Muscle capillarization, O₂ diffusion distance, and Vₒ₂ kinetics in old and young individuals

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Chilibeck, P. D., D. H. Paterson, D. A. Cunningham, A. W. Taylor, and E. G. Noble. Muscle capillarization, O₂ diffusion distance, and Vₒ₂ kinetics in old and young individuals. J. Appl. Physiol. 82(1): 63–69, 1997.—The relationships between muscle capillarization, estimated O₂ diffusion distance from capillary to mitochondria, and O₂ uptake (Vₒ₂) kinetics were studied in 11 young (mean age, 25.9 yr) and 9 old (mean age, 66.0 yr) adults. Vₒ₂ kinetics were determined by calculating the time constants (r) for the phase 2 Vₒ₂ adjustment to and recovery from the average of 12 repeats of a 6-min, moderate-intensity plantar flexion exercise. Muscle capillarization was determined from cross sections of biopsy material taken from lateral gastrocnemius. Young and old groups had similar Vₒ₂ kinetics (r_vₒ₂-on = 44 vs. 48 s; r_vₒ₂-off = 33 vs. 44 s, for young and old, respectively), muscle capillarization, and estimated O₂ diffusion distances. Muscle capillarization, expressed as capillary density or average number of capillary contacts per fiber/average fiber area, and the estimates of diffusion distance were significantly correlated to Vₒ₂-off kinetics in the young (r = -0.68 to -0.83; P < 0.05). We conclude that 1) capillarization and Vₒ₂ kinetics during exercise of a muscle group accustomed to everyday activity (e.g., walking) are well maintained in old individuals, and 2) in the young, recovery of Vₒ₂ after exercise is faster, with a greater capillary supply over a given muscle fiber area or shorter O₂ diffusion distances.

Subjects. Vₒ₂ kinetics during plantar flexion exercise and muscle capillarization of the lateral gastrocnemius were measured in 9 old and 11 young individuals. Subject characteristics are listed in Table 1. Subjects were moderately active but not well trained. Older subjects reported walking as the primary form of exercise. These individuals were recruited from activity classes, where group walking was performed two to three times per week for ~30 min per day. Subjects had been participating in this class for an average of 18 mo (with a range from 9 to 35 mo). Values for maximal O₂ uptake indicated that subjects were of average fitness for their respective age groups (30). All gave informed consent to participate in this study, which was approved by the University Review Board for Research Involving Human Subjects.

Exercise tests. For determination of peak work rate, subjects initially performed exercise tests to fatigue on a custom-built plantar flexion ergometer. This involved pushing on a foot pedal at a frequency of 0.5 Hz to lift a weight attached by a pulley system. Work rate was increased as a ramp function, by continually pumping water into a container attached to the pulley system. Work rate increments averaged 0.3 W/min for old and 0.6 W/min for young subjects.

For determination of Vₒ₂ kinetics, subjects performed 12 6-min square-wave transitions to and from ankle plantar flexion exercise, during three to four separate laboratory visits. The intensity was set at 45% of peak work rate, which averaged 1.6 and 3.4 W for old and young subjects, respectively. This work rate could be considered moderate, as further ramp testing determined that the work rates were below the subjects' P₅₀ intracellular threshold, as determined
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age, yr</th>
<th>Mass, kg</th>
<th>Height, cm</th>
<th>$V_{O2max}$, ml·kg$^{-1}$·min$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>11</td>
<td>25.9 ± 2.1</td>
<td>77.7 ± 16.4</td>
<td>174.5 ± 10.0</td>
<td>43.6 ± 9.3</td>
</tr>
<tr>
<td>Old</td>
<td>9</td>
<td>66.0 ± 6.3</td>
<td>70.7 ± 13.3</td>
<td>165.7 ± 8.8</td>
<td>20.6 ± 2.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. Young group, n = 5 men, 6 women; old group, n = 2 men, 7 women. $V_{O2max}$, maximal O$_2$ uptake.

by $^{31}$P-nuclear magnetic resonance spectroscopy (28). This threshold is considered the point at which anaerobic glycolysis is increased, as evidenced by an increased rate of change in pH (28). A large number of transitions were performed to improve the signal-to-noise ratio (24), in light of the small amplitude of the $V_{O2}$ response with the small-muscle group exercise. Transitions were separated by 6 min of loadless plantar flexion and were initiated manually by the experimenter. Subjects were blinded to the initiation and termination of square-wave changes in work rate.

$V_{O2}$ was measured using a modification of the methods of Babcock et al. (3). Inspired and expired gas flows were measured by using a low-dead space (90-ml) bidirectional turbine (VMM 110, Alpha Technologies) calibrated by a 3.01-liter syringe, and gas concentrations were measured by a mass spectrometer (Airspec 2000 MGR 9N) calibrated against precision-analyzed gas mixtures. Changes in gas concentration were aligned with gas volumes by measuring the time delay for a square-wave bolus of gas passing the turbine to the resulting changes in fractional gas concentrations as measured by the mass spectrometer. Data collected every 20 ms were converted from analog to digital format and stored for later processing by a microcomputer.

Breath-by-breath alveolar gas-exchange data were calculated using the algorithms of Beaver et al. (5). Breath-by-breath data were interpolated to 1 s, with square-wave repeats time aligned and averaged. Averaged responses for each subject were fit, using a first-order (monoexponential) model of the form

$$Y(t) = a(1 - e^{-(t - \tau)/\delta})$$

where $Y$ represents $V_{O2}$ at time (t); a, $\delta$, and $\tau$ are the amplitude, time delay, and time constant of the response, respectively. The monoexponential curves were fit from the start of the phase 2 (20-s) portion of the $V_{O2}$ response (37). Twenty seconds is considered to be a reasonable duration of phase 1 during the adjustment to cycling exercise and represents the transport time for blood-borne gas-exchange signals present in the contracting muscles to be expressed at the lung (37). During cycling exercise, there is a larger cardiovascular, as well as metabolic, readjustment to exercise, compared with plantar flexion exercise. Whether cardiac output and venous return increase at the same rate during the adjustment to both exercise tests and whether phase 1 is the same duration are unknown. We have recently found that heart rate kinetics during the transition to plantar flexion and cycling exercise are similar in young individuals (11). This supports the argument that cardiac output adjustment and phase 1 are similar in the two exercises. Solutions for $a$, $\tau$, and $\delta$ were derived from an iterative optimization computer routine.

Muscle biopsies. Needle biopsy samples were obtained from the lateral head of the right gastrocnemius of each subject. These were oriented longitudinally in embedding media, frozen in liquid N$_2$-cooled isopentane, and stored in liquid N$_2$. Frozen sections were cut, at a width of 10 μm, on a microtome cryostat (Leitz Lauda 1720), mounted on glass coverslips, and analyzed for capillarization by staining with periodic acid-Schiff's reagent, according to the method of Anderson (1). Fiber type composition was determined by using the stain for adenosinetriphosphatase activity after preincubation at pH 4.3, 4.6, and 10.3 (6). Sections were magnified and projected on an image analyzer (Quantimet 520) for counting of capillaries and fibers. The number of muscle fibers in sections averaged 134 ± 63 (range 44–255). Muscle capillarization was expressed as capillary density (total number of capillaries in a section divided by the total area), capillary-to-fiber ratio (C/F; total number of capillaries in a section divided by the number of fibers), the average number of capillaries in contact with each fiber (CC), and the average number of capillaries in contact with each fiber divided by the average fiber area (CC/FA). Average and maximal diffusion distances for $O_{2}$ from capillary to muscle fiber were estimated, using the equations developed by Snyder (35), for capillaries distributed in random arrays.

$$\text{maximal diffusion distance} = \left[ 0.415 - 0.477/ \right] \times \sqrt{\text{average fiber cross-sectional area}}$$

$$\text{average diffusion distance} = \left[ 0.207 + 0.232/ \right] \times (\text{capillary-to-fiber ratio}) \times \sqrt{\text{average fiber cross-sectional area}}$$

These diffusion distances are based on the cumulative frequency of the area of each fiber within a measured distance from a capillary. Maximal diffusion distance is the distance where 95% of the fiber area is served by a capillary, whereas average diffusion distance is the distance where 50% of the fiber area is served by a capillary (36). Equations for random, rather than square or hexagonal, arrays were used, based on our results (see DISCUSSION) for C/F ratio and average number of CC, according to descriptions of arrays by Plyley and Groom (32).

Statistics. All results are expressed as means ± SD. Confidence intervals for parameter estimation of $\tau_{V_{O2}}$ were calculated, based on the $V_{O2}$ response amplitude and the SD of breath-by-breath $V_{O2}$ fluctuation, as described by Lamarra et al. (24). Comparisons of $\tau_{V_{O2}}$ were made using a three-factor analysis of variance (ANOVA) with age (old vs. young) and gender (men vs. women) as between-subjects factors and repeated measures on square-wave transients (on vs. off). Comparisons of fiber types, capillarization, and diffusion distances were made using a two-factor (age × gender) ANOVA. Pearson product correlations were used to compare $\tau_{V_{O2}}$ with measures of muscle capillarization, diffusion distances, and fiber type composition. $P < 0.05$ was accepted as significant.

RESULTS

No effects due to gender were found for any measures. With the inclusion of only two old men, our comparison between genders is weak; this is a limitation of the present study. Using larger groups, others have found differences in capillary measures between genders, with men having a greater capillarization than women (13). For simplicity, all results are presented with subjects grouped by age (old vs. young).
V˙O2-on and -off responses to plantar flexion exercise for an old and young subject, along with monoexponential fits, are depicted in Figs. 1 and 2, respectively. Results for \( t_{V˙O2} \), summarized in Table 2, showed no significant difference in \( t_{V˙O2} \) between young and old, and within age groups no difference was seen between on- and off-kinetics. With use of the equations developed by Lamarra et al. (24), the 95% confidence intervals, determined from the group mean data, for estimation of \( t_{V˙O2} \) (for both on- and off-transients) were \( \pm 11.5 \) s and \( \pm 8.4 \) s for old and young groups, respectively. This is based on the \( V˙O2 \) steady-state amplitudes, which averaged 0.08 and 0.11 l/min for old and young groups, respectively, and the SD of breath-by-breath fluctuations, which averaged 0.067 l/min for both old and young groups.

Muscle capillarization, fiber areas, and diffusion distances are summarized in Table 3. Fiber type composition is summarized in Table 4. No differences were found between old and young groups for any of the variables (\( P > 0.05 \)).

For combined groups, \( \tau V˙O2 \) was generally faster with increased muscle capillarization, with correlations between various measures of muscle capillarization and \( V˙O2 \) kinetics ranging from \(-0.23\) to \(-0.59\) (Table 5). Correlations for capillary density vs. \( \tau V˙O2 \)-off \((-0.48\) and CC/FA vs. \( \tau V˙O2 \)-off \((-0.59\) were significant (\( P < 0.05 \)). The correlation between maximal or average diffusion distance vs. \( \tau V˙O2 \)-off approached significance (\( P = 0.052 \)). For individual groups, capillary density, CC/FA, and diffusion distances were significantly correlated with \( \tau V˙O2 \)-off in the young group only (Table 5).

There were no significant relationships between \( \tau V˙O2 \) and capillarization for the older group (Table 5). Figure 3 shows the individual points for the relationships of \( \tau V˙O2 \)-off kinetics with capillary density, CC/FA, and maximal diffusion distance. Correlations between \( V˙O2 \) kinetics and fiber type composition (% type I) were not significant.

**DISCUSSION**

Our finding that plantar flexion \( V˙O2 \) kinetics were not slower in the group of old subjects may be due to the level of training of the plantar flexors in this population. In an earlier study, which included four of the nine old individuals of the present study, \( V˙O2 \) kinetics during cycle ergometry were slower in the old (9), in agreement with previous results from this laboratory (3, 14). The differences between results may be due to the training of the muscle group tested. The plantar flexors are used to a great extent in everyday activity (e.g., walking) and may be relatively well trained in the old subjects. Babcock et al. (2) have shown that specific training of older subjects can substantially improve the rate of \( V˙O2 \) kinetics to levels similar to those of young fit individuals.

Usually, measurements of \( O2 \) kinetics have been performed during cycle exercise, as opposed to the plantar flexion exercise used in the present study.

**Fig. 1.** A: \( O2 \) uptake (\( V˙O2 \)) during on-transition to ankle plantar flexion in an older individual, along with monoexponential fit during phase 2 (i.e., after 20 s; \( t/V˙O2 = 47 \) s). \( \tau \), Time constant. B: \( V˙O2 \) during off-transition \((t/V˙O2 = 37 \) s).

**Fig. 2.** A: \( V˙O2 \) during on-transition to ankle plantar flexion in a young individual, along with monoexponential fit during phase 2 (i.e., after 20 s; \( \tau V˙O2 = 39 \) s). B: \( V˙O2 \) during off-transition \((\tau V˙O2 = 43 \) s).
We used plantar flexion to compare VO₂ kinetics with capillarization of a specific muscle (the lateral gastrocnemius). Electromyographic (EMG) analyses have shown that cycle exercise involves the recruitment of many muscle groups of the leg (23); therefore, comparison of kinetics with biopsy data of one muscle group (i.e., the vastus lateralis) may be invalid. Results from a previous study, in which we assessed muscle recruitment with magnetic resonance imaging and EMG, demonstrated that the lateral and medial portions of the gastrocnemius are dominant during submaximal ankle plantar flexion, and there is minimal contribution from other muscle groups, such as the soleus or quadriceps (29). We therefore conclude that VO₂ kinetics measured during ankle plantar flexion reflect the exercise of the gastrocnemius and that comparisons with biopsy data from the lateral portion of this muscle are warranted.

Our results for capillarization of the lateral gastrocnemius were very similar to those of both the old and young groups of Coggan et al. (13), with the exception that the C/F ratio of our group was elevated. When capillary data were assessed across studies, it has been suggested that the best measure to use for comparison is C/F ratio, since a measure such as capillary density is highly influenced by muscle fiber size, which in turn may be affected by shrinkage during histochemical preparation techniques that vary from laboratory to laboratory (32). The averages for each of our measures of capillarization tended to be lower in old compared with young groups (Table 3). Whereas differences between our groups were not significant, with a larger sample size, Coggan et al. (13) detected significant differences, with lower levels of capillarization in lateral gastrocnemius of the old group. The subjects of the study of Coggan et al. (13) were described as sedentary, whereas we considered our subjects to be moderately active and involved in walking on a regular basis. The C/F ratio (1.93; Table 3) of our young group was in the middle of the range from the literature for C/F ratios of lateral gastrocnemius for young (range = 1.11–2.51; Refs. 7, 13, 26). C/F ratio of our old group (1.72; Table 3) was similar to the range from two Scandinavian studies (1.48–1.96; Refs. 15 and 16), but as mentioned, it was higher than that of the old subject (1.39 for old men and 0.94 for old women) of Coggan et al. (13). Differences between studies may be due to the training status of groups compared. Muscle capillarization appears to be very sensitive to training in the old, as demonstrated by Coggan et al. (12). Moderate training of older subjects resulted in substantial increases in muscle capillarization, to levels similar to those of their young group (13), despite the old still having substantially lower levels of maximal VO₂. The trained old subjects from the study of Coggan et al. (12) had a C/F ratio (2.08 for men, 1.23 for women) that was closer to that of the old individuals from the present study. The fact that most of our old subjects performed moderate levels of walking on a daily basis could have been a sufficient stimulus to offset any loss of capillarization with aging.

The present data show that measures of capillarization were significantly correlated with VO₂ kinetics only when expressed in relation to fiber area (Table 5, Fig. 1).
3). Capillarization, expressed as CC/FA, had the strongest correlation with V̇O₂ kinetics (Table 5, Fig. 3). This measurement gives an index of capillary supply to fiber area, accounting for the effects of diffusion (31). Estimates of diffusion distances, based on the equations of Snyder (35), differ in that C/F, rather than CC, is used in relation to fiber area. As suggested by Plyley (31), C/F and capillary density are global indices of capillarization and yield little information on the capillary supply of individual fibers. Therefore, CC/FA appears to offer a better assessment of O₂ delivery. Nevertheless, measurement of capillary numbers alone may be an oversimplification. Kinetics of O₂ delivery may also be affected by branching patterns in the capillary network, length of capillary paths, and capillary interconnections (33), all of which would have to be measured in longitudinal as opposed to transverse sections of muscle (33). Another factor may be the rate of capillary recruitment, which has been shown to affect O₂ transport to dog gracilis muscle at the onset of exercise (18). Estimates of diffusion distance from capillary to cell interior, as done in this study, may be inaccurate in assessing O₂ diffusibility. Honig et al. (17) hypothesized that the principal gradient for O₂ diffusion is across the capillary to the sarcolemma, rather than across the muscle cell interior. Tissue gradients for O₂ are small, as myoglobin acts as a buffer to keep PO₂ relatively uniform throughout the muscle cell (17). There may also be diffusion interaction between muscle cells, which would complicate estimates of diffusion from capillaries alone. The PO₂ gradient from inactive to working fibers is substantial, and the surface area for exchange is great compared with that of capillaries (17).

We hypothesized that V̇O₂ kinetics may be related to the degree of muscle capillarization, because it has been shown that kinetics can be highly influenced by changes in peripheral circulation (19, 20). With increased capillarization, diffusion distances from capillary to muscle fiber interior are shorter (36) and there is an increased surface area for O₂ exchange (25), which together should decrease the transport time of O₂ to mitochondria. In the present study, correlations between V̇O₂ kinetics and capillarization per fiber area were in a direction indicating faster kinetics with increased capillarization and shorter diffusion distances (Table 5). The modest correlations between simple measures of capillarization or diffusion distances and V̇O₂ kinetics may be accounted for by the complicating factors described above.

However, our data do reveal two important aspects to consider. First, the findings show a significant relationship between V̇O₂ kinetics and capillarization only in relation to the off-kinetics, not the on-kinetics. Second,
this relationship is significant in the young but not in the old group of subjects.

The finding that capillarization was significantly correlated with $\dot{V}O_2$-off kinetics but not $\dot{V}O_2$-on kinetics suggests that $O_2$ delivery may have a greater influence on $\dot{V}O_2$ recovery than $\dot{V}O_2$ adjustment to exercise. This is supported by Idstrom et al. (22), who found that the rate of recovery of phosphocreatine (PCR) after contractions of perfused rat hindlimb was related to $O_2$ supply through the perfusate, although the rate of PCR breakdown at the start of exercise was not. Here, PCR kinetics are thought to reflect kinetics of muscle $O_2$ consumption (4). Tissue gradients for $O_2$ are small during exercise on-transients due to myoglobin buffering (17); this may prevent $O_2$ transport from limiting during exercise on-transients due to myoglobin buffering (17); this may prevent $O_2$ transport from limiting during exercise on-transients due to myoglobin desaturation and larger $O_2$ gradients during recovery is unknown. If myoglobin desaturates during recovery, $O_2$ diffusion distance from capillary to fiber may affect kinetics of $\dot{V}O_2$.

We hypothesized that capillarization would be lower in the old and that this would result in slow $\dot{V}O_2$ kinetics. However, we found no significant reduction in measures of capillarization in the old and no relationship of capillarization to $\dot{V}O_2$ kinetics. $\dot{V}O_2$ kinetics in the old might be more related to mitochondrial density (27).

The findings of this study indicate the following. 1) $\dot{V}O_2$ kinetics and muscle capillarization are well maintained in the old for a muscle group (the plantar flexors) used extensively during everyday activity. 2) Correlations between $\dot{V}O_2$ kinetics and muscle capillarization are strongest when capillarization is related to fiber area, thus accounting for diffusion distances. 3) Rate of $O_2$ delivery is probably affected by the interaction of many factors (i.e., capillary path lengths, capillary branching, capillary recruitment patterns, and $O_2$ diffusion from adjacent muscle cells), and estimating capacity for $O_2$ delivery from individual measures of capillarization may be an oversimplification. 4) Capillarization has a stronger relationship with $\dot{V}O_2$-off than with $\dot{V}O_2$-on kinetics. 5) Capillarization has a stronger relationship with $O_2$ kinetics in young than in old individuals. Other factors such as mitochondrial density may control $O_2$ kinetics in the old.

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