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ORIGINAL ARTICLE

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The effect of strength training on estimates of mitochondrial density and distribution throughout muscle fibres

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Abstract The purpose of this study was to investigate the effect of strength training (12 weeks, 3 days/week, four lower-body exercises) of young individuals (mean age 23.6 years) on estimates of mitochondrial distribution throughout muscle fibres. A control group (mean age 21.7 years) was followed simultaneously. Skeletal muscle biopsy samples were obtained from the vastus lateralis, pre- and post-training. The regional distribution of subsarcolemmal and intermyofibrillar mitochondrial populations was determined using quantitative histochemical staining of succinate dehydrogenase (SDH) in type I and II muscle fibres. Strength training resulted in significant increases of 26% and 28% in the cross-sectional area of type I and II fibres, respectively ($P < 0.05$). Overall SDH activity decreased by 13% with strength training ($P < 0.05$). The decrease in SDH activity with strength training between fibre types and between subsarcolemmal and intermyofibrillar regions of muscle fibres was not different. Fibre area and SDH activity was unchanged in the control group. We conclude that the muscle hypertrophy associated with strength training results in reduced density of regionally distributed mitochondria, as indicated by the reduction in the activity of SDH.

Key words Subsarcolemmal mitochondria · Intermyofibrillar mitochondria · Succinate dehydrogenase · Exercise

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Introduction

Strength training that results in muscle hypertrophy may have a negative effect on muscle oxidative capacity. Using electron microscopy combined with stereological procedures, it has been shown that the volume density of mitochondria (defined as the volume of mitochondria per unit volume of muscle fibre) decreases as muscle fibres hypertrophy (MacDougall et al. 1979; Luthi et al. 1986). Mitochondrial number remains the same with strength training, but mitochondria are "diluted" as the muscle fibres enlarge. This is reflected in a reduced activity of muscle oxidative enzymes, when enzyme activity is expressed relative to protein content (Tesch et al. 1987).

Although it has been established that mitochondrial volume density is reduced with strength training, little is known about the effects on different populations of mitochondria within muscle fibres. Two populations of mitochondria with different metabolic properties have been identified within muscle fibres. One population is located just beneath the muscle fibre sarcolemma [subsarcolemmal (SS) mitochondria] and the other towards the centre of the muscle fibre [intermyofibrillar (IMF) mitochondria]. SS and IMF mitochondria are thought to have different functional roles since they differ in properties such as oxidative enzyme activities (Krieger et al. 1980; Elander et al. 1985; Martin 1987; Cogswell et al. 1993) and in vitro respiratory rates (Krieger et al. 1980; Cogswell et al. 1993). These different functional roles may help to explain their different rates of adaptation to stressors such as aerobic exercise training (Howald et al. 1985; Chilibeck et al. 1998) and muscle unweighting (Bell et al. 1992). To our knowledge, no study has investigated the effects of strength training on these two different mitochondrial populations.

The purpose of this study was to investigate the effects of strength training on measures of mitochondrial distribution throughout the SS and IMF regions of

muscle fibres. In the present study we used measures of succinate dehydrogenase (SDH) activity throughout the SS and IMF regions of muscle fibres to quantify the populations of mitochondria in these regions (Swatland 1985; Bell et al. 1992; Martin and Edgerton 1992; Chilibeck et al. 1998). It was hypothesized that strength training may result in differential effects on the different mitochondrial subpopulations.

Methods

Subjects

Fifteen subjects gave written informed consent to participate in this study, which was approved by the Faculty of Physical Education and Recreation Research Ethics Committee at the University of Alberta. Subjects were randomly assigned to a strength-training group (five males, three females) and a control group (four females, three males). The strength-training group had a mean (SD) age of 23.6 (2.9) years, a body mass of 73 (2.1) kg, and a height of 177.6 (9.1) cm, and the control group had a mean (SD) age of 21.7 (3.8) years, body mass of 72.6 (12.0) kg, and a height of 173.7 (8.7) cm.

Training

Subjects in the strength-training group trained for 3 days per week for 12 weeks. Training sessions included four lower-body exercises (double leg incline press, single leg knee flexion and extension and double leg calf raises). The intensity and volume of the strength training was monitored and progressively overloaded. The training intensity was increased by approximately 4% every 3 weeks (from a mean of 72% to 84% of the pre-training one-repetition maximum, 1 RM) and the number of sets and repetitions ranged from 2 to 6 and 4 to 12, respectively. The strength-training periodization was accomplished using a computer software package (B.E. Software, Lincoln, Nebraska, U.S.A.).

Strength testing

Strength was assessed with a voluntary 1 RM test for bilateral incline leg press (90-degree knee joint range of motion at a 45-degree incline) and dominant leg unilateral knee extension (90-degree knee joint range of motion). The subjects performed a 5-min warm-up on a cycle ergometer followed by a light set of ten repetitions of the strength exercise to be tested. A second set of eight repetitions was performed at a higher load (approximately 70% of the 1 RM). The subjects then completed two repetition sets of increasing loads, as decided by the judgement of the investigators, until only one repetition could be performed. This required between three and five sets of one to two repetitions. Three minutes of rest was provided between sets and consistent verbal encouragement was given to all subjects.

Muscle biopsy sampling

Skeletal muscle biopsy sampling which was adapted for suction (Evans et al. 1982), was carried out with the subject at rest. Tissue was taken from the lateral aspect of the right quadriceps muscle, before and after the 12-week training program. The tissue was quickly mounted on cork in O.C.T. embedding compound, placed in isopentane cooled to near freezing in liquid nitrogen, and then stored at −70°C for later analyses. Sections were cut from the tissue at a thickness of 6 μm at −20°C, using a Tissue-Tek Cryostat (Miles Laboratories), and then mounted on glass slides and coverslipped. Type I and II fibres were identified after staining sections for myofibrillar ATPase activity using an alkaline incubation at pH 8.6 (Martin et al. 1992). Subsequent serial sections were then stained for SDH activity using the assay described by Martin et al. (1985). Briefly, SDH activity was determined in a medium containing 100 mM phosphate buffer (pH 7.6), 0.75 mM sodium azide, 1 mM 1-methoxyphenazine methylsulphate, 1.5 mM nitro blue tetrazolium, 5.5 mM ethylene diaminetetraacetic acid-disodium salt, and 48 mM succinic acid. The reaction was run at room temperature for 6 min and stopped with repeated washes with distilled water. Sections were then air dried and mounted. For each assay, sections were incubated in medium containing substrate, and serial sections were incubated in identical medium without substrate. These latter sections were used as tissue blanks to correct for non-specific staining that occurred during the reaction. Staining for each individual's pre- and post-training muscle biopsy samples was done within the same assay to decrease inter-assay variability. The regional distribution of SDH activity was determined in sequential circumferential concentric layers of 1 pixel width (0.75 μm) starting from the fibre edge and ending in the centre of the fibre in 16–20 type I and type II fibres from each tissue section, using a PSI-COM 232 computer-assisted image analysis system (Perceptive Systems, League City, Tex., USA). A detailed description of this procedure has been published elsewhere (Castleman et al. 1984; Bell et al. 1992; Martin and Edgerton 1992). Briefly, each pixel was digitized and then converted into an optical density (OD). The difference between the OD due to enzyme-specific staining versus non-specific staining (blanks) was divided by the reaction time to give a mean reaction rate (OD/min) per pixel. The mean SDH activity for SS and IMF regions of individuals fibres was then determined from the pixels spanning these areas. SS and IMF regions of muscle fibres were defined as <5.25 μm and >6.00 μm, respectively, from the fibre edge, and were determined to represent these regions based on previous electron microscope observations of human muscle fibres (Hoppeler et al. 1973, 1985). Previous research in our laboratory has used this technique to indicate SS and IMF regions (Bell et al. 1992; Martin and Edgerton 1992; Chilibeck et al. 1998). For identification of type II fibre subtypes, muscle cross-sections [an average of 235 (78) fibres] were later stained for myosin ATPase activity, with preincubation at pH 4.3, 4.55, and 10.4 (Brooke and Kaiser 1970).

Statistical analyses

Differences in SDH activity between SS and IMF regions; type I and II fibres; trained and control groups; and before and after training were analysed by a four-factor analysis of variance (ANOVA), with one between-groups factor (strength vs control group) and three within-subject factors for time (pre- vs post-training), fibre type (type I vs type II), and region of distribution within fibres (SS vs IMF). Changes in strength measures, muscle fibre cross-sectional area, and fibre type composition were determined with a two-factor ANOVA, with one between-groups factor (strength vs control group) and one within-groups factor for time (before vs after training). Tukey post-hoc tests were performed to determine differences between individual means where main effects were found. All values are expressed as means (SD). The level of statistical significance was set at $P < 0.05$.

Results

The group × time interactions for leg press and knee extension 1-RM strength were significant ($P < 0.05$). The leg press strength of the strength-trained group increased by 53%, from 230 (96) to 352 (113) kg, while that of the control group increased by 21%, from 199 (97) to 241 (100) kg. The knee extension strength of the strength-trained group increased by 34%, from 32 (14) to 43 (14) kg, while that of the control group

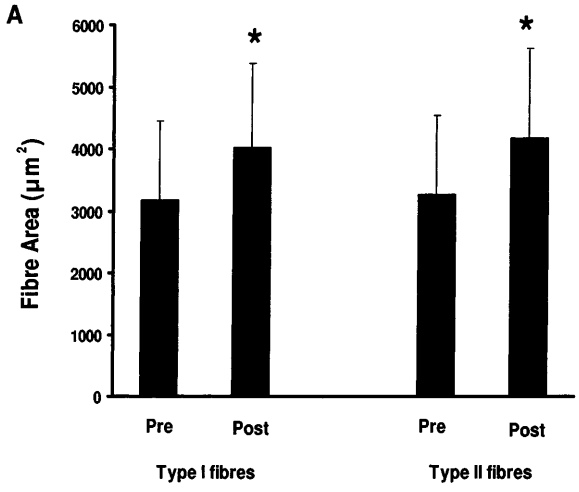


Fig. 1 Muscle fibre cross-sectional areas pre- (*Pre*) and post-training (*Post*) for strength-trained (**A**) and control (**B**) groups. Values are means ± SD. *Significantly different from pre-training mean ($P < 0.05$)

decreased by 17%, from 30 (12) to 25 (9) kg. Post-hoc analysis indicated that the increase in strength over the training program was significant for both lifts in the strength-trained group ($P < 0.05$).

Changes in muscle fibre cross-sectional area are shown in Fig. 1a for the strength-trained group and Fig. 1b for the control group. There was a significant group × time interaction for both type I and type II fibre types ($P < 0.05$), with the fibres areas of the strength-trained group increasing (by 26% and 28% for type I and II fibres, respectively), and the fibre areas of the control groups remaining relatively unchanged (+3% for both type I and II fibres). Post-hoc analyses indicated that the changes in the trained group for both fibre types were significant pre- to post-training ($P < 0.05$).

Examples of muscle fibre cross-sections, stained for SDH activity, are shown in Fig. 2. There was a significant group × time interaction for SDH activity ($P < 0.05$). Post-hoc analysis indicated that muscle SDH activity was significantly reduced after strength training ($P < 0.05$; Fig. 3a). There were no changes in SDH activity in the control group (Fig. 3b). Although strength-training resulted in decreases in overall SDH activity, the effects of training were not different between type I and II fibre types or between the SS and IMF

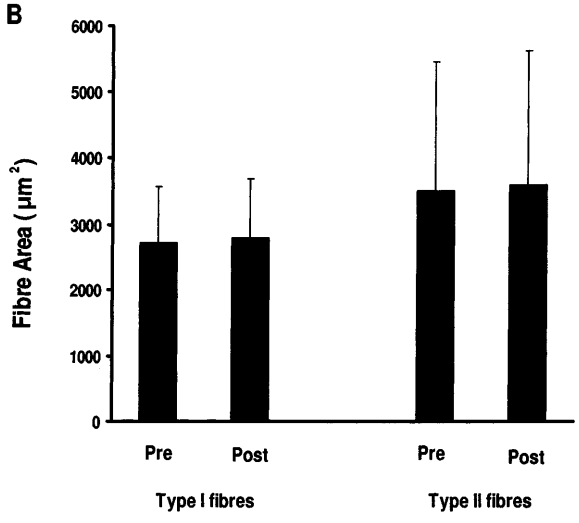
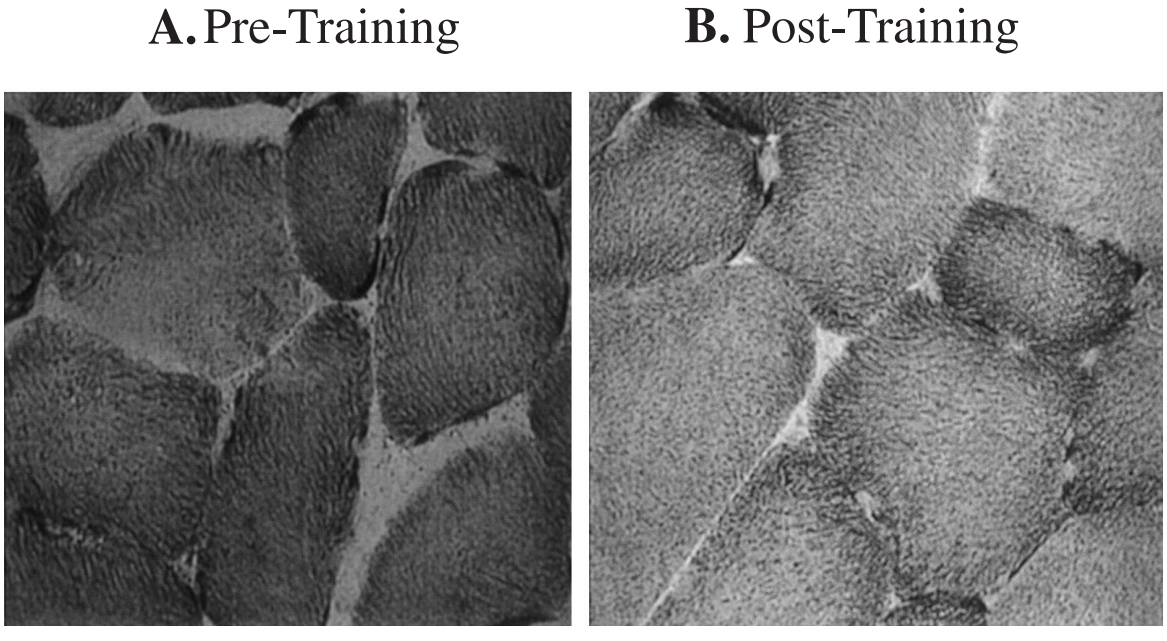


Fig. 2 Muscle fibre cross-sections stained for succinate dehydrogenase activity before (**A**, Pre-training) and after strength training (**B**, Post-training)



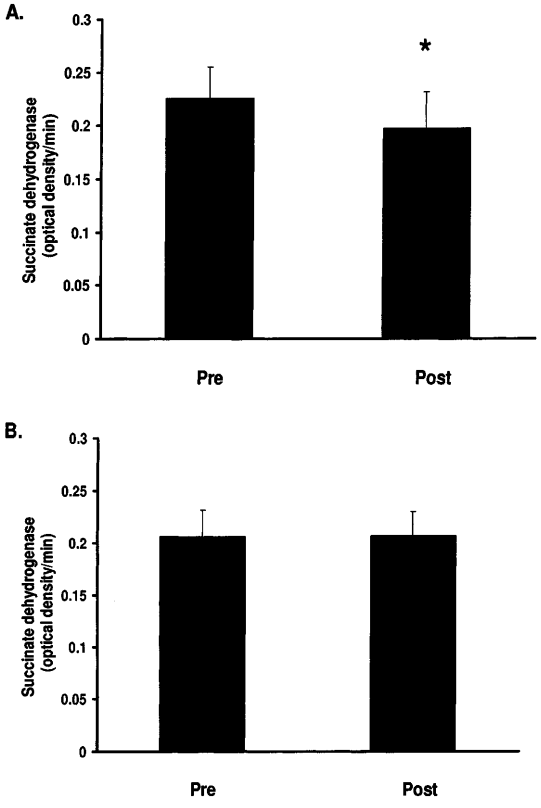


Fig. 3 Succinate dehydrogenase activity pre- and post-training for strength-trained (**A**) and control (**B**) groups. Values are means ± SD. *Significantly different from pre-training mean ($P < 0.05$)

regions of muscle fibres. Table 1 shows the optical density values for SDH activity in the SS and IMF regions of type I and II fibres and the percentage changes after 12 weeks for both groups.

There were no changes in fibre-type composition pre- to post-training in either the strength-trained or control groups. For the strength-trained group, the percentage of fibres classified as type I, IIa, and IIb pre-training was 47 (10)%, 40 (9)%, and 13 (8)%, respectively, compared to post-training values of 53 (6)%, 36 (6)%, and 11 (9)%, respectively. For the control group, the percentage of fibres classified as type I, IIa, and IIb pre-training was 49 (7)%, 38 (7)%, and 13 (5)%, respectively, compared to post-training values of 43 (8)%, 45 (8)%, and 13 (3)%, respectively.

Discussion

We have previously used quantitative histochemical techniques to track changes in mitochondrial distribution based on the regional distribution of SDH staining density in single muscle fibres after endurance training (Chilibeck et al. 1998). The present study is the first to use these techniques to assess changes with strength training. Quantitative histochemical techniques have been shown to reflect accurately biochemical measures of oxidative enzyme activity, as shown by the high correlations that exist between the two types of measurements (Martin et al. 1985). In addition, the technique has allowed us to differentiate between SS and IMF mitochondrial populations and to show independent adaptation due to physical training (Chilibeck et al. 1998) and space flight (Bell et al. 1992).

Table 1 Succinate dehydrogenase activity (optical density/min) of subsarcolemmal and intermyofibrillar regions of type I and II muscle fibres. All values are given as the means (SD)

Muscle fibre region	Pre-training	Post-training	Change (%)
Type I subsarcolemmal			
Strength-trained	0.256 (0.056)	0.215 (0.035)	−16
Control	0.224 (0.039)	0.218 (0.034)	−2.7
Type I intermyofibrillar			
Strength-trained	0.221 (0.039)	0.195 (0.036)	−11.8
Control	0.207 (0.026)	0.202 (0.026)	−2.4
Type II subsarcolemmal			
Strength-trained	0.221 (0.054)	0.194 (0.048)	−12.2
Control	0.200 (0.032)	0.207 (0.030)	+3.5
Type II intermyofibrillar			
Strength-trained	0.201 (0.037)	0.182 (0.038)	−9.5
Control	0.194 (0.022)	0.199 (0.024)	+2.7

Our results indicate that strength training decreases the activity of SDH throughout the SS and IMF regions of both type I and type II muscle fibres. With an increase in fibre cross-sectional area of 26–28% and a decrease in SDH activity of 9–16%, training appears to have stimulated muscle fibre hypertrophy to a greater extent than mitochondrial proliferation, resulting in reduced SDH activity relative to the area of the muscle fibre. The assumption that SDH activity reflects changes in mitochondrial volume density is based on several lines of evidence. First, as pointed out by Hoppeler (1986), if nearly identical exercise protocols with similar gains in aerobic capacity are compared, it is found that the magnitude of changes in SDH (Henriksson and Reitman 1977) is identical to the changes in volume density of mitochondria (Hoppeler et al. 1985; Rosler et al. 1985). In response to endurance training, changes in SDH are greater in the SS compared to IMF regions of muscle fibres (Chilibeck et al. 1998), similar to the pattern of change for mitochondrial volume density (Howald et al. 1985). Secondly, in human muscle, the ratio of mitochondrial volume density between type I and type II fibres (Sjostrom et al. 1982) is the same as the ratio of SDH activity between type I and II fibres (Essen et al. 1975). Finally, both SDH and mitochondrial volume density change with the same time course in response to chronic electrical stimulation (Reichmann et al. 1985).

To our knowledge, this is the first strength-training study to demonstrate effects on mitochondria in SS and IMF regions. Others have used stereological techniques (MacDougall et al. 1979; Luthi et al. 1986) or measures of oxidative enzymes (Tesch 1987) to show that overall mitochondrial volume density is reduced with strength

training. Our results indicate an equivalent dilution of mitochondria in all regions of the muscle fibre, since changes over time between the SS and IMF regions were similar.

The present study also demonstrated that the effect of strength training on mitochondria did not differ between type I and type II fibre populations. This is in agreement with cross-sectional studies, where it has been shown that strength-trained athletes have a lower mitochondrial volume density in type I (Alway et al. 1988, 1991) and type II (Alway et al. 1988) muscle fibres compared to inactive controls. One longitudinal study showed that the volume density of mitochondria in the soleus muscle was lower in type I fibres only of legs that had been trained isometrically at 100% maximal voluntary contraction compared to legs trained at 30% maximal voluntary contraction (Alway 1991). In the present study, the equal degrees of hypertrophy and dilution of SDH activity in both fibre types indicate that the training program was of an intensity that involved substantial recruitment of both type I and II fibre populations (Sale 1987).

The finding that mitochondrial volume density is reduced with fibre hypertrophy is supported by animal studies in which the surgical elimination of synergists has been used to induce muscle hypertrophy (Baldwin et al. 1977, 1981; Chalmers et al. 1992; Stone et al. 1996). Although the stress imposed on the muscle in this situation is completely different than in weight-training (a continuously applied versus intermittently applied mechanical stress) (Baldwin et al. 1981), these tightly controlled animal studies show that muscle fibre size can increase without a concomitant increase in mitochondrial volume. Chalmers et al. (1992) used SDH staining, similar to that used in the present study, of cat plantaris muscle that was surgically overloaded and induced to hypertrophy. Both type I and II fibres showed substantial increases in area, and SDH activity in both SS and IMF regions was significantly reduced. They concluded that "this represents a disproportionate increase in fibre volume relative to mitochondrial volume" (Chalmers et al. 1992). This is in agreement with the findings of the present study, with hypertrophy induced by weight training in humans.

The reduction in mitochondrial volume density with strength training may also be related to an increase in other components of muscle fibres, with a resultant dilution of the mitochondria. A reduced distribution of mitochondria in the centre of muscle fibres (IMF) has been related to an increased glycogen content in this area, described as an "anaerobic core" (Swatland 1984). Strength training results in increased glycogen stores within muscle fibres (MacDougall et al. 1977). This may contribute, at least in part, to a dilution of the mitochondria, especially in the IMF region, with strength training.

In summary, we have shown that SDH activity throughout muscle fibres is reduced with strength training, implying that the mitochondrial volume density is reduced across all regions of the muscle fibre. This may have implications for the supply of energy from mitochondria to the sites of energy utilization in different regions of muscle fibres.

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