Resistance Training Increases Skeletal Muscle Capillarization in Healthy Older Men

LEX B. VERDIJK, TIM SNIJDERS, TANYA M. HOLLOWAY, JANNEAU VAN KRANENBURG, and LUC J. C. VAN LOON

1Nutrim School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre, Maastricht, The Netherlands; and 2Department of Kinesiology and Medical Physics and Applied Radiation Sciences, McMaster University, Hamilton, ON, Canada

ABSTRACT


Purpose: Skeletal muscle capillarization plays a key role in oxygen and nutrient delivery to muscle. The loss of muscle mass with aging and the concept of anabolic resistance have been, at least partly, attributed to changes in skeletal muscle capillary structure and function. We aimed to compare skeletal muscle capillarization between young and older men and evaluate whether resistance-type exercise training increases muscle capillarization in older men. Methods: Muscle biopsies were obtained from the vastus lateralis of healthy young (n = 14, 26 ± 2 yr) and older (n = 16, 72 ± 1 yr) adult men, with biopsies before and after 12 wk of resistance-type exercise training in the older subjects. Immunohistochemistry was used to assess skeletal muscle fiber size, capillary contacts (CC) per muscle fiber, and the capillary-to-fiber perimeter exchange (CFPE) index in type I and II muscle fibers. Results: Type II muscle fibers were smaller in old versus young (4507 ± 268 vs. 6084 ± 497 μm², respectively, P = 0.007). Type I and type II muscle fiber CC and CFPE index were smaller in old compared with young muscle (CC type I: 3.8 ± 0.2 vs. 5.0 ± 0.3; CC type II: 3.2 ± 0.2 vs. 4.2 ± 0.2, respectively; both P < 0.001). Resistance-type exercise training increased type II muscle fiber size only. In addition, CC and CFPE index increased in both the type I (26% ± 9% and 27% ± 8%) and type II muscle fibers (33% ± 7% and 24% ± 6%, respectively; all P < 0.001) after 12 wk resistance training in older men. Conclusions: We conclude that resistance-type exercise training can effectively augment skeletal muscle fiber capillarization in older men. The greater capillary supply may be an important prerequisite to reverse anabolic resistance and support muscle hypertrophy during lifestyle interventions aiming to support healthy aging. Key Words: EXERCISE, AGING, CAPILLARIES, SARCOPENIA

The progressive loss of skeletal muscle mass and strength with aging, termed sarcopenia, results in a decline in functional performance, and an increased risk of developing chronic metabolic diseases. On the myocellular level, the loss of muscle mass with aging is characterized by specific atrophy of the type II muscle fibers and a reduction in type II muscle fiber satellite cell (SC) content (22,36,37). Furthermore, skeletal muscle fiber capillarization has been shown to be reduced in both the type I and type II muscle fibers at a more advanced age (5,13,29).

Skeletal muscle capillarization represents a key determinant of oxygen and nutrient delivery to peripheral muscle tissue. An optimal capillary supply is essential for fulfilling the energy demands of muscle fibers while simultaneously limiting the accumulation of nutrients in the blood through facilitating their peripheral uptake. As such, age-related reductions in capillary supply are likely associated with impairments in oxidative capacity and metabolic homeostasis observed at a more advanced age. In accordance, muscle capillarization has been shown to be correlated with whole-body maximal oxygen consumption (16), insulin sensitivity (15), and glucose tolerance (27) in older adults. Importantly, appropriate muscle capillarization and adequate postprandial perfusion have also been suggested to be essential for the maintenance of skeletal muscle mass.

Age-related reductions in the skeletal muscle protein synthetic response to anabolic stimuli appear to play a key role in the loss of muscle mass with aging (2,7,20). This anabolic resistance can, at least partly, be explained by a reduced insulin-mediated stimulation of muscle perfusion.

Address for correspondence: Lex B. Verdijk, Ph.D., Department of Human Movement Sciences Maastricht University Medical Centre P.O. Box 616, 6200 MD Maastricht, The Netherlands; E-mail: lex.verdijk@maastrichtuniversity.nl.
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in older individuals (11,28). In agreement, adequate muscle perfusion has been shown to be imperative for the postprandial increase in nutritive blood flow and anabolic signaling in skeletal muscle, thereby allowing postprandial muscle protein accretion (31,32). Age-related reductions in capillary density (CD) (15,23) and impairments in endothelial function (33) are thought to be responsible for the reduced responsiveness of the skeletal muscle microvasculature to postprandial insulin release. As such, interventions that may maintain or promote angiogenesis in older adults are of key importance.

Resistance-type exercise training has been well established as an effective interventional strategy to increase muscle mass and function in the elderly (3,8,10). We have previously shown that prolonged resistance-type exercise training in the older population results in specific type II muscle fiber hypertrophy with a concomitant increase in type II muscle fiber SC content (34,37). Furthermore, various metabolic improvements have been shown after resistance-type exercise training in the older population, including increased insulin sensitivity (21). These improvements may be partly explained by increased skeletal muscle capillarization. Whereas endurance-type exercise training has previously been shown to substantially increase skeletal muscle CD (6,12), expansion of the skeletal muscle capillary network is inconsistently reported after prolonged resistance-type exercise training in older adults (9,14,17,40). Therefore, we aimed to compare muscle fiber type-specific differences in vascularization between healthy young and older men and investigate whether age-related changes in skeletal muscle capillarization can be counteracted by prolonged resistance-type exercise training.

**METHODS**

**Subjects.** A total of 16 healthy older (72 ± 1 yr, range 65–83 yr) and 14 healthy young (26 ± 2 yr, range 19–43 yr) adult men participated in this study. All subjects were informed on the nature and possible risks of the experimental procedures, before their written informed consent was obtained. 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, orthopedic limitations, and/or type 2 diabetes. All subjects were living independently and had not participated in any structured exercise training program over the past 2 yr. Among older participants, no problems were reported in normal activities of daily living (e.g., walking, climbing stairs, rising from a chair), and participants did not need any assistive equipment (e.g., using a cane) while walking. Only in older participants, screening included measures of fasting glucose (5.9 ± 0.2 mmol·L⁻¹), 2-h glucose from oral glucose tolerance test (6.3 ± 0.4 mmol·L⁻¹), HbA1c (5.9% ± 0.1%), total cholesterol (6.2 ± 0.3 mmol·L⁻¹), and HDL cholesterol (1.4 ± 0.1 mmol·L⁻¹). Impaired fasting glucose and impaired glucose tolerance were evident in n = 12 and n = 3 older participants, respectively. Medication use in older participants was as follows: antihypertensive medications (n = 1), cholesterol-lowering or lipid-lowering medications (n = 2), medications for prostate or urinary problems (n = 4), or no medications (n = 11). No medical issues were reported in any of the young subjects. All study procedures were performed according to the Declaration of Helsinki and were approved by the Medical Ethics Committee of the Maastricht University Medical Centre.

**Study design.** Skeletal muscle biopsy samples were obtained from the vastus lateralis muscle of healthy young and older men. Immunohistochemical analyses were performed to assess skeletal muscle fiber composition, muscle fiber size, capillary content, and SC content in a fiber type-specific manner. In the older men, muscle biopsies were obtained before and after 12 wk of resistance-type exercise training. In the present study, older subjects from a previously published study (35) were included based on the availability of ample muscle tissue collected to perform all immunohistochemical analyses. Before and after the exercise intervention, anthropometric measurements, strength assessments, and computed tomography (CT) and dual-energy X-ray absorptiometry scans were performed to assess the impact of exercise training on muscle mass and function (35). Subjects were equally divided over the protein group (n = 8) and the placebo group (n = 8) as described in the original article (35).

**Dietary intake and physical activity standardization.** Standardized meals (∼51 kJ·kg⁻¹: 57 energy (En)% carbohydrate, 30 En% fat, and 13 En% protein) were provided to all subjects before the day of muscle biopsy sampling, and subjects were instructed to refrain from strenuous physical activity for 3 d before testing and come to the laboratory by car or public transportation after an overnight fast.

**Muscle biopsies.** Skeletal muscle biopsy samples were collected at baseline in all subjects (and at least 1 wk after the preintervention strength assessment in the elderly), and after 12 wk of resistance-type exercise training in the older subjects (4 d after the final strength assessment). All biopsies were taken in the morning after an overnight fast. After local anesthesia, percutaneous needle biopsies (50–80 mg) were taken from the vastus lateralis muscle of the right leg, approximately 15 cm above the patella (1). The posttraining biopsy in the elderly was performed approximately 2 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. Preintervention and postintervention samples from older subjects 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 A) muscle fiber typing and capillaries, and B) myonuclear and SC content as described previously (13,36). First, antibodies used are directed against MHC-I (A4.840, Developmental Studies Hybridoma Bank, Iowa City, IA; dilution 1:25), laminin (polyclonal rabbit antilaminin; Sigma, Zwijndrecht, The Netherlands; dilution 1:50), CD31 (Dako, Glostrup, Denmark; dilution 1:50), and CD56 (BD Biosciences, San Jose, CA; dilution 1:40). The latter antibody has been extensively used for SC determination in human muscle tissue (18,19,26,34,36). Appropriate secondary antibodies were applied: goat antimouse IgM AlexaFluor 488, goat antirabbit IgG AlexaFluor 350 or 555 (Molecular Probes, Invitrogen, Breda, The Netherlands; dilution 1:500 and 1:125, respectively), Avidin Texas Red, and Avidin-D Probes, Invitrogen, Breda, The Netherlands; dilution 1:500 or 1:125, respectively. Avidin Texas Red, and Avidin-D Probes (Vector Laboratories Inc., Burlingame, CA; dilution 1:333), respectively. Nuclei were stained with 4-,6-diamidino-2-phenylindole (DAPI; Molecular Probes; 0.238 μM). Staining procedures were as follows. After fixation (5 min acetone), slides were air-dried and incubated for 60 min at room temperature with primary antibodies directed against laminin, MHC-I, and CD31; or laminin and CD56, diluted in 0.05% Tween phosphate-buffered saline (PBS). Slides were then washed (3 × 5 min PBS). For optimizing the staining result, slides were then incubated for 30 min with biotinylated goat antimouse IgG for CD31, or horse antimouse IgG for CD56 (Vector; dilution 1:150), diluted in 0.05% Tween-PBS. After another washing step with PBS, slides were then incubated for 30 min with the secondary antibodies, diluted together with DAPI in 0.05% Tween-PBS (DAPI added for the SC slides only). 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 blue, MHC-I in green, and CD31 in red; or laminin in red, CD56 in green, and nuclei in blue.

Image recording and analysis. 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 DAPI UV excitation filter (340–380 nm) for laminin or nuclei, a FITC excitation filter (465–495 nm) for either MHC-I or CD56, and a Texas Red excitation filter (540–580 nm) for CD31 or laminin. Image processing and quantitative analyses were done using Lucia 4.81 software package, as described previously (36). All image recordings and analyses were performed by an investigator blinded to subject coding.

From the “fiber typing and capillaries” 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. For the entire biopsy section (173 ± 15 muscle fibers on average), the fiber perimeter and mean fiber size (cross-sectional area [CSA]) were measured for each type I and type II muscle fiber separately. Fiber circularity was calculated as \((\frac{4\pi \times \text{CSA}}{\text{perimeter}})^2\), and all fibers with circularity below 0.60 were excluded from analysis. No differences in fiber circularity were observed between groups, over time or between fiber types. To quantify muscle fiber capillarization, an artifact-free image was randomly selected, and all fibers in that image were included in the analyses; this process was repeated until a minimum of 25 type I and 25 type II muscle fibers were included per biopsy sample (16). Using the procedures described previously for mixed muscle fiber (16), the following parameters were determined for type I and II muscle fibers separately: 1) the number of capillary contacts (CC); 2) the sharing factor, defined as the number of fibers shared by each capillary (i.e., an average sharing factor was calculated for all capillaries surrounding a type I fiber, and an average sharing factor was calculated for all capillaries surrounding a type II fiber); 3) the capillary-to-fiber (C/Fi) ratio on individual fiber basis, defined as the number of capillaries divided by their sharing factor (i.e., CC/SF; number of “full” capillaries per individual fiber); 4) the capillary-to-fiber perimeter exchange (CFPE) index, defined as C/Fi expressed relative to fiber perimeter (i.e., number of “full” capillaries per 1000 μm of fiber perimeter); and 5) CD defined as C/Fi expressed relative to fiber area (i.e., number of “full” capillaries per square millimeter of fiber area).

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. Fiber typing was determined by matching the serial fiber typing slides. SC were determined at the periphery of each fiber and stained positive for both DNA (DAPI) and CD56, whereas myonuclei were defined as only positive for DAPI. The number of myonuclei and the number of SC per muscle fiber were calculated for the type I and type II muscle fibers separately.

Muscle mass, strength, body composition. In all older subjects, maximum strength was assessed by means of one-repetition maximum (1RM) strength tests on regular leg press and leg extension machines (Technogym, Rotterdam, The Netherlands). After a familiarization trial, 1RM strength was determined before the intervention, after 4 and 8 wk of intervention and after cessation of the intervention program, 2 d after the last exercise session (35). Anatomical CSA of the quadriceps muscle was measured with a CT scanner (IDT 8000, Philips Medical Systems, The Netherlands) before and after cessation of the exercise intervention program, as described previously (35). Directly after CT scanning, body composition was measured with dual-energy X-ray absorptiometry (Lunar Prodigy Advance; GE Healthcare, Madison, WI). Whole-body and regional lean mass and fat mass were determined using the system’s software package enCORE 2005 (version 9.15.00).

Exercise intervention program. All elderly subjects performed supervised, progressive resistance-type exercise training, three times a week for a period of 12 wk. Training consisted of 5 min of warming up on a cycle ergometer, followed by four sets on both the leg press and leg extension machines, and a 5-min cooling-down period on the cycle ergometer. Workload was increased from 60% of 1RM in...
week 1 (10–15 repetitions in each set) to 75%–80% of 1RM (8–10 repetitions) in week 4 and after. Resting periods of 1.5 and 3 min were allowed between sets and exercises, respectively. Workload intensity was adjusted based on 1RM tests (weeks 4 and 8). In addition, workload was increased if more than eight repetitions could be performed in three of four sets. Exercise sessions were always performed in the morning, at the same time of day. On average, subjects attended 35 ± 1 of the 36 scheduled exercise sessions.

Statistics. All data are expressed as means ± SEM. Differences between groups (young vs old) were analyzed by unpaired t-tests. In addition, muscle fiber type-specific variables were analyzed using repeated measures ANOVA with fiber type (type I vs type II) as within subjects factor and group (young vs old) as between subjects factor. In case of significant interaction, separate analyses were performed within groups (i.e., paired t-tests on type I vs type II) or within fiber types (i.e., unpaired t-test on young vs old).

Exercise training-induced changes in the elderly group were analyzed with paired t-tests, comparing prevalues versus postvalues. In addition, two-way repeated-measures ANOVA with training (pre vs post) and fiber type (type I vs type II) as within subjects factors was used to determine training induced changes in muscle fiber type-specific variables. In case of significant interaction, paired t-tests were performed for training effects within type I or type II muscle fiber characteristics. Data from the protein and placebo group as described in the original article (35) were collapsed because protein supplementation did not affect any of the variables (no differences at baseline and no effect on training responses). In support of collapsing all data from older participants, no effects of glucose tolerance or medication use were observed.

Finally, Pearson correlation coefficients were determined to analyze the relation between capillarization data and SC content. All analyses were performed using SPSS version 21.0 (IBM, Chicago, IL). An α-level of 0.05 was used to determine statistical significance.

RESULTS

Subjects. Body weight did not differ between groups (Table 1). However, BMI was elevated in the older (26.6 ± 0.9 kg·m⁻²) compared with the young subjects (22.7 ± 0.6 kg·m⁻²). Both body weight and BMI did not change after prolonged resistance-type exercise training in the older subjects.

Effect of age on muscle fiber morphology. Type I muscle fiber percentage was 50% ± 3% and 54% ± 4% in the old and young, respectively, with no differences between groups. A significant group–fiber type interaction was observed for muscle fiber size (P = 0.002) and muscle fiber perimeter (P = 0.003). No differences were observed for the type I muscle fibers between groups (Table 2). In contrast, type II muscle fiber size (4507 ± 268 μm² vs 6084 ± 497 μm², respectively; P = 0.007) and type II muscle fiber perimeter (283 ± 8 μm vs 321 ± 12 μm, respectively; P = 0.011) were significantly smaller in the old when compared with the young men. In addition, at baseline, muscle fiber size and perimeter were significantly smaller in the type II compared with the type I muscle fibers in the older subjects (Table 2; both P = 0.002).

Effect of age on myonuclear and SC content. For myonuclear content, there was a main effect of fiber type, with lower myonuclear content in the type II versus type I muscle fibers in both groups (P = 0.001). In addition, myonuclear content in both the type I and type II muscle fibers was smaller in old compared with young muscle (P = 0.044). For SC content, a significant group–fiber type interaction was observed (P = 0.039). Separate analyses revealed no differences in type I muscle fiber SC content between groups. In contrast, type II muscle fiber SC content was significantly lower in the old when compared with

### TABLE 1. Subjects’ characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Young, n = 14</th>
<th>Old Pre, n = 16</th>
<th>Old Post, n = 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>26 ± 2</td>
<td>72 ± 1*</td>
<td></td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>73.7 ± 2.1</td>
<td>79.9 ± 2.8</td>
<td>80.1 ± 2.8</td>
</tr>
<tr>
<td>Body height, cm</td>
<td>180 ± 1</td>
<td>174 ± 1*</td>
<td>174 ± 1*</td>
</tr>
<tr>
<td>BMI, kg·m⁻²</td>
<td>22.7 ± 0.6</td>
<td>26.6 ± 0.9*</td>
<td>26.7 ± 0.9*</td>
</tr>
</tbody>
</table>

Data are means ± SEM. *Significantly different from young (P < 0.05).

### TABLE 2. Muscle fiber type-specific characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Young, n = 14</th>
<th>Old Pre, n = 16</th>
<th>Old Post, n = 16</th>
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</thead>
<tbody>
<tr>
<td>Fiber size, μm²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>5773 ± 449</td>
<td>5477 ± 345</td>
<td>5651 ± 351</td>
</tr>
<tr>
<td>Type II</td>
<td>6084 ± 497</td>
<td>4607 ± 268***</td>
<td>5540 ± 334***</td>
</tr>
<tr>
<td>Fiber perimeter, μm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>315 ± 12</td>
<td>310 ± 9</td>
<td>313 ± 10</td>
</tr>
<tr>
<td>Type II</td>
<td>321 ± 12</td>
<td>283 ± 8***</td>
<td>309 ± 10***</td>
</tr>
<tr>
<td>Myonuclei/fiber</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>3.94 ± 0.38</td>
<td>3.12 ± 0.23*</td>
<td>3.45 ± 0.32</td>
</tr>
<tr>
<td>Type II</td>
<td>3.71 ± 0.41**</td>
<td>2.58 ± 0.23**</td>
<td>3.18 ± 0.26**</td>
</tr>
<tr>
<td>SC/fiber</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>0.096 ± 0.009</td>
<td>0.088 ± 0.007</td>
<td>0.096 ± 0.009</td>
</tr>
<tr>
<td>Type II</td>
<td>0.094 ± 0.009</td>
<td>0.051 ± 0.003***</td>
<td>0.089 ± 0.007***</td>
</tr>
<tr>
<td>Sharing factor</td>
<td></td>
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<tr>
<td>Type I</td>
<td>2.65 ± 0.02</td>
<td>2.66 ± 0.03</td>
<td>2.62 ± 0.03</td>
</tr>
<tr>
<td>Type II</td>
<td>2.72 ± 0.04**</td>
<td>2.72 ± 0.03**</td>
<td>2.67 ± 0.02**</td>
</tr>
<tr>
<td>CFi</td>
<td></td>
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</tr>
<tr>
<td>Type I</td>
<td>1.90 ± 0.11</td>
<td>1.44 ± 0.08*</td>
<td>1.80 ± 0.06***</td>
</tr>
<tr>
<td>Type II</td>
<td>1.55 ± 0.09**</td>
<td>1.20 ± 0.07***</td>
<td>1.57 ± 0.17****</td>
</tr>
<tr>
<td>CD</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Type I</td>
<td>250 ± 30</td>
<td>272 ± 15*</td>
<td>237 ± 18**</td>
</tr>
<tr>
<td>Type II</td>
<td>269 ± 20**</td>
<td>276 ± 17</td>
<td>294 ± 14</td>
</tr>
</tbody>
</table>

Data are means ± SEM. Data were analyzed with repeated measures ANOVA with age-group as between subjects factor and fiber type as within subjects factor (young vs old comparison at baseline), or with both time and fiber type as within subjects factor (prevalues vs postvalues in old males).

*Significantly different from young (P < 0.05).

**Significantly different from type I fibers (P < 0.05).

***Significantly different from pretraining (P < 0.05).
the young (0.051 ± 0.003 vs 0.084 ± 0.009 SC/fiber, respectively; P = 0.004). Only in the older subjects, SC content was lower in the type II vs type I muscle fibers (Table 2; P < 0.001).

**Effect of age on muscle fiber capillarization.** A main effect of muscle fiber type showed that the number of CC was significantly smaller in the type II versus type I fibers for both groups (Fig. 1A; P < 0.001). In addition, a main group effect showed that the number of CC was smaller in old compared with young muscle, both in the type I and II muscle fibers (Fig. 1A; P < 0.001). The sharing factor was significantly greater in the type II vs type I muscle fibers (P = 0.004), with no differences between groups (Table 2; P = 0.869). For C/Fi, a main group effect (P = 0.002) as well as a main fiber type effect (P < 0.001) was observed (Table 2). For CFPE index, a significant group–fiber type interaction was observed (P = 0.006). In line with the number of CC and C/Fi, CFPE index was lower in the type II versus type I muscle fibers in both groups (Fig. 1B; P < 0.05). Furthermore, the CFPE index was lower in the older compared with the younger men in the type I muscle fibers (Fig. 1B; P = 0.003). For the type II muscle fibers, only a tendency was observed for a lower CFPE index in the older group (P = 0.076). In line with the CFPE index, a significant group–fiber type interaction was observed for CD (P < 0.001). Only in the young, CD was lower in type II versus type I muscle fibers (Table 2; P < 0.001). Whereas no difference was observed between young and old for type II muscle fiber CD (P = 0.80), type I muscle fiber CD was lower in the older compared with the young men (Table 2; P = 0.023).

**Effects of prolonged resistance-type exercise training in older men.** The results of the 12 wk resistance-type exercise training program on muscle mass and strength in the older subjects are presented in Table 3. Both leg press and leg extension 1RM had increased substantially after training. In accordance, whole body lean mass and leg lean mass had increased and fat percentage had decreased as a result of the 12-wk training program. On the level of the quadriceps, muscle CSA increased from 73.4 ± 2.6 cm² to 80.2 ± 2.4 cm² after 12 wk of training (all P < 0.01).

Remarkably, all age-related differences in muscle fiber characteristics observed in old versus young muscle at baseline (i.e., type II muscle fiber size, perimeter, and SC content; type I and II muscle fiber myonuclear content, CC, C/Fi, and CFPE index) were no longer apparent after 12 wk of resistance-type exercise training in the older men.

Resistance-type exercise training did not affect type I muscle fiber composition in the old subjects (49% ± 3%). A significant training–fiber type interaction (P ≤ 0.001) revealed that after 12 wk of resistance-type exercise training, muscle fiber size and fiber perimeter had increased in the type II fibers only (Table 2; P = 0.004 and P = 0.023, respectively). In line with the fiber size, a significant training–fiber type interaction (P = 0.002) revealed that after training, SC content had increased in the type II muscle fibers only (Table 2; P < 0.001). In addition, there was a tendency for increased myonuclear content after training, but this change did not reach statistical significance (P = 0.085). Whereas at baseline, muscle fiber size, perimeter, and SC content were lower in the type II versus type I muscle fibers, these differences were no longer apparent after the intervention.

For muscle fiber type–specific capillarization, a significant main effect of training and a significant main effect of fiber type were observed. The number of CC increased in both the type I and type II muscle fibers (Fig. 1A; P = 0.001), with no differences between fiber types. Likewise, the C/Fi (Table 2; P = 0.001) and CFPE index (Fig. 1B; P = 0.001) increased in both the type I and type II muscle fibers. Throughout training, the number of CC, C/Fi, and the CFPE index remained lower in the type II versus type I muscle fibers (P < 0.001). The sharing factor did not change with resistance-type exercise training and remained significantly greater in the type II versus type I muscle fibers (P = 0.001). For CD, a significant training–fiber type interaction was observed.

![FIGURE 1—Skeletal muscle fiber capillarization determined as number of CC per fiber (A), and CFPE index (B). *Significantly different from young (P < 0.05). †Significantly different from type I fibers (P < 0.05). ‡Significantly different from pretraining (P < 0.05).](image-url)
observed, showing that the increase in CD only reached significance for the type I muscle fibers \((P = 0.016)\), whereas no significant change was observed for the type II muscle fibers (Table 2; \(P = 0.38\)).

**Correlation analysis.** When not differentiating for muscle fiber type and including all 30 participants in the analyses, a greater number of CC and greater CFPE index were associated with greater SC content \((r = 0.46 \text{ and } r = 0.45, \text{ respectively, } P \leq 0.001)\). Muscle fiber type–specific analyses showed weaker and nonsignificant correlations between type I muscle fiber CC versus SC content (Fig. 2A), and type I CFPE index versus SC content (both \(r = 0.29; P = 0.14\)). In contrast, a significant correlation showed that greater type II muscle fiber CC and CFPE index were associated with greater SC content \((r = 0.47 \text{ and } r = 0.48, \text{ respectively}; P < 0.05; \text{Fig. 2B})\). No correlations were observed between the changes in muscle capillarization and the changes in SC content in response to the 12 wk of resistance-type exercise training in the older males.

**DISCUSSION**

The present study demonstrates that 12 wk of resistance-type exercise training in older men effectively increased muscle fiber size and capillary networks to the level observed in muscle tissue collected in young men. Resistance-type exercise training not only induced type II muscle fiber hypertrophy but also increased the number of CC and the CFPE index in both type I and type II muscle fibers in older men, supporting an increase in oxygen and nutrient uptake and increasing skeletal muscle mass and function despite an advancing age.

It has been well established that the loss of skeletal muscle mass with age results in a decline in functional performance and increases the risk of developing chronic metabolic disease. In agreement with previous work from our laboratory (36,37) as well as others (22), we show that aging is associated with type II muscle fiber atrophy. Resistance-type exercise training is known to represent an effective intervention strategy to increase skeletal muscle mass and function in both young and older adults. Here, we show that 12 wk of resistance-type exercise training increases type II muscle fiber size in older adults, with type II muscle fiber size no longer being different to tissue obtained in healthy young adults. Furthermore, muscle fiber hypertrophy was accompanied by an increase in leg lean mass and leg muscle strength (Table 3). These results are in line with previously published data (10,35,37) and confirm the impact of prolonged resistance-type exercise training on muscle mass and function in the older population.

The maintenance of skeletal muscle fiber size and, as such, muscle mass, relies on the adequate provision of nutrients and growth factors. Hence, skeletal muscle fiber perfusion and/or overall muscle microvascular function likely represent a critical factor in the maintenance of muscle fiber size and function during aging (13,28,33). A decline in microvascular function with aging is likely dependent on both a reduction in endothelial function (33) and a structural loss of skeletal muscle CD (15,23). In the present study, we clearly show that the number of both type I and type II muscle fiber CC is significantly lower in older compared with younger adults (Fig. 1). Apart from the number of CC, the CFPE index was calculated as a measure of the diffusional capacity from the capillary lumen to the muscle cell membrane, partly determining the capacity for blood-tissue exchange of oxygen, nutrients, and growth factors (16). The CFPE index was shown to be substantially lower in both the type I and type II muscle fibers in the older compared with the young adults. Proper muscle perfusion and nutrient (amino acid) delivery to muscle are important factors in facilitating the postprandial stimulation of muscle protein synthesis (28,31,32). The lower capillarization in older versus young muscle is in line with previous findings (5,13,29) and could play an important role in the development of anabolic resistance in senescent muscle, thereby contributing to progressive muscle loss (39).

Whereas endurance-type exercise training has long been recognized as the primary means to stimulate angiogenesis in older adults (6,12), resistance-type exercise is generally applied to increase muscle mass and strength in the older population and, as such, to support healthy aging (24,25). Previous work has shown inconsistent data on the potential of resistance-type exercise training to increase muscle fiber capillarization in older men. Findings from Frontera et al. (9) and Hepple et al. (17) would suggest effective angiogenesis in skeletal muscle tissue after 9–12 wk of
high-intensity resistance exercise (three times per week). In contrast, no significant increase in muscle fiber capillarization was observed after 16 wk of resistance training (two times per week) in older (~65 yr) (14), and 8 wk of resistance training (two times per week) in middle-age (~54 yr) men (40). The discrepancy in the literature on the angiogenic effects of prolonged resistance-type exercise training in the elderly may be attributed to differences in study design, subject selection, and training frequency, intensity, and volume. Furthermore, it could be speculated that any effects may be more likely to become evident when determined in a fiber type–specific manner. It has previously been established that changes in muscle fiber characteristics (i.e., fiber size, SC content) with aging and after resistance training in the elderly are different between type I and type II muscle fibers (34,36–38). Such fiber type specificity may contribute to the observed discrepancy in the literature on the angiogenic effects of resistance-type exercise. Therefore, we specifically aimed to assess whether fiber type–specific differences in skeletal muscle capillarization between young and older men can be counteracted by prolonged resistance-type exercise training, using an established resistance-type exercise protocol with three training sessions a week for a period of 12 wk, focusing on leg press and leg extension exercises only. In line with the original reports from Frontera et al. (9) and Hepple et al. (17), we clearly show that the number of CC per muscle fiber increases by approximately 30% after 12 wk of training. Likewise, the CFPE index was shown to increase by approximately 25% in response to the exercise program. Furthermore, we extend on previous work by showing that the increase in CC and CFPE index is effectively accomplished in both the type I and type II muscle fibers. In support of an angiogenic response, CD did not change or even increased, despite the substantial muscle fiber hypertrophy that was observed. These findings strongly support the contention that muscle tissue in the older men maintains a remarkable level of plasticity and potential decrements in skeletal muscle fiber size and capillarization observed with aging can be attenuated when given an appropriate and continued exercise stimulus.

In addition to the importance of adequate muscle perfusion, muscle fiber repair, growth, and maintenance are known to be dependent on skeletal muscle SC (30). A reduction in muscle SC number and/or function has been suggested to be a key factor in the loss of muscle mass with aging (30,37). We (34,37) and others (38) have previously established that the lower number of type II muscle fiber–associated SC in older versus young adults can be increased with prolonged resistance-type exercise training, facilitating incorporation of newly formed myonuclei, thereby allowing muscle fiber hypertrophy (Table 2). Interestingly, it was recently hypothesized that muscle fiber capillarization may be a plausible factor in governing SC number and/or function (4). Therefore, as a secondary aim, we examined the relation between the number of capillaries and the number of SC in human muscle tissue. We show that greater muscle fiber CC and CFPE index were associated with greater SC content, specifically in the type II muscle fibers. In contrast, no relationships were observed between the increase in SC content and CC/CFPE after 12 wk of resistance-type exercise training. Unfortunately, we were not able to measure the distance between SC and the nearest capillary, which has been suggested to be of importance in regulating the SC myogenic program (4). Future studies are warranted to provide further insight into the relationships between SC content, CD, and fiber size in older muscle.

Given the overall aging of our society, it is of utmost importance to identify effective intervention programs that attenuate the age-related loss of muscle mass and function, as well as impairments in insulin sensitivity, glucose homeostasis, and other metabolic deteriorations. Proper capillary supply of skeletal muscle tissue represents a key player in several physiological processes not only inherent to the muscle itself, but also to whole body metabolic health (15,16,27). Based on its anabolic and angiogenic capacity, increasing both muscle fiber size and capillary networks, we show that resistance-type exercise training may be advocated not only to increase muscle mass and function (24,25) but also to improve the metabolic health in the elderly population (21). Unfortunately, given the number of older participants included in the present study, we were not able to detect such metabolic effects, or to study the relation between (changes in) metabolic profile and the response to training. Furthermore, because muscle biopsies were only collected before and after training, we cannot provide further insight into the timeline of changes in muscle fiber characteristics. Indeed, future work is needed to assess whether, for example, angiogenesis may precede (and thus potentially facilitate) subsequent muscle fiber hypertrophy, or whether it may simply “follow” the increase in fiber size. Finally, we recently reported similar effects of prolonged resistance-type exercise training on muscle mass, strength, function, and metabolic health in elderly women and men (21). However, because we only included older men in the present study, it remains to be established whether skeletal muscle capillarization is also effectively increased in elderly women in response to resistance training.

In conclusion, type II muscle fiber size and types I and II muscle fiber capillarization are lower in older compared with young men. Resistance-type exercise training can effectively augment skeletal muscle fiber size and capillarization in older men. The greater capillary supply may be an important prerequisite to reverse anabolic resistance, support muscle hypertrophy, and improve metabolic health during lifestyle interventions aiming to support healthy aging.

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