Skeletal Muscle Hypertrophy Following Resistance Training Is Accompanied by a Fiber Type–Specific Increase in Satellite Cell Content in Elderly Men

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We determined muscle fiber type–specific hypertrophy and changes in satellite cell (SC) content following a 12-week resistance training program in 13 healthy, elderly men (72 ± 2 years). Leg strength and body composition (dual-energy X-ray absorptiometry and computed tomography) were assessed, and muscle biopsy samples were collected. Leg strength increased 25%–30% after training ($p < .001$). Leg lean mass and quadriceps cross-sectional area increased 6%–9% ($p < .001$). At baseline, mean fiber area and SC content were smaller in the Type II versus Type I muscle fibers ($p < .01$). Following training, Type II muscle fiber area increased from $5,438 \pm 319$ to $6,982 \pm 503 \ \mu m^2$ ($p < .01$). Type II muscle fiber SC content increased from 0.048 ± 0.003 to 0.084 ± 0.008 SCs per fiber ($p < .001$). No changes were observed in the Type I muscle fibers. In older adults, skeletal muscle tissue is still capable of inducing SC proliferation and differentiation, resulting in Type II muscle fiber hypertrophy.

Key Words: Aging—Exercise—Muscle plasticity.

SARCOPENIA is defined as the gradual decline in skeletal muscle mass and strength with aging. On a myocellular level, sarcopenia is characterized by a reduction in the number of both Type I and II muscle fibers (1) and specific Type II muscle fiber atrophy (1–4). In skeletal muscle tissue, satellite cells (SCs) are essential for myofiber repair, maintenance, and growth (5–8). As such, an age-related decline in SC number and/or function could have an important role in the etiology of sarcopenia. Previous studies have either shown a similar (2,9–11) or lower (12,13) SC content in older adults when compared with young adults. Recently, we reported that Type II muscle fiber atrophy in older adults is associated with a specific decline in Type II muscle fiber SC content (4). The latter likely explains the discrepant findings in previous studies that only assessed SC content in mixed muscle tissue.

Resistance type exercise training has been shown to represent an effective interventional strategy to augment muscle mass, strength, and function in older adults (14–19). Previous studies in older adults have shown that the muscle adaptive response to resistance training is characterized by Type II muscle fiber hypertrophy (16–19). For muscle fiber hypertrophy to occur beyond a certain threshold, the addition of new myonuclei is essential. The latter is facilitated by the proliferation of SCs and the differentiation of their progeny into new myonuclei that fuse with existing muscle fibers (7, 8,20). Though the exact signaling pathways leading to muscle hypertrophy following resistance type exercise remain to be elucidated, it seems evident that an increase in the number of SCs is needed to allow the muscle adaptive response to occur. In accordance, some studies (21,22) have reported an upregulation of the number of SCs in mixed muscle tissue following exercise training in older adults. However, others have failed to confirm these findings (9,23). The apparent discrepancy in the literature is likely attributed to the lack of fiber type–specific data on this topic. We hypothesized that resistance type exercise training in older adults increases SC content in a fiber type–specific manner, thereby allowing Type II muscle fiber hypertrophy.

The present study aims to assess the impact of a 3-month resistance type exercise training program on muscle strength, body composition, and muscle fiber type–specific characteristics in healthy, elderly men. Skeletal muscle biopsies were collected before and after completion of the exercise intervention program to determine the impact of resistance type exercise training on muscle fiber type–specific hypertrophy, myonuclei, and SC content in older adults.

METHODS

Participants

A total of 14 healthy elderly men (aged 65–85 years) volunteered to participate in a 12-week resistance type exercise intervention program (three sessions per week). One participant dropped out during the study because of an acute back problem that occurred during gardening. Medical history of all participants was evaluated, and an oral glucose tolerance test (OGTT) and resting electrocardiogram were

performed before selection. Exclusion criteria were defined that would preclude successful participation in the exercise program and included (silent) cardiac or peripheral vascular disease, orthopedic limitations, and/or type 2 diabetes (24). The latter was used as an exclusion criterion as insulin resistance and/or type 2 diabetes can strongly affect the process of muscle mass loss with aging (25). All participants were living independently and had not participated in any structured exercise training program over the past 5 y.

All participants were informed on the nature and possible risks of the experimental procedures, before written informed consent was obtained. All procedures were performed according to the Declaration of Helsinki, and the study was approved by the Medical Ethics Committee of the Academic Hospital, Maastricht, the Netherlands. This study is part of a greater project investigating the clinical benefits of exercise intervention in older adults.

Study Design

After screening, all eligible participants were enrolled in a 12-week resistance type exercise intervention program. Before, during, and after the exercise intervention, anthropometric measurements (height, body mass, leg volume) (26), strength assessments, and computed tomography (CT) and dual-energy X-ray absorptiometry (DEXA) scans were performed and muscle biopsies, blood samples, and dietary intake records were collected.

Dietary Intake and Physical Activity Standardization

Standardized meals (~4.0 MJ: 57 Energy% [En%] carbohydrate, 30 En% fat, and 13 En% protein) were provided to all participants prior to each test day, and participants were instructed to refrain from strenuous physical activity for 3 days prior to testing. Dietary intake was recorded for 2 days prior to muscle biopsy and blood sample collection. These records were used to standardize food intake prior to muscle biopsy and blood sampling 4 days after cessation of the exercise intervention program. On all test days, participants arrived at the laboratory by car or public transportation, following an overnight fast. The latter was confirmed by dietary intake records and further verified by the assessment of basal blood glucose and insulin concentrations and blood lipid profile. To assess potential changes in daily food intake that might have occurred during the intervention period, participants recorded 3-day weighted dietary records (Thursday to Saturday), prior to the onset of the intervention program and in Week 11 of the exercise intervention. Food intake records were analyzed with Eetmeter software 2005, version 1.4.0 (Voedingscentrum, the Hague, the Netherlands).

Strength Assessment

Maximum strength was assessed by means of one-repetition maximum (1RM) strength tests on regular leg press (LP)

and leg extension (LE) machines (Technogym, Rotterdam, the Netherlands). During a familiarization trial, a proper lifting technique was demonstrated and practiced and maximum strength was estimated using the multiple-repetitions testing procedure (27). In an additional session, at least 1 week before muscle biopsy collection, each participant's 1RM was determined as described previously (4). The 1RM tests were repeated following 4 and 8 weeks of intervention and after cessation of the intervention program, 2 days after the last exercise session.

Exercise Intervention Program

Supervised resistance type exercise training was performed three times a week for a period of 12 weeks. Training consisted of 5 minutes of warming-up on a cycle ergometer, followed by four sets on both the LP and LE machines, and 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 at Week 5, four sets of eight repetitions were performed at 75%–80% of 1RM on each machine. Resting periods of 1.5 and 3 minutes were allowed between sets and exercises, respectively. Workload intensity was adjusted based on the 1RM tests (Weeks 4 and 8). In addition, workload was increased if more than eight repetitions could be performed in three out of four sets. Exercise sessions were always performed in the morning, at the same time of day. Participants had breakfast 1.5 hours before each exercise session, and lunch 2 hours after each session. On average, participants attended 35 ± 1 of the 36 scheduled exercise sessions.

CT Scans

Anatomical cross-sectional area (CSA) of the quadriceps muscle was measured with a CT scanner (IDT 8000; Philips Medical Systems, Best, the Netherlands) prior to and after cessation of the exercise intervention program (3 days after strength assessment and prior to muscle biopsy collection). With participants lying supine, legs extended, and their feet secured, a 3-mm-thick axial image (scanning characteristics: 120 kV, 300 mA, rotation time of 0.75 seconds, and field of view of 500 mm) was taken midway between the anterior superior iliac spine and the bottom of the patella. The exact scanning position was measured and marked, to be replicated after cessation of the intervention program. Using the described approach we determined the coefficient of variation for repeated scans to be 0.6%. Images were loaded onto a PC using AGFA IMPAX imaging software, version 5.2 (AGFA Healthcare, Brussels, Belgium). Muscle area of the right leg was selected between −29 and +150 Hounsfield units (28), after which the quadriceps muscle was selected by manual tracing. Quadriceps CSA was calculated using Lucia 4.81 software (Nikon Instruments Europe, Badhoevedorp, the Netherlands). All analyses were

performed by two investigators blinded to participant coding; intraclass correlation coefficients for inter- and intrainvestigator reliability were 0.997 and 0.998, respectively.

DEXA Scans

Directly after CT scanning, body composition and bone mineral content were measured with DEXA (Lunar Prodigy Advance; GE Healthcare, Madison, WI). Whole-body and regional lean mass, fat mass, and bone mineral content were determined using the system's software package enCORE 2005 (version 9.15.00); GE Healthcare.

Blood Samples

To determine glucose homeostasis and exclude insulin-resistant participants and/or participants with diabetes, fasting blood samples were collected prior to the intervention, and 4 days after the strength assessment was performed after completion of the exercise program. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA)–containing tubes and centrifuged at 1,000g and 4°C for 10 minutes. Aliquots of plasma were frozen in liquid nitrogen and stored at −80°C. Plasma samples were analyzed for glucose (COBAS FARA, Uni Kit III; Roche, Basel, Switzerland) and insulin concentrations (Insulin RIA Kit; LINCO Research Inc., St Charles, MO). Blood HbA1c content (3-mL blood sample, EDTA) was analyzed by high-performance liquid chromatography (Bio-Rad Variant II; Bio-Rad, Munich, Germany). Plasma glucose and insulin concentrations from the OGTT were used to estimate whole-body insulin sensitivity using the oral glucose insulin sensitivity index (29).

Muscle Biopsies

Three days prior to the onset of exercise training and 4 days after the postintervention strength assessment, muscle biopsy samples were taken from the right leg of each participant, in the morning after an overnight fast. After local anesthesia, percutaneous needle biopsies (50–80 mg) were taken from the vastus lateralis muscle, ~15 cm above the patella (30). The posttraining biopsy was performed ~1 cm proximal to the pretraining biopsy, at the same depth. Any visible nonmuscle tissue was removed from the biopsy samples, which were then embedded in Tissue-Tek (Sakura Finetek, Zoeterwoude, the Netherlands), immediately frozen in liquid nitrogen–cooled isopentane, and stored at −80°C until further analyses.

Immunohistochemistry

From all biopsies, 5-μm-thick cryosections were cut at −20°C. Pre- and postintervention samples from one participant were mounted together on uncoated glass slides. Care was taken to properly align the samples for cross-sectional fiber analyses. Serial cross-sections were stained for muscle fiber typing (FT) and myocellular SC content as described previously (4,31). First antibodies used are directed against myosin heavy chain (MHC)-I (A4.951, dilution 1:20; Developmental Studies Hybridoma Bank, Iowa City, IA), laminin (polyclonal rabbit anti-laminin, dilution 1:50; Sigma, Zwijndrecht, the Netherlands), and CD56 (dilution 1:40; BD Biosciences, San Jose, CA). The latter antibody has been extensively used for SC determination in human muscle tissue (2,12,21,23,32,33). Appropriate secondary antibodies were applied: goat anti-mouse IgG1 AlexaFluor488 and goat anti-rabbit IgG AlexaFluor555 (dilution 1:500 and 1:200; Molecular Probes, Invitrogen, Breda, the Netherlands, respectively) and Avidin-D (dilution 1:333; Vector Laboratories, Inc., Burlingame, CA), respectively. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI, 0.238 μM; Molecular Probes). Staining procedures were as follows. After fixation (5 minutes acetone), slides were air-dried and incubated for 60 minutes at room temperature with primary antibodies directed against laminin and either MHC-I or CD56, diluted in 0.05% Tween–phosphate-buffered saline (PBS). Slides were then washed (3 × 5 minutes PBS). Slides for FT were then incubated for 30 minutes at room temperature with the appropriate secondary antibodies, diluted together with DAPI in 0.05% Tween-PBS. For optimizing the staining result, slides for SCs were first incubated for 30 minutes with biotinylated horse anti-mouse IgG (dilution 1:150; Vector Laboratories, Inc.), diluted in PBS. After another washing step with PBS, SC slides were then incubated for 10 minutes with the secondary antibodies, diluted together with DAPI in 0.05% Tween-PBS. After a final washing step, all slides were mounted with cover glasses using Mowiol (Calbiochem, Amsterdam, the Netherlands). Staining procedures resulted in laminin stained in red, nuclei in blue, and either MHC-I or CD56 in green (Figure 1).

All images were digitally captured, using fluorescence microscopy with a Nikon E800 fluorescence microscope (Nikon Instruments Europe) coupled to a Basler A113 C progressive scan color CCD camera with a Bayer color filter. Epifluorescence signal was recorded using a Texas Red excitation filter (540–580 nm) for laminin, a DAPI UV excitation filter (340–380 nm) for the nuclei, and a fluorescein isothiocyanate excitation filter (465–495 nm) for either MHC-I or CD56. Image processing and quantitative analyses were done using Lucia 4.81 software package, as described previously (4). All image recordings and analyses were performed by an investigator blinded to participant coding.

From the FT slides, images were captured at a 120× magnification. Laminin was used to determine the basement membrane, and all fibers within each image were identified as Type I or Type II fiber (Figure 1). Within each image, the number of fibers, the mean fiber cross-sectional area (CSA), the number of myonuclei per fiber, and the mean fiber area per myonucleus (fiber CSA/no. of myonuclei) were measured for the Type I and Type II muscle fibers separately. To obtain the true number of myonuclei (ie, not SC nuclei), the
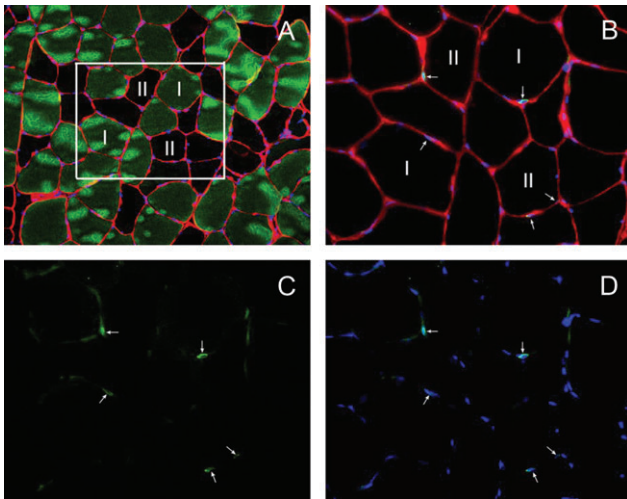
Figure 1. Muscle fiber type–specific analysis for satellite cell (SC) content. (A) myosin heavy chain-I + laminin + 4,6-diamidino-2-phenylindole (DAPI) staining from serial cross-section of images (B–D); the marked area represents the same area as shown in frames (B–D); (B) CD56 + laminin + DAPI staining; (C) CD56 staining; (D) CD56 + DAPI staining. Arrows indicate the SCs. Numbers indicate muscle fiber Type I and II.

number of nuclei was corrected for the number of SCs counted on the SC slides. Fiber circularity was calculated as $(4\pi \cdot CSA)/(perimeter)^2$; all fibers with circularity below 0.60 were excluded from analysis (17). No differences in fiber circularity were observed over time or between fiber types, validating the comparison of pre- and posttraining data on muscle fiber CSA.

From the SC slides, images were captured at a 240× magnification to allow clear determination of SC localization. Laminin was used to visualize cell borders. FT was determined by matching the serial FT slides. SCs were determined at the periphery of each fiber and stained positive for both DNA (DAPI) and CD56 (Figure 1). The number of SCs per muscle fiber, the percentage of SCs [no. of SCs/(no. of SCs + no. of myonuclei) × 100], and the number of SCs per fiber area (in square millimeters) were calculated for the Type I and Type II muscle fibers separately. A mean total of 328 ± 31 and 254 ± 37 muscle fibers were analyzed in each participants' pre- and postintervention biopsy sample, respectively.

Statistics

All data are expressed as means ± SEMs. Differences between pre- and postintervention values were analyzed with Student's paired *t* tests. In addition, two-way repeated measures analysis of variance with time (pre vs post) and fiber type (Type I vs Type II) as within-participants factors was used to determine training-induced changes in muscle fiber type–specific variables. In case of significant interaction, Student's paired *t* tests were performed for time effects within Type I or Type II muscle fiber characteristics, and/or effects of muscle fiber type within the pre- or postintervention values. All analyses were performed using SPSS

Table 1. Participants' Characteristics

	Before	After
Body mass (kg)	80.2 ± 3.4	80.1 ± 3.4
Height (m)	1.71 ± 0.01	1.71 ± 0.01
BMI (kg/m^2)	27.4 ± 1.1	27.4 ± 1.1
Leg volume (L)	8.2 ± 0.5	8.3 ± 0.5*
Glucose (mmol/L)	5.6 ± 0.2	5.5 ± 0.1
HbA1c (%)	5.8 ± 0.1	5.7 ± 0.1
OGIS (mL/min/m^2)	368 ± 22	382 ± 19
1RM LE (kg)	88 ± 4	111 ± 5*
1RM LP (kg)	170 ± 8	210 ± 10*

Notes: Data are means ± SEMs; BMI, body mass index; OGIS, oral glucose insulin sensitivity index (29); 1RM, one-repetition maximum; LE, leg extension; LP, leg press.
* Significantly different compared with before the intervention ($p < .05$).

version 13.0 (SPSS, Chicago, IL). An α-level of 0.05 was used to determine statistical significance.

RESULTS

Participants

Participants' characteristics at baseline and after intervention are provided in Table 1. Participants' age averaged 72 ± 2 years. Total body mass, height, and body mass index did not change over the intervention period. Fasting blood glucose and insulin concentrations and HbA1c contents were within normal range for healthy individuals and did not change over time (Table 1).

Muscle Strength

Strength (1RM) increased significantly from pre- to postintervention for both the LE and LP exercises, from 88 ± 4 to 111 ± 5 kg and from 170 ± 8 to 210 ± 10 kg, respectively ($p < .001$). Repeated measures analysis revealed that the increase in 1RM strength was statistically significant for each 4-week interval during the intervention period for both exercises (data not shown).

Body Composition and Muscle Mass

Whole-body lean mass tended to increase throughout the intervention period (from 57.4 ± 1.6 to 58.0 ± 1.7 kg; $p = .062$). Total fat mass decreased significantly ($p < .01$), resulting in a significant decline in whole-body fat percentage ($p < .01$; Table 2). Leg lean mass increased from 18.3 ± 0.5 to 19.3 ± 0.5 kg ($p < .001$). In accordance, fat percentage in the legs had also decreased following the exercise intervention ($p < .05$; Table 2). No changes were observed in whole-body and regional bone mineral content (data not shown).

CT scans of the upper leg, performed prior to and after the exercise program, revealed a substantial $8.8\% \pm 1.4\%$ increase in quadriceps' anatomical CSA following the intervention program, from 75.9 ± 3.7 to 82.4 ± 3.9 cm^2 ($p < .001$).

Table 2. Body Composition and Muscle Mass

	Before	After
Lean mass (kg)	57.4 ± 1.6	58.0 ± 1.7
Fat mass (kg)	19.6 ± 2.4	19.0 ± 2.4*
Body fat (%)	23.6 ± 2.2	22.9 ± 2.2*
Leg lean mass (kg)	18.3 ± 0.5	19.3 ± 0.5*
Leg fat (%)	18.9 ± 2.3	18.3 ± 2.3*
CSA (cm^2)	75.9 ± 3.7	82.4 ± 3.9*

Notes: Data are means ± SEMs; CSA, cross-sectional area of the right quadriceps muscle.

*Significantly different compared with before the intervention ($p < .05$).

Dietary Records

Analysis of the 3-day dietary records before and after 11 weeks of intervention did not show any changes in total daily energy intake (9.2 ± 0.6 vs 9.1 ± 0.4 MJ/day, respectively). Likewise, macronutrient composition of the diet did not change during the intervention period, and averaged 50 ± 2, 33 ± 2, and 17 ± 1 En% before intervention and 52 ± 2, 32 ± 2, and 16 ± 1 En% after 11 weeks of intervention, for carbohydrate, fat, and protein, respectively. Daily protein intake averaged 1.1 ± 0.1 g/kg body mass/day, both before and after 11 weeks of intervention.

Muscle Fiber Type Composition and Fiber Area

At baseline, the proportion of Type I and Type II muscle fibers was 47% ± 4% and 53% ± 4%, and the proportion of muscle area occupied by Type I and II fibers was 52% ± 4% and 48% ± 4%, respectively. Muscle fiber type percentage, expressed either as fiber number or area occupied by Type I and Type II muscle fibers, did not show any changes following 3 months of exercise intervention (Table 3).

For muscle fiber CSA, a significant "Time × Fiber type" interaction was observed. At baseline, Type II muscle fiber CSA was smaller than Type I muscle fiber CSA (Table 3, $p < .01$). However, the difference in fiber type CSA was no longer apparent after the intervention period, with a mean Type I and II muscle fiber CSA of 6,881 ± 493 and 6,982 ± 503 μm^2, respectively (Table 3). In contrast to the Type I fibers, Type II muscle fiber CSA significantly increased following exercise intervention from 5,438 ± 319 to 6,982 ± 503 μm^2 ($p < .01$).

Myonuclear and SC Content

At baseline, the number of myonuclei per muscle fiber was higher in the Type I versus Type II muscle fibers (3.6 ± 0.2 vs 2.8 ± 0.2, respectively; $p < .01$). A significant Time × Fiber type interaction revealed that after the exercise intervention, differences in myonuclear content were no longer apparent between the Type I and II muscle fibers (3.4 ± 0.3 vs 3.3 ± 0.8, respectively). In contrast to the Type I fibers, the number of myonuclei per Type II muscle fiber tended to increase from 2.8 ± 0.2 to 3.3 ± 0.3 during the intervention program ($p = .091$). Mean fiber area per myonucleus was similar for the Type I and II muscle fibers at baseline and did not change over time (Table 4).

For all SC variables, significant Time × Fiber type interactions were observed. The number of SCs per muscle fiber at baseline was lower for the Type II fibers compared with the Type I fibers (0.048 ± 0.003 vs 0.089 ± 0.006, respectively; $p < .001$). For the percentage of SCs and the number of SCs per square millimeter, analysis also revealed a significantly lower SC content in the Type II versus Type I fibers at baseline (Table 4). In contrast to the Type I muscle fibers, significant increases were observed in the Type II muscle fiber SC content after 3 months of exercise intervention (Figure 2, Table 4). The number of SCs per Type II muscle fiber increased substantially from 0.048 ± 0.003 to 0.084 ± 0.008 ($p < .001$), the percentage of SC increased from 1.8 ± 0.1 to 2.6 ± 0.3 ($p < .01$), and the number of SCs per square millimeter of Type II fiber area increased from 9.2 ± 0.8 to 11.9 ± 0.9 ($p < .01$). In accordance, after the intervention program, no differences in SC content were observed between the Type I and II muscle fibers for any of the SC variables (Figure 2, Table 4).

DISCUSSION

The present study shows that 3 months of resistance type exercise training augments muscle mass, reduces fat mass, and increases muscle strength in healthy, elderly men. The observed skeletal muscle hypertrophy is shown to be specific for the Type II muscle fibers and accompanied by a specific increase in Type II muscle fiber SC content.

Aging is associated with the loss of muscle mass, strength, and functional capacity (34–36). It has been well established that resistance type exercise training represents a feasible and effective interventional strategy to counteract sarcopenia in various elderly populations (14–19,21,22, 34,37,38). The resistance exercise program implemented in the present study was shown to be feasible, and resulted in a high compliance and adherence rate, with 35 ± 1 out of 36

Table 3. Muscle Fiber Type Composition

	Before Intervention			After Intervention		
	Fiber%	CSA (μm^2)	CSA%	Fiber%	CSA (μm^2)	CSA%
Type I	47 ± 4	6,635 ± 354	52 ± 4	47 ± 3	6,881 ± 493	46 ± 3
Type II	53 ± 4	5,438 ± 319*	48 ± 4	53 ± 3	6,982 ± 503†	54 ± 3

Notes: Data are means ± SEMs; CSA: fiber cross-sectional area; CSA%: percentage area occupied by muscle fiber type.

*Significantly different compared with the Type I muscle fibers ($p < .01$).

†Significantly different compared with before the intervention ($p < .01$).

Table 4. Myonuclear and SC Content

	Before	After
Number of nuclei per fiber		
I	3.6 ± 0.2	3.4 ± 0.3
II	2.8 ± 0.2*	3.3 ± 0.3
Fiber area per nucleus (μm^2)		
I	1,918 ± 133	2,096 ± 118
II	2,029 ± 159	2,149 ± 148
Number of SCs per fiber		
I	0.089 ± 0.006	0.096 ± 0.011
II	0.048 ± 0.003*	0.084 ± 0.008†
SC %		
I	2.6 ± 0.3	2.7 ± 0.3
II	1.8 ± 0.1*	2.6 ± 0.3†
Number of SCs per square millimeter		
I	13.9 ± 1.2	12.9 ± 1.2
II	9.2 ± 0.8*	11.9 ± 0.9†

Notes: Data are means ± SEMs; SC, satellite cells; SC%, number of SCs as a percentage of the total number of nuclei (ie, number of myonuclei + number of SCs); I, Type I muscle fibers; II, Type II muscle fibers.

*Significantly different compared with the Type I muscle fibers ($p < .01$).

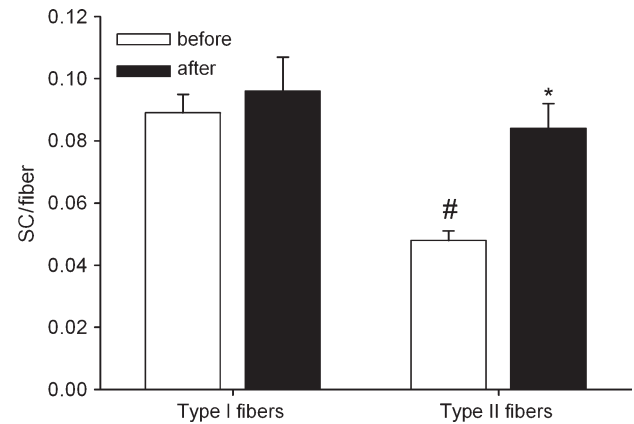†Significantly different compared with before the intervention ($p < .01$).

Figure 2. Mean number of satellite cells (SC) in the Type I and Type II muscle fibers before and after the exercise intervention program. #Significantly different compared with the Type I muscle fibers ($p < .01$); *significantly different compared with before the intervention ($p < .01$).

sessions being performed by the participants. In accordance, we observed 25%–30% improvements in 1RM strength for the LE and LP exercises. This increase in muscle strength is similar to previously published studies (17,18,22). Skeletal muscle strength has been positively correlated with muscle mass and muscle CSA (34,35). In accordance, we observed a concomitant increase in leg lean mass (6% ± 1%) and quadriceps CSA (9% ± 1%; Table 2). The latter clearly shows the efficacy of the 12-week exercise program to increase muscle mass and strength in healthy, elderly men.

Previous studies by other groups (1–3) as well as from our laboratory (4) have shown that the loss of muscle mass with aging is largely attributed to the specific atrophy of Type II muscle fibers. In the present study, we confirm a substantially smaller Type II muscle fiber area when compared with Type I muscle fibers at baseline in the elderly men (Table 3). Furthermore, we observed a significantly lower myonuclear and SC content in the Type II versus Type I muscle fibers prior to intervention (Figure 2, Table 4). The latter is consistent with our recent findings showing a lower SC content in Type II muscle fibers in muscle tissue from elderly versus young men (4). In the present study, no young control group was included, and Type II muscle fiber characteristics at baseline could only be compared with Type I muscle fibers, which were relatively large in the present study. However, the latter was probably due to the lower fiber circularity as compared with our previous study (0.71 vs 0.74) (4). In addition, interindividual variability was large in the present study, with fiber size ranging from 3,700 to 9,000 μm^2. Despite these findings, specific Type II muscle fiber atrophy was still apparent when the present data were compared with the young control group in our previous study (4). Moreover, we (4) and others (33) have previously shown that SC content is similar between Type I and Type II muscle fibers in young adults. Therefore,

we argue that the lower fiber size and SC content of the Type II versus Type I muscle fibers at baseline in the present study are consistent with and indicative of the specific reduction of Type II muscle fiber size and SC content with aging. Similar fiber type–specific changes in SC content have also been observed in animal studies (39). These data might suggest a causal relationship between a decline in myonuclear and/or SC content and muscle fiber type–specific atrophy. The lower number of SCs might be insufficient to support myofiber maintenance and, as such, contribute to the gradual loss of muscle mass with aging (39). Reduced neuromuscular activity (due to a more sedentary lifestyle and/or functional disability) and/or impaired neuronal input might represent important factors contributing to the age-related decline in myonuclear and SC number and activity and the associated reduction in muscle fiber size (20,40). More research is warranted to elucidate the potential role of the decline in Type II muscle fiber SC content in the etiology of sarcopenia.

To further investigate the proposed functional relationship between the reduced SC content in the Type II versus Type I muscle fibers and the accompanying smaller Type II muscle fiber size in elderly men, it is of substantial clinical relevance to assess whether strategies that induce muscle fiber hypertrophy are associated with an increase in muscle fiber SC content. This would provide important novel information, offering new targets for the evaluation of effective nutritional, pharmaceutical, and/or exercise interventions to combat sarcopenia in older adults. Resistance type exercise training has been established as an effective therapeutic strategy to induce muscle hypertrophy in older adults and has been associated with a more pronounced hypertrophy of the Type II muscle fibers (16–19). The latter is supported by the present findings, as we observed a 28% ± 6% increase in Type II muscle fiber CSA following 12 weeks of training (Table 3). Notably, differences in fiber size between Type I and II muscle fibers as observed prior to intervention were no longer apparent after completing the exercise intervention program (Table 3).

There has been much discrepancy in the literature on the proposed impact of resistance type exercise training on myonuclear and/or SC content in muscle tissue in older adults (9,21–23). The apparent discrepancy is likely attributed to the lack of fiber type–specific data on muscle fiber hypertrophy/atrophy and the associated changes in myonuclear and/or SC content. In the present study, the specific hypertrophy of Type II muscle fibers after training was accompanied by a substantial 76% ± 15% increase in Type II muscle fiber SC content (Figure 2, Table 4). In addition, the number of myonuclei per Type II muscle fiber tended to increase from 2.8 ± 0.2 to 3.3 ± 0.3 ($p = .091$), whereas the fiber area per myonucleus was maintained throughout the intervention period ($p = .531$; Table 4). These findings are in line with the rationale that myofiber hypertrophy is facilitated by increased translational activity of preexisting myonuclei and/or the incorporation of newly formed myonuclei (11,41,42). Consequently, our findings suggest that the maximum amount of cytoplasm that can be controlled by one myonucleus, referred to as the "myonuclear domain" ceiling (23,43), was reached during the intervention program. Therefore, SC proliferation and the subsequent incorporation of their differentiated progeny (ie, newly formed myonuclei) was essential to allow further Type II muscle fiber hypertrophy. In that respect, it was surprising that we only observed a tendency toward an increase in Type II muscle fiber myonuclear content ($p = .091$). However, as described by Petrella and coworkers (42) in their recent article, the myofiber response to an exercise program can be highly variable. By using cluster analysis, the authors reported that participants with extreme myofiber hypertrophy also showed the largest increases in SC content and both myonuclear number and domain size (42). In accordance, interindividual variability has an important role in the apparent discrepancy between significant myofiber hypertrophy and nonsignificant changes in myonuclear content and/or domain as observed in the present and previous studies (9,23). Supposedly, some participants might have relied more heavily on expansion of the myonuclear domain, whereas most participants were able to incorporate new myonuclei. In the present study, we observed a nonsignificant 25% ± 12% increase in the number of myonuclei per Type II muscle fiber. The latter tends to be in line with the 26% increase observed by Petrella and coworkers (42). Because only pre- and postintervention biopsy samples were collected, we can only speculate on the proposed time line between an increase in SC number, the incorporation of new myonuclei, and subsequent muscle fiber hypertrophy. However, in vitro data and rodent studies indicate that an increase in SC number and subsequent incorporation of new myonuclei precedes muscle hypertrophy (reviewed in (20)).

Considering the large interindividual variability in the hypertrophy response and the relations between the changes in myofiber size, SC content, and myonuclear content, it is interesting to speculate which factors might be responsible for the apparent differential responses. Previous research implicated that the myogenic regulatory factors (myogenin, MyoD, myf-5, and MRF4) and various growth agents such as insulin-like growth factor-I, mechano growth factor, and hepatocyte growth factor have a role in the (load-mediated) hypertrophy and in SC activation and/or proliferation and differentiation (reviewed in (6,44)). However, most data are derived from in vitro and acute in vivo studies and, as such, little is known on the potential role these agents have in the response to prolonged exercise training. However, as we restricted ourselves to the assessment of the impact of exercise training on muscle fiber characteristics, we can only speculate on this matter.

This is the first study to show that the relatively lower Type II muscle fiber size and SC content in older adults can be specifically enhanced following 3 months of resistance type exercise training. Though the exact signaling pathways leading to SC-induced muscle fiber hypertrophy as well as the reasons for interindividual response variability in these pathways remain to be elucidated, these findings might open up novel opportunities for evaluating the efficacy of nutritional, pharmaceutical, and/or exercise-based interventional strategies to counteract the loss of muscle mass, strength, and function with aging.

We conclude that prolonged resistance type exercise training effectively increases muscle mass and strength in healthy, elderly men. The relative Type II muscle fiber atrophy and the associated lower SC content in Type II versus Type I muscle fibers in older adults can be reversed by prolonged resistance type exercise training. The latter indicates that muscle tissue in older adults is still capable of inducing SC proliferation, differentiation, and fusion of new myonuclei into existing muscle fibers, resulting in Type II muscle fiber hypertrophy.

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REFERENCES

1. Lexell J, Taylor CC, 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*. 1988;84:275–294.
2. Dreyer HC, Blanco CE, Sattler FR, Schroeder ET, Wiswell RA. Satellite cell numbers in young and older men 24 hours after eccentric exercise. *Muscle Nerve*. 2006;33:242–253.
3. Larsson L, Sjodin B, Karlsson J. Histochemical and biochemical changes in human skeletal muscle with age in sedentary males, age 22–65 years. *Acta Physiol Scand*. 1978;103:31–39.

4. Verdijk LB, Koopman R, Schaart G, Meijer K, Savelberg HH, van Loon LJ. Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly. *Am J Physiol Endocrinol Metab*. 2007;292:E151–E157.

5. Mauro A. Satellite cell of skeletal muscle fibers. *J Biophys Biochem Cytol*. 1961;9:493–495.

6. Hawke TJ, Garry DJ. Myogenic satellite cells: physiology to molecular biology. *J Appl Physiol*. 2001;91:534–551.

7. Moss FP, Leblond CP. Nature of dividing nuclei in skeletal muscle of growing rats. *J Cell Biol*. 1970;44:459–462.

8. Moss FP, Leblond CP. Satellite cells as the source of nuclei in muscles of growing rats. *Anat Rec*. 1971;170:421–435.

9. Hikida RS, Walsh S, Barylski N, Campos G, Hagerman FC, Staron RS. Is hypertrophy limited in elderly muscle fibers? A comparison of elderly and young strength-trained men. *Basic Appl Myol*. 1998;8:419–427.

10. Roth SM, Martel GF, Ivey FM, et al. Skeletal muscle satellite cell populations in healthy young and older men and women. *Anat Rec*. 2000;260:351–358.

11. Sinha-Hikim I, Cornford M, Gaytan H, Lee ML, Bhasin S. Effects of testosterone supplementation on skeletal muscle fiber hypertrophy and satellite cells in community-dwelling older men. *J Clin Endocrinol Metab*. 2006;91:3024–3033.

12. Kadi F, Charifi N, Denis C, Lexell J. Satellite cells and myonuclei in young and elderly women and men. *Muscle Nerve*. 2004;29:120–127.

13. Renault V, Thornell LE, Eriksson PO, Butler-Browne G, Mouly V. Regenerative potential of human skeletal muscle during aging. *Aging Cell*. 2002;1:132–139.

14. Fiatarone MA, O'Neill EF, Ryan ND, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med*. 1994;330:1769–1775.

15. Frontera WR, Meredith CN, O'Reilly KP, Knuttgen HG, Evans WJ. Strength conditioning in older men: skeletal muscle hypertrophy and improved function. *J Appl Physiol*. 1988;64:1038–1044.

16. Charette SL, McEvoy L, Pyka G, et al. Muscle hypertrophy response to resistance training in older women. *J Appl Physiol*. 1991;70:1912–1916.

17. Kosek DJ, Kim JS, Petrella JK, Cross JM, Bamman MM. Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults. *J Appl Physiol*. 2006;101:531–544.

18. Martel GF, Roth SM, Ivey FM, et al. Age and sex affect human muscle fibre adaptations to heavy-resistance strength training. *Exp Physiol*. 2006;91:457–464.

19. Singh MA, Ding W, Manfredi TJ, et al. Insulin-like growth factor I in skeletal muscle after weight-lifting exercise in frail elders. *Am J Physiol*. 1999;277(1 pt 1):E135–E143.

20. Allen DL, Roy RR, Edgerton VR. Myonuclear domains in muscle adaptation and disease. *Muscle Nerve*. 1999;22:1350–1360.

21. 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*. 2007;17:34–42.

22. Roth SM, Martel GF, Ivey FM, et al. Skeletal muscle satellite cell characteristics in young and older men and women after heavy resistance strength training. *J Gerontol A Biol Sci Med Sci*. 2001;56:B240–B247.

23. Petrella JK, Kim JS, Cross JM, Kosek DJ, Bamman MM. Efficacy of myonuclear addition may explain differential myofiber growth among resistance-trained young and older men and women. *Am J Physiol Endocrinol Metab*. 2006;291:E937–E946.

24. Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 2006;29(suppl 1):S43–S48.

25. Park SW, Goodpaster BH, Strotmeyer ES, et al. Decreased muscle strength and quality in older adults with type 2 diabetes: the health, aging, and body composition study. *Diabetes*. 2006;55:1813–1818.

26. Jones PR, Pearson J. Anthropometric determination of leg fat and muscle plus bone volumes in young male and female adults. *J Physiol*. 1969;204:63P–66P.

27. Mayhew JL, Prinster JL, Ware JS, Zimmer DL, Arabas JR, Bemben MG. Muscular endurance repetitions to predict bench press strength in men of different training levels. *J Sports Med Phys Fitness*. 1995;35:108–113.

28. Mitsiopoulos N, Baumgartner RN, Heymsfield SB, Lyons W, Gallagher D, Ross R. Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. *J Appl Physiol*. 1998;85:115–122.

29. Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. *Diabetes Care*. 2001;24:539–548.

30. Bergstrom J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest*. 1975;35:609–616.

31. Koopman R, Zorenc AH, Gransier RJ, Cameron-Smith D, van Loon LJ. Increase in S6K1 phosphorylation in human skeletal muscle following resistance exercise occurs mainly in type II muscle fibers. *Am J Physiol Endocrinol Metab*. 2006;290:E1245–E1252.

32. Charifi N, Kadi F, Feasson L, Denis C. Effects of endurance training on satellite cell frequency in skeletal muscle of old men. *Muscle Nerve*. 2003;28:87–92.

33. Kadi F, Charifi N, Henriksson J. The number of satellite cells in slow and fast fibres from human vastus lateralis muscle. *Histochem Cell Biol*. 2006;126:83–87.

34. Fiatarone MA, Marks EC, Ryan ND, Meredith CN, Lipsitz LA, Evans WJ. High-intensity strength training in nonagenarians. Effects on skeletal muscle. *JAMA*. 1990;263:3029–3034.

35. Frontera WR, Hughes VA, Fielding RA, Fiatarone MA, Evans WJ, Roubenoff R. Aging of skeletal muscle: a 12-yr longitudinal study. *J Appl Physiol*. 2000;88:1321–1326.

36. Lexell J. Human aging, muscle mass, and fiber type composition. *J Gerontol A Biol Sci Med Sci*. 1995;50:11–16.

37. Esmarck B, Andersen JL, Olsen S, Richter EA, Mizuno M, Kjaer M. Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. *J Physiol*. 2001;535(pt 1):301–311.

38. Hikida RS, Staron RS, Hagerman FC, et al. Effects of high-intensity resistance training on untrained older men. II. Muscle fiber characteristics and nucleo-cytoplasmic relationships. *J Gerontol A Biol Sci Med Sci*. 2000;55:B347–B354.

39. Shefer G, Van de Mark DP, Richardson JB, Yablonka-Reuveni Z. Satellite-cell pool size does matter: defining the myogenic potency of aging skeletal muscle. *Dev Biol*. 2006;294:50–66.

40. Delbono O. Neural control of aging skeletal muscle. *Aging Cell*. 2003;2:21–29.

41. Edgerton VR, Roy RR. Regulation of skeletal muscle fiber size, shape and function. *J Biomech*. 1991;24(suppl 1):123–133.

42. Petrella JK, Kim JS, Mayhew DL, Cross JM, 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;104:1736–1742.

43. Cheek DB. The control of cell mass and replication. The DNA unit—a personal 20-year study. *Early Hum Dev*. 1985;12:211–239.

44. Dhawan J, Rando TA. Stem cells in postnatal myogenesis: molecular mechanisms of satellite cell quiescence, activation and replenishment. *Trends Cell Biol*. 2005;15:666–673.