhou S. Intra- and intermuscu-lar variation in human quadriceps femoris architecture assessed in vivo. J Anat 2006;209: 289-310.
28. Blomstrand E and Ekblom B. The needle biopsy tech-nique for fibre type determination in human skeletal muscle--a methodological study. Acta Physiol Scand 1982;116: 437-442.
29. Bodine SC, Latres E, Baumhueter S, et al. Identification of ubiquitin ligases required for skeletal muscle atro-phy. Science 2001;294: 1704-1708.
30. Bodine SC, Stitt TN, Gonzalez M, et al. Akt/mTOR pathway is a crucial regulator of skeletal muscle hyper-trophy and can prevent muscle atrophy in vivo. Nat Cell Biol 2001;3: 1014-1019.
31. Boesen AP, Dideriksen K, Couppe C, et al. Effect of growth hormone on aging connective tissue in muscle and tendon: gene expression, morphology, and function following immobilization and rehabilitation. J Appl Physiol (1985), 2014;116: 192-203.
32. Boesen AP, Dideriksen K, Couppe C, et al. Tendon and skeletal muscle matrix gene expression and functional responses to immobilisation and rehabilitation in young males: effect of growth hormone administration. J Physiol 2013;591: 6039-6052.
33. Booth FW and Gollnick PD. Effects of disuse on the structure and function of skeletal muscle. Med Sci Sports Exerc 1983;15: 415-420.
34. Booth FW and Seider MJ. Early change in skeletal mus-cle protein synthesis after limb immobilization of rats. J Appl Physiol 1979;47: 974-977.
35. Brooke MH and Kaiser KK. Muscle fiber types: How many and what kind? Arch Neurol 23: 369-379, 1970.
36. Brooks SV and Faulkner JA. Contraction-induced injury: recovery of skeletal muscles in young and old mice. Am J Physiol 1990;258: C436-C442.
37. Brown M and Hasser EM. Differential effects of re-duced muscle use (hindlimb unweighting) on skeletal muscle with aging. Aging (Milano) 8: 99-105, 1996.
38. Bruunsgaard H and Pedersen BK. Age-related inflam-matory cytokines and disease. Immunol Allergy Clin North Am 23: 15-39, 2003.
39. Buchman AS, Wilson RS, Boyle PA, et al. Change in mo-tor function and risk of mortality in older persons. J Am Geriatr Soc 55: 11-19, 2007.
40. Carlson BM, Borisov AB, Dedkov EI, et al. Effects of long-term denervation on skeletal muscle in old rats. J Gerontol A Biol Sci Med Sci 57: B366-B374, 2002.
41. Carlson BM, Dedkov EI, Borisov AB and Faulkner JA. Skeletal muscle regeneration in very old rats. J Gerontol A Biol Sci Med Sci 56: B224-B233, 2001.
42. Carlson ME and Conboy IM. Loss of stem cell regenera-tive capacity within aged niches. Aging Cell 6: 371-382, 2007.
43. Carlson ME, Suetta C, Conboy MJ, et al. Molecular aging and rejuvenation of human muscle stem cells. EMBO Mol Med 1: 381-391, 2009.
44. Chakravarthy MV, Davis BS and Booth FW. IGF-I re-stores satellite cell proliferative potential in immobi-lized old skeletal muscle. J Appl Physiol 89: 1365-1379, 2000.
45. Chen YW, Gregory CM, Scarborough MT, et al. Tran-scriptional pathways associated with skeletal muscle disuse atrophy in humans. Physiol Genomics 31: 510-520, 2007.
46. Clarkson PM, Kroll W and Melchionda AM. Age, Isomet-ric Strength, Rate of Tension Development and Fiber Type Composition. Journals of Gerontology 36: 648-653, 1981.
47. Conboy IM, Conboy MJ, Wagers AJ, et al. Rejuvenation of aged progenitor cells by exposure to a young system-ic environment. Nature 433: 760-764, 2005.
48. Convertino VA, Bloomfield SA and Greenleaf JE. An overview of the issues: Physiological effects of bed rest and restricted physical activity. Medicine and Science in Sports and Exercise 29: 187-190, 1997.
49. Covinsky KE, Palmer RM, Fortinsky RH, et al. Loss of in-dependence in activities of daily living in older adults hospitalized with medical illnesses: Increased vulnera-bility with age. Journal of the American Geriatrics Soci-ety 51: 451-458, 2003.
50. Cuthbertson D, Smith K, Babraj J, et al. Anabolic signal-ing deficits underlie amino acid resistance of wasting, aging muscle. FASEB J 19: 422-424, 2005.
51. D'Antona G, Pellegrino MA, Adami R, et al. The effect of ageing and immobilization on structure and function of human skeletal muscle fibres. J Physiol 2003;552: 499-511.
52. Dargent-Molina P, Favier F, Grandjean H, et al. Fall-related factors and risk of hip fracture: the EPIDOS pro-spective study. [published erratum appears in Lancet 1996 Aug 10;348(9024):416
53. de Boer MD, Maganaris CN, Seynnes OR, et al. Time course of muscular, neural and tendinous adaptations to 23 day unilateral lower-limb suspension in young men. J Physiol 2007;583: 1079-1091.
54. de Boer MD, Selby A, Atherton P, et al. The temporal responses of protein synthesis, gene expression and cell signalling in human quadriceps muscle and patellar tendon to disuse. J Physiol 2007;585: 241-251.

55. de Boer MD, Seynnes OR, di Prampero PE, et al. Effect of 5 weeks horizontal bed rest on human muscle thick-ness and architecture of weight bearing and non-weight bearing muscles. Eur J Appl Physiol 2008;104(2):401-7.
56. de Rekeneire N, Visser M, Peila R, et al. Is a fall just a fall: correlates of falling in healthy older persons. The Health, Aging and Body Composition Study. J Am Geri-atr Soc 2003;51: 841-846.
57. de Vos NJ, Singh NA, Ross DA, et al. Optimal load for increasing muscle power during explosive resistance training in older adults. J Gerontol A Biol Sci Med Sci 2005;60: 638-647.
58. Deschenes MR, Britt AA and Chandler WC. A compari-son of the effects of unloading in young adult and aged skeletal muscle. Med Sci Sports Exerc 2001;33: 1477-1483.
59. Deschenes MR, Holdren AN and McCoy RW. Adapta-tions to short-term muscle unloading in young and aged men. Med Sci Sports Exerc 2008;40: 856-863.
60. Deschenes MR, Will KM, Booth FW and Gordon SE. Un-like myofibers, neuromuscular junctions remain stable during prolonged muscle unloading. J Neurol Sci 2003;210: 5-10.
61. Desplanches D, Mayet MH, Sempore B and Flandrois R. Structural and functional responses to prolonged hindlimb suspension in rat muscle. J Appl Physiol 1987;63: 558-563.
62. Dirks A and Leeuwenburgh C. Apoptosis in skeletal mus-cle with aging. Am J Physiol Regul Integr Comp Physiol 2002;282: R519-R527.
63. Dirks ML, Hansen D, Van AA, Dendale P and van Loon LJ. Neuromuscular electrical stimulation prevents muscle wasting in critically ill comatose patients. Clin Sci (Lond) 2015;128: 357-365.
64. Doherty TJ. Invited review: Aging and sarcopenia. J Appl Physiol 2003;95: 1717-1727.
65. Dreyer HC, Blanco CE, Sattler FR, et al. Satellite cell numbers in young and older men 24 hours after eccen-tric exercise. Muscle Nerve 2006;33: 242-253.
66. Dupont-Versteegden EE. Apoptosis in muscle atrophy: relevance to sarcopenia. Exp Gerontol 2005;40: 473-481.
67. Dupont-Versteegden EE, Strotman BA, Gurley CM, et al. Nuclear translocation of EndoG at the initiation of dis-use muscle atrophy and apoptosis is specific to myonu-clei. Am J Physiol Regul Integr Comp Physiol 291: R1730-R1740, 2006.
68. Esmarck B, Andersen JL, Olsen S, Richter EA, Mizuno M and Kjaer M. Timing of postexercise protein intake is important for muscle hypertrophy with resistance train-ing in elderly humans. J Physiol 535: 301-311, 2001.
69. Farrell M and Richards JG. Analysis of the Reliability and Validity of the Kinetic Communicator Exercise De-vice. Medicine and Science in Sports and Exercise 18: 44-49, 1986.
70. Felson DT, Lawrence RC, Dieppe PA, et al. Osteoarthri-tis: new insights. Part 1: the disease and its risk factors. Ann Intern Med 133: 635-646, 2000.
71. Fiatarone MA, Marks EC, Ryan ND, Meredith CN, Lipsitz LA and Evans WJ. High-intensity strength training in no-nagenarians. Effects on skeletal muscle. JAMA 263: 3029-3034, 1990.
72. Fleming BE, Wilson DR and Pendergast DR. A portable, easily performed muscle power test and its association with falls by elderly persoms. Arch Phys Med Rehabil 72: 886-889, 1991.
73. Foss NB, Kristensen MT and Kehlet H. Prediction of postoperative morbidity, mortality and rehabilitation in hip fracture patients: the cumulated ambulation score. Clin Rehabil 20: 701-708, 2006.
74. Gallegly JC, Turesky NA, Strotman BA, Gurley CM, Pe-terson CA and Dupont-Versteegden EE. Satellite cell regulation of muscle mass is altered at old age. J Appl Physiol 97: 1082-1090, 2004.
75. Gandevia SC, Macefield G, Burke D et al. Voluntary acti-vation of human motor axons in the absence of muscle afferent feedback. The control of the deafferented hand. Brain 113 (Pt 5): 1563-1581, 1990.
76. Gibson JN, Halliday D, Morrison WL, et al. Decrease in human quadriceps muscle protein turnover consequent upon leg immobilization. Clin Sci (Lond) 72: 503-509, 1987.
77. Gibson JN, Smith K and Rennie MJ. Prevention of disuse muscle atrophy by means of electrical stimulation: maintenance of protein synthesis. Lancet 2: 767-770, 1988.
78. Glover EI, Phillips SM, Oates BR, Tang JE, Tarnopolsky MA, Selby A, Smith K and Rennie MJ. Immobilization induces anabolic resistance in human myofibrillar pro-tein synthesis with low and high dose amino acid infu-sion. J Physiol 586: 6049-6061, 2008.
79. Goldspink G. Cellular and molecular aspects of muscle growth, adaptation and ageing. Gerodontology 15: 35-43, 1998.
80. Goldspink G and Harridge SD. Growth factors and mus-cle ageing. Exp Gerontol 39: 1433-1438, 2004.
81. Goldspink G and Yang SY. Effects of activity on growth factor expression. Int J Sport Nutr Exerc Metab 11 Suppl: S21-S27, 2001.
82. Gomes MD, Lecker SH, Jagoe RT, Navon A and Goldberg AL. Atrogin-1, a muscle-specific F-box protein highly ex-pressed during muscle atrophy. Proc Natl Acad Sci U S A 98: 14440-14445, 2001.
83. Gopinath SD and Rando TA. Stem cell review series: ag-ing of the skeletal muscle stem cell niche. Aging Cell 7: 590-598, 2008.
84. Gram M, Vigelso A, Yokota T, et al. Two weeks of one-leg immobilization decreases skeletal muscle respirato-ry capacity equally in young and elderly men. Exp Ger-ontol 58: 269-278, 2014.
85. Grounds MD. Age-associated changes in the response of skeletal muscle cells to exercise and regeneration. Ann N Y Acad Sci 854: 78-91, 1998.
86. Guccione AA, Felson DT and Anderson JJ. Defining ar-thritis and measuring functional status in elders:

meth-odological issues in the study of disease and physical disability. Am J Public Health 80: 945-949, 1990.
87. Guerra B, Gomez-Cabrera MC, Ponce-Gonzalez JG, et al. Repeated muscle biopsies through a single skin inci-sion do not elicit muscle signaling, but IL-6 mRNA and STAT3 phosphorylation increase in injured muscle. J Appl Physiol (1985) 110: 1708-1715, 2011.
88. Guralnik JM, Ferrucci L, Simonsick EM, et al. Lower-Extremity Function in Persons Over the Age of 70 Years As A Predictor of Subsequent Disability. New England Journal of Medicine 332: 556-561, 1995.
89. Hakkinen K, Alen M and Komi PV. Changes in Isometric Force-Time and Relaxation-Time, Electromyographic and Muscle-Fiber Characteristics of Human Skeletal-Muscle During Strength Training and Detraining. Acta Physiologica Scandinavica 125: 573-585, 1985.
90. Hakkinen K, Kraemer WJ, Kallinen M, et al. Bilateral and unilateral neuromuscular function and muscle cross- sectional area in middle-aged and elderly men and women. J Gerontol A Biol Sci Med Sci 51: B21-B29, 1996.
91. Hakkinen K, Kraemer WJ, Newton RU and Alen M. Changes in electromyographic activity, muscle fibre and force production characteristics during heavy re-sistance/power strength training in middle-aged and older men and women. Acta Physiologica Scandinavica 171: 51-62, 2001.
92. Hakkinen K, Newton RU, Gordon SE, McCormick M, Volek JS, Nindl BC, Gotshalk LA, Campbell WW, Evans WJ, Hakkinen A, Humphries BJ and Kraemer WJ. Changes in muscle morphology, electromyographic ac-tivity, and force production characteristics during pro-gressive strength training in young and older men. J Gerontol A Biol Sci Med Sci 53: B415-B423, 1998.
93. Hakkinen K, Newton RU, Gordon SE, McCormick M, Volek JS, Nindl BC, Gotshalk LA, Campbell WW, Evans WJ, Hakkinen A, Humphries BJ and Kraemer WJ. Changes in muscle morphology, electromyographic ac-tivity, and force production characteristics during pro-gressive strength training in young and older men. Jour-nals of Gerontology Series A-Biological Sciences and Medical Sciences 53: B415-B423, 1998.
94. Hakkinen K, Pastinen UM, Karsikas R and Linnamo V. Neuromuscular performance in voluntary bilateral and unilateral contraction and during electrical stimulation in men at different ages. Eur J Appl Physiol 70: 518-527, 1995.
95. Halkjaer-Kristensen J and Ingemann-Hansen T. Varia-tions in single fibre areas and fibre composition in nee-dle biopsies from the human quadriceps muscle. Scand J Clin Lab Invest 41: 391-395, 1981.
96. Hameed M, Lange KH, Andersen JL, Schjerling P, Kjaer M, Harridge SD and Goldspink G. The effect of recom-binant human growth hormone and resistance training on IGF-I mRNA expression in the muscles of elderly men. J Physiol 555: 231-240, 2004.
97. Hameed M, Orrell RW, Cobbold M, et al. Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. J Physiol 547: 247-254, 2003.
98. Harridge SDR, Bottinelli R, Canepari M, et al. Whole-muscle and single-fibre contractile properties and myo-sin heavy chain isoforms in humans. Pflugers Archiv-European Journal of Physiology 432: 913-920, 1996.
99. Harridge SDR, Kryger A and Stensgaard A. The effects of strength training on muscle size and force production in elderly people aged 85-97 years. Journal of Physiolo-gy-London 509P: 42P-43P, 1998.
100. Harridge SDR, Kryger A and Stensgaard A. Knee exten-sor strength, activation, and size in very elderly people following strength training. Muscle & Nerve 22: 831-839, 1999.
101. Hartkopp A, Harridge SD, Mizuno M, Ratkevicius A, Quistorff B, Kjaer M and Biering-Sorensen F. Effect of training on contractile and metabolic properties of wrist extensors in spinal cord-injured individuals. Muscle Nerve 27: 72-80, 2003.
102. Haruna Y, Suzuki Y, Kawakubo K, Yanagibori R and Gunji A. Decremental reset in basal metabolism during 20-days bed rest. Acta Physiol Scand Suppl 616: 43-49, 1994.
103. Hauer K, Specht N, Schuler M, Bartsch P and Oster P. In-tensive physical training in geriatric patients after se-vere falls and hip surgery. Age and Ageing 31: 49-57, 2002.
104. Herbert RD and Gandevia SC. Changes in pennation with joint angle and muscle torque: in vivo measure-ments in human brachialis muscle. J Physiol 484 (Pt 2): 523-532, 1995.
105. Heslop L, Beauchamp JR, Tajbakhsh S, Buckingham ME, Partridge TA and Zammit PS. Transplanted primary ne-onatal myoblasts can give rise to functional satellite cells as identified using the Myf5nlacZl+ mouse. Gene Ther 8: 778-783, 2001.
106. Hespel P, Op't EB, Van LM, et al. Oral creatine supple-mentation facilitates the rehabilitation of disuse atro-phy and alters the expression of muscle myogenic fac-tors in humans. J Physiol 536: 625-633, 2001.
107. Hill AG and Hill GL. Metabolic response to severe injury. Br J Surg 85: 884-890, 1998.
108. Hirsch CH, Sommers L, Olsen A, et al. The natural histo-ry of functional morbidity in hospitalized older patients. J Am Geriatr Soc 38: 1296-1303, 1990.
109. Holsgaard LA, Caserotti P, Puggaard L and Aagaard P. Reproducibility and relationship of single-joint strength vs multi-joint strength and power in aging individuals. Scand J Med Sci Sports 17: 43-53, 2007.
110. Hortobagyi T, Dempsey L, Fraser D, et al. Changes in muscle strength, muscle fibre size and myofibrillar gene expression after immobilization

and retraining in humans. J Physiol 524 Pt 1: 293-304, 2000.
111. Husby VS, Helgerud J, Bjorgen S, et al. Early maximal strength training is an efficient treatment for patients operated with total hip arthroplasty. Arch Phys Med Re-habil 90: 1658-1667, 2009.
112. Hvid L, Aagaard P, Justesen L, Bayer ML, Andersen JL, Ortenblad N, Kjaer M and Suetta C. Effects of aging on muscle mechanical function and muscle fiber morphol-ogy during short-term immobilization and subsequent retraining. J Appl Physiol (1985) 109: 1628-1634, 2010.
113. Hvid LG, Ortenblad N, Aagaard P, Kjaer M and Suetta C. Effects of ageing on single muscle fibre contractile function following short-term immobilisation. J Physiol 589: 4745-4757, 2011.
114. Hvid LG, Suetta C, Aagaard P, Kjaer M, Frandsen U and Ortenblad N. Four days of muscle disuse impairs single fiber contractile function in young and old healthy men. Exp Gerontol 48: 154-161, 2013.
115. Izquierdo M, Ibanez J, Gorostiaga E, et al. Maximal strength and power characteristics in isometric and dy-namic actions of the upper and lower extremities in middle-aged and older men. Acta Physiol Scand 167: 57-68, 1999.
116. Jakobsen TL, Husted H, Kehlet H et al. Progressive strength training (10 RM) commenced immediately af-ter fast-track total knee arthroplasty: is it feasible? Dis-abil Rehabil 2012;34: 1034-1040.
117. Jakobsen TL, Kehlet H, Husted H, et al. Early progressive strength training to enhance recovery after fast-track total knee arthroplasty: a randomized controlled trial. Arthritis Care Res 2014;66: 1856-1866.
118. Janssen I, Heymsfield SB and Ross R. Low relative skel-etal muscle mass (sarcopenia) in older persons is asso-ciated with functional impairment and physical disabil-ity. Journal of the American Geriatrics Society 2002;50: 889-896,.
119. Jones SW, Hill RJ, Krasney PA, et al. Disuse atrophy and exercise rehabilitation in humans profoundly affects the expression of genes associated with the regulation of skeletal muscle mass. FASEB J 2004;18: 1025-1027.
120. Joulia D, Bernardi H, Garandel V, et al. Mechanisms in-volved in the inhibition of myoblast proliferation and differentiation by myostatin. Exp Cell Res 286: 263-275, 2003.
121. Kadi F, Bonnerud P, Eriksson A and Thornell LE. The ex-pression of androgen receptors in human neck and limb muscles: effects of training and self-administration of androgenic-anabolic steroids. Histochem Cell Biol 113: 25-29, 2000.
122. Kadi F, Charifi N, Denis C and Lexell J. Satellite cells and myonuclei in young and elderly women and men. Mus-cle Nerve 29: 120-127, 2004.
123. Kadi F, Charifi N, Denis C, et al. The behaviour of satel-lite cells in response to exercise: what have we learned from human studies? Pflugers Arch 2005;451: 319-327.
124. Kadi F, Charifi N and Henriksson J. The number of satel-lite cells in slow and fast fibres from human vastus lat-eralis muscle. Histochem Cell Biol 126: 83-87, 2006.
125. Kadi F, Schjerling P, Andersen LL, et al. The effects of heavy resistance training and detraining on satellite cells in human skeletal muscles. J Physiol 558: 1005-1012, 2004.
126. Kamel HK, Iqbal MA, Mogallapu R, et al. Time to ambu-lation after hip fracture surgery: relation to hospitaliza-tion outcomes. J Gerontol A Biol Sci Med Sci , 2003;58: 1042-1045.
127. Kawakami Y, Muraoka Y, Kubo K, et al. Changes in mus-cle size and architecture following 20 days of bed rest. J Gravit Physiol 2000;7: 53-59.
128. Kehlet H. Future perspectives and research initiatives in fast-track surgery. Langenbecks Arch Surg 391: 495-498, 2006.
129. Kehlet H and Wilmore DW. Multimodal strategies to improve surgical outcome. Am J Surg 183: 630-641, 2002.
130. Kellis E and Baltzopoulos V. The effects of antagonist moment on the resultant knee joint moment during iso-kinetic testing of the knee extensors. European Journal of Applied Physiology and Occupational Physiology 76: 253-259, 1997.
131. Kent-Braun JA and Ng AV. Specific strength and volun-tary muscle activation in young and elderly women and men. J Appl Physiol 87: 22-29, 1999.
132. Kim JS, Kosek DJ, Petrella JK, Cross JM and Bamman MM. Resting and load-induced levels of myogenic gene transcripts differ between older adults with demon-strable sarcopenia and young men and women. J Appl Physiol 99: 2149-2158, 2005.
133. Klass M, Baudry S and Duchateau J. Age-related decline in rate of torque development is accompanied by lower maximal motor unit discharge frequency during fast contractions. J Appl Physiol 104: 739-746, 2008.
134. Klitgaard H, Mantoni M, Schiaffino S, Ausoni S, Gorza L, Laurent-Winter C, Schnohr P and Saltin B. Function, morphology and protein expression of ageing skeletal muscle: a cross-sectional study of elderly men with dif-ferent training backgrounds. Acta Physiol Scand 140: 41-54, 1990.
135. Kortebein P. RE: Effect of short-term hospitalization on functional capacity in patients not restricted to bed. Am J Phys Med Rehabil 87: 425-426, 2008.
136. Kortebein P. Rehabilitation for hospital-associated de-conditioning. Am J Phys Med Rehabil 88: 66-77, 2009.
137. Kortebein P, Ferrando A, Lombeida J, Wolfe R and Evans WJ. Effect of 10 days of bed rest on skeletal muscle in healthy older adults. JAMA 297: 1772-1774, 2007.
138. Kortebein P, Ferrando A, Lombeida J, Wolfe R and Evans WJ. Effect of 10 days of bed rest on skeletal muscle in healthy older adults. JAMA 297: 1772-1774, 2007.

139. Kortebein P, Symons TB, Ferrando A, Paddon-Jones D, Ronsen O, Protas E, Conger S, Lombeida J, Wolfe R and Evans WJ. Functional impact of 10 days of bed rest in healthy older adults. J Gerontol A Biol Sci Med Sci 63: 1076-1081, 2008.
140. Kosek DJ, Kim JS, Petrella JK, et al. Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myo-genic mechanisms in young vs. older adults. J Appl Physiol 101: 531-544, 2006.
141. Kraemer WJ, Mazzetti SA, Nindl BC, et al. Effect of re-sistance training on women's strength/power and oc-cupational performances. Medicine and Science in Sports and Exercise 33: 1011-1025, 2001.
142. Kryger AI and Andersen JL. Resistance training in the oldest old: consequences for muscle strength, fiber types, fiber size, and MHC isoforms. Scand J Med Sci Sports 17: 422-430, 2007.
143. Kuang S, Kuroda K, Le GF and Rudnicki MA. Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell 129: 999-1010, 2007.
144. Kvorning T, Andersen M, Brixen K, Schjerling P, Suetta C and Madsen K. Suppression of testosterone does not blunt mRNA expression of myoD, myogenin, IGF, myo-statin or androgen receptor post strength training in humans. J Physiol 578: 579-593, 2007.
145. Labarque VL, Eijnde BO and Van LM. Effect of immobili-zation and retraining on torque-velocity relationship of human knee flexor and extensor muscles. Eur J Appl Physiol 86: 251-257, 2002.
146. Lange KH, Andersen JL, Beyer N, et al. GH administra-tion changes myosin heavy chain isoforms in skeletal muscle but does not augment muscle strength or hyper-trophy, either alone or combined with resistance exer-cise training in healthy elderly men. J Clin Endocrinol Metab 87: 513-523, 2002.
147. LeBlanc A, Gogia P, Schneider V, Krebs J, Schonfeld E and Evans H. Calf muscle area and strength changes af-ter five weeks of horizontal bed rest. Am J Sports Med 16: 624-629, 1988.
148. LeBlanc A, Rowe R, Evans H, West S, Shackelford L and Schneider V. Muscle atrophy during long duration bed rest. Int J Sports Med 18 Suppl 4: S283-S285, 1997.
149. Lecker SH, Jagoe RT, Gilbert A, Gomes M, Baracos V, Bailey J, Price SR, Mitch WE and Goldberg AL. Multiple types of skeletal muscle atrophy involve a common pro-gram of changes in gene expression. FASEB J 18: 39-51, 2004.
150. Leger B, Cartoni R, Praz M, et al. Akt signalling through GSK-3beta, mTOR and Foxo1 is involved in human skel-etal muscle hypertrophy and atrophy. J Physiol 2006;576: 923-933.
151. Lexell J, Downham DY, Larsson Y, Bruhn E and Morsing B. Heavy-resistance training in older Scandinavian men and women: short- and long-term effects on arm and leg muscles. Scand J Med Sci Sports 5: 329-341, 1995.
152. Lexell J and Taylor CC. Variability in muscle fibre areas in whole human quadriceps muscle: how to reduce sampling errors in biopsy techniques. Clin Physiol 9: 333-343, 1989.
153. Lexell J and Taylor CC. Variability in muscle fibre areas in whole human quadriceps muscle: effects of increas-ing age. J Anat 174: 239-249, 1991.
154. Lexell J, Taylor CC and Sjostrom M. What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. J Neurol Sci 84: 275-294, 1988.
155. Lindle RS, Metter EJ, Lynch NA, et al. Age and gender comparisons of muscle strength in 654 women and men aged 20-93 yr. J Appl Physiol 83: 1581-1587, 1997.
156. Lindstrom M, Pedrosa-Domellof F and Thornell LE. Sat-ellite cell heterogeneity with respect to expression of MyoD, myogenin, Dlk1 and c-Met in human skeletal muscle: application to a cohort of power lifters and sedentary men. Histochem Cell Biol 134: 371-385, 2010.
157. Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408, 2001.
158. Mackey AL, Esmarck B, Kadi F, et al. Enhanced satellite cell proliferation with resistance training in elderly men and women. Scand J Med Sci Sports 17: 34-42, 2007.
159. Mackey AL, Kjaer M, Charifi N, Henriksson J, Bojsen-Moller J, Holm L and Kadi F. Assessment of satellite cell number and activity status in human skeletal muscle biopsies. Muscle Nerve 40: 455-465, 2009.
160. Madsen OR, Bliddal H, Egsmose C and Sylvest J. Iso-metric and isokinetic quadriceps strength in gonarthro-sis; inter- relations between quadriceps strength, walk-ing ability, radiology, subchondral bone density and pain. Clin Rheumatol 14: 308-314, 1995.
161. Madsen OR, Lauridsen UB, Hartkopp A and Sorensen OH. Muscle strength and soft tissue composition as measured by dual energy x-ray absorptiometry in wom-en aged 18-87 years. Eur J Appl Physiol Occup Physiol 75: 239-245, 1997.
162. Magaziner J, Simonsick EM, Kashner TM, Hebel JR and Kenzora JE. Predictors of functional recovery one year following hospital discharge for hip fracture: a prospec-tive study. J Gerontol 45: M101-M107, 1990.
163. Malmgren LT, Jones CE and Bookman LM. Muscle fiber and satellite cell apoptosis in the aging human thy-roarytenoid muscle: a stereological study with confocal laser scanning microscopy. Otolaryngol Head Neck Surg 125: 34-39, 2001.
164. Mammucari C, Milan G, Romanello V, et al. FoxO3 con-trols autophagy in skeletal muscle in vivo. Cell Metab 6: 458-471, 2007.
165. Marcinek DJ, Schenkman KA, Ciesielski WA, Lee D and Conley KE. Reduced mitochondrial coupling in

vivo alters cellular energetics in aged mouse skeletal muscle. J Physiol 569: 467-473, 2005.
166. Marzetti E, Carter CS, Wohlgemuth SE, Lees HA, Gio-vannini S, Anderson B, Quinn LS and Leeuwenburgh C. Changes in IL-15 expression and death-receptor apop-totic signaling in rat gastrocnemius muscle with aging and life-long calorie restriction. Mech Ageing Dev 130: 272-280, 2009.
167. Marzetti E, Wohlgemuth SE, Lees HA, Chung HY, Gio-vannini S and Leeuwenburgh C. Age-related activation of mitochondrial caspase-independent apoptotic signal-ing in rat gastrocnemius muscle. Mech Ageing Dev 129: 542-549, 2008.
168. Mauro A. Satellite cell of skeletal muscle fibers. J Bio-phys Biochem Cytol 9: 493-495, 1961.
169. McCroskery S, Thomas M, Maxwell L, Sharma M and Kambadur R. Myostatin negatively regulates satellite cell activation and self-renewal. J Cell Biol 162: 1135-1147, 2003.
170. McPherron AC, Lawler AM and Lee SJ. Regulation of skeletal muscle mass in mice by a new TGF-beta super-family member. Nature 387: 83-90, 1997.
171. Merton PA. Voluntary strength and fatigue. J Physiol 123: 553-564, 1954.
172. Metter EJ, Talbot LA, Schrager M and Conwit R. Skeletal muscle strength as a predictor of all-cause mortality in healthy men. J Gerontol A Biol Sci Med Sci 57: B359-B365, 2002.
173. Mezzogiorno A, Coletta M, Zani BM, Cossu G and Moli-naro M. Paracrine stimulation of senescent satellite cell proliferation by factors released by muscle or myotubes from young mice. Mech Ageing Dev 70: 35-44, 1993.
174. Mikkelsen UR, Langberg H, Helmark IC, et al. Local NSAID infusion inhibits satellite cell proliferation in human skeletal muscle after eccentric exercise. J Appl Physiol (1985) 107: 1600-1611, 2009.
175. Mitsiopoulos N, Baumgartner RN, Heymsfield SB, Lyons W, Gallagher D and Ross R. Cadaver validation of skele-tal muscle measurement by magnetic resonance imag-ing and computerized tomography. J Appl Physiol (1985) 85: 115-122, 1998.
176. Moritani T and deVries HA. Neural factors versus hyper-trophy in the time course of muscle strength gain. Am J Phys Med 58: 115-130, 1979.
177. Morse CI, Thom JM, Birch KM and Narici MV. Changes in triceps surae muscle architecture with sarcopenia. Acta Physiol Scand 183: 291-298, 2005.
178. Morse CI, Thom JM, Mian OS, Birch KM and Narici MV. Gastrocnemius specific force is increased in elderly males following a 12-month physical training pro-gramme. Eur J Appl Physiol 2006.
179. Morse CI, Thom JM, Mian OS, Muirhead A, Birch KM and Narici MV. Muscle strength, volume and activation following 12-month resistance training in 70-year-old males. Eur J Appl Physiol 95: 197-204, 2005.
180. Moss FP and Leblond CP. Nature of dividing nuclei in skeletal muscle of growing rats. J Cell Biol 44: 459-462, 1970.
181. Moss FP and Leblond CP. Satellite cells as the source of nuclei in muscles of growing rats. Anat Rec 170: 421-435, 1971.
182. Narici M. Human skeletal muscle architecture studied in vivo by non-invasive imaging techniques: functional significance and applications. J Electromyogr Kinesiol 9: 97-103, 1999.
183. Narici M and Cerretelli P. Changes in human muscle ar-chitecture in disuse-atrophy evaluated by ultrasound imaging. J Gravit Physiol 5: 73-74, 1998.
184. Narici MV and de Boer MD. Disuse of the musculo-skeletal system in space and on earth. Eur J Appl Physiol 111: 403-420, 2011.
185. Narici MV, Maganaris CN, Reeves ND and Capodaglio P. Effect of aging on human muscle architecture. J Appl Physiol (1985) 95: 2229-2234, 2003.
186. Newman AB, Kupelian V, Visser M, Simonsick EM, Goodpaster BH, Kritchevsky SB, Tylavsky FA, Rubin SM and Harris TB. Strength, but not muscle mass, is asso-ciated with mortality in the health, aging and body composition study cohort. J Gerontol A Biol Sci Med Sci 61: 72-77, 2006.
187. Olson EN, Brennan TJ, Chakraborty T, Cheng TC, Cserjesi P, Edmondson D, James G and Li L. Molecular control of myogenesis: antagonism between growth and differen-tiation. Mol Cell Biochem 104: 7-13, 1991.
188. Paddon-Jones D, Sheffield-Moore M, Cree MG, et al. At-rophy and impaired muscle protein synthesis during prolonged inactivity and stress. J Clin Endocrinol Metab 2006.
189. Pearson SJ, Young A, Macaluso A, et al. Muscle function in elite master weightlifters. Med Sci Sports Exerc 34: 1199-1206, 2002.
190. Petrella JK, Kim JS, Cross JM, Kosek DJ and Bamman MM. Efficacy of myonuclear addition may explain dif-ferential myofiber growth among resistance-trained young and older men and women. Am J Physiol Endo-crinol Metab 291: E937-E946, 2006.
191. Petrella JK, Kim JS, Mayhew DL, Cross JM and Bamman MM. Potent Myofiber Hypertrophy During Resistance Training in Humans is Associated with Satellite Cell-Mediated Myonuclear Addition: A Cluster Analysis. J Appl Physiol 2008.
192. Phillips T and Leeuwenburgh C. Muscle fiber specific apoptosis and TNF-alpha signaling in sarcopenia are at-tenuated by life-long calorie restriction. FASEB J 19: 668-670, 2005.
193. Pistilli EE, Jackson JR and Alway SE. Death receptor-associated pro-apoptotic signaling in aged skeletal muscle. Apoptosis 11: 2115-2126, 2006.
194. Pistilli EE, Siu PM and Alway SE. Interleukin-15 respons-es to aging and unloading-induced skeletal muscle at-rophy. Am J Physiol Cell Physiol 292: C1298-C1304, 2007.
195. Portegijs E, Sipila S, Alen M, Kaprio J, Koskenvuo M, Tiainen K and Rantanen T. Leg extension power

asym-metry and mobility limitation in healthy older women. Arch Phys Med Rehabil 86: 1838-1842, 2005.
196. Portegijs E, Sipila S, Pajala S, Lamb SE, Alen M, Kaprio J, Kosekenvuo M and Rantanen T. Asymmetrical lower ex-tremity power deficit as a risk factor for injurious falls in healthy older women. J Am Geriatr Soc 54: 551-553, 2006.
197. Psilander N, Damsgaard R and Pilegaard H. Resistance exercise alters MRF and IGF-I mRNA content in human skeletal muscle. J Appl Physiol 95: 1038-1044, 2003.
198. Raj IS, Bird SR and Shield AJ. Reliability of ultraso-nographic measurement of the architecture of the vastus lateralis and gastrocnemius medialis muscles in older adults. Clin Physiol Funct Imaging 2012;32: 65-70.
199. Rantanen T, Harris T, Leveille SG, et al. Muscle strength and body mass index as long-term predictors of mortal-ity in initially healthy men. J Gerontol A Biol Sci Med Sci 2000;55: M168-M173,.
200. Rantanen T, Volpato S, Ferrucci L, et al. Handgrip strength and cause-specific and total mortality in older disabled women: exploring the mechanism. J Am Geri-atr Soc 2003;51: 636-641.
201. Rasch A, Bystrom AH, Dalen N et al. Reduced muscle radiological density, cross-sectional area, and strength of major hip and knee muscles in 22 patients with hip osteoarthritis. Acta Orthop 2007;78: 505-510.
202. Raue U, Slivka D, Jemiolo B, et al. Myogenic Gene Ex-pression at Rest and Following a Bout of Resistance Ex-ercise in Young (18-30 y) and Old (80-89 y) Women. J Appl Physiol 2006;101(1):53-9.
203. Reardon K, Galea M, Dennett X, Choong P et al. Quadri-ceps muscle wasting persists 5 months after total hip arthroplasty for osteoarthritis of the hip: a pilot study. Internal Medicine Journal 2001;31: 7-14.
204. Reardon KA, Davis J, Kapsa RM, Choong P and Byrne E. Myostatin, insulin-like growth factor-1, and leukemia inhibitory factor mRNAs are upregulated in chronic hu-man disuse muscle atrophy. Muscle Nerve 2001;24: 893-899.
205. Reeves ND, Maganaris CN and Narici MV. Ultraso-nographic assessment of human skeletal muscle size. Eur J Appl Physiol 2004;91: 116-118.
206. Reeves ND, Maganaris CN and Narici MV. Ultraso-nographic assessment of human skeletal muscle size. Eur J Appl Physiol 2004;91: 116-118.
207. Reeves ND, Maganaris CN and Narici MV. Plasticity of dynamic muscle performance with strength training in elderly humans. Muscle Nerve 2005;31: 355-364.
208. Reeves ND, Narici MV and Maganaris CN. Myotendi-nous plasticity to ageing and resistance exercise in hu-mans. Exp Physiol 2006;91: 483-498.
209. Renault V, Thornell LE, Eriksson PO, Butler-Browne G and Mouly V. Regenerative potential of human skeletal muscle during aging. Aging Cell 1: 132-139, 2002.
210. Rennie MJ, Selby A, Atherton P, Smith K, Kumar V, Glover EL and Philips SM. Facts, noise and wishful thinking: muscle protein turnover in aging and human disuse atrophy. Scand J Med Sci Sports 20: 5-9, 2010.
211. Rennie MJ, Wackerhage H, Spangenburg EE and Booth FW. Control of the size of the human muscle mass. An-nu Rev Physiol 66: 799-828, 2004.
212. Roos MR, Rice CL, Connelly DM and Vandervoort AA. Quadriceps muscle strength, contractile properties, and motor unit firing rates in young and old men. Muscle Nerve 22: 1094-1103, 1999.
213. Roth SM, Ivey FM, Martel GF, Lemmer JT, Hurlbut DE, Siegel EL, Metter EJ, Fleg JL, Fozard JL, Kostek MC, Wernick DM and Hurley BF. Muscle size responses to strength training in young and older men and women. J Am Geriatr Soc 49: 1428-1433, 2001.
214. Roth SM, Martel GF, Ivey FM, Lemmer JT, Metter EJ, Hurley BF and Rogers MA. Skeletal muscle satellite cell populations in healthy young and older men and wom-en. Anat Rec 260: 351-358, 2000.
215. Rutherford OM and Jones DA. Measurement of fibre pennation using ultrasound in the human quadriceps in vivo. Eur J Appl Physiol Occup Physiol 65: 433-437, 1992.
216. Sacheck JM, Hyatt JP, Raffaello A, Jagoe RT, Roy RR, Edgerton VR, Lecker SH and Goldberg AL. Rapid disuse and denervation atrophy involve transcriptional changes similar to those of muscle wasting during systemic dis-eases. FASEB J 21: 140-155, 2007.
217. Sager MA, Franke T, Inouye SK, Landefeld CS, Morgan TM, Rudberg MA, Sebens H and Winograd CH. Func-tional outcomes of acute medical illness and hospitali-zation in older persons. Arch Intern Med 156: 645-652, 1996.
218. Sandri M. Signaling in muscle atrophy and hypertrophy. Physiology (Bethesda) 23: 160-170, 2008.
219. Sandri M, Lin J, Handschin C, Yang W, Arany ZP, Lecker SH, Goldberg AL and Spiegelman BM. PGC-1alpha pro-tects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. Proc Natl Acad Sci U S A 103: 16260-16265, 2006.
220. Sandri M, Sandri C, Gilbert A, Skurk C, Calabria E, Picard A, Walsh K, Schiaffino S, Lecker SH and Goldberg AL. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle at-rophy. Cell 117: 399-412, 2004.
221. Sashika H, Matsuba Y and Watanabe Y. Home program of physical therapy: Effect on disabilities patients with total hip arthroplasty. Archives of Physical Medicine and Rehabilitation 77: 273-277, 1996.
222. Sheehan SM and Allen RE. Skeletal muscle satellite cell proliferation in response to members of the fibroblast growth factor family and hepatocyte growth factor. J Cell Physiol 181: 499-506, 1999.

223. Shefer G, Rauner G, Yablonka-Reuveni Z and Benayahu D. Reduced satellite cell numbers and myogenic capaci-ty in aging can be alleviated by endurance exercise. PLoS ONE 5: e13307, 2010.
224. Sherrington C and Lord SR. Home exercise to improve strength and walking velocity after hip fracture: A ran-domized controlled trial. Archives of Physical Medicine and Rehabilitation 1997;78: 208-212.
225. Shih CH, Du YK, Lin YH and Wu CC. Muscular recovery around the hip joint after total hip arthroplasty. Clin Or-thop 1994;115-120.
226. Sicard-Rosenbaum L, Light KE and Behrman AL. Gait, lower extremity strength, and self-assessed mobility af-ter hip arthroplasty. Journals of Gerontology Series A-Biological Sciences and Medical Sciences 2002;57: M47-M51.
227. Simoneau JA, Lortie G, Boulay MR, Thibault MC and Bouchard C. Repeatability of fibre type and enzyme ac-tivity measurements in human skeletal muscle. Clin Physiol 1986;6: 347-356.
228. Singh MA, Ding W, Manfredi TJ, Solares GS, O'Neill EF, Clements KM, Ryan ND, Kehayias JJ, Fielding RA and Evans WJ. Insulin-like growth factor I in skeletal muscle after weight-lifting exercise in frail elders. Am J Physiol 1999;277: E135-E143.
229. Sipila S and Suominen H. Effects of strength and endur-ance training on thigh and leg muscle mass and compo-sition in elderly women. J Appl Physiol 1995;78: 334-340.
230. Siu PM, Pistilli EE, Butler DC and Alway SE. Aging influ-ences cellular and molecular responses of apoptosis to skeletal muscle unloading. Am J Physiol Cell Physiol 2005;288: C338-C349.
231. Siu PM, Pistilli EE, Murlasits Z et al. Hindlimb unloading increases muscle content of cytosolic but not nuclear Id2 and p53 proteins in young adult and aged rats. J Appl Physiol 2006;100: 907-916.
232. Skelton DA, Greig CA, Davies JM et al. Strength, power and related functional ability of healthy people aged 65-89 years. Age Ageing 1994;23: 371-377.
233. Skelton DA, Kennedy J and Rutherford OM. Lower limb muscle power in frequently falling community-dwelling women aged 65 and over. Journal of Aging and Physical Activity 1999;7: 281-282.
234. Skelton DA, Kennedy J and Rutherford OM. Explosive power and asymmetry in leg muscle function in fre-quent fallers and non-fallers aged over 65. Age and Ageing 2002;31: 119-125.
235. Sleivert GG and Wenger HA. Reliability of measuring isometric and isokinetic peak torque, rate of torque de-velopment, integrated electromyography, and tibial nerve conduction velocity. Arch Phys Med Rehabil 1994;75: 1315-1321.
236. Sonn U and Asberg KH. Assessment of activities of daily living in the elderly. A study of a population of 76-year-olds in Gothenburg, Sweden. Scand J Rehabil Med 1991;23: 193-202.
237. Stevens JE, Walter GA, Okereke E, et al. Muscle adapta-tions with immobilization and rehabilitation after ankle fracture. Med Sci Sports Exerc 2004;36: 1695-1701.
238. Strasser H, Tiefenthaler M, Steinlechner M, et al. Age dependent apoptosis and loss of rhabdosphincter cells. J Urol 2000;164: 1781-1785.
239. Strojnik V and Komi PV. Neuromuscular fatigue after maximal stretch-shortening cycle exercise. J Appl Phys-iol 1998;84: 344-350.
240. Suetta C, Aagaard P, Magnusson SP, Andersen LL, Sipila S, Rosted A, Jakobsen AK, Duus B and Kjaer M. Muscle size, neural activation and rapid force characteristics in elderly men and women - effects of unilateral long-term disuse due to hip-osteoarthritis. J Appl Physiol 2006.
241. Suetta C, Aagaard P, Rosted A, et al. Training-induced changes in muscle CSA, muscle strength, EMG, and rate of force development in elderly subjects after long-term unilateral disuse. J Appl Physiol 2004;97: 1954-1961.
242. Suetta C, Frandsen U, Jensen L, et al. Aging affects the transcriptional regulation of human skeletal muscle disuse atrophy. PLoS ONE 2012;7: e51238.
243. Suetta C, Magnusson SP, Rosted A, et al. Resistance training in the early postoperative phase reduces hospi-talization and leads to muscle hypertrophy in elderly hip surgery patients--a controlled, randomized study. J Am Geriatr Soc 2004;52: 2016-2022.
244. Swanson CE, Day GA, Yelland CE, et al. The manage-ment of elderly patients with femoral fractures. A ran-domised controlled trial of early intervention versus standard care. Med J Aust 169: 515-518, 1998.
245. Tesch PA, von WF, Gustafsson T, et al. Skeletal muscle proteolysis in response to short-term unloading in hu-mans. J Appl Physiol (1985) 2008;105: 902-906.
246. Thelen DG, Schultz AB, Alexander NB et al. Effects of age on rapid ankle torque development. Journals of Gerontology Series A-Biological Sciences and Medical Sciences 51: M226-M232, 1996.
247. Tracy BL, Ivey FM, Hurlbut D, et al. Muscle quality. II. Ef-fects Of strength training in 65- to 75-yr-old men and women. J Appl Physiol 1999;86: 195-201.
248. Trudelle-Jackson E, Emerson R and Smith S. Outcomes of total hip arthroplasty: A study of patients one year postsurgery. Journal of Orthopaedic & Sports Physical Therapy 2002;32: 260-267.
249. Trudelle-Jackson E and Smith SS. Effects of a late-phase exercise program after total hip arthroplasty: a random-ized controlled trial. Arch Phys Med Rehabil 2004;85: 1056-1062.
250. Urso ML, Clarkson PM and Price TB. Immobilization ef-fects in young and older adults. Eur J Appl Physiol , 2006;96: 564-571.
251. Van CM, Duchateau J and Hainaut K. Changes in single motor unit behaviour contribute to the increase in con-traction speed after dynamic

training in humans. J Phys-iol 1998;513 (Pt 1): 295-305.
252. Vandervoort AA. Aging of the human neuromuscular system. Muscle Nerve 2002;25: 17-25.
253. Vandervoort AA and McComas AJ. Contractile changes in opposing muscles of the human ankle joint with ag-ing. J Appl Physiol 1986;61: 361-367.
254. Verdijk LB, Koopman R, Schaart G, et al. Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly. Am J Physiol Endocrinol Metab 2007;292: E151-E157.
255. Verney J, Kadi F, Charifi N, et al. Effects of combined lower body endurance and upper body resistance train-ing on the satellite cell pool in elderly subjects. Muscle Nerve 2008;38: 1147-1154.
256. Viitasalo JT, Saukkonen S and Komi PV. Reproducibility of measurements of selected neuromuscular perfor-mance variables in man. Electromyogr Clin Neurophys-iol 1980;20: 487-501.
257. Visser M, Harris TB, Fox KM, et al. Change in muscle mass and muscle strength after a hip fracture: relation-ship to mobility recovery. J Gerontol A Biol Sci Med Sci 2000;55: M434-M440.
258. Wall BT, Dirks ML, Snijders T, et al. Substantial skeletal muscle loss occurs during only 5 days of disuse. Acta Physiol (Oxf) 2014;210: 600-611.
259. Walsh M, Woodhouse LJ, Thomas SG et al. Physical im-pairments and functional limitations: a comparison of individuals 1 year after total knee arthroplasty with con-trol subjects. Phys Ther 1998;78: 248-258.
260. Welle S, Brooks A and Thornton CA. Senescence-related changes in gene expression in muscle: similarities and differences between mice and men. Physiol Genomics 2001;5: 67-73.
261. Welle S, Totterman S and Thornton C. Effect of age on muscle hypertrophy induced by resistance training. J Gerontol A Biol Sci Med Sci 1996;51: M270-M275.
262. Wennerberg D. Reliability of an isokinetic dorsiflexion and plantar flexion apparatus. Am J Sports Med 1991;19: 519-522.
263. Whitman SA, Wacker MJ, Richmond SR and Godard MP. Contributions of the ubiquitin-proteasome pathway and apoptosis to human skeletal muscle wasting with age. Pflugers Arch 2009;450: 437-446.
264. Wozniak AC and Anderson JE. Nitric oxide-dependence of satellite stem cell activation and quiescence on nor-mal skeletal muscle fibers. Dev Dyn 2007;236: 240-250.
265. Wyszomierski SA, Chambers AJ and Cham R. Knee strength capabilities and slip severity. J Appl Biomech 2009;25: 140-148,.
266. Yang SY and Goldspink G. Different roles of the IGF-I Ec peptide (MGF) and mature IGF-I in myoblast prolifera-tion and differentiation. FEBS Lett 2002;522: 156-160.
267. Zarzhevsky N, Carmeli E, Fuchs D, et al. Recovery of muscles of old rats after hindlimb immobilisation by ex-ternal fixation is impaired compared with those of young rats. Exp Gerontol 2001;36: 125-140.
268. Zhao J, Brault JJ, Schild A, et al. FoxO3 coordinately ac-tivates protein degradation by the autophag-ic/lysosomal and proteasomal pathways in atrophying muscle cells. Cell Metab 2007;6: 472-483.