Enhanced satellite cell proliferation with resistance training in elderly men and women

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In addition to the well-documented loss of muscle mass and strength associated with aging, there is evidence for the attenuating effects of aging on the number of satellite cells in human skeletal muscle. The aim of this study was to investigate the response of satellite cells in elderly men and women to 12 weeks of resistance training. Biopsies were collected from the m. vastus lateralis of 13 healthy elderly men and 16 healthy elderly women (mean age 76 ± 3 years) before and after the training period. Satellite cells were visualized by immunohistochemical staining of muscle cross-sections with a monoclonal antibody against neural cell adhesion molecule (NCAM) and counterstaining with Mayer’s hematoxylin. Compared with the pre-training values, there was a significant increase (P < 0.05) in the number of NCAM-positively stained cells per fiber post-training in males (from 0.11 ± 0.03 to 0.15 ± 0.06; mean ± SD) and females (from 0.11 ± 0.04 to 0.13 ± 0.05). These results suggest that 12 weeks of resistance training is effective in enhancing the satellite cell pool in skeletal muscle in the elderly.

It is well documented that aging is associated with a progressive loss of muscle mass and strength (for a review, see Doherty, 2003), due to a combination of denervation, loss of muscle fibers and reduced muscle fiber cross-sectional area (CSA) (Lexell, 1993, 1995; Porter et al., 1995; Shavlakadze & Grounds, 2003), as well as a lower regenerative efficiency of older muscle (Grounds, 1998). While the function of satellite cells in generating new muscle fibers and providing new myonuclei to existing fibers is well established (Schultz & McCormick, 1994; Hawke & Garry, 2001), alterations in the response of satellite cells with aging are not so well documented. Whereas some studies have reported similar proportions of satellite cells in young and elderly individuals (Hikida et al., 1998; Roth et al., 2000a), there is mounting evidence for reduced numbers of satellite cells in the elderly (Renault et al., 2002; Sajko et al., 2004; Kadi et al., 2004a), although whether this limits the capacity to form new muscle in vivo is unclear.

Studies investigating the effects of physical activity on satellite cell behavior in humans do not all lead to the same conclusion, but the majority have revealed a positive regulatory effect of exercise on the number of satellite cells and their activity levels in young populations. Significant increases in the proportion of satellite cells have been detected in young men 4 and 8 days after a single bout of maximal exercise (Cramer et al., 2004b), and significant increases have been reported as early as 24 h after one bout of maximal eccentric contractions (Dreyer et al., 2005), demonstrating the capacity of skeletal muscle to activate satellite cells to proliferate rapidly in response to this type of exercise. Ninety days of resistance training have also been shown to have a positive effect on the number of satellite cells in young men (Kadi et al., 2004b). Regular repeated training bouts over 8–16 weeks of strength training did not influence the satellite cell population in a study where both young and elderly men were studied (Hikida et al., 1998). In contrast, Roth et al. (2001) found a significant increase in the satellite cell proportion in young and old individuals in groups with both men and women in response to 9 weeks of unilateral knee-extension training. The effects of endurance training on the modulation of the satellite cell pool in old men were investigated by Charifi et al. (2003), where a significant increase was observed following a 14-week cycle ergometer training program. In young individuals, it appears that both acute bouts of exercise and long-term training may result in proliferation of satellite cells, whereas some disparity exists concerning the response of satellite cells to resistance training in the elderly.
Furthermore, with the exception of the study by Roth et al. (2001), there is a lack of information on gender differences and the response to training in the elderly. Finally, to our knowledge, this has not yet been investigated by immunohistochemical means. The main aim of this study therefore was to investigate the response of satellite cells in skeletal muscle from old men and women to a period of resistance training. Satellite cells were identified in the present study using an antibody against neural cell adhesion molecule (NCAM), which, in skeletal muscle, also stains nerves, neuromuscular junctions and the cytoplasm of an occasional myofiber, all of which are easily recognized, making this a highly suitable marker of satellite cells. Several previous studies have also used this antibody to identify satellite cells in frozen human muscle sections (Kadi & Thornell, 2000; Charifi et al., 2003; Crameri et al., 2004b; Kadi et al., 2004a, b) – for a more detailed overview, readers are referred to an excellent recent review on this topic (Kadi et al., 2005).

**Materials and methods**

**Subjects and training**

Thirteen healthy elderly men and 16 healthy elderly women (age range 70–82 years) volunteered for this study (Table 1). All subjects gave written informed consent for the study, which was approved by the Ethics Committees of the Municipalities of Copenhagen and Frederiksberg (ref KF01-012/01), and confirmed to the Declaration of Helsinki. Participants followed a resistance training program similar to that described by Esmarck et al. (2001). Briefly, training was carried out three times a week for 12 weeks and involved three dynamic strength exercises: unilateral horizontal seated leg press, unilateral seated knee extension and unilateral prone-lying knee flexion. At the beginning of the training period, the number of repetitions in each set was 15; this was reduced every 2 weeks for the first 6 weeks to 12, 10 and finally down to eight repetitions. For the last 6 weeks, the number of repetitions was maintained at eight. Correspondingly, the intensity increased from 15 to 12, 10 and 8 repetitions maximum (RM), i.e. ~62%, 70%, 75% and 80% of 1 RM, respectively. The maximum voluntary isometric force of the quadriceps was measured at an angle of 2.09 rad (120°) of knee flexion.

**Muscle biopsies**

Two muscle biopsies were collected from each volunteer: one before the training started and one 2–3 days after the last training session. Biopsies were obtained from the mid-portion of m. vastus lateralis using a standard needle biopsy technique. On extraction, the sample was mounted and frozen by immersion in isopentane, pre-cooled to approximately –160 °C by liquid nitrogen. Samples were stored at –80 °C pending analyses.

**Immunohistochemistry**

**Satellite cells**

Serial transverse sections (10 μm) were cut at –24 °C using a cryostat and mounted on SuperFrost Plus slides (Menzel-Gläser, Braunschweig, Germany). Satellite cells were visualized by immunohistochemical staining using an NCAM antibody (CD56, cat. no. 347740; Becton Dickinson, San Jose, California, USA), following a previously described protocol (Charifi et al., 2003). Briefly, sections were blocked with 1.5% normal horse serum (S-2000; Vector Labs, Peterborough, UK) for 20 min and incubated with the primary antibody (1:100 dilution) overnight at 4 °C. Unbound antibody was removed by washing, and the sections were covered with biotinylated goat anti-mouse secondary antibody diluted to 1:200 (E0433; DakoCytomation Denmark A/S, Glostrup, Denmark). After washing, the Vectastain Elite ABC reagent (PK-6100; Vector Labs) was applied, according to the kit instructions. Diaminobenzidine (DAB) substrate-chromogen for peroxidase was used to visualize the bound antibody, producing brown staining of satellite cells (Fig. 1). A light blue staining of the nuclei on the same sections was achieved by brief immersion in Mayer’s hematoxylin (~ 3 s). Satellite cells were counted using light microscopy with high magnification (× 40 objective) based on the following criteria: NCAM-positive staining around the border of the cell, containing a nucleus and located at the periphery of a myofiber. In each section, the numbers of myofibers, NCAM-positive cells and myonuclei were counted. The same person (A. M.) performed all counting and was blinded to the subject’s identity and sample time point until all counting was completed. Counts were made from the whole cross-section (523 ± 297 fibers, mean ± SD; 148–1572, range) excluding any areas of freeze-damaged or longitudinally oriented fibers. At the beginning, NCAM-positive cells were counted twice on the same section but there were no sub-

![Fig. 1. Image of satellite cell staining using neural cell adhesion molecule antibody on m. vastus lateralis cross-section from a healthy elderly individual. Areas of brown Diaminobenzidine staining correspond to areas of antibody binding, and the light blue hue of nuclei is due to hematoxylin staining; × 40 magnification.](image-url)
stantial differences between counts, so a single count was considered reliable. NCAM-positive cell numbers were expressed in two ways: relative to fiber number, or relative to myonuclear number \( \text{[NCAM-positive cells/(myonuclei+}
\text{NCAM-positive cells)] \times 100} \).

**Antibodies**

Double immunohistochemical staining was also carried out to identify fibers expressing (1) neonatal myosin and/or embryonic myosin, and (2) vimentin and/or NCAM. The neonatal and embryonic myosins were investigated as indicators of fibers undergoing regeneration. In addition to staining satellite cells, the NCAM antibody also stains the cytoplasm of fibers that are regenerating or have become denervated (Winter & Bornemann, 1999; Gosztonyi et al., 2001). Vimentin stains fibroblasts and epithelial cells, so capillaries are clearly visible, but the reason for using this antibody was that it also stains the cytoplasm of regenerating fibers (Winter & Bornemann, 1999; Vaarinen et al., 2001). The antibodies used were as follows: neonatal myosin (NCL-MHCn; Novocastra, Newcastle upon Tyne, UK), embryonic myosin (F1.652; Developmental Studies Hybridoma Bank, Iowa City, Iowa, USA), Vimentin (clone V9, category number M0725; Dako-Cytomation Denmark A/S) and NCAM as used for the identification of satellite cells (CD56, category number 347740; Becton Dickinson). After blocking, sections were incubated overnight at 4 °C with the first primary antibody, followed by Alexa Fluor 488 goat anti-mouse secondary antibody (Molecular Probes category number A11029; Invitrogen A/S, Taastrup, Denmark). Before application of the second primary antibody, any remaining free binding sites on the first primary antibody were blocked with goat anti-mouse Fab fragment (category number 115-007-003; Jackson ImmunoResearch Europe Ltd., Cambridgeshire, UK). Visualization of the second primary antibody was achieved by application of Alexa Fluor 568 goat anti-mouse secondary antibody (Molecular Probes category number A11031; Invitrogen A/S). Sections were mounted with Molecular Probes ProLong Gold antifade reagent with DAPI (category number P36935; Invitrogen A/S).

**Fiber typing**

Muscle fiber type was determined from frozen transverse sections stained according to a standard adenosine triphosphatase (ATPase) staining method (Brooke & Kaiser, 1970). Sections were pre-incubated at pH 4.37, 4.55 or 10.30 before incubation in ATP solution. The staining for each fiber was classified as light, intermediate or dark. Type I, IIa, IIx and IIc fibers were identified for each subject pre- and post-training as follows: type I fibers appeared light at pH 10.3 and dark at pH 4.55 and 4.37, while type IIa fibers exhibited a reversed staining pattern. Type IIx fibers were dark at pH 10.3 and 4.55, but light at 4.37. Type IIc fibers were identified as those that showed intermediate staining under all three pH conditions. Measurement of fiber area was aided by immunohistochemical staining of the fiber membrane with a monoclonal mouse anti-dystrophin antibody (dys2; Novocastra Laboratories). Sections were incubated with the primary antibody overnight at 4 °C and visualization of antibody binding was achieved using the same secondary system as described above for satellite cell staining. Analysis was performed using Tumor image analysis software (Scan Beam, Hadsund, Denmark) on images acquired by a JVC color video camera (JVC Ltd., Yokohama, Japan) mounted on a Zeiss AxioLab microscope (Carl Zeiss, Oberkochen, Germany). A mean of 238 fibers (range, 70–456) was analyzed from each biopsy.

**Magnetic resonance imaging (MRI) scanning**

CSA of m. quadriceps femoris was measured by MR scanning before and after the training period as described previously (Kongsgaard et al., 2004), but with scanning carried out at three points on the thigh (10, 20 and 30 cm from the tibia plateau). CSA of lean muscle tissue was calculated from scanning images. The 20-cm point corresponded most closely to the area from which the muscle biopsy was obtained.

**Statistical analysis**

Data were analyzed with SPSS standard version 10.0.5 (SPSS, Chicago, Illinois, USA). Kurtosis and skewness profiles revealed non-normal distribution of data, necessitating the use of non-parametric statistical analyses. The Kruskal–Wallis test was used to compare results for males and females. For comparing pre- and post-training values, the Wilcoxon signed-rank test for matched pairs was applied. Differences were considered statistically significant at P<0.05. Data are presented as means ± standard deviations (SDs).

**Results**

**Muscle characteristics**

The results from force measurements, MRI scans and muscle fiber cross-section analysis are presented in Table 2. Maximum voluntary contractile (MVC) force increased significantly compared with pre-
training values in males (14%) and females (14%); the relative increase was similar between genders. MRI scanning revealed a small increase of approximately 3.5% in quadriceps CSA in response to training. Compared with pre-training values, MRI-determined CSA increased significantly in females at the 10, 20 and 30 cm points measured. The males also showed significant increases with training at 10 and 20 cm, but only showed a tendency at 30 cm. Relative changes with training were not significantly different in males compared with females. However, all absolute values, both pre- and post-training, displayed significant differences between males and females ($P<0.05$).

There were no significant differences between the pre- and post-training biopsies in the percentage of type I, IIa, IIx or IIc fibers, and no significant change in fiber area was observed with training for any fiber type (data not shown). Mean fiber area (MFA) data [(area type I + area type IIa)/2] from the pre- and post-training biopsies were also similar. Spearman's correlation analysis revealed a significant positive relationship between MFA and MRI scanning CSA at the 20 and 30 cm measurement points, both before and after exercise. Correlation coefficients ranged from 0.511 to 0.693 ($P<0.01$) for all correlations.

Positive staining for neonatal myosin was found in four subjects post-training (range 1–3 fibers). There was only one pre-training biopsy where a single positively stained fiber for neonatal myosin was observed. No positive staining for embryonic myosin was observed in any of the biopsies. While blood vessels and satellite cells showed strong vimentin staining, some fibers exhibited a fine network of vimentin staining across the cytoplasm. This was seen in 11 subjects pre-training and 11 subjects post-training, with no relationship between the two time points. Fibers showing cytoplasmic staining for NCAM were present in 24 of the pre-training and 22 of the post-training biopsies. From the double staining for NCAM and vimentin, it was observed that a few fibers stained positive for both (range 1–5); these fibers were observed in nine subjects pre-training and 10 post-training, with no relationship between the two time points. For two subjects, the same neonatal positive fibers identified on an adjacent section were also positive for vimentin and NCAM.

### Satellite cells and myonuclei

The results for satellite cells and myonuclei are presented in Table 3. Pooled data revealed a 27% increase in the number of NCAM-positive cells per fiber over the training period when compared with pre-training values ($P<0.05$). The increase was significant for both males and females (Fig. 2). NCAM-positive cell number as expressed relative to the total number of nuclei increased in males only. Analysis of the relative changes in this variable using the Kruskal–Wallis test revealed a trend toward a larger change in male subjects compared with female subjects ($P = 0.095$). There was no relationship between age and, either satellite cell number pre-training, or the response of satellite cells to training. Myonuclear number per fiber cross-section showed a significant increase post-training for female subjects, but not for males (Table 3). There was no difference between males and females for relative changes in these variables. The number of central myonuclei, i.e. those not in their normal location at the periphery of the muscle fiber, did not change with training (Table 3). However, a significantly higher percentage of fibers with central nuclei were observed pre-training in males compared with females. A similar pattern was observed when the number of central nuclei was expressed relative to total myonuclear...
number. The difference between males and females was not present after training and was largely attributed to two male subjects, in whom 22% and 15% of fibers displayed central nuclei. There was no other sign of pathological abnormality in the biopsies from these subjects.

Discussion

The main finding of this study was a significant increase in the number of satellite cells, identified by NCAM-positive staining, which was found in response to 12 weeks of resistance training for both elderly males and elderly females. To our knowledge, this is the first study to investigate satellite cells in elderly men and women by immunohistochemical means and our results are in agreement with previous studies using electron microscopy (Roth et al., 2001) and immunohistochemistry in younger populations (Kadi & Thornell, 2000; Kadi et al., 2004b). Furthermore, the magnitude of increase in NCAM-positive cells per fiber seen in the current study (27%) is not dissimilar to that reported for young men (31%) in a strength training study by Kadi et al. (2004b). An important new finding in the present study is that females adapt similarly to males in response to resistance training with regard to satellite cell number.

An indication of the effectiveness of the training program in the present study was a 14% improvement in MVC force for both men and women. Furthermore, MR scanning revealed significant increases in quadriceps CSA in both males and females and, not surprisingly, CSA at the three measurement points on the thigh was higher for men when compared with women, both pre- and post-training. MFA as determined from dystrophin-stained biopsy sections also revealed larger myofiber areas for males than females pre- and post-training. There was, however, no change in fiber areas over the training period. This was somewhat surprising given that a significant positive relationship was found for MFA and MR scanning CSA for the 20 and 30 cm scan sites. While determination of fiber areas from biopsy sections is widely used as a reliable assessment of hypertrophy, increasing variation in fiber area with age has also been reported (Lexell & Taylor, 1991), and it cannot be excluded that this contributed to our findings. One important difference between these two variables is the calculation of CSA for the whole quadriceps muscle group in the case of MR scanning compared with a biopsy sample from the m. vastus lateralis portion of the quadriceps, from which MFA is calculated. It has been shown that differences exist in the recruitment and activity patterns for the different portions of the quadriceps muscle, in particular, the rectus femoris muscle (Weidman et al., 1991; Richardson et al., 1998; Krustrup et al., 2004). It is therefore possible that the CSA data obtained from MR scans in the present study are reflective of hypertrophy in other portions of the quadriceps muscle as well as the vastus lateralis. Furthermore, the change in CSA as determined from MR scanning in the present study was small (~ 3.5%) and it is possible that, for the participants in this study, 12 weeks were not long enough to observe marked hypertrophy at the light microscopy level. The increase in MVC force, together with the MR data,
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nonetheless provides evidence that the training pro-
gram used in the present study was effective in
improving quadriceps strength, concomitant with
hypertrophy.

In addition to examining the number of NCAM-
positive cells expressed per fiber, the number of
NCAM-positive cells relative to myonuclear number
was also analyzed. The percentage change between
pre- and post-training biopsies in this variable dis-
played a trend toward a larger change in men
compared with women. The lack of change in the
relative number of NCAM-positive cells in female
subjects is explained by the fact that while the
number of NCAM-positive cells per fiber increased,
so too did the myonuclear number (Table 3). Like-
wise, the significant change in the relative number
of NCAM-positive cells in male subjects is reflective
of a combination of the increase in NCAM-positive
cells per fiber and the similar numbers of myonuclei
before and after training. The study by Roth et al.
(2001) only provides details of changes in satellite cell
number expressed in this way, i.e. relative to the
number of myonuclei, where they reported signifi-
cant increases in the satellite cell proportion with
resistance training in old men and women. Further-
more, comparison between our data and theirs is
difficult given the different methods of analysis, i.e.
counting satellite cells from NCAM-immunohisto-
chemical stained muscle cross-sections in our case,
compared with analysis of longitudinally oriented
fibers using electron microscopy (Roth et al., 2001).
Despite this, the results from the present study are in
general agreement with those reported by Roth et al.
(2001), suggesting that satellite cells in the elderly
have the capacity to respond in a manner not unlike
that observed in younger adults, at least when
evaluated from more long-term training studies
(Roth et al., 2001; Charifi et al., 2003). With respect
to acute exercise, the number of NCAM-positive cells
has been reported to increase by twice as much in
young men compared with old men as early as 24 h
after a single bout of exercise (Dreyer et al., 2005,
unpublished, abstract presented at XXXV Inter-
national Congress of Physiological Sciences, San
Diego, California, USA). Later time points were
not examined, however, so it is not possible to say
whether satellite cells in the elderly do not have the
capacity to respond to this type of exercise at the
same magnitude as younger individuals or whether it
simply reflects a slower time response in the elderly.
It has been reported that, compared with young men,
elderly men have an attenuated response of me-
chano-growth factor (MGF) production with acute
exercise (Hameed et al., 2003). As it has been
suggested that MGF is involved in satellite cell
proliferation, at least in C2/C12 cells (Yang & Gold-
spink, 2002), a blunted response of this growth factor
could partly explain differences in satellite cell re-
sponses in young and elderly individuals. The similar
enhancement of the pool of NCAM-positive cells in
the elderly participants in the present study com-
pared with that of younger men (Kadi et al., 2004b)
suggests that with prolonged and repeated exposure
to resistance training, skeletal muscle in the elderly
develops the capacity to activate satellite cells on a
level similar to younger adults.

Why would satellite cell number increase? Satellite
cells are classically associated with three main roles,
outlined in Fig. 3: (1) to generate new fibers in the
case of myofiber development or to repair damaged
segments of mature myofibers in response to injury,
(2) to contribute new myonuclei to the existing
muscle fibers as they undergo hypertrophy and (3)
to generate new daughter cells in order to maintain
or add to the satellite cell pool (Schultz & McCor-
mick, 1994; Hawke & Garry, 2001). In the study
presented here, new fiber formation was investigated
by immunostaining for neonatal and embryonic
myosins, recognized markers of developing fibers
(Ecob-Prince et al., 1989; Cho et al., 1993; Kadi &
Thornell, 1999). The neonatal staining provided
evidence of new fiber formation in only four subjects
following the training period, suggesting that the fate
of the new satellite cells was probably not to form
new fibers. No positive staining for embryonic myo-
sin was observed in any of the biopsies. It should be
noted, however, that is not clear how long the
neonatal or embryonic myosins persists in newly
formed developing or regenerated myofibers, so it is
possible that many more fibers experienced regeneration
during the 12-week training period. Further-
more, it is important to realize that neonatal and
embryonic myosins have also been shown to be
present in muscle cytoplasm following denervation
(Schiaffino et al., 1988), suggesting caution when
interpreting staining of the developmental myosin
phenotypes. Cytoplasmic staining for vimentin and
NCAM was also assessed, as these have also been
proposed as useful markers for regenerating fibers
(Winter & Bornemann, 1999). The finding of some
fibers that were positive for vimentin, NCAM and
neonatal myosin supports this theory; however, there
were many fibers positive for NCAM or vimentin
alone. In two subjects, a high percentage of fibers
(6.5% and 2.9%) with a fine network-like pattern of
vimentin staining across the cytoplasm was observed.
ATPase staining of sections cut in close proximity
revealed that these were type II fibers, which ap-
ppeared to be atrophic from their relatively small size
and angular shape. Similar to the early myosins,
cytoplasmic NCAM staining was also expressed in
denervated fibers as well as during regeneration
(Winter & Bornemann, 1999; Gosztonyi et al.,
2001), and it is thus possible that vimentin, along
with other markers usually associated with development or regeneration, may also appear transiently in response to fiber denervation or degeneration. It is therefore difficult to draw any firm conclusions from the NCAM, vimentin and neonatal staining in the present study with regard to the proportion of satellite cells involved in repairing damaged myofibers.

An important factor to consider with regard to satellite cells and exercise is muscle damage. Greater proliferation of NCAM-positive cells has recently been reported following a single bout of maximal eccentric exercise induced by electrical stimulation compared with voluntary muscle contraction (Cramer et al., 2004a). In this study, there was evidence of myofiber necrosis in the muscle from the electrically stimulated leg, but not in the voluntary leg, suggesting more satellite cell proliferation in the presence of muscle damage. It could be hypothesized, thus, that with regular training such as in a strength training program, susceptibility to myofiber damage (Ploutz-Snyder et al., 2001; Kim et al., 2005), and consequently “unnecessary” satellite cell activity, is reduced. Investigation into the effects of long-term training on satellite cells, however, has revealed a 70% higher content of NCAM-positive cells in the trapezius muscle of high-level power lifters who had adhered to strength training for several years, when compared with control subjects (Kadi et al., 1999). This suggests that the response of satellite cells to training is heightened further with more long-term training, such as in the present study, and does not appear to become more “efficient.” Unlike the use of electron microscopy, the use of an anti-NCAM antibody to identify satellite cells does not allow determination of their activation status. It is also important to consider that anti-NCAM antibodies may not label all satellite cells and it is possible that the activation status of satellite cells influences the staining pattern. Nonetheless, the data presented here suggest that satellite cells were induced to proliferate in response to training and some of the new daughter cells have been prevented from differentiating and remain as satellite cells. Alternatively, it is possible that two different populations of satellite cells exist, as suggested by Vaittinen et al. (2001), and that only one of these was activated with the training in the present study, resulting in the observed proliferation.

The finding of a difference in the number of centrally located myonuclei, i.e. those nuclei not in the usual location at the periphery of the myofiber, between men and women in the pre-training biopsy is in accordance with a similar finding by Roth et al. (2001). Furthermore, in line with the present study, Roth et al. (2001) did not find any change in the proportion of central nuclei with training. The presence of central nuclei is traditionally recognized as an indication of regeneration in response to muscle damage, at least in rodent muscle (Carlson & Faulkner, 1983; Carlson et al., 2001), whereas fusion of satellite cells with a mature hypertrophying myofiber does not result in central nuclei. A difference between genders in the number of fibers with central nuclei was present only in the pre-training biopsy in our study, suggesting that no damage was sustained by the muscle as a result of the training but may instead be reflective of previous muscle damage – repair cycles. It has been suggested, at least from animal studies, that females are more resistant to exercise-induced muscle damage than males (Amelink & Bar, 1986; Komulainen et al., 1999), a difference that has been attributed to the influence of estrogen (Tidus, 2005). Evidence in humans, however, is sparse and contradictory (Rinard et al., 2000; Stupka et al., 2000; Clarkson & Hubal, 2001; Tidus, 2005). A higher resistance to muscle fatigue has been reported for females compared with males (Clark et al., 2005), which could also serve to protect women from muscle damage compared with men performing the same exercise task. In skeletal muscle of elderly women, however, a higher occurrence of ultrastructural muscle damage has been reported following strength training when compared with elderly men and younger men and women (Roth et al., 1999, 2000b), suggesting greater susceptibility to exercise-induced damage in this population. No change in the number of centrally located myonuclei was observed with training, however (Roth et al., 2001), possibly because either the timing of the last biopsy was too early for regeneration of damaged areas to be completed, or the presence of centrally located nuclei is not an accurate indicator of regeneration of damaged fibers in elderly individuals. It is possible that the greater proportion of fibers with central nuclei observed in the male subjects in the present study is an indication of more frequent experience of muscle damage in the past. A detailed training history of the subjects was unfortunately not recorded, rendering it difficult to draw any firm conclusions regarding our observations on central nuclei in this study.

In summary, the main finding of the present study was a significant increase in the number of NCAM-positively stained cells in skeletal muscle in both elderly men and women in response to a period of regular resistance training. This suggests that this type of training can successfully induce proliferation of satellite cells in elderly individuals and, furthermore, that men and women adapt similarly with regard to the response of satellite cells to resistance training. The purpose of the new satellite cells was unclear, but it can be hypothesized that proliferation of satellite cells is a normal physiological response to exercise in order to prepare the muscle for adaptation.
in the case of further stimulus, and that where this stimulus is absent, numbers of satellite cells eventually return to resting levels.

**Perspectives**

The potential for exercise to alleviate the adverse effects of aging on skeletal muscle is a growing area of interest due to implications for the elderly in terms of functional capacity in daily life. Satellite cells are a valuable resource for muscle maintenance and regeneration and there is evidence for the attenuating effects of aging on satellite cell numbers, prompting many questions about the regenerative potential of skeletal muscle in the elderly and the possible role of satellite cells in alleviating the rate of progression of sarcopenia. In the present study, the proliferative capacity of satellite cells, as identified by NCAM-positive staining, in elderly individuals in response to resistance training is shown to be similar to previous reports for younger populations (Kadi et al., 2004b).

This is encouraging in the context of the age-related decline in muscle mass and function, demonstrating that despite reduced muscle mass and reduced numbers of satellite cells, the remaining satellite cells can be induced to proliferate. Furthermore, we report here that males and females adapt similarly in response to this type of training with regard to satellite cells. Further work is required to determine the downstream effects of satellite cell proliferation on muscle function.

**Keywords:** satellite cells, NCAM, aging, gender, resistance training.

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