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ORIGINAL ARTICLE

Speeding of VO_2 kinetics in response to endurance-training in older and young women

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Abstract The goal of this study was to examine the time-course of changes in oxygen uptake kinetics (τVO_{2p}) during step-transitions from 20 W to moderate-intensity cycling in response to endurance-training in older (O) and young (Y) women. Six O (69 ± 7 years) and 8 Y (25 ± 5 years) were tested pre-training, and at 3, 6, 9, and 12 weeks of training. VO_{2p} was measured breath-by-breath using a mass spectrometer. Changes in deoxygenated-hemoglobin concentration of the vastus lateralis (Δ[HHb]) were measured by near-infrared spectroscopy in Y (but this was not possible in O). VO_{2p} and Δ[HHb] were modeled with a mono-exponential. Training was performed on a cycle-ergometer three times per week for 45 min at ~70% of VO_{2peak}. Pre-training τVO_{2p} was greater ($p < 0.05$) in O (55 ± 16 s) than Y (31 ± 8 s). After 3 weeks training, τVO_{2p} decreased ($p < 0.05$) in both O (35 ± 12 s) and Y (22 ± 4 s). A pre-training "overshoot" in the normalized Δ[HHb]/VO_{2p} ratio relative to the subsequent steady-state level (interpreted as a mismatch of local O_2 delivery to muscle VO_2) was observed in Y. Three weeks of training resulted in that "overshoot" being abolished. Thus there was a training-induced speeding of VO_2 kinetics in O and Y. In the Y this appeared to be the result of improved matching of local O_2 delivery to muscle VO_2. In O, inadequate systemic O_2 distribution (as indirectly expressed by the arterial-venous O_2 difference/VO_{2p} ratio) seemed to play a role for the initial slower rate of adjustment in VO_{2p}.

Keywords Aging · O_2 distribution · Near-infrared spectroscopy · Exercise

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Introduction

The study of pulmonary O_2 uptake (VO_{2p}) kinetics provides insight into the potential factors regulating mitochondrial oxidative phosphorylation. In healthy individuals, the rate at which VO_2 adjusts to a new energetic demand is predominantly controlled intracellularly (Grassi 2001; Poole et al. 2007) but may be constrained by the rate of delivery of O_2 to the active muscle fibers (Hughson et al. 2001; Tschakovsky and Hughson 1999).

Older individuals have a longer Phase II VO_{2p} time constant (τVO_{2p}) during the on-transient of moderate-intensity exercise compared to younger adults (Bell et al. 1999; DeLorey et al. 2005). Endurance training has been demonstrated to result in faster VO_{2p} kinetics in both older (Babcock et al. 1994a; Bell et al. 2001) and young (Phillips et al. 1995) healthy men. In a recent study we reported that after only 3 weeks of endurance training the adjustment of VO_2 at the onset of exercise became faster in both the older and young males and that this short training time was enough for older adults to achieve a VO_2 kinetics response similar to that observed in the young individuals before the start of training (Murias et al. 2010b). In that study we also reported that during transitions to moderate-intensity exercise, a better matching of microvascular O_2 distribution

within the active muscles seemed to be responsible for the speeding of VO_{2p} kinetics in response to training (Murias et al. 2010b).

Surprisingly, there is little information on VO_2 kinetics in women. Gurd et al. (2007) examined the effects of menstrual cycle on τVO_{2p} in young women, and Stathokostas et al. (2008) studied the effects of hormone replacement therapy on τVO_{2p} in older women. However, to our knowledge, there is no information on changes in the rate of adjustment of VO_{2p} kinetics in response to endurance training in older and young women. Interestingly, recent studies suggest that during steady-state exercise blood flow distribution may differ depending on gender and age (Parker et al. 2008) such that older women showed reduced leg blood flow. More specifically, unlike older men (Proctor et al. 2003b), normally active older women have a blunted vascular conductance and hyperemic response (Parker et al. 2008; Proctor et al. 2003a), whereas young female adults show a femoral blood flow and vascular conductance that is even better than that observed in young male adults (Parker et al. 2007). Additionally, cardiovascular adaptations to endurance training in older (Spina et al. 1993) but not in young (Murias et al. 2010a) women have been shown to rely mainly on a wider arterial-venous O_2 difference (a-vO_{2diff}) with no training-related increases in maximal cardiac output. This markedly different cardiovascular response in older compared to young women could affect the matching of O_2 delivery to O_2 utilization and thus, the rate of adjustment of VO_{2p} in response to a step-transition in the moderate-intensity domain by either affecting intracellular processes or O_2 delivery.

Based on the contention that older and young women represent the lower and upper ends of the spectrum in terms of vascular responsiveness to exercise and considering that the matching of O_2 distribution to muscle VO_2 (VO_{2m}) within the active muscles has been shown to play an important role in rate of adjustment of oxidative phosphorylation (Murias et al. 2010b), and that endurance training has been demonstrated to speed VO_{2p} kinetics in young and old men (Murias et al. 2010b), we sought to determine the time-course of adjustment for phase II VO_{2p} kinetics in older and younger women during a 12-week endurance training program. We hypothesized that: (1) older women would have a slower phase II VO_{2p} kinetics compared to their younger counterparts at any testing time; (2) endurance training would result in speeding of phase II VO_{2p} kinetics in both older and young women with the majority of the change occurring during the first 3 weeks of training; (3) the speeding of VO_{2p} kinetics in response to endurance training in older women would not be enough to reduce τVO_{2p} to the values observed in the young women before training started.

Methods

Subjects

Six older (O) (69 ± 7 years; mean ± SD) and 8 young (Y) (25 ± 5 years) adult women volunteered and gave written consent to participate in the study. Descriptive and baseline data from these subjects were given in a previous report examining central and peripheral adaptations to endurance training in the same women (Murias et al. (2010a); the reader is referred to this paper for further information on increases in maximal VO_{2p}, cardiac output and a-vO_{2diff}). All procedures were approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects. All subjects were non-obese (body mass index $\leq$30 kg/m^2), non-smokers, and were physically active but none of them had been involved in any type of endurance training program for at least 12 months prior to the study. Additionally, no subjects were taking medications that would affect the cardiorespiratory or hemodynamic responses to exercise. Subjects had no history of cardiovascular, respiratory or musculoskeletal diseases, and older women were medically screened by a physician and underwent a maximal exercise stress test.

Protocol

Before training began, subjects reported to the laboratory on two separate occasions. On day one, a maximal cycle ergometer ramp test (O 15–20 W/min; Y 25 W/min) was performed (Lode Corival 400; Lode B.V., Groningen, Holland) for determination of peak VO_2 (VO_{2peak}) and the estimated lactate threshold (θ_L). θ_L was defined as the VO_2 at which CO_2 output (VCO_2) began to increase out of proportion in relation to VO_2 with a systematic rise in minute ventilation-to-VO_2 ratio and end-tidal PO_2 whereas minute ventilation-to-VCO_2 ratio and end-tidal PCO_2 were stable. After this test, subjects returned to the laboratory on a different day to perform step transitions in work rate (WR) from 20 W to a moderate-intensity WR that elicited a VO_2 corresponding to 90% θ_L. Each subject performed two repetitions of the following protocol: 6 min cycling at 20 W, 6 min at 90% θ_L, 8 min at 20 W, and 6 min at 90% θ_L; transitions were performed continuously and each repetition was separated by 30 min rest with the subject sitting on a chair. Identical procedures were repeated after 3, 6, 9, and 12 weeks of training. However, since the step-transitions were performed at the same absolute power output as the initial tests, the order for the ramp test and the step-transitions was assigned randomly. At least 24 h were allowed between the ramp-test and the step transitions.

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 intensity was adjusted at 3 week intervals to reflect changes in fitness. During the first 10 weeks, each session consisted of continuous training (CT) for 45 min at a power output that elicited ~70% of the VO_{2peak} observed during the incremental ramp test. During the final 2 weeks of training (6 exercise sessions), to maintain an emphasis on increasing intensity, some subjects performed high-intensity interval training (HIT) which consisted of 10–12 exercise bouts each lasting 1-min at 90–100% of the peak power output achieved during the previous incremental ramp test, each separated by 1-min resting recovery.

Measurements

Gas-exchange measurements were similar to those previously described (Babcock et al. 1994b). Briefly, inspired and expired flow rates were measured using a low dead space (90 mL) bidirectional turbine (Alpha Technologies VMM 110) which was calibrated before each test by using a syringe of known volume. Inspired and expired gases were sampled continuously (every 20 ms) at the mouth and analyzed for concentrations of O_2, CO_2, and N_2 by mass spectrometry (Perkin Elmer MGA-1100) after calibration with precision-analyzed gas mixtures. Changes in gas concentrations 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 transferred to a computer, which aligned concentrations with volume information to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated by using algorithms of Beaver et al. (1981).

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

Local muscle oxygenation profiles of the quadriceps vastus lateralis muscle were made with NIRS (Hamamatsu NIRO 300, Hamamatsu Photonics, Hamamatsu, Japan). Optodes were placed on the belly of the muscle midway between the lateral epicondyle and greater trochanter of the femur. The optodes were housed in an optically dense plastic holder and secured on the skin surface with tape and then covered with an optically dense, black vinyl sheet, thus minimizing the intrusion of extraneous light. The thigh was wrapped with an elastic bandage to minimize movement of the optodes.

The manner in which the NIRS-derived signal is collected and analyzed has been explained by DeLorey et al. (2003). Given the uncertainty of the optical path length in the vastus lateralis at rest and during exercise, NIRS data are presented as delta (Δ) arbitrary units (a.u.). The NIRS-derived signal was zero set prior to the onset of exercise while subjects were quietly seated on the cycle ergometer. The raw attenuation signals (in optical density units) were transferred to a computer for later analysis. Changes in light intensities were recorded continuously at 2 Hz.

Data analysis

VO_2 data were filtered by removing aberrant data points that lay outside 4 SD of the local mean. The data for each transition then were linearly interpolated to 1 s intervals and time-aligned such that time zero represented the onset of exercise. Data from each transition were ensemble-averaged to yield a single, averaged response for each subject. This transition was further time-averaged into 10 s bins to provide a single time-averaged response for each subject. The on-transient response for VO_2 was modeled using a mono-exponential of the form:

$$Y_{(t)} = Y_{\mathrm{Bsln}} + \mathrm{Amp}\left(1 - e^{-(t-\mathrm{TD})/\tau}\right), \quad (1)$$

where $Y_{(t)}$ represents VO_2 at any time (t); Y_{Bsln} is the baseline VO_2 during 20 W cycling; Amp is the steady-state increase in VO_2 above the baseline value; τ is the time-constant defined as the duration of time for VO_2 to increase to 63% of the steady-state increase; and TD is the time delay (such that the model is not constrained to pass through the origin). The phase I–phase II transition was constrained to 20 s. Data were modeled from the beginning of phase II to 4 min (240 s) of the step-transition with the assurance that steady-state had been attained. The model parameters were estimated by least-squares non-linear regression (Origin, OriginLab Corp., Northampton, MA, USA) in which the best fit was defined by minimization of the residual sum of squares and minimal variation of residuals around the Y-axis ($Y = 0$). The 95% confidence interval (CI) for the estimated time constant was determined after preliminary fit of the data with Bsln, Amp, and TD constrained to the best-fit values and the τ allowed to vary.

HR data were determined form the R–R interval on a second-by-second basis and edited and modeled in the same manner as described above for VO_2 data. The on-transient HR response was modeled from the onset of

exercise to 240 s using the mono-exponential model described in Eq. 1.

The NIRS-derived Δ[HHb] data were time-aligned and ensemble-averaged to 5 s bins to yield a single response for each subject. The Δ[HHb] profile is described as consisting of a time delay at the onset of exercise, followed by an increase in the signal with an "exponential-like" time-course (DeLorey et al. 2003). The time delay for the Δ[HHb] response (TD-Δ[HHb]) was determined using second-by-second data as the duration between the onset of exercise and the first point at which the Δ[HHb] signal started to systematically increase. The Δ[HHb] data were modeled from the end of the TD-Δ[HHb] to 90 s of the transition using an exponential model as described in Eq. 1. As described by duManoir et al. (2010), different fitting strategies (i.e. 90–180 s) resulted in minimal differences in the group average estimates of τ[HHb]. In the present analysis, examination of the model fit and residuals showed the early exponential increase in Δ[HHb] and the steady-state Δ[HHb] were well-characterized by a fit to 90 s; whereas longer fitting windows risked fitting the steady-state at the expense of a poorer fitting of the early transient. The τΔ[HHb] described the time course for the increase in Δ[HHb], while the overall time course of Δ[HHb] from the onset of exercise was described by the effective Δ[HHb] ($\tau'\Delta[HHb] = TD\text{-}\Delta[HHb] + \tau\Delta[HHb]$).

The second-by-second Δ[HHb] and VO_{2p} data were normalized for each subject (0–100% of the response). The normalized VO_{2p} was left-shifted by 20 s to account for the phase I–phase II transition so that the beginning of phase II VO_{2p}, which has been previously described to coincide with muscle VO_2 within 10% (Grassi et al. 1996) coincided with the onset of exercise. Normalized and time-aligned data were further averaged into 5 s bins for statistical comparison of the rate of adjustment for Δ[HHb] and ΔVO_{2p}. Additionally, an overall normalized $\Delta[HHb]/\Delta VO_{2p}$ ratio for the adjustment during the exercise on-transient was derived for each individual as the average value from 20 to 150 s into the transition. The start point was selected to be 20 s to begin beyond the physiological TD-Δ[HHb] derived from NIRS. An end point of 150 s was selected as the normalized $\Delta[HHb]/VO_2$ data versus time showed a steady-state.

Statistics

Data are presented as means ± SD. Paired and unpaired *t* tests and a two-way repeated measures analysis of variance (ANOVA) were used to determine statistical significance for the dependent variables. A Tukey post hoc analysis was used when significant differences were found for the main effects of each dependent variable. Pearson product moment correlation coefficients were used to determine the degree of association between key variables. The ANOVA and correlation coefficients were analyzed by SPSS Version 15.0, (SPSS Inc., Chicago, IL, USA). Statistical significance was declared when $p < 0.05$.

Results

Subject characteristics and pre-training peak exercise values are listed in Table 1. Compliance to the training program was 94 ± 4% (28/30 training sessions) and 98 ± 3% (29/30 training sessions) in O and Y, respectively, with all subjects completing at least 90% of the programmed training sessions. As noted in 'methods' groups were split after the ninth week of training; however, since training type (i.e., continuous vs. interval) did not affect any kinetic parameter, data were combined. The training program resulted in a significant increase in VO_{2peak} in O and Y (Murias et al. 2010a).

VO_2 kinetics

The phase II VO_2 time constant (τVO_{2p}) was greater ($p < 0.05$) in O (55 ± 16 s) than in Y (31 ± 8 s). The τVO_{2p} decreased by approximately 30–35% ($p < 0.05$) after 3 weeks training in both O (35 ± 12 s) and Y (22 ± 4 s) with a further decrease observed at week 9 of training compared to week 3 (O 32 ± 12 s; Y 18 ± 5 s) (Table 2); the τVO_{2p} observed after 3 weeks of training in O was similar to that observed in Y pre-training. There was no testing time by age interactions reflecting a similar time-course for the decrease in τVO_{2p} in O and Y over the course of training.

The VO_{2p} amplitude was lower in O (0.29 ± 0.08 L min^{-1}) compared with Y (0.57 ± 0.12 L min^{-1}) reflecting the lower work rate (WR) in O (O 47 ± 6 W; Y 73 ± 9 W). The VO_{2p} amplitudes were unchanged over the course of training reflecting the unchanged WR at each testing time, and resulted in a similar VO_{2p} gain ($\Delta VO_{2p}/\Delta WR$) in O (10.0 ± 1.4 mL min^{-1} W^{-1}) and Y (10.6 ± 1.0 mL min^{-1} W^{-1}) over the course of training.

HR kinetics

The τHR was greater ($p < 0.05$) in O compared with Y. After 3 weeks training τHR decreased from 62 ± 21 to 53 ± 13 s in O and from 45 ± 13 to 31 ± 11 s in Y, with a further decrease in τHR ($p < 0.05$) observed after 6 weeks training (O 47 ± 12 s; Y 22 ± 7 s) (Table 2).

There was no relationship between the decrease in τHR and the decrease in τVO_2 from pre- to 3 weeks training for either O ($r = 0.19$; $p > 0.05$) or Y ($r = 0.46$; $p > 0.05$). In O, τHR was larger than τVO_{2p} at week 3 of training

Table 1 Pre-training subject characteristics and peak exercise responses

	n	Age (years)	Height (cm)	Body mass (kg)	Peak WR (W)	Peak HR (bpm)	VO_{2peak} (L min^{-1})	VO_{2peak} (mL kg^{-1} min^{-1})
Older	6	69 ± 7	163 ± 3	72 ± 6	119 ± 15	150 ± 18	1.73 ± 0.25	22.9 ± 2.3
Young	8	25 ± 5*	166 ± 5	66 ± 15	211 ± 26*	187 ± 3*	2.65 ± 0.34*	41.2 ± 4.7*

Values are means ± SD

n no. of subjects; *WR* work rate; *HR* heart rate; VO_{2peak} peak O_2 uptake

* $p < 0.05$ compared to old

Table 2 Kinetics parameters for VO_2, HR, in O and Y women and Δ[HHb] in Y women from pre-training through post-training

	Pre-training	Week 3	Week 6	Week 9	Post-training
Phase II τVO_{2p} (s)					
O[#]	55 ± 16	35 ± 12*	35 ± 10*	32 ± 12*,†	33 ± 8*
Y	31 ± 8	22 ± 4*	17 ± 5*	17 ± 5*,†	17 ± 4*
Phase II τVO_{2p} 95% CI (s)					
O[#]	8 ± 4	6 ± 2	7 ± 2	5 ± 1	6 ± 3
Y	3 ± 1	3 ± 1	2 ± 1	3 ± 1	3 ± 1
τHR (s)					
O[#]	62 ± 21	53 ± 13*,‡	47 ± 12*,†	41 ± 11*,†	41 ± 14*,†
Y	45 ± 13‡	31 ± 11*	22 ± 7*,†	26 ± 9*,†,‡	22 ± 4*,†,‡
τΔ[HHb] (s)					
Y	17 ± 3	15 ± 7	12 ± 3	13 ± 7	11 ± 4
TD-Δ[HHb] (s)					
Y	8 ± 4	7 ± 1	8 ± 2	8 ± 2	9 ± 1
τ′Δ[HHb] (s)					
Y	25 ± 5	22 ± 7	20 ± 3	21 ± 7	21 ± 3‡

Values are means ± SD

CI confidence interval; *HR* heart rate; *HHb* deoxygenated hemoglobin; τ time constant of response; *TD* time delay; τ′Δ[HHb], effective τΔ[HHb], sum of τΔ[HHb] and TD Δ[HHb]

There were no significant testing time by age interactions. *Significantly different from pre-training values ($p < 0.05$); †Significantly different from week 3 ($p < 0.05$); ‡Significantly different from Phase II τVO_{2p} ($p < 0.05$); #Significantly different from Y ($p < 0.05$)

($p < 0.05$). In Y τHR was larger than τVO_{2p} pre-training, at week 9 and post-training ($p < 0.05$; p values for week 3 and week 6 = 0.06 and 0.09, respectively).

Δ[HHb] kinetics

Due to technical issues related to NIRS data acquisition in the older women, NIRS data were only available for the young women. In Y, after the step increase in work rate, a TD-Δ[HHb] of 8 ± 2 s was observed pre-training. No change in TD-Δ[HHb] occurred as a result of training (Table 2). The amplitude of the increase in Δ[HHb] was also unaffected by training (e.g., pre-training: 4 ± 3 a.u.; post-training: 3 ± 2 a.u.). The τΔ[HHb] was similar across testing times. The overall change of the effective Δ[HHb] ($\tau'\Delta[HHb] = TD\text{-}\Delta[HHb] + \tau\Delta[HHb]$) was not affected by training (Table 2). In fact, the $\tau'\Delta$[HHb] adjustment progressed from being faster (albeit not significant) to being significantly slower than the adjustment of τVO_{2p} (Table 2). Although the $\tau'\Delta$[HHb] was not significantly greater than τVO_{2p}, the normalized $\Delta[HHb]/\Delta VO_{2p}$ displayed a significant pre-training transient "overshoot" relative to the subsequent steady state level (Fig. 1 panels 1, 2) that was abolished during the subsequent testing times (Fig. 1 panels 1, 2). The reductions in τVO_{2p} with training in Y were closely associated with a lowered normalized $\Delta[HHb]/\Delta VO_{2p}$ ratio during the exercise on-transient ($r = 0.97$, $p < 0.05$; Fig. 2).

Discussion

The main goal of this study was to investigate the time-course of adaptation of τVO_{2p} induced by endurance training in O and Y women. The main findings were as follows: (1) older women had slower VO_{2p} kinetics than young women in response to step-transitions in work rate within the moderate intensity domain; (2) the τVO_{2p} was reduced as a consequence of endurance training in both age groups, with the greatest reduction in τVO_{2p} seen within the first 3 weeks of training; (3) contrary to our hypothesis after 3 weeks training in older women, the τVO_{2p} was reduced to the extent that it was similar to that observed in the younger women prior to the start of training; (4) in young women, the reduction in τVO_{2p} with an unchanged $\tau'\Delta$[HHb] resulted in a reduction in the $\Delta[HHb]/\Delta VO_{2p}$ "overshoot" during the on-transient relative to the subsequent steady-state level, suggesting that the matching of microvascular O_2 distribution to O_2 utilization was improved, thus requiring less reliance on O_2 extraction to support the muscle O_2 requirement during the exercise transient after 3 weeks of training.

This is the first study to explore the rate of adaptation with training of VO_{2p} kinetics in O and Y women. Previous studies have shown that endurance training results in a faster VO_{2p} kinetics in both O (Babcock et al. 1994a;

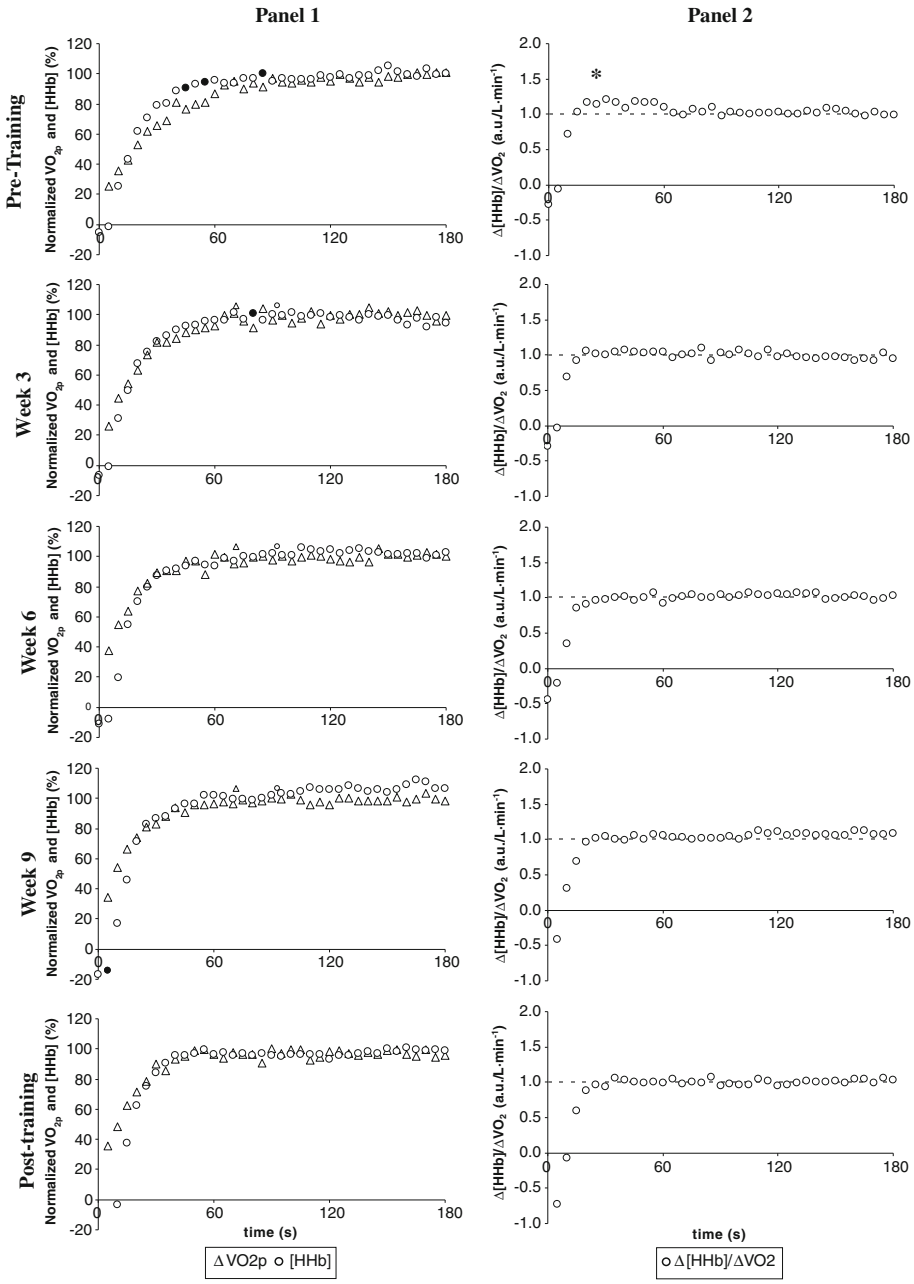

Fig. 1 **Panel 1** group mean profiles for the adjustment of Δ[HHb] (*circles*) and VO_{2p} (*triangles*; left shifted such that data from phase I VO_{2p} was not included) during the step-transition in work rate in young women from pre- to post-training. *Filled circles* denote time points at which the relative increase of Δ[HHb] is greater than the relative increase of VO_{2p} ($p < 0.05$). **Panel 2** group mean profiles for the adjustment of the normalized Δ[HHb]/ΔVO_{2p} ratio during the step-transition in work rate in young women from pre- to post-training. *Δ[HHb]/ΔVO_{2p} significantly different from 1.0 ($p < 0.05$)

Bell et al. 2001; Murias et al. 2010b) and Y (McKay et al. 2009; Murias et al. 2010b; Phillips et al. 1995) men. These changes were shown to occur as quickly as 2 days (McKay et al. 2009) or 4 days (Phillips et al. 1995) after the start of a endurance training program, with improvements continuing for 2–4 weeks of training but with no further changes observed thereafter (up to 12 weeks) (Murias et al. 2010b; Phillips et al. 1995). The findings of the present study demonstrate that the training-induced adaptations reported in older and young men are also seen in O and Y women, with reductions in τVO_{2p} occurring rapidly and continuing for $\sim$30 days after the start of an endurance training program.

It was hypothesized previously that a greater τVO_{2p} in older individuals could be related to a mismatch between O_2 distribution and O_2 utilization within the active muscles (DeLorey et al. 2004). Recently, we proposed that an improved microvascular O_2 distribution (as represented by a reduction in the transient Δ[HHb]/ΔVO_{2p} "overshoot" relative to the subsequent steady-state level) was responsible for the reduction in τVO_{2p} in O and Y men (Murias et al. 2010b). This transient "overshoot" in the Δ[HHb]/ΔVO_{2p} ratio (values >1.0) is consistent with a greater microvascular fractional O_2 extraction per unit VO_{2p} compared to the exercise steady-state (values = 1.0), and reflects a poorer matching of O_2 distribution relative to

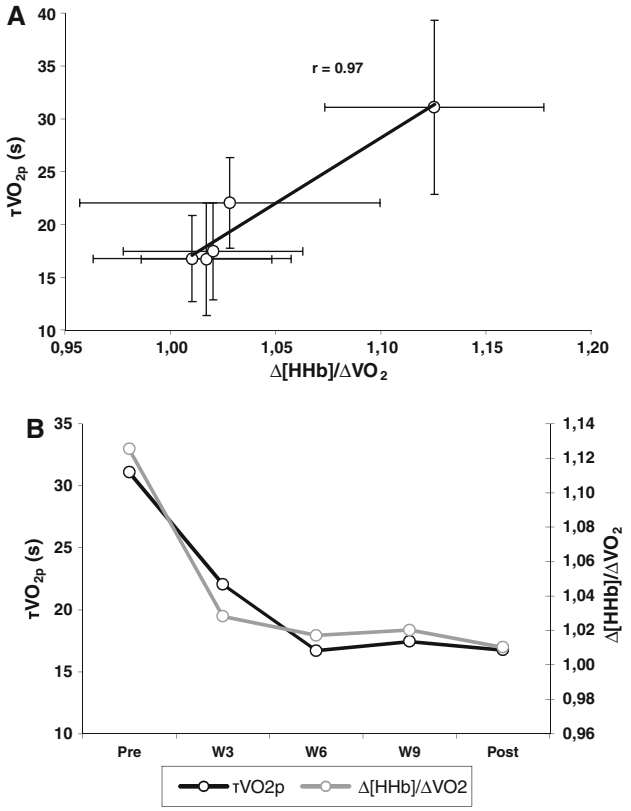

Fig. 2 **a** Correlation between changes in the normalized Δ[HHb]/ΔVO_{2p} ratio and τVO_{2p} in response to training for Y; **b** time course of changes in the normalized Δ[HHb]/ΔVO_{2p} ratio and τVO_{2p} in Y

muscle O_2 utilization in the area of the NIRS probe. Pre-training data in Y women showed that with a tendency for a smaller τ'Δ[HHb] compared to τVO_{2p} there was a small but significant transient "overshoot" in Δ[HHb]/ΔVO_{2p} (relative to steady-state values) during the transition to moderate-intensity exercise (Table 2; Fig. 1 panels 1, 2), but from 3 weeks to the end of training, the "overshoot" was abolished in young women (Table 2; Fig. 1 panels 1, 2). The reduction in τVO_{2p} early in the response to training without a concomitant reduction in τ'Δ[HHb] suggests that training resulted in improved matching of O_2 distribution to O_2 utilization that likely contributed to the faster adjustment of VO_{2m} in young women. Indeed, post-training τ'Δ[HHb] was actually greater than τVO_{2p} suggesting a well matched microvascular O_2 distribution to O_2 utilization during the on-transient through the steady-state (Table 2). It is acknowledged that an overshoot in the pre-training Δ[HHb] signal itself was not evident. It is important to notice that those studies that have shown an undershoot in the PO_{2mv} (also reflecting O_2 extraction analogous to the Δ[HHb] overshoot) were performed in single cell preparations or muscles composed of specific fiber types (Behnke et al. 2005; Hogan 2001; McDonough et al. 2005). In the human muscle mosaic, it is likely that some of the fibers in the area of NIRS interrogation showed an overshoot in the Δ[HHb] (active fibers for which the increase in blood flow and O_2 delivery has not adequately adjusted and greater extraction is needed to meet the O_2 demand) that is not observed in the overall Δ[HHb] response (because some inactive fibers may be supplied by blood flow, and some active fibers may have adequate blood flow and limited need for increased extraction to meet their VO_2 demand). It is not surprising that in the present study the Δ[HHb] adjusts with a similar time-course at different testing times. Once the initial calculated TD (likely reflecting a matched or even surplus blood flow for a given O_2 demand) is overcome, then a drop in intracellular PO_2 with the rapid increase in O_2 extraction (reflected in the Δ[HHb] signal) will be required to achieve the O_2 demand regardless of the magnitude of the decrease in the intracellular PO_2 (Walsh et al. 2005). It is unclear from the NIRS-derived signal what the actual magnitude of that increase in O_2 extraction is but, as long as the intracellular PO_2 does not drop below critical levels (which is not expected to happen in the moderate-intensity domain), an overshoot in Δ[HHb] is not to be expected. An overshoot in the Δ[HHb] signal is also unlikely since the area of inspection for the NIRS-derived signal is assumed to be composed by both active and inactive fibers that have different metabolic properties (i.e., type I and type II fibers). As such, using the Δ[HHb]/ΔVO_{2p} ratio provides an indication of blood flow distribution such that the rate of adjustment for O_2 extraction can be compared to the rate of adjustment for VO_2 and thus inferences about the matching of blood flow (O_2) distribution to O_2 utilization can be made. However, the reader should note that validation of the Δ[HHb]/ΔVO_{2p} ratio as an index of O_2 distribution within the tissue in humans awaits the development of technologies that allow for appropriate measures of O_2 delivery (and its rate of adjustment) within the muscle.

The training-induced changes in VO_{2p} and Δ[HHb] seen in young women in the present study are similar to those training-induced changes reported for young men in our previous study (Murias et al. 2010b) and confirm a high correlation and similar time course of changes in the normalized Δ[HHb]/ΔVO_{2p} ratio and τVO_{2p} (Fig. 2) that further support the notion that an improved O_2 distribution within the microvasculature plays a major role in the changes observed in τVO_{2p}. Parker et al. (2007, 2008) reported that during steady-state exercise leg blood flow and vascular conductance were greater in young women compared to young men and to older women. Taken together, these data support the idea that younger women have a greater blood flow that could facilitate matching O_2 delivery to O_2 utilization, such that at a given VO_2, O_2 extraction and a-vO_{2diff} are reduced.

A limitation of the present study is that we were unable to detect any NIRS-derived signals in the older women. A methodological consideration related to the use of NIRS is that propagation of light through the tissue is influenced not only by the muscle but also by the subcutaneous fat. A thick adipose tissue layer observed in the O group [caliper-derived measurement of adipose thickness in the thigh in O in this study was $\sim 37 \pm 7$ mm, whereas in young women thigh skinfold thickness is reported as being ~ 20 mm (Clasey et al. 1999)] results in greater "scattering" of light within the adipose tissue layer and less light returning to or detected by the receiving optode, thus lack of signal and erroneous measurements. Nevertheless, as part of the overall study of endurance training in older and young women we measured cardiac output (Q), by open circuit acetylene method during the steady-state of moderate-intensity exercise [these data were detailed in another paper (Murias et al. 2010a)]. In relation to the present analysis the calculated a-vO_{2diff} per VO_{2p} (a-vO_{2diff}/VO_{2p}), reflecting whole body O_2 extraction for a given VO_{2p}, can be used as a proxy for muscle O_2 extraction, and thus potentially provide an indication of muscle perfusion during state-state exercise. Interestingly, in the moderate-intensity steady-state a-vO_{diff}/VO_{2p} was significantly higher in O compared to Y at any testing time throughout the training program (e.g., pre-training: O 41 ± 12 mL 100 mL^{-1} blood (L min O_2)$^{-1}$; Y 22 ± 4 mL 100 mL^{-1} blood (L min O_2)$^{-1}$; post-training: O 40 ± 12 mL 100 mL^{-1} blood (L min O_2)$^{-1}$; Y 22 ± 4 mL 100 mL^{-1} blood (L min O_2)$^{-1}$). Acknowledging that this measure is based on systemic cardiac output and does not provide information during the transition to exercise, a greater reliance on O_2 extraction (i.e., a wider a-vO_{2diff}) for a given steady-state VO_2 during moderate-intensity exercise in older women suggests that blood flow to the active muscles may have been compromised. A reduced leg blood flow and vascular responsiveness during steady-state exercise and a greater reliance on O_2 extraction in older compared to young women has previously been reported (Parker et al. 2008; Proctor et al. 2003a).

The rate of adjustment of τHR is often used as a proxy of central delivery of O_2 to the tissues. In this study, τHR was smaller after 3 weeks of training and then further decreased after 6 weeks of training in both O and Y women. Nevertheless, there was no relationship between changes in τHR and τVO_{2p} in either O or Y women. Also, young women had a significantly slower τHR pre-training, at week 9, and post-training compared to the τVO_{2p} adjustment; however, the Δ[HHb]/ΔVO_{2p} ratio (likely indicating a better matching of O_2 distribution to O_2 utilization) was improved with training suggesting that microvascular blood flow distribution during the exercise on-transient may have improved. In this regard, it has been shown that an enhanced endothelium and flow-mediated vasodilatory response occurs in response to acute and chronic exercise (Haram et al. 2006), and this early alteration in vascular control may correspond to the early change in τVO_{2p}.

The mitochondrial content of ADP likely provides an important signal for activation of oxidative phosphorylation at the onset of exercise with the PCr shuttle (Whipp and Mahler 1980) serving as a spatial and temporal buffer attenuating the increase in intracellular ADP and thus slowing the adjustment of oxidative phosphorylation. Activation of intracellular enzymes to provide Acetyl CoA and electrons (in the form of reducing equivalents) to the tricarboxylic acid cycle and electron transport chain may also influence the rate of adjustment of muscle VO_2 kinetics (Grassi et al. 1998a, b). Nevertheless, data from the present [and also from our previous (Murias et al. 2010b)] study suggest that VO_2 kinetics become faster through an improved microvascular O_2 distribution, as indicated by the measure of a better matched microvascular deoxygenation relative to the rate of adjustment of VO_2. In young women it appears that short-term training can remove any constraint imposed by O_2 delivery resulting in a τVO_2 in the ~ 20 s range, whereas in older women the τVO_2 was reduced from 55 to 33 s, but the constraint to the rate of adjustment of VO_2 kinetics was not fully resolved.

In conclusion, this study demonstrated that pulmonary O_2 uptake (and muscle O_2 utilization) adjusted more rapidly in both O and Y women after only 3 weeks of endurance training with no significant changes observed thereafter. Additionally, 3 weeks of training resulted in the τVO_{2p} in O women being similar to that observed in Y women pre-training. Inequalities in O_2 distribution may contribute to the initial slower rate of adjustment in τVO_{2p} in both O and Y women. Although these data do not preclude that the fundamental control to VO_{2p} kinetics may be attributed to intracellular factors that were not measured in this study, they suggest that O_2 distribution appears to be a constraint in those with "slow" kinetics or indeed VO_2 kinetics of $> \sim 20$ s.

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