Long-term strength training reverses the effects of aging on skeletal muscle of healthy elderly men.

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Abstract

Introduction: Aging is related to a gradual decline in skeletal muscle mass, which is associated with morphological modifications such as reduced muscle fiber cross-sectional area and satellite cell content. Data also suggest that a short-term strength training period can be an effective instrument to rejuvenate these morphological parameters and to restore muscle mass. Therefore, the aim of this study is to investigate the effects of one year progressive strength training on fiber type-specific morphological parameters (fiber type composition, fiber area, satellite cell content, myonuclear number and domain) in skeletal muscle of elderly men.

Methods: Thirteen healthy elderly men (age range, 66–77 years) were randomly assigned into training (T) (n=7) and control (C) (n=6) groups. 52 weeks of progressive strength training was performed. Before and after the training, muscles biopsies were collected from the middle part of the vastus lateralis by percutaneous needle biopsy technique. Muscle biopsies were examined for muscle fiber type composition, fiber type-specific hypertrophy and alterations in satellite cell content, myonuclear content and domain using immuno-histochemistry.

Results: At baseline, myonuclear content and mean fiber area was larger in type I fibers compared to type II fibers (p<0.05). No statistically significant differences were found in fiber type composition, mean fiber area, satellite cell content and myonuclear domain between T and C groups at baseline. By the end of the training period, fiber area was increased by 59% (p<0.05) in type I and 71% (p<0.05) in type II. Satellite cell content, myonuclear content and myonuclear domain were increased after training in type I by 58% (p<0.05), 33% (p<0.05), and 20% (p<0.05), respectively. Similar increases in satellite cell content (+65%; p <0.05), myonuclear content (+36%; p <0.05) and myonuclear domain (+25%; p<0.05) were seen in type II fibers.

Conclusion: The current study reported that long-term strength training is an excellent tool to prevent sarcopenia. It is demonstrated that skeletal muscle in elderly is capable to enhance satellite cell and myonuclear content, which contributed to muscle hypertrophy.

Key words: Aging, Muscle mass, Muscle plasticity, Long-term strength training.
Aging is related to a gradual deterioration of strength, functional capacity and muscle mass (Thompson et al., 1994; Aagaard et al., 2007; Verdijk et al., 2009). A longitudinal study in healthy men reported a reduction of about one-sixth of the quadriceps muscle mass during one decade, from the age of 65 to 75 years (Frontera et al., 2000). Reduction in muscle mass is usually linked with a reduction in cross-sectional area (CSA) of individual fibers and can also be due to decrement in number of fibers (Kirkendall et al., 1998).

Age-related reduction in specific type II fiber CSA was found in human (Lexell et al., 1988; Rowan et al., 2011; Verdijk et al., 2007) and animal skeletal muscles (Brack et al., 2005). It has also been shown that aging has a negative impact on type II fiber proportion (Verdijk et al., 2007).

Satellite cells have an important role in repair and growth of skeletal muscles as they can provide new myonuclei to the growing fiber (Grounds et al., 2002; Kadi & Thornell, 2000; Petrella et al., 2006). Reduction in satellite cell content associated with aging could result into a decline of muscle mass (Verdijk et al., 2009). Studies exploring the effect of aging on satellite cell content and number of myonuclei have demonstrated equivocal conclusions. A few studies (Kadi et al., 2004b; Verdijk et al., 2007) reported a marked reduction in satellite cell content in elderly as compared with young adults. However, other studies (Hikida et al., 2000; Roth et al., 2000) have found no significant effect of aging on satellite cell content. Studies examining the effect of aging on myonuclei demonstrated that older adults have similar (Hikida et al., 1998) or higher (Kadi et al., 2004b; Verdijk et al., 2007) myonuclear content than young adults. Collectively, these modifications in morphological parameters could lead to a marked reduction in strength and muscle mass, which can lead to impaired mobility and reduced functional independence in elderly.

At present, strength training is considered as an excellent tool to gain muscle strength (Fiatarone et al., 1994; Hakkinen et al., 1998; Trappe et al., 2002), power (Kotzamanidis et al., 2005) and mass (Fiatarone et al., 1994; Hakkinen et al., 1998) in elderly. In young, data from long-term as well as short-term strength training studies reported a significant effect of strength training on the morphological parameters of the skeletal muscle. However, in elderly, only data from short-term (12 to 16 weeks) strength training studies is available with ambiguous conclusions. In young adults with long
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experience in strength training an important type II fiber hypertrophy occurred together with an augmentation in satellite cell and myonuclear content (Kadi et al., 1999). Similarly, 10 to 16 weeks of strength training in young adults also induced a marked augmentation in fast fiber proportion (Kadi et al., 2000), fiber CSA (Kadi & Thornell, 2000), satellite cell and myonuclear content (Kadi & Thornell, 2000; Olsen et al., 2006). A positive correlation was also found between the fiber CSA and myonuclear content (Kadi et al., 1999; Hikida et al., 1998) and between the satellite cell and myonuclear content in young adults (Kadi & Thornell, 2000).

In elderly, 12 to 16 weeks of strength training induced a significant improvement in fiber CSA (Hikida et al., 2000; Hikida 1998; Kosek et al., 2006; Hagerman et al., 2000; Kryger et al., 2007) but no significant changes in satellite cell content, myonuclear content (Hikida et al., 2000; Hikida 1998), myonuclear domain (Hikida et al., 2000) and fiber type composition (Lange et al., 2002). However, after 12 to 14 weeks of strength training in elderly, some studies showed a significant improvement in satellite cell (Verdijk et al., 2009; Verney et al., 2008) and myonuclear content (Verdijk et al., 2009). Hikida et al. (1998) conducted strength training in eight healthy elderly men (age 65±6) for 16 weeks and reported significant improvements in CSA of both type I and type II fiber. However, no marked improvements were reported in satellite cell and myonuclear content (Hikida et al. (1998). On the other hand, Verdijk et al. (2009) have investigated the fiber type-specific impact of 12 weeks strength training in 14 healthy elderly men (age 73±4years) and demonstrated type II-specific augmentations in the fiber CSA (28%), satellite cell content (75%) and myonuclear content (17%) in the type II.

These conflicting findings may be due to dissimilar training protocols, study designs, participant characteristics, muscle analyzed and analyzing techniques (Hikida et al., 1998, Verney et al., 2008, Verdijk et al., 2009).

To our knowledge, the effects of a long-term strength training period of one year on morphological parameters of skeletal muscle in elderly men have not been investigated yet. Collectively, to what extent long-term strength training could affect fiber type distribution, fiber CSA, satellite cells, number of myonuclei and myonuclear domain in elderly is not known. Moreover, the existence of differences in the response of slow and fast fibers after one year strength training in elderly is unknown.

Therefore, the aim of this study is to investigate the effects of one year progressive
strength training on fiber type-specific morphological parameters (fiber type composition, fiber area, satellite cell content, myonuclear number and domain) in skeletal muscle of elderly men. We hypothesized that one year of strength training can reverse the effects of aging on skeletal muscle of healthy elderly men.

**Material and methods:**

**Study design:**

The current study is a randomized controlled intervention with a training and control group. This intervention consisted in a period of 52 weeks of resistance training, split into 2 equal periods of 26 weeks. In the first period of 26 weeks, whole body strength exercises were performed with frequency of 2 days/week. Rest intervals (≥2 days) were allowed between 2 consecutive strength training sessions. In the 2nd period of 26 weeks, strength training was split into upper and lower body training sessions with a frequency of 3 days per weeks. After a period of familiarization, each period of 26 weeks comprised three specific training goals with the aim to achieve hypertrophy, strength and hypertrophy and strength and power. All measurements were performed at baseline and at the end of training period.

**Subjects and ethics:**

Thirteen healthy elderly men (age range, 66-77 years) participated in the current study. Participants were randomized into a training (T) and a control (C) group. Subject’s characteristics are demonstrated in table 1. Subjects were included in this project after examination by physician. They had no cardio-pulmonary or hormonal disorders and had not participated in any strength or aerobic training programme during the year before the participation in this study.

<table>
<thead>
<tr>
<th></th>
<th>T group (n=7)</th>
<th>C group (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.8±1.35 (67-70)</td>
<td>69.1±4.16 (66-77)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.4±4.8 (167.5-177.5)</td>
<td>180.3±5.5 (170.5-186.5)</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>81.6±10.7 (71.3-99.1)</td>
<td>81.9±7.4 (72.3-89.4)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>27.5±6.5 (15.6-36.6)</td>
<td>29.2±4.2 (23.7-36.4)</td>
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</table>

Table 1 Subject characteristics in T and C group.

Data is demonstrated as mean, ± SD and range.

After being informed on the requirements involved in the study, all subjects provided an oral and written consent prior to participation in the experiment. All procedures adopted in the study were performed in accordance with the principles of the declaration of Helsinki. An ethical application was sent to the ethical
review board and the study received ethical approval.

**Strength training programme:**

Leg press, knee flexion and extension exercises were used for lower limbs while bench press, triceps pushdown and elbow flexion exercises were used for the upper body. Repetition maximum (RM) testing was performed to determine the individual load for lower and upper body exercises. 1RM is the maximum weight which a person could lift once through the entire range of motion. Progressive resistance exercises in terms of increasing intensity and duration were introduced for achieving the specific goals. To achieve hypertrophy, the training was carried out with the loads of 60-80% of 1RM with 10-12 RM/set. A short resting time (1.5-2 min.) was allowed between the three sets. To achieve strength and hypertrophy, training was carried out with a load of 70-90% of 1RM with 6-8RM/set. A relatively longer resting time (3min.) was allowed between the three sets. To achieve strength and power, training was carried out at a higher load of 100-130% of 1RM with maximum possible speed. A three minute rest interval was allowed between the three sets. The average strength exercise programme lasts about 70 to 90 minutes and the whole strengthening training protocol was supervised by a qualified exercise specialist.

**Muscle biopsies:**

Muscle biopsies were taken from middle-portion of vastus lateralis muscle from all subjects at baseline and after the training period of one year by using the percutaneous needle biopsy technique. Xylocaine (2%) was used as local anesthethesia. After anaesthetizing, the 5 mm needle was introduced into the muscle belly and 80 milligram muscle sample was taken with the aid of suction. The post training muscle biopsies were taken 2-cm away from the site of first biopsy taken. Muscle biopsies were cleaned and embedded in embedded medium (Tissue-TEK, Miles Inc., Elkhart, IN) and frozen in isopentene at -160 ºC. The samples were stored at -80 ºC until further analysis.

**Immuno-histochemistry:**

A cryostat was used to section muscle biopsies into 7 µm thick cross-section at -20 ºC. The cross- sections were placed over glass slides and care was taken not to stretch or break the samples. The cross-sections were air dried, washed in PBS and incubated in normal serum for about 20 minutes. Two consecutive sections were used to determine fiber type
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composition, fiber area, satellite cell content and myonuclear content.

The first section was stained with the monoclonal antibody (mAb) against Pax-7 (Pax7, Developmental Studies Hybridoma Bank) followed by a second incubation in a mixture of two mAbs against type I muscle fibres (A4.840, Developmental Studies Hybridoma Bank) and laminin (2E8, Developmental Studies Hybridoma Bank). Pax-7 is a widely used marker for satellite cells (Mackey et al. 2009; Verdijk et al., 2007). Laminin is a marker for the basal lamina of muscle fibres and A4:840 is a marker for type I muscle fibres (Kadi et al. 1998). Visualization of antigen-antibody binding for Pax7 was performed by successive incubations in a biotinylated antimouse secondary antibody (Vector Laboratories), the vectastain ABC reagent (Vector Laboratories) and the diamino-benzidine substrate kit for per-oxidase (Vector Laboratories), which gives a brown staining. Visualization of A4.840 and 2E8 antibody mixture was achieved by incubation with Alexa Fluor 488 goat anti mouse secondary antibody (Invitrogen A/S), which gives a green-fluorescent staining. Myonuclei were visualized using mounting medium containing DAPI (Invitrogen A/S). This first step allows the visualization on the same section of Pax7+ satellite cells (brown staining) using light microscopy and green-fluorescent-stained basal lamina and type I muscle fibers using fluorescent microscopy (figure, 1A, 2, 3). Using the A4:840 antibody, type II fibres were unstained.

The second section was employed to further characterize muscle fiber types. The section was stained with the mAb against N2:261 (Developmental Studies Hybridoma Bank), which strongly stains type IIA fibres, while type IIX fibres were unstained (Verney et al., 2008). Visualization of the antigen-antibody binding was achieved by successive incubations in a biotinylated anti-mouse secondary antibody (Vector Laboratories), the vectastain ABC reagent (Vector Laboratories) and the diamino-benzidine substrate kit for per-oxidase (Vector Laboratories), which gives a brown staining (figure, 1B).

The cross-sections were analyzed under light microscopy (Nikon Eclipse, E400, Netherland) at high magnification and images were captured using a digital camera (Spot insight, diagnostic instrument, Sterling heights, Michigan). Magnification x20 was used for measurement of fiber area and magnification x40 was used for counting myonuclei and satellite cells (Kadi et al., 2004a). The number of myonuclei and the
satellite cells were counted three times by the same observer (MMQ). The first counting was used for statistical analysis as there were no significant variations between the three readings. Sigma pro-5 software was used for the measurement of muscle fibers area in \( \mu m^2 \) by tracking manually the margins of the individual muscle fiber. The counting of number of fibers was done by using photographed image of the whole muscle cross-sections.

Figure 1- Identification of different fiber types on two consecutive muscle cross-sections.

(A) Staining of a 7\( \mu m \) muscle cross-section with A4:840.

(B) Staining of a 7\( \mu m \) muscle cross-section with N2:261

Figure 2- Muscle fiber type-specific analysis of satellite cells-Pax-7 positive stained satellite cell appeared brown as shown in circle.

Figure 3- Muscle fiber type-specific analysis of myonuclei-DAPI positive stained myonuclei appeared blue as shown in circle.
For each muscle biopsy, the whole muscle cross-section was analyzed for fiber type distribution and frequency of satellite cells/fiber. (In training group, on average, 1022 muscle fibers per muscle biopsy were analyzed at pre-training and 321 muscle fibers per muscle biopsy at post-training, whereas in control group, 769 muscle fibers per muscle biopsy were analyzed at pre and 215 fibers per muscle biopsy at post-study). One hundred and fifty randomly selected fibers were used for the measurement of fiber cross-sectional area ($\mu m^2$) and fifty fibers were used to determine the myonuclear content. The myonuclear domain was calculated with the help of the following formula.

$$\text{Myonuclear domain} = \frac{\text{fiber area}}{\text{number of myonuclei/fiber}}$$

(Verdijk et al., 2007; Verney et al., 2008).

**Statistical analysis:**

Data is demonstrated as mean, standard deviation and range. Percentage changes were calculated for all morphological parameters. Data was analyzed by using one way anova with repeated measurement. Un-paired t-test was used to analyze the differences in morphological parameters at baseline between T and C group. The Pearson correlation coefficient was used for evaluating the association between satellite cell content, myonuclear content and mean fiber area. SPSS software, (version 16) for personal computer was used for statistical work. p value ($<0.05$) was set as statistical significance.

**Results:**

**Comparison between training and control group at baseline:**

No statistically significant differences were found in morphological parameters between the baseline values of the T and C groups (Table-2). At baseline, type I fiber had more myonuclear content compared to type II fibers in both T ($p = 0.002$) and C groups ($p = 0.008$). Type I fibers tended to have larger fiber CSA compared to type II fibers in T ($p = 0.03$) and C group ($p = 0.1$) at baseline. However, we noticed that at baseline there were no statistically significant differences in satellite cell content between type I and type II fibers.

**Effect of strength training on fiber type composition:**

Vastus lateralis muscle is made of slow (Type I) and fast (Type IIA and IIAX) fibers. Type I (53.8%) and type IIA (43.6%) represent the main fiber types while type IIAX fibers (2.55%) are rare (Table-2). As the type IIAX fibers were rare, they were not used in statistical analysis. After the study period,
there were no significant alterations in fiber type distribution in C and T groups (Table-3, 4).

**Effect of strength training on muscle fiber area:**

One year strength training significantly affected muscle fiber area. Muscle fiber area increased by 59% in type I fibers (p = 0.000) and 71% in type II fibers (p =0.001; Table-3) in T group. No significant differences were seen in the response of type I and type II fibers to strength training. In C, there were no significant changes in the fiber CSA of type I fibers after the study period. Interestingly, type II fiber CSA decreased by 14% (p =0.012; Table-4) by the end of study period.

**Effect of strength training on myonuclei content:**

There was a significant impact of resistance training on myonuclear content in both type I and type II fibers in T group. Myonuclei per fiber increased by 33% in type I fibers (p =0.000) and 36% in type II fibers (p =0.000) after one year of strength training (Table-3). In C, no statistically significant alterations were seen in myonuclear content at the end of study period (Table-4).

**Effect of strength training on satellite cell content:**

There was a significant impact of strength training on the frequency of satellite cells/fiber in T group. Satellite cell content increased by 58% (p =0.001) in type I and 65% (p =0.000) in type II fibers after one year of strength training. No significant differences were found between the response of type I and type II in T group (Table-3). In C, no statistically significant changes were seen in satellite cell content in type I fibers. Interestingly, satellite cell content in type II fibers decreased by 13% (p=0.000) at the end of study period (Table-4).

**Effect of strength training on myonuclear domain (MND):**

There was a significant impact of strength training on the myonuclear domain in both type I and type II fibers after the strength training programme. MND expanded by 20% in type I (p =0.01) and 25% in type II fibers (p =0.027; Table-3). No difference was seen between the response of type I and type II fibers. In C, no significant changes were seen in MND of type I fiber, whereas, in type II fibers MND was significantly reduced by 15% (p= 0.008) at the end of study period (Table-4).
**Table 2.** Fiber type composition, mean fiber area, number of myonuclei/fiber, satellite cells/fiber and myonuclear domain at baseline in T and C groups. Data is demonstrated as mean, ±SD and range. ¹ Significance difference compared to type IIA (p<0.05).

<table>
<thead>
<tr>
<th>Type</th>
<th>Fiber type composition (%)</th>
<th>Mean fiber area (um²)</th>
<th>No of myonuclei/fiber (m/f)</th>
<th>No of satellite cells/fiber (s/f)</th>
<th>Myonuclear Domain (MND)</th>
</tr>
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<tbody>
<tr>
<td>Type I</td>
<td></td>
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<tr>
<td>T group</td>
<td>53.8±7 (41-65)</td>
<td>4732±471 (3939-5349)</td>
<td>2.61±0.21 (2.24-2.84)</td>
<td>0.047±0.02 (.032-.058)</td>
<td>1826±249 (1515-2219)</td>
</tr>
<tr>
<td>C group</td>
<td>55.4±6 (46-66)</td>
<td>5589±1411 (3826-7844)</td>
<td>2.64±0.17 (2.36-2.8)</td>
<td>0.0497±.004 (.044-0.056)</td>
<td>2106±449 (1773-2801)</td>
</tr>
<tr>
<td>Type IIA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T group</td>
<td>43.6±4 (38-56)</td>
<td>4006±650 (3046-4953)</td>
<td>2.28±0.9 (2.14-2.38)</td>
<td>0.0452±.008 (.033-.056)</td>
<td>1767±331 (1313-1966)</td>
</tr>
<tr>
<td>C group</td>
<td>41.9±5 (34-53)</td>
<td>4626±600 (3622-5157)</td>
<td>2.33±0.16 (2.12-2.52)</td>
<td>0.0484±.005 (.042-.056)</td>
<td>1988±174 (1646-2120)</td>
</tr>
<tr>
<td>Type IIA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T group</td>
<td>2.55±1 (1.5-3)</td>
<td>3442±789 (2015-4414)</td>
<td>2.2±0.13 (2.14-2.34)</td>
<td>--</td>
<td>1585±428 (1310-2207)</td>
</tr>
<tr>
<td>C group</td>
<td>2.68±1 (1.7-3.5)</td>
<td>3987±769 (2984-4952)</td>
<td>2.2±0.22 (1.98-2.5)</td>
<td>--</td>
<td>1814±303 (1420-2128)</td>
</tr>
</tbody>
</table>

**Table 3.** Effect of one year resistance training on fiber type composition, mean fiber area, number of myonuclei/fiber, satellite cells/fiber and myonuclear domain in T group. Data is demonstrated as mean, ±SD and range.

* Significantly different compared to type I baseline (p<0.05).
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Table 4. Effect of one year study period on fiber type composition, mean fiber area, number of myonuclei/fiber, satellite cells/fiber and myonuclear domain in C group. Data is demonstrated as mean, ±SD and range.

<table>
<thead>
<tr>
<th></th>
<th>Type I</th>
<th>Post-training</th>
<th>Type II</th>
<th>Post-training</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber type composition(%)</td>
<td>55.4±6(46-64)</td>
<td>56.6±7.6(45-67)</td>
<td>44.6±6(35-53)</td>
<td>43.4±7.6(33-54)</td>
</tr>
<tr>
<td>Mean fiber area (um2)</td>
<td>5589±1411(3826-7844)</td>
<td>5571±1379(3729-7563)</td>
<td>4626±600(3622-5157)</td>
<td>3950±527(3148-4518)</td>
</tr>
<tr>
<td>No of myonuclei/fiber (m/f)</td>
<td>2.64±0.17(2.36-2.8)</td>
<td>2.66±0.15(2.38-2.84)</td>
<td>2.33±0.16(2.12-2.52)</td>
<td>2.35±0.16(2.12-2.58)</td>
</tr>
<tr>
<td>No of satellite cells/fiber(s/f)</td>
<td>0.0497±0.004(.044-.056)</td>
<td>0.0454±0.004(.039-.052)</td>
<td>0.0484±0.005(.042-.056)</td>
<td>0.0421±0.006(.035-.052)</td>
</tr>
<tr>
<td>Myonuclear Domain (MND)</td>
<td>2106±449(1773-2801)</td>
<td>2071±419(1567-2664)</td>
<td>1988±174(1646-2120)</td>
<td>1687±196 (1380-1958)</td>
</tr>
</tbody>
</table>

Significantly different compared to type II baseline (p<0.05)

Relationship between the fiber CSA, myonuclear and satellite cell content:

A strong positive correlation was observed between the fiber CSA and myonuclear content in type I (r =0.76; p = 0.001) and type II fibers (r =0.74; p =0.002) in T group. Similarly in C, a positive correlation was found between fiber CSA and myonuclear content in type I (r =0.75; p = 0.04) fibers. However, no statistically significant correlation was found in type II fibers (r =0.5; p = 0.08) in C group (Fig. 4A).

Myonuclear and satellite cell content were positively correlated in type I (r = 0.69; p = 0.005) and type II (r = 0.81; p = 0.000) fibers in T group. However in C, no statistically significant correlation was found between the myonuclear and satellite cell content in type I and type II fibers (Fig. 4B).
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Figure 4A: Relationship between the mean fiber CSA (μm$^2$) and myonuclear content in type I and type II fibers of vastus lateralis muscles in C and T groups. $r = 0.77; p = 0.000$

Figure 4B: Relationship between the mean myonuclear and satellite cell content in type I and type II fibers of vastus lateralis muscles in C and T group. $r = 0.72; p = 0.000$
Discussion:

This is the first study examining the impact of one year strength training on muscle fiber composition, fiber CSA, satellite cells per fiber, number of myonuclei per fiber and myonuclear domain in skeletal muscle of healthy elderly men. The important finding was a significant improvement in fiber CSA, satellite cell and myonuclear content in both type I and type II fibers after one year of strength training.

Effect of strength training on muscle fiber area:

Aging induces diverse morphological changes in the skeletal muscle (Kirkendall et al., 1998; Kadi et al., 2004b; Verdi jk et al., 2007; Verdi jk et al., 2009; Rowan et al., 2011), which result into a decline of muscle mass, strength and power and functional capability (Aagaard et al., 2007; Verdi jk et al., 2009). Very limited options are available to counteract the aging effects on skeletal muscles. Strength training is considered as an appropriate way to counteract and rejuvenate the morphological alterations associated with aging and to induce skeletal muscle hypertrophy (Fiatarone et al., 1994; Nelson et al., 1994; Hakkininen et al., 1998; Kotzamanidis et al., 2005). This is evidenced by our current data that demonstrated a significant improvement in the fiber CSA of both type I and type II fibers. Our results are in accordance with many earlier studies analyzing the impact of resistance training on fiber CSA in elderly adults (Hikida et al., 1998; Hakkininen et al., 1998; Hikida et al., 2000). In contrast, some studies (Verdi jk et al., 2009) demonstrated a preferential increment in type II fiber CSA. These contradictory results may be due to differences in the duration of the strength training program between our study and that of Verdi jk et al. (2009). In elderly, type I fibers may take longer time to adapt to resistance training than type II fibers. A strength training period of 12 weeks duration reported only an increment in type II fiber CSA (Verdi jk et al., 2009). However, strength training of 16 weeks duration demonstrated a remarkable improvement in fiber CSA of both type I and type II fibers (Hikida et al., 1998; Hikida et al., 2000). Hikida et al. (1998), have taken 8 healthy elderly men and introduced 16 weeks of strength training and demonstrated that fiber CSA significantly increased by 46% (from 3887 µm² to 5657µm²) in type I and by 43% (from 4146 µm² to 5529 µm²) in type II fibers. On the other hand, Verdi jk et al. (2009) used a period of 12 weeks strength training and found significant improvement in type II

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fiber CSA (28%; from 5438 µm² to 6982 µm²) with no significant improvement in type I fiber CSA. In young adults, the studies examining the effects of strength training on muscle fiber area revealed the same conclusion that type II shows rapid response compared to type I fibers. In young adults, it also seems that type II fiber area adapts faster than that of type I fibers. For example, Coyle et al. (1981) used a period of 6 weeks of strength exercises in 22 young males and found more significant improvements in type II fiber CSA (11.2%) compared to type I fiber (5.1%).

We have reported that at baseline mean fiber CSA of type II fiber is smaller compared to type I fibers. This is in consistent with previous studies (Verdijk et al., 2007; Verdijk et al., 2010). In contrast, studies on young adults (Hikida et al., 1998) have showed that fiber CSA of type II fibers is larger compared to type I fibers. This discrepancy in the literature may be due to type II fibers-specific atrophic changes, which are associated with aging. Comparative morphological studies examining the muscle fiber characteristics between young and elderly adults have reported that type II fibers in young are larger than type II fibers in elderly (Hakkinen et al., 1998; Verdijk et al., 2007). Verdijk et al., (2007) have taken 8 elderly adults (76±1 year) and 8 young adults (20±1 years) and examined their muscle fiber characteristic. They have found no difference in type I fiber CSA between the elderly (5471 µm²) and young adults (5589 µm²). However, elderly have small type II fiber CSA (4451 µm²) compared with fiber CSA (6162 µm²) of young adults. It seems that type II fibers are more prone to age-related atrophic changes.

We have reported a specific reduction in fiber CSA of type II fiber, in accordance with the finding of previous studies (Lexell et al., 1988; Brack et al., 2005). The preferential atrophy in type II fibers can be explained by several factors. We argue that, physical activity is reduced with increasing age and type II fibers seems to require intense physical activity to maintain their size, failure to which result into reduction of fiber size.

Effect of strength training on fiber type composition:

We found that vastus lateralis is composed of 53% of type I and 47% of type II fibers, which is in accordance with previous available data (Verdijk 2007; Hakkininen 1998; Verdijk et al., 2010). No significant modifications were seen in fiber type distribution after one year of strength training, which is accordance with the findings of published studies (Hakkininen 1998; Hikida et
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al., 1998; Hikida et al., 2000; Verdijk et al., 2009). However, a few studies on young adults (Staron et al., 1990; Kadi et al., 2000) reported significant changes in type II proportion after 10 to 20 weeks of resistance training. Kadi et al. (2000) investigated the effects of ten weeks of resistance training on fiber type composition in 30 young women (age, 38.7±5.5 years). They have compared trapezius muscle biopsies before and after the training and found a significant enhancement in type II proportion (13%) with concurrent reduction in type I fibers proportion. Contrarily, current and other studies (Hakkininen et al., 1998; Hikida et al., 2000; Verdijk et al., 2009) reported no alteration in fiber type composition after strength training. Hakkininen et al. (1998) used progressive strength training for ten weeks in elderly men (age, 61±4 years) and found no significant alteration in fiber type composition. It seems that in elderly, type II fibers are less responsive to strength training compared to young. The participants in our and other studies (Hakkininen 1998; Hikida et al., 1998; Verdijk et al., 2009) were elderly compared to those studies (Kadi et al., 2000).

Effect of strength training on myonuclear and satellite cell content:

We have demonstrated that fiber CSA is directly proportional to the myonuclear and satellite cell content. This is in accordance with the previous studies on this subject (Kadi et al., 1999; Hikida et al., 1998; Kadi & thornell, 2000). As the muscle fiber CSA increases, this association is secured by enhancing myonuclear content to muscle fiber by satellite cells proliferation (Hikida et al., 1998).

At baseline, we have found that type I had more myonuclei than type II, which is consistent with published data (Hikida et al., 1997; Verdijk et al., 2009; Verdijk 2007, Verdijk et al 2010). This indicates that larger the fiber CSA is as it is the case for type I muscle fibers in elderly vastus lateralis, the greater myonuclear pool. We have showed that myonuclear content increased significantly in both type I (33%) and type II (36%) fibers in a manner proportional to the increase in fiber CSA. No study exists examining the long-term strength training impact on fiber type-specific number of myonuclei/fiber. Only a few studies (with duration of 12-16 weeks) are available and a preferential augmentation of myonuclei in type II fibers was demonstrated (Hikida et al.,
Strength training reverses the effects of aging (Verdijk et al., 2009). Previously, it has been showed that existing myonuclei have some extra capacity to induce skeletal muscle hypertrophy and limited amount of hypertrophy can be attainable without induction of new myonuclei (Kadi et al., 2004a). But in case of extensive hypertrophy, acquisition of new myonuclei may be needed (Kadi & thornell et al., 2000).

In present study, we have found similar (Petrella et al., 2006) or lower (Verney et al., 2008) baseline values of myonuclei/fiber in vastus lateralis muscles in elderly. The differences between studies may be due to differences in age of the participants as it has been suggested that myonuclear content increases with aging (Kadi et al., 2004b; Verdijk et al., 2007). The participants in Verney et al. (2008) were older (Average age, 75 years) compared to our study (Average age, 67 years). It is now accepted that the number of myonuclei does not decrease with increasing age (Hikida et al., 1998; Kadi et al., 2004b). The question why atrophy takes place and whether the functional capacity of myonuclei is compromised can be raised. We argue that specific threshold in the form of strength exercises is required to maintain the functional capacity of the myonuclei.

Muscle regeneration, repair and hypertrophy rely on the satellite cell content (Grounds et al., 2002; Petrella et al., 2008). We have showed that satellite cell content increased significantly in both type I (58%) and type II (65%) fibers in consensus with the existing data (Verney et al., 2008). No data is available reporting the long-term impact of resistance training on satellite cell content in elderly. Only a few studies (12-16 weeks) are available exploring the impact of strength training on satellite cell content in elderly (Hikida et al., 1998, Verney et al., 2008; Verdijk et al., 2009). In elderly skeletal muscles, the duration of training and functional demand of muscle sampled are important factors that can affect the response of satellite cell content to strength training. Verney et al. (2008) have investigated the effects of 14 weeks of strength training on satellite cell content in deltoid muscles of ten healthy elderly men (age, 73±4 years). They have found significant improvement in satellite cell content in both type I (27%) and type II (63%) fibers. However, a few researchers (Verdijk et al., 2009) reported only increment in type II fibers satellite cell content. Verdijk et al. (2009) have studies satellite cells in vastus lateralis muscle biopsies after 12 weeks of strength training in 14 healthy elderly men (age range, 65-85
years) and reported preferential enhancement in satellite cell content (75%) of type II fibers. It is obvious from the above mentioned studies that type II fibers shows rapid response compared to type I fibers.

In the current study, no differences was reported between the baseline values of satellite cell content in type I fibers versus type II fibers, in accordance with the existing data (Kadi et al., 2006; Verney et al., 2008). However, a few researchers (Verdijk et al., 2009) have found that type I fibers have more satellite cell content versus type II fibers. This discrepancy may be due to the fact that type II fibers are more prone to age-related decline of satellite cell content compared to type I fibers (Verdijk et al., 2009). The participants in Verdijk et al. (2009) were older (Average age, 77 years) compared to participants (Average age, 67 years) of our study.

A few studies (Verdijk et al., 2007; Verdijk et al., 2009) reported that a decline in satellite cell content takes place primarily in type II fibers. Likewise, we have observed a decline in satellite cell content preferentially in type II fibers. It is suggested that decreased physical activity negatively influences the satellite cell content (Kadi et al., 2004b). Reduced physical activity in elderly may be one of the reasons for reduction of satellite cell content in elderly. We argue that satellite cell content in type II fibers require a certain threshold in the form of weight training for sustaining their existence and functional capacity.

**Effect of strength training on myonuclear domain:** Myonuclear domain is used to describe the part of cytoplasm regulated by one myonucleus (Mantilla et al., 2008). The myonuclear domain expands proportionally with the increase in the fiber CSA (Olsen et al., 2006). In the current study, the range of myonuclear domain values varied from 1767 µm² to 2202 µm², which is similar to the previously available data (Verney et al., 2008). We showed that myonuclear domain expands significantly in both type I (20%) and type II fibers (25%) after one year of strength training, in accordance with the published data (Mantilla et al., 2008). No studies are available studying long-term strength training effect on myonuclear domain in elderly. However, a few researchers (Hikida et al., 1998; Hikida et al., 2000; Petrella et al., 2006) studied the impact of 16 weeks of resistance training in elderly and reported no significant alteration in myonuclear domain. The intensity of training, functional capacity of the existing myonuclei and the capability of satellite cell to give rise to myonuclei are important factors that can influence the
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myonuclear domain. It has been suggested that up to 30% increment in muscle fiber size can be accomplished without significant inclusion of extra myonuclei (Hikida et al., 1998; Hikida et al., 2000; Petrella et al., 2006). Therefore, when the working limit of existing myonuclei exceeded, satellite cells contributed more myonuclear content to provide genetic machinery for skeletal muscle growth (Verdijk et al., 2009).

We have reported that myonuclear domain is decreased in type II fibers, in accordance with the findings of the available data on aging (Kadi et al., 2004b). Small myonuclear domain implies that one myonucleus rules over less volume of cytoplasm. Type II fibers have more potential to increase their fiber size (Verdijk et al., 2009) that may be due to small myonuclear domain. So, in response of strength training, type II fibers respond rapidly.

**Strength and limitations of the study:**

Long duration (one year), randomization and control are the strength of our study. Small sample size, availability of less number of myofibers to examine in the control group at post training time point was our limitation. Furthermore, the study is also lacking proper information regarding diet plans of the participants. It is very important because diet (protein supplementation) considerably influences the skeletal muscle mass.

**Conclusions:**

In short, we conclude from the above discussion that long-term resistance training is an effective way to combat age-related deterioration of muscle mass. We have demonstrated that one year strength training has a very positive effect on mean fiber area, satellite cell and myonuclear content in both fiber types in healthy elderly men. Muscle fiber area is directly proportional to the satellite cell and myonuclear content. Satellite cells incorporation gives rise to new myonuclei which results into the hypertrophy. A huge budget can be saved by managing sarcopenia and long-term strength training is an excellent available option to combat sarcopenia.
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