Adaptations in Capillarization and Citrate Synthase Activity in Response to Endurance Training in Older and Young Men

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The time-course of adaptation in cardiorespiratory fitness, measures of capillarization, and citrate synthase (CS) activity were examined in seven older (O; 69 ± 7 years) and seven young (Y; 22 ± 1 years) men pre-, mid-, and posttraining during a 12-week endurance training program. Training was performed on a cycle ergometer three times per week for 45 minutes at ~70% of maximal VO$_{2\text{max}}$ (VO$_{2\text{max}}$ and maximal cardiac output increased similarly from pre- to posttraining in O and Y (p < .05), and maximal a-vO$_{2\text{diff}}$ was greater (p < .05) posttraining in O and Y. CS was elevated at mid- and posttraining compared with pretraining in both O and Y (p < .05). Indices of capillarization increased 30%–40% in O and 20%–30% in Y and were elevated at posttraining compared with pre- and midtraining in both groups (p < .05). This study showed that both O and Y undertaking similar endurance training displayed capillary angiogenesis and improved mitochondrial respiratory capacity.

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MAXIMAL O$_2$ uptake (VO$_{2\text{max}}$) declines progressively in older individuals (1–4). Although maximal aerobic power is unlikely to limit normal activities of daily living in healthy young adults, the age-associated decline in VO$_{2\text{max}}$ has been shown to impact mobility and functional abilities of daily life and is linked to decreased quality of life and loss of independence in the older population (2,5).

Endurance training has been shown to increase VO$_{2\text{max}}$ in both older (6–8) and young (9,9) men. Although limitations to further increase maximal cardiac output (Q$_{\text{max}}$) are often postulated as the main factor constraining additional increases in VO$_{2\text{max}}$ with exercise training (10), distribution of blood flow to the exercising muscle as well as the capacity of the “aerobic machinery” for utilizing the O$_2$ delivered to generate ATP are important factors regulating the adjustment of whole-body VO$_2$. Approximately 2/3 of the increase in VO$_{2\text{max}}$ with training in older and young men has been demonstrated to be related to increments in Q$_{\text{max}}$ with the remaining 1/3 of the adaptation explained by a widened maximal arterial–venous O$_2$ difference (a-vO$_{2\text{diff}}$) (7,8). In terms of peripheral mechanisms, early studies (11–13) suggested that older adults may not adapt with increases in mitochondrial respiratory enzyme levels or muscle capillarization. Nonetheless, this may have been due to the “low intensity” of training because subsequent studies have shown improvements in the muscle capillary supply as well as oxidative enzymes activity in response to endurance training in older (14–18) and young men (18–20). However, direct comparison of the adaptations of older and young men undertaking a similar exercise training program are limited, and no studies to date have examined the time-course or whether the rate or magnitude of training-induced adaptations for capillarization and oxidative enzymes activity might differ with age. This is important because it is often assumed that older adults take longer to adapt to a given stimulus; therefore, training programs have frequently started at low levels and progressed slowly with the duration of a training intervention presumed to require longer than in young (21). Additionally, knowledge of the time-course of adaptations (eg, central vs peripheral) may allow endurance training interventions to appropriately target specific mechanisms of adaptation throughout the exercise training program.

Therefore, the main goal of this study was to examine the changes from pre- to mid- and posttraining in measures of capillarization and citrate synthase (CS) activity in response to a 12-week endurance training program in older (O) and young (Y) male adults. Based on previous studies reporting a similar widening of the maximal arterial–venous O$_2$
difference during the time-course of a 12-week endurance training intervention in older and young men (8), we hypothesized that both older and young individuals would show improvements in measures of capillarization and CS activity throughout the endurance training program.

METHODS

Subjects

Seven O (69 ± 7 years; mean ± SD) and seven Y (22 ± 1 years) men volunteered and gave written consent to participate in the study. Participants in this study were a subset of subjects from a previous study (8) who agreed to have muscle samples obtained from their vastus lateralis muscle. All procedures were approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects. All participants were nonobese (body mass index ≤ 30 kg/m²), nonsmokers, and were physically active (both age groups in this study had baseline VO₂max above the mean predicted for age-matched populations [22, 23]) but none had been involved in any type of endurance training program for at least 12 months prior to the study. Additionally, no participants were treated for hypertension or high cholesterol and no participants were taking medications that would affect the cardiorespiratory or hemodynamic responses to exercise. Older participants had no history of cardiovascular, respiratory, or musculoskeletal diseases were medically screened by a physician and underwent a maximal exercise stress test.

Protocol

Before the commencement of training, participants performed a maximal cycle ergometer ramp test to exhaustion (O 15–20 W/min; Y 25 W/min; on a Lode Corival 400 cycle ergometer; Lode B.V., Groningen, Holland) for determination of VO₂max. Within 5 minutes after completion of this test, participants performed a constant-load cycling exercise to volitional fatigue at 85% of the peak power output (POpeak) achieved during the ramp incremental test. This protocol [described in (24)] was implemented to confirm the attainment of VO₂max and to determine Qmax. Participants were instructed to indicate when they thought they were ∼30 seconds from exhaustion. At that point, verbal encouragement increased and within ∼15 seconds the measurement of Q was performed. VO₂max was defined as the highest VO₂ observed for an average of 20 consecutive seconds during either the ramp test to exhaustion or the 2- to 3-minute constant load at 85% of POpeak. Similar procedures were repeated after 3, 6, 9, and 12 weeks of training.

Training

The endurance training program consisted of three exercise sessions per week on a stationary cycle ergometer (Monark Ergomedic 874E; Monark Exercise AB, Varberg, Sweden) for a total duration of 12 weeks. Training work rate was adjusted based on the results obtained during the latest incremental ramp test to reflect changes in fitness level as assessed by changes in the work rate associated with changes in VO₂max. Each session consisted of continuous training for 45 minutes at a power output that elicited ∼70% of the VO₂max observed during the latest incremental ramp test. During the testing weeks (that included two testing sessions and a muscle biopsy performed on separate days), participants were asked to complete at least one training session.

Measurements

Gas exchange measurements were similar to those previously described (25). Briefly, inspired and expired flow rates were measured using a low dead space (90 mL) bidirectional turbine (Alpha Technologies VMM 110), which was calibrated prior to each test with a syringe of known volume. Inspired and expired gases were sampled continuously (every 20 ms) at the mouth and analyzed for concentrations of O₂, CO₂, nitrogen (N₂), acetylene (C₂H₂), and helium (He) by mass spectrometry (Perkin Elmer MGA-1100) after calibration with precision-analyzed gas mixtures. Breath-by-breath alveolar gas exchange was calculated by using algorithms of Beaver and colleagues (26).

Heart rate was monitored continuously by electrocardiogram using PowerLab (ML132/ML880; ADInstruments, Colorado Springs, CO) with a three-lead arrangement. Data were recorded using LabChart v4.2 (ADInstruments) on a separate computer.

Q Measurements

Q was measured using the acetylene (C₂H₂) open circuit inert gas wash-in method and analyzed using custom data acquisition software. This technique was described and validated previously (27). Briefly, a pneumotachograph (Hans Rudolph Model 3800, Kansas City, MO; transducer, Validyne MP45-871, Northridge, CA) was attached to a non-rebreathing Y valve (Hans Rudolph 7900), which was connected to a manual valve that allowed switching inspired gases between room air and a bag containing a mixture of C₂H₂ (0.7%), O₂ (21%), He (9%), and balance N₂. Changes in gas concentrations were aligned with gas volumes by measuring the time delay for fractional changes of the gases to occur. Throughout the submaximal and peak exercise measurements of Q, participants were asked to continue their normal breathing pattern when the source of inspired air was switched to the bag containing the gas mixture, and after 10 breaths, the protocol was terminated. Data analysis for the calculation of Q was performed immediately after each maneuver using equations reported previously (27). The a-vO₂diff was calculated from the Fick equation as: a-vO₂diff (mLO₂/100 mL⁻1 blood) = VO₂ (L·min⁻¹)/Q (L·min⁻¹) × 100.
**Muscle Sampling**

Before the start of the training as well as mid- and posttraining, two muscle biopsy samples were obtained from the vastus lateralis muscle of each participant under resting conditions using a standard 6-mm Bergstrom needle biopsy and usual technique (28). Pretraining biopsies and those during the exercise training program were obtained at least 36 hours (in most cases ~48 hours) after the latest exercise training or testing session. A single biopsy site was prepared by making an incision through the skin to the deep fascia under local anesthesia (2% lidocaine [lignocaine] without adrenaline) with the participant resting on an examining table.

**Histology**

Cork-mounted biopsy portions were sectioned with a cryostat at ~20°C to a thickness of 10 μm and stored for no longer than 1 week at ~80°C. As previously described (29), sections were fixed at room temperature for 5 minutes in Guth and Samaha (30) fixative followed by incubation in lead-ATPase–staining medium (31) for 1 hour at 37°C in a heated shaker bath.

**Capillary Morphometry**

Morphometric analysis was performed by a single-blinded observer. Muscle sections were viewed with a light microscope and images were captured with a digital camera (Nikon Coolpix 990, Nikon Eclipse E400) at a magnification of 200×. Images were then viewed in Sigma Scan Pro 5.0 (SPSS sciences) for analysis. As performed previously (29), a frame was embedded within the image to prevent sampling bias. Indices of analysis included fiber cross-sectional area (CSA), fiber perimeter (P), capillary contacts (CC), individual capillary-to-fiber ratio (C/Fi), capillary-to-fiber perimeter exchange (CPFE) index, and capillary density (CD; the reader is referred to Hepple and colleagues (17,32) for detailed information on the interpretation of these measures of capillarization). An average of 43 ± 9 fibers were analyzed for each sample.

**Citrate Synthase Activity**

Citrate synthase activity (CS) was measured with the method of Srere as described previously (33) with modification for microplate analysis. Portions of biopsy were thawed, weighed, and homogenized for 20 seconds with an electric adaptable homogenizer (VDI 25; VWR International) on ice in 20 volumes (1:20 weight/volume) of homogenizing buffer (100 mM KPO$_4$ + 5 mM EDTA + 5 mM ethylene glycol tetraacetic acid, pH = 7.4). Homogenates were then vortexed, subjected to three freeze–thaw cycles, and then further diluted to a 1:400 ratio. Citrate synthase activity was measured by following the production of the mercaptide ion spectrophotometrically at a wavelength of 412 nm. Twenty microliters of homogenate was added in triplicates for each sample to a 96-well plate with each well containing 170 μL of reaction mixture (100 mM Tris + 30 mM acetyl CoA + 10 mM 5.5′-dithiobis[2-nitrobenzoic acid]). Baseline reactivity was measured to account for endogenous thiol and deacetylase activity and subtracted from enzyme activity initiated by the addition of 10 mM oxaloacetic acid. Net citrate synthase activity was expressed as pmol × min$^{-1}$ × g$^{-1}$ wet weight. Homogenate protein concentration was assessed using the BioRad protein assay with bovine serum albumin as the standard and measured spectrophotometrically at 595 nm. Citrate synthase activity was then expressed as pmol × min$^{-1}$ × mg protein$^{-1}$.

**Statistics**

Data are presented as means ± SD. Independent $t$ tests and repeated measures analysis of variance were used to determine statistical significance for the dependent variables. The analysis of variance model was described as $S_{14} \times T_{13} \times A_2$ such that subjects (S; number of subjects) are crossed with testing time (T; three testing times: pretraining, Week 6, and posttraining) and age (A; older and young adults). A Tukey post hoc analysis was used when significant differences were found for the main effects of each dependent variable. The analysis of variance was performed using SPSS Version 16.0 (SPSS Inc., Chicago, IL). Statistical significance was declared when $p < .05$.

**Results**

Participants body mass (O, 80 ± 6 kg; Y, 79 ± 9 kg) and height (O, 1.8 ± 0.1 m; Y, 1.8 ± 0.1 m) were similar in both groups ($p > .05$). Body mass was not affected by the endurance training program. Adherence to the training program was 94% ± 1% (28/30 training sessions) and 95% ± 1% (29/30 training sessions) in O and Y, respectively. Each participant completed at least 90% of the programmed training sessions (range: O = 27–29 sessions; Y = 27–30 sessions).

Table 1 summarizes the changes in training PO throughout the exercise training program as well as peak exercise values in response to training. Training PO, VO$_{\text{peak}}$, VO$_{\text{2max}}$, and $Q_{\text{max}}$ progressively increased from pre- to posttraining in both O and Y. No testing time by age interactions was detected. Maximal a-vO$_{\text{2diff}}$ was larger ($p < .05$) posttraining compared with pretraining in O and Y (Table 1) with a trend toward significance from pretraining to midtraining values in both groups ($p = .07$).

CS was measured in seven O and seven Y; however, due to technical issues related to the size of the muscle samples and quality of the collected tissue, capillary morphometry was only obtained at all three time points from four O and four Y men. Figures 1 and 2 depict changes in CS activity and capillary morphometry in response to training. CS expressed as μmol·min$^{-1}$·g wet weight$^{-1}$ (Table 1) or normalized as...
Table 1. Training PO and Maximal Exercise Responses for PO, VO₂, Q̇, a-VO₂diff, and CS Activity in O and Y from Pretraining Through Posttraining

<table>
<thead>
<tr>
<th>Training PO (Watts)</th>
<th>Pretraining</th>
<th>Week 6</th>
<th>Posttraining</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>94 (33)</td>
<td>105 (33)*</td>
<td>113 (33)*</td>
</tr>
<tr>
<td>Y</td>
<td>182 (34)</td>
<td>202 (39)*</td>
<td>207 (33)*</td>
</tr>
<tr>
<td>P0peak (Watts)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>186 (47)</td>
<td>204 (46)*</td>
<td>215 (51)*</td>
</tr>
<tr>
<td>Y</td>
<td>317 (43)</td>
<td>361 (48)*</td>
<td>380 (53)*</td>
</tr>
<tr>
<td>VO₂max (L/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>2.27 (0.53)</td>
<td>2.57 (0.58)*</td>
<td>2.89 (0.47)*</td>
</tr>
<tr>
<td>Y</td>
<td>3.84 (0.51)</td>
<td>4.23 (0.48)*</td>
<td>4.47 (0.36)*</td>
</tr>
<tr>
<td>VO₂max (mL·kg⁻¹·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>28.7 (7.6)</td>
<td>32.5 (8.2)*</td>
<td>36.5 (7.0)*</td>
</tr>
<tr>
<td>Y</td>
<td>48.5 (6.4)</td>
<td>52.9 (6.8)*</td>
<td>55.8 (5.9)*</td>
</tr>
<tr>
<td>Q̇O₂peak (L/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>16.9 (3.3)</td>
<td>18.6 (4.5)*</td>
<td>20.3 (4.0)*</td>
</tr>
<tr>
<td>Y</td>
<td>26.1 (2.9)</td>
<td>27.5 (2.3)*</td>
<td>28.4 (2.0)*</td>
</tr>
</tbody>
</table>

Notes: Values are means ± SD. P0peak = peak power output; VO₂max = maximal O₂ uptake; Q̇O₂max = maximal cardiac output; Maximal a-VO₂diff = maximal arterial–venous O₂ difference; CS = citrate synthase activity.
*Significantly different from midtraining values (p < .05); †significantly different from Y (p < .05).

µmol·min⁻¹·mg protein⁻¹ (Figure 1) was significantly elevated at mid- and posttraining compared with pretraining values in both O (48%) and Y (67%) men. Muscle CSA (µm²); O pre- 4356 ± 752, mid- 4607 ± 707, post- 4839 ± 982; Y pre- 5933 ± 313, mid- 6515 ± 302, post- 6709 ± 399 and P (µm); O pre- 275.4 ± 27.7, mid- 283.7 ± 22.4, post- 291.1 ± 31.0; Y pre- 317.6 ± 9.4, mid- 328.9 ± 14.6, post- 335.9 ± 35.5 were not affected by training in either group. CD was significantly increased from pre- to posttraining in O (28%) and Y (17%). CC, C:F, and CFPE were elevated at posttraining compared with pre- and midtraining in O and Y (Figure 2). There was a trend toward significance from pre- to midtraining for CC (p = .06) and C:F (p = .07) in both groups.

Discussion

The main findings of this study were as follows: (a) Older and young men showed increases in CS activity, CC, C:F, and CD in response to an endurance training protocol accompanying the significant increases in VO₂max in both groups and (b) increase in CS (significant by 6 weeks of training) and changes in capillarization occurred within the same time frame in older and young men.

This study examined the time-course of adaptations of older and young men to a 12-week endurance training program of similar characteristics (relative intensity [70% VO₂max and regular progression of intensity], frequency [three training sessions per week], and duration [45 minutes per session]) with biopsy measures at pretraining, 6, and 12 weeks. Given this similar relative physiologic stressor of exercise training in older and young men, these present data showed changes in measures of capillarization and CS activity occurring at the same testing times in older and young men. Previous studies of muscle respiratory capacity have shown that muscle of older humans of up to ~70 years of age retains the ability to adapt with ~40% increases in enzyme activities with 8 (13) and 12 weeks (34) of training. Regarding specifically CS activity, as a marker of mitochondrial content, previous studies in older adults showed improvement with a long- (9–12 months) (16) and short-term (12 and 16 weeks) (12,35) exercise training program. In these previous studies, no analysis of the time-course of adaptations with exercise training was made and only one of them directly compared older and young individuals (35). The present data allowed direct comparison of O and Y and showed that the increase in CS occurred as early as 6 weeks into the training with increases (of ~30%) main accompanying the significant increases in VO₂max, CFPE, and CD in response to an endurance training protocol accompanying the significant increases in VO₂max in both groups and (b) increase in CS (significant by 6 weeks of training) and changes in capillarization occurred within the same time frame in older and young men.

Figure 1. Resting citrate synthase activity (n = 7 per group) in O and Y at pre-, mid-, and posttraining. *Significantly different from pretraining values (p < .05).
differences were not apparent for CS normalized by protein content.

Additionally, the present findings regarding capillarization extend the recent observation that increases in capillarization with training are present not only in young but also in older men (18). Gavin and colleagues (18) reported that 8 weeks of aerobic exercise training of 1-hour duration at 65% \(\text{VO}_2\text{max}\), with increases of \(\text{VO}_2\text{max}\) of approximately 4.0–4.9, 5.4–5.9, and 6.3–7.0 for each group, respectively, were associated with significant increases in capillary density and capillary-to-fiber ratio.

Figure 2. Resting capillary morphometry (n = 4 per group) in O and Y at pre-, mid-, and posttraining. *Significantly different from pretraining values (p < .05); †significantly different from midtraining values (p < .05).
10%, resulted in similar increases in capillarization in older men (age 64 years) compared with young men. A trend toward exercise-induced skeletal muscle angiogenesis in older men was observed in the present study with changes occurring by 6 weeks ($p = .06$) and with significant increases observed to 12 weeks. Gavin and colleagues (18) reported a training-related increase in capillarization to be $\sim 25\%$ in young and $18\%$ in older men; our data show a similar $20\%$–$30\%$ increase in various measures of capillarization in $Y$ and an increase of $30\%$–$40\%$ in $O$. In comparison, men in the present study were somewhat older ($\sim 69$ years) and the exercise program was of higher intensity and continuous progression resulting in a $27\%$ increase in VO$_{2\text{max}}$.

Earlier studies reported increases of $21\%$ (in CD) (16) with long-term training and $39\%$ (17) after an 18-week aerobic training program. The results of these studies of exercise training in older adults suggest that higher intensity exercise training and progression is associated with greater gains in capillarization. What is novel from the present study is that with exercise training, the improvements in the measures of oxidative capacity (CS) and various indices of capillarization in older men were shown to occur at the same testing times as seen in young individuals. The increase in measures of capillarization reflect a better O$_2$ delivery and potential for improved distribution, with increases in CFPE index indicating a larger surface area available for O$_2$ exchange, suggesting an increased oxygen flux capacity between the capillaries and muscle fibers (17), and the increased CS activity representing a potential for increased O$_2$ utilization. These positive adaptations in the periphery are important for the training-related increase in VO$_{2\text{max}}$, which has been shown to be a strong predictor of function in older individuals (2).

It has been demonstrated that CS activity (at least in the vastus lateralis muscle or in animals of an age comparable with the participants in the present study) (36–38) and capillarization (37,39) are similar in older and young adults, although some found that capillarization (40) and CS activity (40,41) were lower in older compared with young men. Indeed, it has been postulated that capillaries decline only in truly sedentary older people (42). Taking into account that the older men in the present study had an average age of 69 years and were community dwelling, recreationally active individuals, it would be reasonable not to expect major structural changes in capillarization. If that is true, the smaller fiber perimeter and fiber cross-sectional area observed in older men compared with young men [consistent with age-related sarcopenia (43)] would likely result in facilitated O$_2$ conductance from the capillaries to the mitochondria.

Although this study was well powered to determine changes in VO$_{2\text{max}}$, Q$_{\text{max}}$, and maximal a-VO$_{2\text{diff}}$ in older and young individuals, data should be interpreted with caution in consideration of the larger variability inherent to biopsy histochemical and biochemical measurements, in addition to the limitation that samples from only four older and four young men were of sufficient quality to perform the histology analyses at all three time points (pre-, mid-, and posttraining). In this regard, given the variance in the biopsy data in order to detect a significant difference between older and young participants would require very large sample sizes (>25 participants per group). In addition, the present findings are applicable to healthy active groups; although participants in the present study were not chronically trained, both age groups in this study at baseline were above the mean VO$_{2\text{max}}$ predicted for age-matched populations (22,23). Notwithstanding this limitation, we observed significant improvements in CS and capillarization in both age groups as a result of the training, suggesting that at this age, there is no impairment in the capacity for adaptation of the muscle “aerobic machinery”. In this respect, our data are similar to data in the rodent literature where endurance training programs have been shown effective in inducing adaptations of the muscle “aerobic machinery” in rodents of similar relative ages to the humans we studied (44,45).

However, it should also be noted that data from the oldest old (>85 years or “frailty” in humans; senescence in rats) indicate a reduced magnitude of the increase in VO$_{2\text{max}}$ with training (46) and a markedly impaired muscle response to both resistance (47,48) and endurance exercise (46,49,50) stimuli. As such, our data are not at odds with these significant impairments at more advanced ages.

In conclusion, this study showed that changes in measures of capillarization and citrate synthase activity in a small sample of older and young men occurred at similar testing times in response to a “vigorous” 12-week endurance training program. These adaptations to endurance training in the periphery accompanied a greater central (Q$_{\text{max}}$) capacity; a greater capillarization would allow for a larger O$_2$ delivery and increased O$_2$ flux, whereas increased CS activity may reflect a greater mitochondrial respiratory capacity, both of which likely contribute to the greater O$_2$ extraction in maximal exercise. These peripheral adaptations in the O$_2$ transport and utilization system are important given the established relationship between aerobic fitness and functional independence and health of older adults (51).

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**References**


