



# The decline in skeletal muscle mass with aging is mainly attributed to a reduction in type II muscle fiber size



Rachel Nilwik, Tim Snijders, Marika Leenders, Bart B.L. Groen, Janneau van Kranenburg, Lex B. Verdijk, Luc J.C. van Loon\*

Department of Human Movement Sciences, NUTRIM School for Nutrition, Toxicology and Metabolism, Maastricht University Medical Centre +, Maastricht, The Netherlands

## ARTICLE INFO

### Article history:

Received 31 October 2012

Received in revised form 7 February 2013

Accepted 11 February 2013

Available online 17 February 2013

Section Editor: Christiaan Leeuwenburgh

### Keywords:

Sarcopenia

Atrophy

Exercise training

Hypertrophy

Type II muscle fibers

## ABSTRACT

**Background:** The loss of skeletal muscle mass with aging has been attributed to a decline in muscle fiber number and muscle fiber size.

**Objective:** To define to what extent differences in leg muscle cross-sectional area (CSA) between young and elderly men are attributed to differences in muscle fiber size.

**Methods:** *Quadriceps* muscle CSA and type I and type II muscle fiber size were measured in healthy young ( $n=25$ ;  $23 \pm 1$  y) and older ( $n=26$ ;  $71 \pm 1$  y) men. Subsequently, the older subjects performed 6 months of resistance type exercise training, after which measurements were repeated. Differences in *quadriceps* muscle CSA were compared with differences in type I and type II muscle fiber size.

**Results:** *Quadriceps* CSA was substantially smaller in older versus young men ( $68 \pm 2$  vs  $80 \pm 2$  cm<sup>2</sup>, respectively;  $P < 0.001$ ). Type II muscle fiber size was substantially smaller in the elderly vs the young (29%;  $P < 0.001$ ), with a tendency of smaller type I muscle fibers ( $P = 0.052$ ). Differences in type II muscle fiber size fully explained differences in *quadriceps* CSA between groups. Prolonged resistance type exercise training in the elderly increased type II muscle fiber size by  $24 \pm 8\%$  ( $P < 0.01$ ), explaining  $100 \pm 3\%$  of the increase in *quadriceps* muscle CSA (from  $68 \pm 2$  to  $74 \pm 2$  cm<sup>2</sup>).

**Conclusion:** Reduced muscle mass with aging is mainly attributed to smaller type II muscle fiber size and, as such, is unlikely accompanied by substantial muscle fiber loss. In line, the increase in muscle mass following prolonged resistance type exercise training can be attributed entirely to specific type II muscle fiber hypertrophy.

Crown Copyright © 2013 Published by Elsevier Inc. All rights reserved.

## 1. Introduction

Aging is accompanied by a progressive loss of skeletal muscle mass and strength, known as sarcopenia, which leads to reduced functional capacity and an increased risk of developing chronic metabolic disease. The decline in muscle mass is most prominent in the lower limbs (Janssen et al., 2000). On a muscle tissue level, senescent muscle has been characterized by muscle fiber loss, muscle fiber atrophy, and fiber type grouping (Doherty, 2003; Koopman, 2011; Lexell et al., 1988).

Previous work has reported a decline in thigh muscle cross-sectional area (CSA) of 25–40% over the lifespan (Klitgaard et al., 1990; Lexell et al., 1988). This seems to be in line with other studies reporting a ~30% reduction in leg muscle mass (Fleg and Lakatta, 1988; Janssen et al., 2000). Several mechanisms have been proposed to play a role in the etiology of age-related muscle loss. These include increased levels of nuclear apoptosis (Buford et al., 2010; Degens and

Alway, 2006; Marzetti et al., 2012) and oxidative stress (Johnston et al., 2008), muscle fiber denervation (Degens and Alway, 2006), and reduced satellite cell content and/or regenerative potential (Buford et al., 2010). However, it remains to be established to what extent these mechanisms lead to muscle fiber loss or atrophy.

Although a fundamental underlying process remains to be identified, many studies have reported a substantial decrease in muscle fiber size in elderly subjects when compared with young controls (Dreyer et al., 2006; Larsson et al., 1978; Verdijk et al., 2007). This reduction in muscle fiber size has been shown to be fiber type specific, with 10–40% smaller type II fibers observed in muscle tissue collected from elderly compared with young controls. In contrast, type I muscle fiber size seems to remain largely unaffected during the aging process (Larsson et al., 1978; Martel et al., 2006; Snijders et al., 2009; Verdijk et al., 2007). Less information is available on the proposed decline in muscle fiber number with aging. Previous reports suggesting this are either based on excised human muscle (Lexell et al., 1988) or on a variety of animal models, with inconsistent findings (Brown, 1987; Sheard and Anderson, 2012; Wanagat et al., 2001). Thus, it remains unclear whether the substantial loss of skeletal muscle mass with aging is mainly attributed to muscle fiber loss or (type II specific) muscle fiber atrophy.

\* Corresponding author at: Department of Human Movement Sciences, Faculty of Health, Medicine and Life Sciences, Maastricht University Medical Centre +, P.O. Box 616, 6200 MD Maastricht, The Netherlands. Tel.: +31 43 3881397; fax: +31 43 3670976.

E-mail address: [L.vanLoon@maastrichtuniversity.nl](mailto:L.vanLoon@maastrichtuniversity.nl) (L.J.C. van Loon).

Many studies have focused on possibilities to counteract sarcopenia. Prolonged resistance type exercise training is a well-established effective strategy to increase muscle mass and strength in the elderly (Frontera et al., 1988; Narici et al., 2004; Peterson et al., 2010; Roth et al., 2001). Improvements of up to 15% in *quadriceps* muscle CSA have been achieved within 16 weeks (Frontera et al., 1988; Narici et al., 2004; Verdijk et al., 2009a). Furthermore, resistance type exercise training is inherently associated with the predominant recruitment of type II muscle fibers (Hodson-Tole and Wakeling, 2008), resulting in a 20–30% increase in type II muscle fiber size (Frontera et al., 1988; Martel et al., 2006; Verdijk et al., 2009a). This selective type II muscle fiber hypertrophy is accompanied by a fiber type specific increase in satellite cell content (Verdijk et al., 2009a).

Whether changes in muscle mass due to aging or following exercise training can be entirely attributed to changes in (type II) muscle fiber size remains a topic of debate. This debate has been difficult to resolve; due to obvious methodological limitations, it is challenging to differentiate between changes in muscle fiber number and muscle fiber size. Our laboratory has assessed numerous histological muscle samples collected from young and elderly subjects, in which quite substantial differences in fiber size between groups were observed (Kuipers et al., 1993; Leenders et al., 2013; Snijders et al., 2012; Verdijk et al., 2009a, 2010, 2012). In agreement, strong correlations were observed between muscle mass and (type II) muscle fiber size (Leenders et al., 2013; Verdijk et al., 2010). As such, our previous work has led us to hypothesize that type II muscle fiber atrophy is mainly responsible for the observed loss of muscle mass with aging. In line, we hypothesize that type II specific muscle fiber hypertrophy is responsible for the observed increase in skeletal muscle mass following prolonged resistance type exercise training in elderly men.

To gain insight as to the contribution of differences in muscle fiber size versus muscle fiber number to overall differences in leg muscle mass, we collected muscle biopsies as well as upper leg CT scans to compare muscle fiber characteristics and *quadriceps* muscle CSA between a cohort of young ( $n = 25$ ) and older ( $n = 26$ ) healthy men. Subsequently, the older men participated in a 6 month resistance type exercise training program, after which additional muscle biopsies and CT scans were performed. This study is the first to show that differences in *quadriceps* muscle mass between young and older men are mainly attributed to smaller type II muscle fibers, with no indication of substantial differences in muscle fiber numbers. In addition, the training-induced increase in muscle CSA can be entirely attributed to type II muscle fiber hypertrophy.

## 2. Methods

### 2.1. Subjects

A total of 25 healthy young ( $23 \pm 1$  y) and 26 healthy older ( $71 \pm 1$  y) men participated in the present study. All subjects were recruited through advertisements in local newspapers. Medical history of all subjects was evaluated. Exclusion criteria were defined that would preclude successful participation in the exercise program, and included (silent) cardiac or peripheral vascular disease and orthopedic limitations. Furthermore, as insulin resistance and/or type 2 diabetes have been associated with a more progressive loss of muscle mass and strength with aging, type 2 diabetes patients were excluded from participation (ADA, 2012). All subjects were living independently and had no history of participating in any structured exercise training program for at least 2 years. All subjects were informed about the nature and possible risks of the experimental procedures before written informed consent was obtained. This study was approved by the Medical Ethical Committee of the Maastricht University Medical Centre<sup>+</sup>. The study is part of a greater project investigating the clinical benefits of exercise intervention in the elderly.

### 2.2. Study design

After inclusion in the study, whole-body DXA scans and CT scans of the upper leg were performed, and muscle biopsies from the *vastus lateralis* muscle were collected in all subjects. In the elderly, additional DXA scans, CT scans, and muscle biopsies were collected after 6 months of resistance type exercise training.

*Quadriceps* muscle CSA and muscle fiber sizes were compared between groups, to assess the impact of differences in type I and type II muscle fiber size on differences in whole-muscle CSA between young and older men. In the elderly, changes in *quadriceps* muscle CSA following resistance type exercise training were associated with changes in type I and type II muscle fiber size following training. This allowed us to assess the impact of type I and/or type II muscle fiber hypertrophy on the increase in muscle CSA in the older men.

### 2.3. DXA and CT scans

Body composition was determined in all subjects at baseline, and after the training intervention in the elderly. Whole-body and leg lean mass and fat mass were determined by DXA (Hologic, Discovery A, QDR Series, Bradford, MA, USA) using the system's software package Apex version 2.3.

Anatomic cross-sectional area of the *quadriceps* muscle was assessed by CT scanning (Brilliance 64, Philips Medical Systems, Best, The Netherlands) at baseline for all subjects. Additional CT scans were performed post-training in the older subjects. The scanning characteristics were as follows: 120 kV, 300 mA, rotation time of 0.75 s, and a field of view of 500 mm. While the subjects were supine with their legs extended and their feet secured, a 3-mm thick axial image was taken 15 cm proximal to the base of the patella. The exact scanning position was measured and marked for replication at subsequent visits.

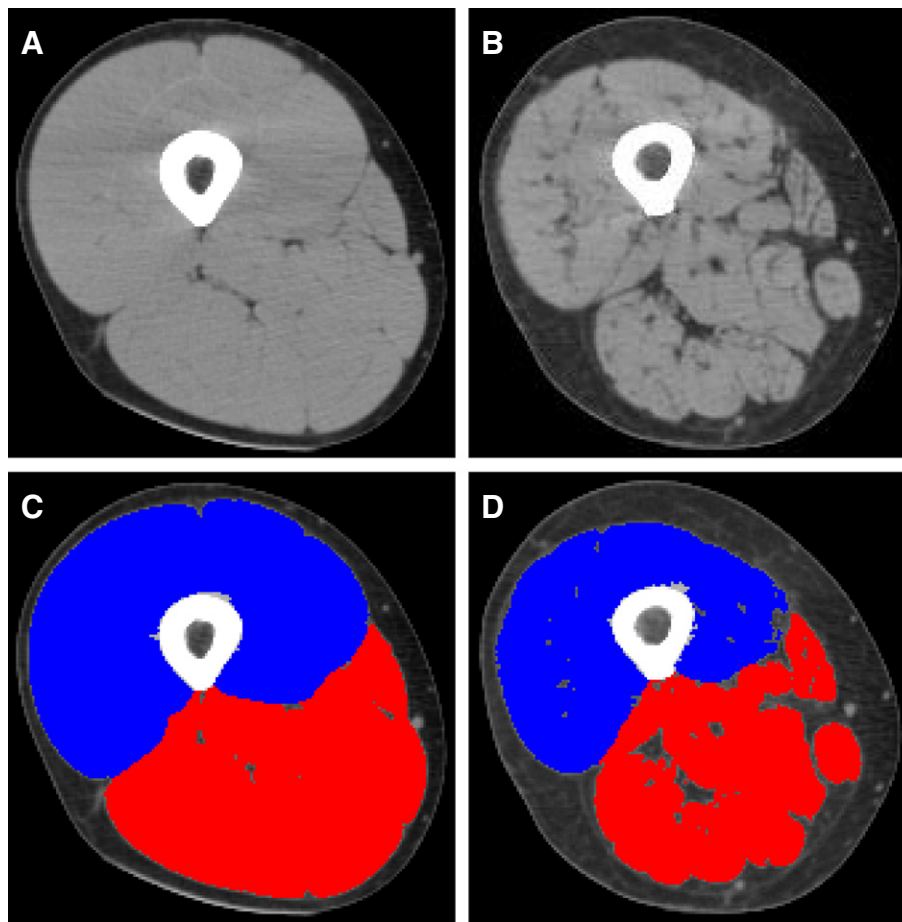
On the obtained CT images, muscle tissue was defined by threshold values of 0–100 Hounsfield Units (Strandberg et al., 2010), and the *quadriceps* muscle was selected by manual tracing using ImageJ software (version 1.45d, National Institute of Health, Maryland, USA) (Goodpaster et al., 2000). Representative examples of CT scan analysis are presented in Fig. 1. All analyses were performed by 2 investigators blinded to subject coding; intraclass correlation coefficients for inter- and intra-investigator reliability were 1.000 and 0.997, respectively.

### 2.4. Muscle biopsies

Skeletal muscle biopsies were collected at baseline in all subjects, and after 6 months of resistance type exercise training in the elderly subjects (4 days after strength testing). All biopsies were taken in the morning after an overnight fast. Under local anesthesia, percutaneous needle biopsies were collected from the *vastus lateralis* muscle of the right leg (Tarnopolsky et al., 2011). Any visible non-muscle tissue was removed immediately, and samples were embedded in Tissue-Tek (Sakura Finetek, Zoeterwoude, The Netherlands), frozen in liquid nitrogen-cooled isopentane, and stored at  $-80$  °C until further analysis.

### 2.5. Immunohistochemistry

Of all muscle tissue samples, 5  $\mu$ m-thick cryosections were cut at  $-20$  °C. All samples from one subject were mounted together on an uncoated glass slide. Care was taken to properly align the samples for cross-sectional fiber analysis. Sections were stained for muscle fiber typing as described in detail previously (Verdijk et al., 2009a). In short, slides were incubated with primary antibodies against MHC-I (A4.840, Developmental Studies Hybridoma Bank, Iowa City, IA) and laminin (polyclonal laminin, Sigma, Zwijndrecht, The Netherlands). After washing, appropriate secondary antibodies were applied (goat anti-mouse IgM Alexa Fluor 555 and goat anti-rabbit IgG AlexaFluor 647, respectively, Molecular Probes, Invitrogen, Breda, The Netherlands).



**Fig. 1.** Representative images of CT scans of the upper leg in a young (A) and elderly (B) subject. Quadriceps (blue) and whole thigh muscle area (blue + red) in the young (C) and elderly subject (D) are illustrated below.

Images were visualized and automatically captured at  $10\times$  magnification with a fluorescent microscope equipped with an automatic stage (IX81 motorized inverted microscope, Olympus, Hamburg, Germany). The camera was centered and focused on each section, after which the microscope was programmed to automatically capture a series of images to record the entire section. All recordings were then stitched together to obtain a single image of the entire biopsy section, including both fluorescent channels (a tetramethyl rhodamine isothiocyanate excitation filter [540–570 nm] for MHC-I, and a Cy5 excitation filter [590–650 nm] for laminin). Using ImageJ software (version 1.45f, National Institute of Health, Maryland, USA), individual fibers were localized using the laminin outline, and a Region of Interest (ROI) list was created listing all individual fibers. Muscle fiber size ( $\mu\text{m}^2$ ) and fiber type (I or II) were determined for each individual muscle fiber (see Fig. 2 for examples of type I and II fibers in muscle sections from a young and older subject). As a measure of fiber circularity, form factors were calculated by using the following formula:  $(4\pi \cdot \text{CSA}) / (\text{perimeter})^2$ . Fiber circularity was significantly lower in the older men when compared to young (0.71 and 0.74, respectively;  $P < 0.001$ ), and did not change during the course of the training intervention. On average,  $385 \pm 19$  and  $407 \pm 20$  muscle fibers were analyzed in the biopsy samples collected at baseline and after 6 months of exercise intervention, respectively. These numbers are sufficient to reliably determine muscle fiber characteristics (Mackey et al., 2009).

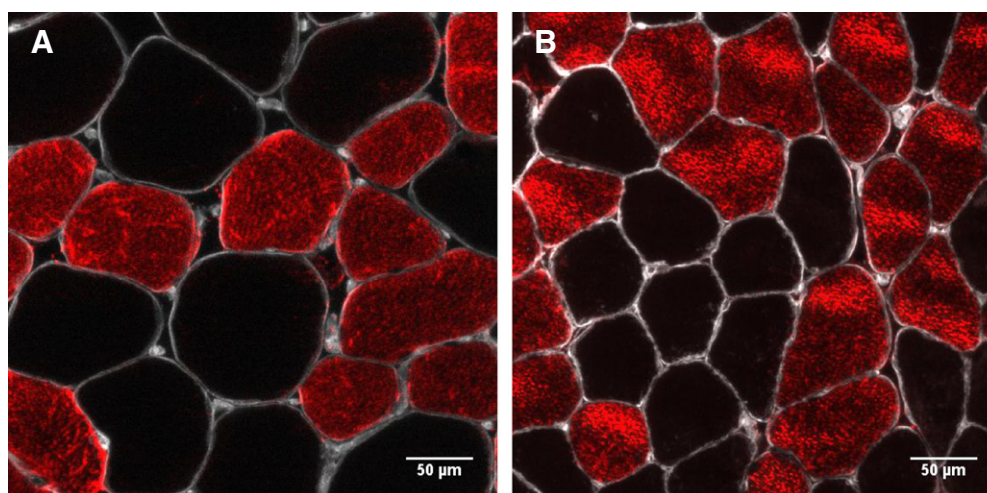
#### 2.6. Screening and strength assessment

During a screening day, basal blood samples were collected for blood HbA1c content determination, and a resting electrocardiogram

was performed in the older subjects. Subjects involved in the training program were familiarized with the weight lifting machines (leg press, leg extension, chest press, horizontal row, lat pull down, and biceps curl; Technogym, Rotterdam), and maximum strength was estimated using the multiple repetition testing procedure (Mayhew et al., 1995). During a subsequent visit, muscle strength was assessed by one-repetition maximum (1RM) strength tests on the leg press and leg extension machines (Verdijk et al., 2009b). 1RM tests were repeated after 4, 8, 12, 16, and 20 weeks of intervention and 2 days after the last training session of the intervention program.

#### 2.7. Exercise training intervention program

The elderly subjects participated in a supervised resistance type exercise training program, which consisted of 3 sessions per week, for 6 months. Training consisted of warming-up on a cycle ergometer, followed by 4 sets of 8–10 repetitions on both the leg press and leg extension machines. In addition, 3 sets were performed on the chest press and horizontal row, and (alternating): vertical lat pull down and abdominals, or biceps curl and triceps extension; followed by a 5-minute cooling-down period on the cycle ergometer. During the first 4 weeks of training, the workload was increased from 60% of 1RM (10–15 repetitions in each set) to 75% of 1RM (8–10 repetitions). Starting in week 5, 4 sets of 8 repetitions were performed at 75–80% of 1RM on the leg press and leg extension. For the upper body exercises, 2 sets were increased to 3 sets starting in week 5. Resting periods of 1.5 and 3 min were allowed between sets and exercises, respectively. Workload intensity was adjusted based on the 1RM tests (performed at 4, 8, 12, 16, and 20 weeks) to allow a progressive increase in



**Fig. 2.** Examples of immunohistochemical stainings of muscle tissue cross sections taken from a biopsy in a young (A) and elderly (B) subject, with type I fiber MHC in red, and basement membrane (laminin) in gray.

resistance (Kraemer and Fry, 1995). In addition, workload was increased if more than 8 repetitions could be performed in 3 out of 4 sets. Subjects attended  $90 \pm 1\%$  of the scheduled exercise sessions.

### 2.8. Data analysis

All data are expressed as means  $\pm$  SEM. For each subject, *quadriceps* muscle CSA was multiplied by the type I and type II muscle fiber area percentage (divided by 100) to obtain the area of the muscle occupied by type I and type II muscle fibers. These areas were divided by mean type I and type II muscle fiber size, respectively, to calculate the theoretical number of type I and type II muscle fibers.

Differences in whole-muscle CSA, muscle fiber size, number, and fiber type distribution between young and older men were analyzed using independent samples t-tests. Resistance type exercise training-induced changes in muscle strength and *quadriceps* CSA in the older men were determined using paired samples t-tests. For changes in fiber size, repeated measures ANOVA was performed with time (pre- and post-training) and fiber type (type I and type II fibers) as within subject factors. When a significant time  $\times$  fiber type interaction was observed, post-hoc analyses (paired samples t-tests) were performed to determine training effects on type I and type II muscle fiber size separately.

Pearson rank correlation coefficients ( $r$ ) were calculated to determine whether greater type I and type II muscle fiber size was associated with greater muscle strength, in the older men, both for baseline values and for their training-induced changes. For all statistical tests, significance was set at  $P < 0.05$ . All calculations were performed using SPSS version 19.0 (Chicago, IL).

## 3. Results

### 3.1. Subjects

Subjects' characteristics are presented in Table 1. All subjects were healthy normoglycemic males, although blood HbA1c content was higher in the older men when compared with the young ( $P < 0.01$ ).

### 3.2. Age-related differences

Whereas whole-body lean mass did not differ between the young and older men ( $62.7 \pm 1.4$  and  $62.2 \pm 1.1$  kg, respectively;  $P > 0.05$ ), leg lean mass was significantly lower in the elderly compared with the young ( $9.9 \pm 0.2$  vs  $11.0 \pm 0.3$  kg, respectively;  $P < 0.01$ ; Table 1).

In accordance, *quadriceps* muscle CSA was 14% smaller in the older men ( $68 \pm 2$  cm<sup>2</sup>) versus the young ( $80 \pm 2$  cm<sup>2</sup>;  $P < 0.001$ ; Fig. 3A).

Mean muscle fiber size was  $\sim 20\%$  smaller in the older men compared with the young ( $P < 0.001$ ), which is in line with the group differences in *quadriceps* muscle CSA. Type II muscle fiber size was substantially smaller in the elderly vs the young ( $5050 \pm 198$  vs  $7136 \pm 309$   $\mu\text{m}^2$ , respectively;  $P < 0.001$ ), with a tendency for smaller type I muscle fibers ( $P = 0.052$ ; Fig. 3B). Also, fiber type distribution differed between groups, with a slightly lower percentage of type II fibers in the older men ( $46 \pm 3\%$ ) compared with the young ( $56 \pm 3\%$ ;  $P = 0.01$ ). Similarly, the percentage of muscle area occupied by type II muscle fibers was significantly lower in the older men when compared with the young ( $43 \pm 3$  vs  $58 \pm 3\%$  of muscle area, respectively;  $P < 0.05$ ). As a consequence, the absolute muscle area occupied by type II muscle fibers was significantly smaller in the older vs the young men ( $29 \pm 2$  vs  $46 \pm 2$  cm<sup>2</sup>, respectively;  $P < 0.001$ ). The calculated number of fibers in the *quadriceps* muscle did not differ between groups and averaged  $119 \pm 4 \cdot 10^4$  and  $127 \pm 3 \cdot 10^4$  in the young and older subjects, respectively ( $P = 0.13$ ).

### 3.3. Resistance type exercise training

Six months of resistance type exercise training in the older men increased whole body and leg lean mass by  $1.7 \pm 0.4\%$  and  $3.2 \pm 0.7\%$ , respectively ( $P < 0.001$ ). Leg press and leg extension strength increased with  $25 \pm 2$  and  $41 \pm 3\%$ , respectively ( $P < 0.001$  for both). In accordance, *quadriceps* muscle CSA increased from  $68 \pm 2$  to  $74 \pm 2$  cm<sup>2</sup> ( $P < 0.001$ ; Fig. 4A).

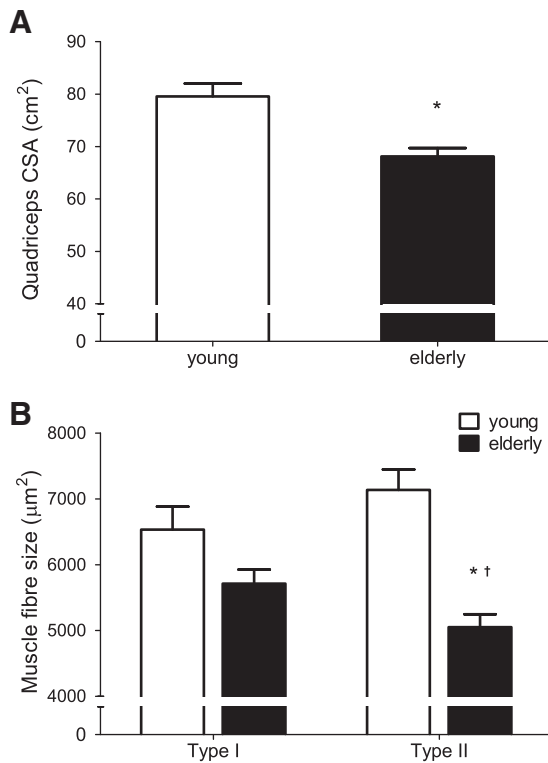
On a myocellular level, no changes were observed in muscle fiber type composition following the 6 months of resistance type exercise

**Table 1**  
Subjects' characteristics.

	Young men ( $n = 25$ )	Older men ( $n = 26$ )
Age (yr)	$23 \pm 1$	$70 \pm 1$
Height (m)	$1.85 \pm 0.01$	$1.77 \pm 0.01^a$
Weight (kg)	$79.8 \pm 1.7$	$84.7 \pm 1.8$
BMI (kg/m <sup>2</sup> )	$23.4 \pm 0.5$	$27.1 \pm 0.5^a$
HbA1c (%)	$5.2 \pm 0.1$	$5.5 \pm 0.1^a$
Lean body mass (kg)	$62.7 \pm 1.4$	$62.2 \pm 1.1$
Fat %	$22.2 \pm 2.3$	$23.2 \pm 0.9$
Lean leg mass (kg)	$11.0 \pm 0.3$	$9.9 \pm 0.2^a$

Values are means  $\pm$  SEM. BMI: body mass index.

<sup>a</sup> Significantly different compared with young men ( $P < 0.05$ ).



**Fig. 3.** Quadriceps muscle cross-sectional area (CSA; A) and type I and II muscle fiber size (B) in the young and elderly men. \* Significantly different from young men ( $P < 0.05$ ). † Significantly different from type I muscle fiber size ( $P < 0.05$ ).

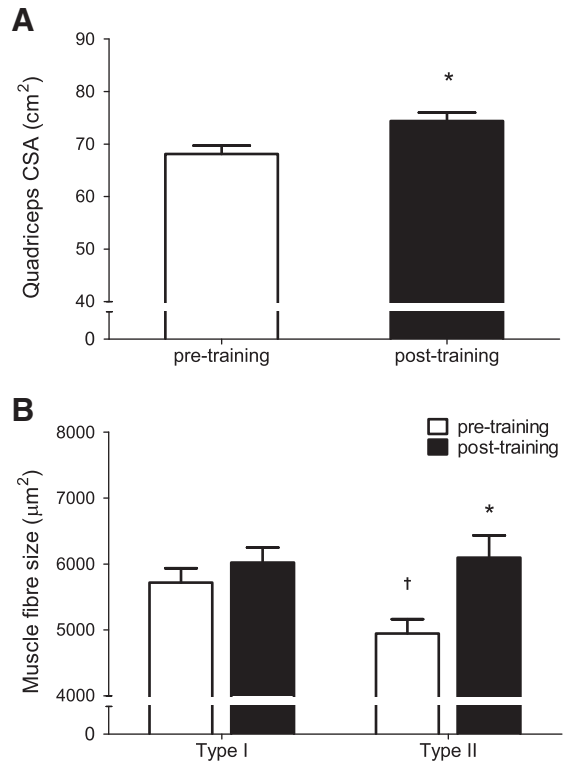
training. For muscle fiber size, a significant time  $\times$  fiber type interaction was observed. Type II muscle fiber size increased with  $24 \pm 8\%$ , from  $5050 \pm 198 \mu\text{m}^2$  at baseline to  $6096 \pm 338 \mu\text{m}^2$  following exercise training ( $P < 0.01$ ). In contrast, type I muscle fiber size did not change significantly throughout the intervention ( $P = 0.25$ , Fig. 4B). At baseline, type II muscle fibers were significantly smaller than type I muscle fibers in the elderly subjects ( $P < 0.05$ ). This difference was no longer apparent after prolonged exercise training ( $P = 0.86$ ; Fig. 4B).

The calculated number of muscle fibers in the *quadriceps* muscle averaged  $127 \pm 3 \cdot 10^4$  and  $128 \pm 6 \cdot 10^4$  before and after exercise training in the older men ( $P = 0.94$ ). This translated to  $68 \pm 4 \cdot 10^4$  vs  $69 \pm 5 \cdot 10^4$  type I muscle fibers and  $59 \pm 4 \cdot 10^4$  vs  $59 \pm 4 \cdot 10^4$  type II muscle fibers, pre- and post-training, respectively ( $P > 0.90$  for both fiber types). As no significant changes were observed in muscle fiber type composition, total number of fibers, or type I muscle fiber size, we calculated the cumulative increase in type II muscle fiber area, in the entire *quadriceps* muscle. This resulted in a total increase in type II muscle fiber area that was  $100 \pm 3\%$  of the observed increase in *quadriceps* CSA following exercise training. In other words, type II muscle fiber hypertrophy explained  $100 \pm 3\%$  of the measured increase in *quadriceps* CSA. When changes in type I muscle fiber size were also taken into account, the cumulative muscle fiber hypertrophy was higher ( $113 \pm 5\%$ ) than the measured increase in muscle CSA.

Interestingly, correlations were observed at baseline between type II muscle fiber size and leg press ( $r = 0.62$ ;  $P < 0.01$ ) and leg extension strength ( $r = 0.38$ ;  $P = 0.028$ ). In contrast, no significant correlations were observed between changes in muscle fiber size and changes in muscle strength following the resistance type exercise training program.

#### 4. Discussion

The present study shows that *quadriceps* muscle CSA is 14% smaller in elderly compared with young men. In addition, type II muscle fiber size was 29% smaller in the elderly versus the young. As the



**Fig. 4.** Quadriceps muscle cross sectional area (CSA; A) and type I and type II muscle fiber size (B) at baseline and after 6 months of resistance type exercise training in the elderly men. \* Significantly different from baseline ( $P < 0.05$ ). † Significantly different from type I fiber size ( $P < 0.05$ ).

calculated number of fibers in the *quadriceps* muscle did not differ between young and older men, these results suggest that type II specific differences in fiber size are responsible for the differences in muscle mass between age groups. Furthermore, prolonged resistance type exercise training in the older men resulted in a 9% increase in *quadriceps* CSA, a change that could be explained entirely by specific type II muscle fiber hypertrophy.

Since muscle mass is dependent on muscle fiber size and number, the decline in muscle mass with aging may be due to the loss of muscle fibers as well as a decline in muscle fiber size. Although several mechanisms have been proposed to contribute to the age-related loss of muscle mass (Buford et al., 2010; Degens and Alway, 2006; Johnston et al., 2008; Marzetti et al., 2012), a direct relation with muscle fiber loss and/or muscle fiber atrophy remains to be established. To gain further insight in the etiology of sarcopenia, we assessed to what extent differences in type I and type II muscle fiber size can explain the differences in *quadriceps* muscle CSA between young and older men. We observed a 14% smaller *quadriceps* muscle CSA in the older men when compared with the young (Fig. 3A). In line, type II muscle fiber size was 29% smaller in the elderly when compared with the young (Fig. 3B), which is consistent with previous findings showing 10–40% smaller type II muscle fibers in older populations (Dreyer et al., 2006; Klitgaard et al., 1990; Kosek et al., 2006; Verdijk et al., 2007). Furthermore, the percentage of type II muscle fibers was slightly lower in the elderly, indicating a possible muscle fiber type shift with aging (Klitgaard et al., 1990). Taking into account this minor difference in muscle fiber type distribution, our data clearly show that differences in leg muscle CSA between young and older men can be fully explained by differences in type II muscle fiber size. Obviously, this finding does not leave much room for a substantial reduction in muscle fiber number as a factor contributing to the lower muscle mass in the older population. In fact, the calculated number of muscle fibers in the *quadriceps* muscle was remarkably

similar between groups, with  $119 \pm 4 \cdot 10^4$  and  $127 \pm 3 \cdot 10^4$  muscle fibers in the young and older subjects, respectively.

To further investigate the impact of type II muscle fiber size as the main factor responsible for changes in muscle mass, the elderly subjects were included in a 6-month resistance type exercise training program. *Quadriceps* muscle CSA and type I and type II muscle fiber size were assessed prior to and after the training program. Prolonged resistance type exercise training strongly increased both leg lean mass and leg muscle strength, as has been shown previously (Frontera et al., 1988; Leenders et al., 2013; Lemmer et al., 2000; Macaluso and De Vito, 2004; Verdijk et al., 2009a). At baseline, leg muscle strength correlated well with type II muscle fiber size. However, no correlations were observed between training-induced changes in muscle fiber size and muscle strength. This is in line with previous findings, and likely due to the large inter-individual variability in the response to prolonged resistance type exercise training (Peterson et al., 2010; Verdijk et al., 2010). The observed 9% increase in quadriceps muscle CSA (Fig. 4A) is in line with previous reports showing increases in quadriceps CSA ranging between 8 and 15% following 10–16 weeks of exercise training (Frontera et al., 1988; Kosek et al., 2006; Kryger and Andersen, 2007; Narici et al., 2004; Verdijk et al., 2009a). On a myocellular level, we observed a substantial 24% increase in type II muscle fiber size and an 8% increase in type I muscle fiber size that did not reach statistical significance (Fig. 4B). This type II specific muscle fiber hypertrophy is likely attributed to the typical muscle recruitment patterns during the high-intensity resistance type exercise activities applied in the training intervention (Hodson-Tole and Wakeling, 2008). Previous studies have also reported an increased type II muscle fiber size ranging between 20 and 40% after 12–16 weeks of resistance type exercise training in elderly subjects (Hikida et al., 2000; Kryger and Andersen, 2007; Verdijk et al., 2009a). In the present study, we extend on previous work by comparing the measurements of both *quadriceps* muscle CSA and type I and type II muscle fiber size within the same subjects. The increase in type II muscle fiber size could explain  $100 \pm 3\%$  of the larger *quadriceps* muscle CSA that was observed following prolonged training. When also taking into account the (non-significant) increase in type I muscle fiber size, the total increase in fiber area would have explained  $113 \pm 5\%$  of the observed increase in quadriceps muscle CSA. Clearly, the training-induced increase in type II muscle fiber size seems to be largely responsible for the observed increase in quadriceps muscle CSA. As such, our data indicate that differences in muscle CSA, either associated with aging, or in response to prolonged resistance type exercise training, are mainly attributed to differences in type II muscle fiber size.

The immunohistochemical staining approach employed in the present study does not allow us to assess differences in MHC coexpression. Although the clinical significance of a change in MHC coexpression is not unequivocal (Andersen et al., 1999; Klitgaard et al., 1990; Verdijk et al., 2007; Williamson et al., 2000), such analyses may provide further insight into the prevalence of potential shifts in muscle fiber type distribution. Furthermore, due to the cross-sectional nature of the present study, we cannot prove that type II muscle fiber atrophy has occurred over the lifespan in the elderly subjects. In fact, type II muscle fiber size observed in the older subjects was rather large (i.e.  $5050 \mu\text{m}^2$ ) when compared with some previous findings (Dreyer et al., 2006; Lexell et al., 1988; Martel et al., 2006). Nonetheless, muscle fiber size in the present study falls well within the range generally reported in literature for healthy young and older men (i.e.  $4000\text{--}8000 \mu\text{m}^2$ ) (D'Antona et al., 2003; Kosek et al., 2006; Verdijk et al., 2007, 2009a). Moreover, type II muscle fiber size in the elderly was not only smaller when compared with the young, but also when compared with their own type I muscle fiber size. These findings strongly support that specific type II muscle fiber atrophy has occurred in the older subjects (Larsson et al., 1978; Lexell et al., 1988).

There seems to be a general belief that besides a decline in muscle fiber sizes, reduced muscle fiber numbers contribute substantially to the loss of muscle mass with aging. This assumption has been based

predominantly on work performed in animal models. Whereas some have reported a decline in muscle fiber number with aging (Faulkner et al., 2007; Sheard and Anderson, 2012), others have failed to confirm these findings (Brown, 1987; Sheard and Anderson, 2012). Lexell et al. (1988) were the first to perform muscle fiber counts in excised human *vastus lateralis* muscle, and reported a 40% lower muscle fiber number in older men when compared to young controls. Such large differences in muscle fiber numbers, combined with the observed reduction in type II muscle fiber size of 25–30% (Lexell et al., 1988), do not seem realistic and are at odds with the ~25% loss of whole-muscle CSA observed previously (Doherty, 2003; Lang et al., 2010). In agreement, others have failed to find evidence for reduced fiber numbers in older individuals (Klein et al., 2003), and over time (McCall et al., 1996; Young et al., 1982). The present study extends on these previous reports by, for the first time, combining both cross-sectional and longitudinal observations of muscle mass and muscle fiber size in a muscle group known to be strongly affected by sarcopenia in humans. Although we did not directly measure muscle fiber numbers, our data clearly show that changes in muscle CSA related to aging and exercise training can be entirely attributed to changes in type II muscle fiber size. Even when considering a potential (minor) fiber type shift, it is unlikely that changes in muscle fiber number contribute substantially to the observed changes in muscle mass with aging and in response to resistance type exercise training.

The clinical relevance of the current findings lies in the ongoing search for effective intervention strategies to prevent or treat sarcopenia. This loss of muscle mass and strength with aging predisposes to disability, frailty, and eventually, loss of independence. Over the last few years much research has focused on the effect of exercise, nutrition and pharmacological interventions to stimulate muscle growth (Lang et al., 2010; Verdijk et al., 2009a). The efficacy of interventions designed to combat sarcopenia depends on their ability to affect those processes that play a role in the etiology of sarcopenia (Dirks and Leeuwenburgh, 2005; Faulkner et al., 2007). In this study, we provide evidence to suggest that in healthy men, type II muscle fiber atrophy and hypertrophy are responsible for age-related muscle loss and training-induced gain of muscle mass, with little or no contribution from changes in muscle fiber number. Clearly, research aiming to develop effective exercise, nutritional, or pharmaceutical interventions to prevent and/or treat muscle loss with aging should focus on increasing type II muscle fiber size (Candow et al., 2012; Lang et al., 2010; Tieland et al., 2012). Interestingly, we have recently shown that resistance type exercise training effectively increases type II muscle fiber size in both genders (Leenders et al., in press), suggesting that men and women benefit equally from the same standardized exercise program. Nonetheless, as we included only men in the present study, we cannot extend our observations to both genders. Furthermore, research is warranted to elucidate the physiological mechanisms underlying specific type II muscle fiber atrophy with aging. Such information will provide novel leads to develop more effective intervention strategies to counteract the age-related loss of muscle mass and function.

We conclude that age-related reductions in muscle mass can be mainly attributed to specific type II muscle fiber atrophy, with no substantial decline in muscle fiber numbers. Furthermore, muscle mass can be effectively increased through prolonged resistance type exercise training, which can be entirely attributed to specific type II specific muscle fiber hypertrophy. Reversing or preventing type II muscle fiber atrophy should therefore be considered the primary target for the development of effective intervention strategies to prevent or treat sarcopenia.

## Acknowledgments

We greatly acknowledge the expert technical assistance of Mario Losen. We greatly appreciate the enthusiastic support of all subjects who volunteered to participate in this study.

## References

- American Diabetes Association, 2012. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 35 (Suppl 1), S64–S71.
- Andersen, J.L., Terzis, G., Kryger, A., 1999. Increase in the degree of coexpression of myosin heavy chain isoforms in skeletal muscle fibers of the very old. *Muscle Nerve* 22, 449–454.
- Brown, M., 1987. Change in fibre size, not number, in ageing skeletal muscle. *Age Ageing* 16, 244–248.
- Buford, T.W., Anton, S.D., Judge, A.R., Marzetti, E., Wohlgenuth, S.E., Carter, C.S., Leeuwenburgh, C., Pahor, M., Manini, T.M., 2010. Models of accelerated sarcopenia: critical pieces for solving the puzzle of age-related muscle atrophy. *Ageing Res. Rev.* 9, 369–383.
- Candow, D.G., Forbes, S.C., Little, J.P., Cornish, S.M., Pinkoski, C., Chilibeck, P.D., 2012. Effect of nutritional interventions and resistance exercise on aging muscle mass and strength. *Biogerontology* 13, 345–358.
- D'Antona, G., Pellegrino, M.A., Adami, R., Rossi, R., Carlizzi, C.N., Canepari, M., Saltin, B., Bottinelli, R., 2003. The effect of ageing and immobilization on structure and function of human skeletal muscle fibres. *J. Physiol.* 552, 499–511.
- Degens, H., Alway, S.E., 2006. Control of muscle size during disuse, disease, and aging. *Int. J. Sports Med.* 27, 94–99.
- Dirks, A.J., Leeuwenburgh, C., 2005. The role of apoptosis in age-related skeletal muscle atrophy. *Sports Med.* 35, 473–483.
- Doherty, T.J., 2003. Invited review: aging and sarcopenia. *J. Appl. Physiol.* 95, 1717–1727.
- Dreyer, H.C., Blanco, C.E., Sattler, F.R., Schroeder, E.T., Wiswell, R.A., 2006. Satellite cell numbers in young and older men 24 hours after eccentric exercise. *Muscle Nerve* 33, 242–253.
- Faulkner, J.A., Larkin, L.M., Clafin, D.R., Brooks, S.V., 2007. Age-related changes in the structure and function of skeletal muscles. *Clin. Exp. Pharmacol. Physiol.* 34, 1091–1096.
- Fleg, J.L., Lakatta, E.G., 1988. Role of muscle loss in the age-associated reduction in VO<sub>2</sub> max. *J. Appl. Physiol.* 65, 1147–1151.
- Frontera, W.R., Meredith, C.N., O'Reilly, K.P., Knuttgen, H.G., Evans, W.J., 1988. Strength conditioning in older men: skeletal muscle hypertrophy and improved function. *J. Appl. Physiol.* 64, 1038–1044.
- Goodpaster, B.H., Thaete, F.L., Kelley, D.E., 2000. Composition of skeletal muscle evaluated with computed tomography. *Ann. N. Y. Acad. Sci.* 904, 18–24.
- Hikida, R.S., Staron, R.S., Hagerman, F.C., Walsh, S., Kaiser, E., Shell, S., Hervey, S., 2000. Effects of high-intensity resistance training on untrained older men. II. Muscle fiber characteristics and nucleocytoplasmic relationships. *J. Gerontol. A Biol. Sci. Med. Sci.* 55, B347–B354.
- Hodson-Tole, E.F., Wakeling, J.M., 2008. Motor unit recruitment patterns 2: the influence of myoelectric intensity and muscle fascicle strain rate. *J. Exp. Biol.* 211, 1893–1902.
- Janssen, I., Heymsfield, S.B., Wang, Z.M., Ross, R., 2000. Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *J. Appl. Physiol.* 89, 81–88.
- Johnston, A.P., De Lisio, M., Parise, G., 2008. Resistance training, sarcopenia, and the mitochondrial theory of aging. *Appl. Physiol. Nutr. Metab.* 33, 191–199.
- Klein, C.S., Marsh, G.D., Petrella, R.J., Rice, C.L., 2003. Muscle fiber number in the biceps brachii muscle of young and old men. *Muscle Nerve* 28, 62–68.
- Klitgaard, H., Mantoni, M., Schiaffino, S., Ausoni, S., Gorza, L., Laurent-Winter, C., Schnohr, P., Saltin, B., 1990. Function, morphology and protein expression of ageing skeletal muscle: a cross-sectional study of elderly men with different training backgrounds. *Acta Physiol. Scand.* 140, 41–54.
- Koopman, R., 2011. Dietary protein and exercise training in ageing. *Proc. Nutr. Soc.* 70, 104–113.
- Kosek, D.J., Kim, J.S., Petrella, J.K., Cross, J.M., Bamman, M.M., 2006. Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults. *J. Appl. Physiol.* 101, 531–544.
- Kraemer, W., Fry, A., 1995. Strength testing: development and evaluation of methodology. In: Maud, P., Foster, C. (Eds.), *Physiological Assessment of Physical Fitness*. Human Kinetics, Leeds.
- Kryger, A.L., Andersen, J.L., 2007. 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.
- Kuipers, H., Peeze Binkhorst, F.M., Hartgens, F., Wijnen, J.A., Keizer, H.A., 1993. Muscle ultrastructure after strength training with placebo or anabolic steroid. *Can. J. Appl. Physiol.* 18, 189–196.
- Lang, T., Streeter, T., Cawthon, P., Baldwin, K., Taaffe, D.R., Harris, T.B., 2010. Sarcopenia: etiology, clinical consequences, intervention, and assessment. *Osteoporos. Int.* 21, 543–559.
- Larsson, L., Sjodin, B., Karlsson, J., 1978. Histochemical and biochemical changes in human skeletal muscle with age in sedentary males, age 22–65 years. *Acta Physiol. Scand.* 103, 31–39.
- Leenders, M., Verdijk, L.B., van Der Hoeven, L., van Kranenburg, J., Nilwik, R., Wodzig, W.K., Senden, J.M., Keizer, H.A., van Loon, L.J., 2013. Protein supplementation during resistance-type exercise training in the elderly. *Med. Sci. Sports Exerc.* 45 (3), 542–552.
- Leenders, M., Verdijk, L.B., van der Hoeven, L., van Kranenburg, J., Nilwik, R., van Loon, L.J., in press. Elderly men and women benefit equally from prolonged resistance-type exercise training. *J. Gerontol. A Biol. Sci. Med. Sci.*
- Lemmer, J.T., Hurlbut, D.E., Martel, G.F., Tracy, B.L., Ivey, F.M., Metter, E.J., Fozard, J.L., Fleg, J.L., Hurley, B.F., 2000. Age and gender responses to strength training and detraining. *Med. Sci. Sports Exerc.* 32, 1505–1512.
- Lexell, J., Taylor, C.C., Sjostrom, M., 1988. 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.
- Macaluso, A., De Vito, G., 2004. Muscle strength, power and adaptations to resistance training in older people. *Eur. J. Appl. Physiol.* 91, 450–472.
- Mackey, A.L., Kjaer, M., Charifi, N., Henriksson, J., Bojsen-Moller, J., Holm, L., Kadi, F., 2009. Assessment of satellite cell number and activity status in human skeletal muscle biopsies. *Muscle Nerve* 40, 455–465.
- Martel, G.F., Roth, S.M., Ivey, F.M., Lemmer, J.T., Tracy, B.L., Hurlbut, D.E., Metter, E.J., Hurley, B.F., Rogers, M.A., 2006. Age and sex affect human muscle fiber adaptations to heavy-resistance strength training. *Exp. Physiol.* 91, 457–464.
- Marzetti, E., Calvani, R., Bernabei, R., Leeuwenburgh, C., 2012. Apoptosis in skeletal myocytes: a potential target for interventions against sarcopenia and physical frailty – a mini-review. *Gerontology* 58, 99–106.
- Mayhew, J.L., Prinster, J.L., Ware, J.S., Zimmer, D.L., Arabas, J.R., Bembem, M.G., 1995. Muscular endurance repetitions to predict bench press strength in men of different training levels. *J. Sports Med. Phys. Fitness* 35, 108–113.
- McCall, G.E., Byrnes, W.C., Dickinson, A., Pattany, P.M., Fleck, S.J., 1996. Muscle fiber hypertrophy, hyperplasia, and capillary density in college men after resistance training. *J. Appl. Physiol.* 81, 2004–2012.
- Narici, M.V., Reeves, N.D., Morse, C.I., Maganaris, C.N., 2004. Muscular adaptations to resistance exercise in the elderly. *J. Musculoskelet. Neuronal. Interact.* 4, 161–164.
- Peterson, M.D., Rhea, M.R., Sen, A., Gordon, P.M., 2010. Resistance exercise for muscular strength in older adults: a meta-analysis. *Ageing Res. Rev.* 9, 226–237.
- Roth, S.M., Ivey, F.M., Martel, G.F., Lemmer, J.T., Hurlbut, D.E., Siegel, E.L., Metter, E.J., Fleg, J.L., Fozard, J.L., Kostek, M.C., Wernick, D.M., Hurley, B.F., 2001. Muscle size responses to strength training in young and older men and women. *J. Am. Geriatr. Soc.* 49, 1428–1433.
- Sheard, P.W., Anderson, R.D., 2012. Age-related loss of muscle fibres is highly variable amongst mouse skeletal muscles. *Biogerontology* 13, 157–167.
- Snijders, T., Verdijk, L.B., van Loon, L.J., 2009. The impact of sarcopenia and exercise training on skeletal muscle satellite cells. *Ageing Res. Rev.* 8, 328–338.
- Snijders, T., Verdijk, L.B., Beelen, M., McKay, B.R., Parise, G., Kadi, F., van Loon, L.J., 2012. A single bout of exercise activates skeletal muscle satellite cells during subsequent overnight recovery. *Exp. Physiol.* 97 (6), 762–773.
- Strandberg, S., Wretling, M.L., Wredmark, T., Shalabi, A., 2010. Reliability of computed tomography measurements in assessment of thigh muscle cross-sectional area and attenuation. *BMC Med. Imaging* 10, 18.
- Tarnopolsky, M.A., Pearce, E., Smith, K., Lach, B., 2011. Suction-modified Bergstrom muscle biopsy technique: experience with 13,500 procedures. *Muscle Nerve* 43, 717–725.
- Tieland, M., Dirks, M.L., van der Zwaluw, N., Verdijk, L.B., van de Rest, O., de Groot, L.C., van Loon, L.J., 2012. Protein supplementation increases muscle mass gain during prolonged resistance-type exercise training in frail elderly people: a randomized, double-blind, placebo-controlled trial. *J. Am. Med. Dir. Assoc.* 13 (8), 713–719.
- Verdijk, L.B., Koopman, R., Schaart, G., Meijer, K., Savelberg, H.H., van Loon, L.J., 2007. Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly. *Am. J. Physiol. Endocrinol. Metab.* 292, E151–E157.
- Verdijk, L.B., Gleeson, B.G., Jonkers, R.A., Meijer, K., Savelberg, H.H., Dendale, P., van Loon, L.J., 2009a. Skeletal muscle hypertrophy following resistance training is accompanied by a fiber type-specific increase in satellite cell content in elderly men. *J. Gerontol. A Biol. Sci. Med. Sci.* 64, 332–339.
- Verdijk, L.B., van Loon, L., Meijer, K., Savelberg, H.H., 2009b. One-repetition maximum strength test represents a valid means to assess leg strength in vivo in humans. *J. Sports Sci.* 27, 59–68.
- Verdijk, L.B., Snijders, T., Beelen, M., Savelberg, H.H., Meijer, K., Kuipers, H., Van Loon, L.J., 2010. Characteristics of muscle fiber type are predictive of skeletal muscle mass and strength in elderly men. *J. Am. Geriatr. Soc.* 58, 2069–2075.
- Verdijk, L.B., Dirks, M.L., Snijders, T., Prompers, J.J., Beelen, M., Jonkers, R.A., Thijssen, D.H., Hopman, M.T., Van Loon, L.J., 2012. Reduced satellite cell numbers with spinal cord injury and aging in humans. *Med. Sci. Sports Exerc.* 44, 2322–2330.
- Wanagat, J., Cao, Z., Pathare, P., Aiken, J.M., 2001. Mitochondrial DNA deletion mutations colocalize with segmental electron transport system abnormalities, muscle fiber atrophy, fiber splitting, and oxidative damage in sarcopenia. *FASEB J.* 15, 322–332.
- Williamson, D.L., Godard, M.P., Porter, D.A., Costill, D.L., Trappe, S.W., 2000. Progressive resistance training reduces myosin heavy chain coexpression in single muscle fibers from older men. *J. Appl. Physiol.* 88, 627–633.
- Young, A., Hughes, I., Round, J.M., Edwards, R.H., 1982. The effect of knee injury on the number of muscle fibres in the human quadriceps femoris. *Clin. Sci. (Lond.)* 62, 227–234.