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Pulmonary oxygen uptake and muscle deoxygenation kinetics during recovery in trained and untrained male adolescents

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Abstract

Previous studies have demonstrated faster pulmonary oxygen uptake ($\dot{V}o_2$) kinetics in the trained state during the transition to and from moderate-intensity exercise in adults. Whilst a similar effect of training status has previously been observed during the on-transition in adolescents, whether this is also observed during recovery from exercise is presently unknown. The aim of the present study was therefore to examine $\dot{V}o_2$ kinetics in trained and untrained male adolescents during recovery from moderate-intensity exercise. 15 trained (15 ± 0.8 yrs, $\dot{V}o_2$ max: 54.9 ± 6.4 mL.kg^{-1}.min^{-1}) and 8 untrained (15 ± 0.5 yrs, $\dot{V}o_2$ max: 44.0 ± 4.6 mL.kg^{-1}.min^{-1}) male adolescents performed two 6-minute exercise off-transitions to 10W from a preceding "baseline" of exercise at a workload equivalent to 80% lactate threshold; $\dot{V}o_2$ (breath-by-breath) and muscle deoxyhaemoglobin (near-infrared spectroscopy) were measured continuously. The time constant of the fundamental phase of $\dot{V}o_2$ off-kinetics was not different between trained and untrained (Trained: 27.8 ± 5.9 s vs Untrained: 28.9 ± 7.6 s, *P*=0.71). However the time constant (Trained: 17.0 ± 7.5 s vs Untrained: 32 ± 11 s, *P*<0.01) and mean response time (Trained: 24.2 ± 9.2 s vs Untrained: 34 ± 13 s, *P*=0.05) of muscle deoxyhaemoglobin off-kinetics was faster in the trained subjects compared to the untrained subjects. $\dot{V}o_2$ kinetics was unaffected by training status; the faster muscle deoxyhaemoglobin kinetics in the trained subjects thus indicates slower blood flow kinetics during recovery from exercise compared to the untrained subjects.

Key words: $\dot{V}o_2$ off-kinetics, near-infrared spectroscopy, muscle deoxyhaemoglobin, training, asymmetry

Introduction

During the transition to and from exercise at an intensity below the lactate threshold (i.e. moderate-intensity exercise), the kinetics of pulmonary oxygen uptake proceed via a time-course that can be effectively modelled as a single exponential function with a time delay reflecting the muscle-to-lung transit time (Ozyener et al. 2001). The kinetics of the pulmonary oxygen uptake response at the on- and offset of exercise is of interest because they are generally interpreted to represent the kinetics of muscle oxygen consumption kinetics (Barstow et al. 1990; Grassi et al. 1996; Koga et al. 2005; Krustrup et al. 2009; Rossiter et al. 2002), though how strong this relationship is at the offset of exercise has recently been questioned (Krustrup et al. 2009).

The kinetics of the pulmonary oxygen uptake response to exercise has been extensively studied in adults and children (Armstrong and Barker 2009; Jones and Poole 2005). In general, children are considered to possess faster pulmonary oxygen uptake kinetics than adults during the transition to moderate-intensity exercise (Armstrong and Barker 2009; Fawkner et al. 2002). Children are biologically defined as being pre-pubescent and thus their physiology has not been influenced by the changing hormonal milieu that puberty brings. By contrast, adults are typically described as being post-pubertal with an altered anatomy and physiology reflective of the full time-course of pubertal effects. Adolescents therefore occupy a transitional phase where they are no longer children since they have been subject to some of the effects of an as yet incomplete puberty. Despite this, far less attention in this field has been given to adolescents. Of the few studies to examine adolescents, Cooper et al. (Cooper et al. 1985) showed similar time constants for pulmonary oxygen uptake kinetics for boys and adolescent males but slower kinetics in the adolescent females compared to the girls, although there was evidence of distinctly lower aerobic fitness in the adolescent females which may have been a confounding factor. On the other hand, Lai et al. (Lai et al. 2008) reported similar time courses for the on- and off-kinetics of pulmonary oxygen uptake in adolescents as compared to previously published data in adults. Furthermore, it has recently been shown that during the 2-year period between the ages of 14 and 16 years old the fundamental phase of pulmonary oxygen uptake kinetics slows and the contribution of the slow component increases during heavy intensity exercise (Breese et al. 2010), therefore becoming characteristic of the adult response to exercise. We have recently demonstrated faster pulmonary oxygen uptake kinetics during the transition to moderate-intensity exercise in a group of trained male adolescents as compared to an untrained control group (Marwood et al. 2010) which

is a feature of the trained state both in adults and children (Jones and Koppo 2005; Winlove et al. 2010). However, it is presently unknown whether training status speeds pulmonary oxygen uptake off-kinetics in adolescents.

The recovery of pulmonary oxygen uptake from exercise to baseline levels is considered to reflect the recovery of muscle phosphocreatine to pre-exercise levels (Barker et al. 2008; Rossiter et al. 2002). Therefore an understanding of the time course of the recovery of pulmonary oxygen consumption at the off-set of exercise is important because of the potentially deleterious effects of incomplete muscle phosphocreatine recovery on subsequent exercise performance (Ferguson et al. 2010; Vanhatalo and Jones 2009). In the adult population, training has been shown to result in both faster muscle phosphocreatine (Forbes et al. 2008; Takahashi et al. 1995; Yoshida 2002) and pulmonary oxygen uptake (Fukuoka et al. 2002; Fukuoka et al. 2006) kinetics during recovery from moderate-intensity exercise, suggestive of enhanced oxidative capacity of the exercising muscle (Barker et al. 2008; Paganini et al. 1997; Rossiter et al. 2002). The favourable adaptations to oxygen delivery, mitochondrial density and oxidative enzyme activity and thus aerobic fitness that appropriate training permits (Holloszy and Coyle 1984; Murias et al. 2010; Phillips et al. 1995; Russell et al. 2002) are likely to explain the faster recovery kinetics relative to the untrained state. However, to our knowledge no previous study has examined the effect of training status on pulmonary oxygen uptake kinetics during recovery from exercise in adolescents. Prolonged, high intensity interval training, the habitual form of training undertaken by the trained subjects in the present study, brings about significant enhancements in aerobic fitness in adolescents (Chamari et al. 2005; McMillan et al. 2005) as has been shown in adults (Krustrup et al. 2010). The purpose of the present study was therefore to examine the effect of training status on the off-kinetics of pulmonary oxygen uptake in a group of trained and untrained male adolescents; these subjects were participants in our recent analysis of their pulmonary oxygen uptake on-kinetics (Marwood et al. 2010). We hypothesised that, in line with previous data in adults, the trained subjects would exhibit faster pulmonary oxygen uptake off-kinetics as compared to the untrained controls. To gain insight into the physiological basis of any effects of training status on the off-kinetics of pulmonary oxygen uptake we also examined the kinetics of muscle deoxygenation via the deoxyhaemoglobin signal from near-infrared spectroscopy.

Methods

Subjects

Fifteen trained (15 ± 0.8 yrs) and eight untrained (15 ± 0.5 yrs) male adolescents volunteered to take part in the study. All subjects were in good health and taking no medications that would influence cardiovascular or muscular function. These subjects were participants in a previously published study examining the on-kinetics response to exercise (Marwood et al. 2010); the present study represents an analysis of the off-transition from these exercise bouts. By self-assessment, the two groups were matched for maturity status (Tanner stage range 2-4, for trained and untrained subjects, see table 1 for subjects' physical characteristics). Informed written parental consent and subjects' assent were obtained prior to participation. The study was approved by an institutional research ethics committee.

Thirteen of the trained subjects were soccer players from an English Premier League club youth academy. These subjects reported an average of 7.4 ± 2.2 years of training, currently practicing 9.9 ± 1.4 months yearly, 6.1 ± 1.9 hours a week and playing in competitive matches for 6.9 ± 1.8 years. A further two subjects reporting seven hours of weekly cycle and martial arts training, respectively. The untrained group consisted of eight subjects who reported little regular physical activity and limited recreational sports participation.

Experimental protocol

Subjects visited the laboratory on two occasions, separated by 3 – 7 days. At each visit, subjects were asked to have refrained from strenuous exercise in the preceding 48 hours and to be 3 hours post-prandial. At the first visit, subjects performed an incremental exercise test to volitional exhaustion on a cycle ergometer at 60rpm (Lode Excalibur Sport, Groningen, The Netherlands). The test consisted of 3-minute increments of 35W commencing from an initial workload of 35W. Maximal oxygen uptake ($\dot{V}o_2$ max) was defined as the mean of the two highest 30-s average values during the final stage of exercise. Additionally, the lactate threshold was independently estimated via the v-slope method (i.e. gas exchange threshold) and confirmatory inspection of the ventilatory equivalent and end-tidal pressure plots for oxygen uptake and carbon dioxide production (Beaver et al. 1986) by two of the authors. At the second visit to the laboratory, subjects completed two 6-minute square-wave exercise transitions to 80% of the workload which elicited the gas exchange threshold ("loaded phase") immediately followed by six minutes of exercise at 10W ("unloaded phase"). Subjects maintained a cadence of 60 – 65 rpm throughout the exercise transitions. Each exercise

transition was separated by a minimum 1 hour of rest during which time subjects' body composition was assessed via air-displacement plethysmography (BodPod, Life Measurement Inc., Concord, USA).

Measurements

Throughout all exercise bouts heart-rate was measured every 5-s via short-range telemetry (Polar S610, Kempele, Finland). Expired air was measured breath-by-breath via standard open circuit techniques with minute ventilation assessed via pneumotachometer (Zan 600, Oberthulba, Germany). During the square-wave exercise bouts, continuous non-invasive measurements of muscle deoxygenation status were also made via near-infrared spectroscopy (OxiplexTS, ISS, Champaign, USA). The OxiplexTS uses light at wavelengths of 690 and 830 nm and is a frequency-domain multidistance system which enables direct measurement of the scattering, and therefore absorption coefficient, of NIR-light. Hence absolute values (μM) of oxyhaemoglobin (HbO_2), deoxyhaemoglobin (*HHb*) and total haemoglobin (*THb*) concentration in the interrogated tissue are produced. Light source–detector separation distances of 1.50 – 3.04 cm for each wavelength were utilised with cell water concentration assumed constant at 70%. The extent of the contribution which haemoglobin and myoglobin make to the near-infrared signal is presently unclear, therefore for the purposes of the present study the abbreviations [HbO_2], [*HHb*] & [*THb*] refer to the combined signal due to haemoglobin and myoglobin. For the present study, data was sampled at 2 Hz. The flexible probe was placed longitudinally along the belly of the left vastus lateralis midway between the greater trochanter and the lateral condyle of the tibia and marked with washable pen such that the probe could be placed in the same position for the second exercise bout. The probe was held firmly in place by elastic Velcro strapping and movement of the optical fibres during cycling limited by taping them to an adjacent table. Following each trial, indentations of the probe on the subject's skin were inspected to confirm that the probe had not moved, which was the case for every exercise transition. The NIRS probe was calibrated prior to each testing session using a calibration block of known absorption and scattering coefficients. Calibration was then cross-checked using a second block of known but distinctly different absorption and scattering coefficients. Each of these procedures was according to the manufacturer's recommendations.

Pulmonary oxygen uptake off-kinetic analysis

Abnormal breaths due to coughs and swallows were first removed from the pulmonary oxygen uptake ($\dot{V}o_2$) data to prevent skewing of the underlying response. The criterion for removal of these breaths was those

that were different to the mean of the adjacent four data points by more than three times the standard deviation of those four points. Each dataset was then interpolated second-by-second between 0 – 360 s; the end of the loaded phase defined as 0 s. The two datasets were then ensemble averaged to produce a single response for each subject. The first 15 s of the ensemble dataset were then removed (Barker et al. 2008), having first confirmed visually that this removed all phase 1 data. The remaining dataset (i.e. up until the end of the unloaded phase) was fitted to a mono-exponential curve (Origin, Microcal, Northampton, MA, USA) with a delay relative to the end of the loaded phase of exercise of the form:

$$\dot{V}o_{2(t)} = \dot{V}o_{2(b)} + A_{Vo_2} * (1 - e^{-(t - TD_{Vo_2}) / \tau_{Vo_2}})$$

Where $\dot{V}o_{2(b)}$ is the baseline $\dot{V}o_2$ during the loaded phase taken from the on-transition modelling process, (see Marwood et al. 2010), A_{Vo_2} is the asymptotic amplitude of the fundamental (phase 2) response, TD_{Vo_2} is a time delay relative to the offset of the loaded phase and τ_{Vo_2} is the time constant for the fundamental component of the response. Steady state (phase 3) oxygen uptake ($\dot{V}o_{2(SS)}$) during the unloaded phase is therefore calculated as $\dot{V}o_{2(b)}$ - A_{Vo_2} and the mean response time (MRT_{VO_2}) was defined as τ_{Vo_2} + TD_{Vo_2}.

NIRS kinetic analysis

The [*HHb*] response to exercise was modelled in a similar fashion to $\dot{V}o_2$ kinetics; firstly the two datasets for each subject were ensemble averaged to produce a single dataset for each subject with data points at 0.5 s intervals (i.e. 2 Hz). Secondly, the point at which [*HHb*] starts to decrease following a short delay (typically 5 – 10 s) relative to the end of the loaded phase was identified. This time-point was identified by utilising a double linear regression plot at each time point within the first 20 s of the ensemble dataset and, using custom written software and the Solver function in Microsft Excel, determining the time-point at which the sum of error squared was minimised. This technique assumes linear characteristics of the data in the first few seconds following the onset of the decrease in [*HHb*], which given the short time frame appears reasonable, despite the entire response being exponential in nature. From this point up until the time at which the $\dot{V}o_2$ data achieved 98% of its final value, (t = 4 τ_{Vo_2} + TD_{Vo_2}, i.e. effective steady state) the data

were fitted to a mono-exponential curve (Origin, Microcal) with a delay relative to the onset of exercise of the form:

$$HHb(t) = HHb_{(b)} + A_{HHb} * (1 - e^{-(t - TD_{HHb})/\tau_{HHb}})$$

Where $HHb_{(b)}$ is the baseline [*HHb*] measured during the final 30s of the loaded phase, A_{HHb} is the asymptotic amplitude of the "phase 2" response, TD_{HHb} is a time delay relative to the offset of the loaded phase and τ_{Hb} is the time constant for the response. The absolute value of [*HHb*] during "phase 3" ($HHb_{(\phi 3)}$) of the response (at t = 4 τ_{Vo_2} + TD_{Vo_2}) is therefore calculated as $HHb_{(b)}$ - A_{HHb} and the mean response time (MRT_{HHb}) was defined as τ_{HHb} +. TD_{HHb}. It is not certain whether the processes underlying the [*HHb*] response during the off-transition are exponential in nature. However, visual inspection of the data and reference to previous literature (Ferreira et al. 2005) suggests that a monoexponential decay model provides a reasonable estimate of the time course of muscle deoxygenation during this "primary" phase of the [*HHb*] off-kinetic response.

Heart-rate kinetics

Heart-rate kinetics was modelled via a similar monoexponential function as for $\dot{V}o_2$ and [*HHb*] kinetics but with the response constrained to start at the offset of exercise (t=0), i.e. no delay term (Chin et al. 2007). "Baseline" heart-rate ($HR_{(b)}$) was taken as the steady state heart-rate during the loaded phase taken from the on-transition modelling process (see Marwood et al. 2010), with steady state heart-rate ($HR_{(ss)}$) calculated as the difference between the baseline heart-rate and the amplitude (A_{HR}) of the response. The time constant of the response was defined as τ_{HR}.

Statistics

Comparisons between groups of $\dot{V}o_2$, [*HHb*] and heart-rate kinetics were made using an independent two-tailed t-test with homogeneity of variance checked via Levene's test. [*THb*] and [*HbO*$_2$] data were analysed by a 2-way ANOVA with repeated measures (group x time) using the mean data at 30s intervals throughout

the recovery period. Comparisons of $\dot{V}O_2$, [*HHb*] and heart-rate kinetics with the on-transition data (Marwood et al. 2010) were made using a paired t-test. Data are presented as mean ± standard deviation and statistical significance accepted at the $P \leq 0.05$ level.

Results

$\dot{V}O_2$ off-kinetics

Representative plots of an untrained and trained subject are presented in figure 1 with exponential curve fit and residuals shown. The 95% confidence interval for τ_{Vo_2} was 2.94 ± 0.94 s for the trained subjects and 3.6 ± 1.0 s for the untrained subjects. Neither the time constant (*P*=0.71) nor mean response time (*P*=0.13) for the off-kinetics of oxygen uptake were different between the trained and untrained subjects (table 2).

NIRS off-kinetics

The time constant (*P*<0.01) and mean response time (*P*=0.05) of [*HHb*] kinetics was shorter in the trained versus untrained subjects, (table 2). [*HHb*] at baseline and during phase 3 of the off-transition was also higher in the trained subjects, (*P*<0.01, table 2); when corrected for oxygen uptake (i.e. *HHb* / $\dot{V}O_2$), these differences remained (*P*=0.04, *P*<0.01). Representative plots of [*HHb*] kinetics are shown in figure 2, the 95% confidence interval for τ_{HHb} was 1.34 ± 0.32 s for the trained subjects and 3.7 ± 2.0 s for the untrained subjects. Group mean responses of [*THb*] are shown in figure 3. Total haemoglobin concentration was significantly higher throughout recovery in trained versus untrained subjects (main effect group, *P*<0.01, figure 3), however a similar time-courses was observed during recovery for the trained and untrained subjects (group x time interaction, *P*=0.1, figure 3). A similar pattern to that of the [*THb*] data was seen for [HbO_2] data in both trained and untrained subjects.

Heart-rate off-kinetics

Neither the time constant (Trained: 46 ± 13 vs Untrained: 45 ± 19 bpm, *P*=0.87), baseline (Trained: 137 ± 10 vs Untrained: 132 ± 12 bpm, *P*=0.30), amplitude (Trained: 36 ± 10 vs Untrained: 29 ± 5 bpm, *P*=0.10), or steady state value (Trained: 101 ± 10 vs Untrained: 103 ± 14 bpm, *P*=0.80), for heart-rate kinetics were different between groups. The 95% confidence interval for τ_{HR} was 6.0 ± 2.0 s (Trained) and 8.8 ± 4.4 s (Untrained).

Comparison with on-kinetics

For comparison, data from the on-transition obtained from a previous study (Marwood et al. 2010) which the present subjects were also participants in are presented in table 3. For trained subjects, the time constant of the off-transition of pulmonary oxygen uptake kinetics was significantly greater than the on-transition (P<0.01, tables 2 & 3), but there was no difference between the off- and on-transition for the untrained subjects. In contrast, the mean response time of pulmonary oxygen uptake kinetics was less than during the off- versus on-transition for both trained and untrained subjects (P=0.04, P<0.01 respectively, tables 2 & 3). This effect appeared to be primarily dictated by a shorter time delay of the oxygen uptake response (see tables 2 & 3). For the [*HHb*] data, the time constant and mean response time of [*HHb*] kinetics were both significantly greater during the off-transition compared to the on-transition for both trained (P<0.01, P=0.01) and untrained (P<0.01, P=0.01) subjects (see tables 2 & 3). The rate constant of heart-rate kinetics was greater during the off- versus on-transition in the trained subjects, but unchanged between the on- and off-transition in the untrained subjects.

Discussion

The purpose of the present study was to examine the effects of training status on the off-kinetics of pulmonary oxygen uptake in adolescents. We hypothesised that the trained subjects would possess faster pulmonary oxygen uptake kinetics during recovery from exercise. However, contrary to this hypothesis, there was no difference in the off-kinetics of pulmonary oxygen uptake between the trained and untrained groups. Relatively few previous studies have evaluated the effects of training on the off-kinetics of pulmonary oxygen uptake (Billat et al. 2002; Fukuoka et al. 2002; Fukuoka et al. 2006); these studies were limited to adult populations.

To our knowledge, the present study is the first to examine the effects of training status on the off-kinetics of pulmonary oxygen uptake in adolescents. The adolescent period represents a transitional phase of growth between child and adulthood which may provide a departure from the response to exercise training as a child or adult. For example, the impact of aerobic training on $\dot{V}o_2$ peak has been suggested to be blunted in midpubescence as compared to the pre- and post-pubertal period (Weber et al. 1976). In contrast, in a longitudinal study, marked responses of $\dot{V}o_2$ peak to training were observed only in a period close to peak

height velocity (Kobayashi et al. 1978). The effect of exercise training on pulmonary oxygen uptake kinetics (a parameter related to aerobic fitness) in adolescents therefore deserves attention since the outcome cannot be assumed based on data from children and / or adults. In the present study, the time course of pulmonary oxygen uptake kinetics in the present study was similar to previous studies in adults (i.e. ~30s, Brittain et al. 2001; Ozyener et al. 2001; Paterson and Whipp 1991). However, this congruence of the adult and present adolescent data does not apparently extend to the effect of training status since in the present study pulmonary oxygen uptake kinetics was unchanged with training status, whereas previous studies in adults have shown faster pulmonary oxygen uptake kinetics in recovery following exercise training (Billat et al. 2002; Fukuoka et al. 2002; Fukuoka et al. 2006).

The cross-sectional study design we employed potentially limits the conclusions that can be drawn from the data as the two groups of subjects may have been pre-disposed to possess similar pulmonary oxygen uptake kinetics to each other. However, the trained group reported 6 – 7 hours of training a week over a number of years, with thirteen of the fifteen trained subjects being part of a structured training programme with a professional football club in the English Premier League. The type of training associated with football (i.e. prolonged, high intensity aerobic training) brings about significant enhancements in aerobic fitness in adolescents (Chamari et al. 2005; McMillan et al. 2005). In comparison, the untrained group reported little or no regular physical activity and limited recreational exercise. Reference to table 1 objectively demonstrates the different training status of the two groups where, for example, the $\dot{V}o_2$ max was ~11 ml.kg^{-1}.min^{-1} higher in the trained as compared to the untrained subjects. Therefore we are confident that the two groups' training status was distinctively different from each other.

We hypothesised that pulmonary oxygen uptake kinetics would be faster during recovery from moderate-intensity exercise in the trained versus untrained subjects. This hypothesis was based in part on the findings of similar studies in adults (Fukuoka et al. 2002; Fukuoka et al. 2006), and the assumption that pulmonary oxygen uptake kinetics reflects muscle oxygen consumption kinetics (Grassi et al. 1996; Koga et al. 2005; Krustrup et al. 2009; Rossiter et al. 2002), which has been shown to be faster during recovery in the trained state (as indicated by muscle phosphocreatine kinetics, Forbes et al. 2008; Takahashi et al. 1995; Yoshida 2002). Given the distinction in training status between our two groups, it is therefore perhaps surprising that there was no difference in the off-kinetics of pulmonary oxygen uptake between the trained and untrained

adolescents. Our data therefore imply that muscle oxygen consumption kinetics during recovery from moderate-intensity exercise is unaffected by training status in male adolescents, in contrast to our recent analysis of the on-transition in this population (Marwood et al. 2010). However, at the off-transition there is a paucity of data regarding the agreement between muscle oxygen consumption and pulmonary oxygen uptake kinetics. Good agreement between muscle phosphocreatine and pulmonary oxygen uptake kinetics during recovery from exercise has been demonstrated (Rossiter et al. 2002). However, pulmonary oxygen uptake kinetics was recently shown to be significantly longer than, and unrelated to, direct measurements of muscle oxygen consumption kinetics during the recovery from exercise (Krustrup et al. 2009). These data suggest that the otherwise close relationship between pulmonary oxygen uptake and muscle oxygen consumption kinetics may be dissociated in the recovery period (Krustrup et al. 2009). That we could find no effect of training status on pulmonary oxygen uptake kinetics during recovery from exercise is supportive of this contention.

One explanation offered by Krustrup *et al.* (Krustrup et al. 2009) for the disparity between muscle and pulmonary oxygen uptake kinetics during recovery is that whilst muscle energy turnover declines rapidly in recovery, energy turnover in the rest of the body takes longer to recover, thus causing prolonged recovery kinetics of pulmonary oxygen uptake relative to those of muscle oxygen consumption. The effects of elevated pulmonary ventilation, cardiac work, deep body temperature, lactate clearance and gluconeogenesis may all be important in this regard, particularly at higher absolute work-rates even within the moderate-intensity domain which necessitate greater absolute perturbations to ventilation and cardiac work and an increased, albeit transient, reliance upon non-oxidative metabolism and thus lactate production (Cerretelli et al. 1979). The relative importance of these factors during recovery from moderate-intensity exercise is not clear, however some of these effects may play a role in determining the symmetry, or lack thereof, of pulmonary oxygen uptake kinetics at the on- and off-transition from exercise (Brittain et al. 2001; Rossiter et al. 2005). It is therefore interesting to note that in comparing the present off-transition data with the data at the on-transition that was recently published for this group of trained and untrained male adolescents (Marwood et al. 2010) it can be seen (table 3) that the untrained subjects demonstrated on-off symmetry whereas the trained subjects, exercising at a higher absolute workload within the moderate-intensity domain, had asymmetric pulmonary oxygen uptake kinetics. A number of studies of the transition to and from upright cycle exercise within the moderate-intensity domain in untrained, albeit healthy, subjects

have demonstrated similar time-courses for pulmonary oxygen uptake kinetics, i.e. on-off symmetry (Cleuziou et al. 2004; Ozyener et al. 2001; Paterson and Whipp 1991). However, previous studies in trained subjects have demonstrated on-off asymmetry in the pulmonary oxygen uptake kinetic response (Fukuoka et al. 2002; Kilding et al. 2005; Kilding et al. 2006; Kilding et al. 2007). Of particular note is the study by Fukuoka et al. (Fukuoka et al. 2002) where previously untrained adults undertook a 90 day training programme. At baseline these subjects demonstrated on-off symmetry; however whilst the training programme speeded the pulmonary oxygen uptake kinetics during both the off- and on-transition, these effects were much more marked in the on-transition resulting in an apparent on-off asymmetry at the end of the training programme. It is therefore tempting to speculate that the extended moderate-intensity work-rate range that endurance training permits amplifies the expression of on-off asymmetry for pulmonary oxygen uptake kinetics that may not be discriminable at lower work-rate increments. However such a hypothesis remains to be tested.

[HHb] kinetics

The deoxyhaemoglobin (*HHb*) signal from near-infrared spectroscopy (NIRS) reflects the balance between oxygen supply and oxygen demand in the interrogated tissue. In the present study, the initial time delay of [*HHb*] was greater in the trained versus untrained subjects, before proceeding with significantly faster kinetics during "phase II" of the response. Nevertheless, the overall kinetics of [*HHb*] were significantly faster in the trained versus untrained subjects. Given the similar time course of pulmonary oxygen uptake off-kinetics between groups, this suggests a slower blood flow response in recovery that, relative to the untrained subjects, will maximise conductive and diffusive oxygen flux (Behnke et al. 2009). However, notwithstanding that pulmonary oxygen uptake kinetics was not different between groups, this result does not necessarily imply that oxygen delivery was limiting to pulmonary oxygen uptake kinetics during recovery in the untrained subjects. Were this to be the case an initial overshoot in the [*HHb*] response may have been expected, indicative of a mismatch in the blood flow-to-muscle oxygen consumption relationship in favour of muscle oxygen consumption (Barstow et al. 1990; Behnke et al. 2009). Furthermore, in healthy subjects pulmonary oxygen uptake kinetics has been shown to be unaffected by changes in inspired oxygen fraction during recovery from exercise (Hughson and Kowalchuk 1995). Nonetheless it is interesting to note that as early in life as adolescence, the untrained state is apparently characterised by faster blood flow recovery kinetics as compared to the trained state.

It is noteworthy that the exercise baseline and steady state of [*HHb*] was significantly higher in the trained versus the untrained subjects, which remained following a correction for oxygen uptake, (P=0.04, P<0.01). This may suggest a greater oxygen extraction for a given oxygen uptake in the trained subjects and thus a blunted blood flow. However, given the enhanced estimated capillary blood flow kinetics during the on-transition in the trained subjects (Marwood et al. 2010), this seems unlikely. Therefore an alternative explanation is a differential blunting of the near-infrared signal between groups due to the effects of subcutaneous adipose tissue thickness at the site of interrogation. Since fat acts as a low-scattering constant absorber of near-infrared light an increased subcutaneous fat thickness has the potential to blunt the interrogative depth of near-infrared light into the interrogated tissue and therefore reduce the detectable change in haemoglobin oxygenation in response to exercise (Bhambhani 2004; Van Beekvelt et al. 2001). Though we did not measure skinfold thickness at the thigh, the significantly higher percentage body fat in the untrained subjects may also have manifested itself as increased subcutaneous fat at the site of interrogation and could therefore explain the tendency for lower exercise baseline and steady state values of [*HHb*] even when corrected for oxygen uptake. Furthermore, in the vastus lateralis a slight predominance of slow twitch fibres has been shown in the deeper regions of the muscle tissue (Lexell et al. 1983). Therefore a greater penetrative depth of near-infrared light would likely favour faster microvascular oxygen pressure (McDonough et al. 2004) and thus [*HHb*] kinetics (Ferreira et al. 2005) during recovery and may explain some of the differences between groups. However, whether depth differences in muscle fibre type composition exist in the adolescent population is unknown. Additionally, aside from potential differences in muscle fibre type composition, differences in the absolute values of [*HHb*] is not expected to affect determination of the kinetic response. There were also differences between groups in the extent of the exercise recovery hyperaemic response as indicated by [*THb*] (see figure 3) which may also have been related to the available interrogative depth of NIR light as a consequence of differences in the depth of subcutaneous fat at the site of interrogation. These differences may have impacted upon the [*HHb*] data since the [*HHb*] signal is in part made up of the arterial microcirculation. However, in healthy populations arterial blood is ~99% saturated, therefore increases in the [*THb*] signal due to increases in arterial blood volume at the site of interrogation will have limited effects on the [*HHb*] signal outside of its role in indicating the balance between oxygen supply and demand. Taken together, whilst the potentially blunted interrogative depth of the near-infrared light may have impacted on the [*HHb*] kinetics due to fibre type differences at

different tissue depths and differences in blood volume, the marked differences in recovery profiles of [*HHb*] between groups are likely to reflect the underlying physiology of these trained and untrained adolescent subjects

In summary the present study did not show any effect of training status on the kinetics of pulmonary oxygen uptake during the recovery from moderate-intensity exercise in a group of well trained and untrained male adolescents. These data are in contrast to our analysis of these subjects' pulmonary oxygen uptake kinetics during the on-transition to exercise (Marwood et al. 2010) and taken together may therefore be supportive of recent work demonstrating a relationship between muscle oxygen consumption and pulmonary oxygen uptake kinetics during the transition to, but not the recovery from exercise (Krustrup et al. 2009). The present study also demonstrated significantly faster muscle deoxyhaemoglobin recovery kinetics in the trained versus untrained subjects which, in combination with the pulmonary oxygen uptake kinetic data, suggests slower blood flow kinetics during recovery in the trained subjects.

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Table 1: Summary of physical characteristics of subjects

	Trained	Untrained
Age (yrs)	15 ± 0.8	15 ± 0.5
%. Tanner stage 2	13.3	12.5
%. Tanner stage 3	33.3	25.0
%. Tanner stage 4	53.3	62.5
Height (cm)	168 ± 7	173 ± 8
Mass (kg)	56.5 ± 9.9	61.3 ± 8.8
% Body Fat**	10.3 ± 5.8	17.1 ± 4.9
VO_2max ($L.min^{-1}$)*	3.10 ± 0.62	2.70 ± 0.35
VO_2max ($mL.kg^{-1}.min^{-1}$)**	54.9 ± 6.4	44.0 ± 4.6
VO_2 at LT ($L.min^{-1}$)*	2.11 ± 0.52	1.70 ± 0.24
VO_2 at LT ($mL.kg^{-1}.min^{-1}$)**	36.6 ± 6.2	29.0 ± 3.2
% VO_2max at LT**	69.4 ± 6.9	60.3 ± 6.1

*Trained versus untrained, $P<0.05$; **Trained versus untrained, $P<0.01$.

Table 2. Off-kinetic characteristics of $\dot{V}O_2$, [*HHb*] and heart-rate data in trained and untrained subjects

	VO_2 ($l.min^{-1}$)		[*HHb*] (μM)		Heart-rate (bpm)	
Parameter	Trained	Untrained	Trained	Untrained	Trained	Untrained
Baseline	1.78 ± 0.33**	1.38 ± 0.30	37 ± 15**	18 ± 13	137 ± 10	132 ± 12
Amplitude	1.17 ± 0.25**	0.79 ± 0.21	18 ± 12	8 ± 12	36 ± 10	29 ± 5
Absolute value at phase 3	0.61 ± 0.15	0.59 ± 0.10	18.8 ± 4.7**	9.0 ± 2.8	101 ± 10	103 ± 14
Time delay (s)	7.6 ± 5.6	9.9 ± 5.7	7.2 ± 3.6*	2.0 ± 6.8		
Time constant (s)	27.8 ± 5.9	28.9 ± 7.6	17.0 ± 7.5*	32 ± 11	46 ± 13	45 ± 19
Mean response time (s)	35.5 ± 4.9	38.8 ± 4.4	24.2 ± 9.2*	34 ± 13		

*Trained versus untrained, $P \leq 0.05$; **Trained versus untrained, $P \leq 0.01$.

Table 3. On-transition kinetics of $\dot{V}O_2$, [*HHb*] and heart-rate data in trained and untrained subjects. Data is from a previous study (Marwood et al. 2010) which the present subjects were also participants in.

	VO_2 (l.min^{-1})		[*HHb*] (μM)		Heart-Rate (bpm)	
Parameter	Trained	Untrained	Trained	Untrained	Trained	Untrained
Amplitude	1.14 ± 0.25	0.77 ± 0.23	12.5 ± 8.5$^{\#\#}$	4.7 ± 5.5	44 ± 10$^{\#\#}$	38 ± 6$^{\#\#}$
Baseline[1]	0.64 ± 0.13	0.59 ± 0.10	22.2 ± 7.1	12.0 ± 6.2	92 ± 10$^{\#\#}$	93 ± 12$^{\#\#}$
Time delay (s)	16.2 ± 3.4$^{\#\#}$	14.9 ± 5.3	7.3 ± 2.2	7.9 ± 1.9		
Time constant (s)	22.8 ± 7.2$^{\#\#}$	30.4 ± 8.7	9.8 ± 3.1$^{\#\#}$	10.0 ± 2.6$^{\#\#}$	37 ± 10$^{\#}$	49 ± 13
Mean response time (s)	38.9 ± 6.2$^{\#}$	45.3 ± 5.8$^{\#}$	17.0 ± 2.4$^{\#\#}$	17.8 ± 2.3$^{\#\#}$		

[1]Compared with absolute values at phase 3 during the off-transition. $^{\#}$Off- versus on-kinetics, $P \leq 0.05$; $^{\#\#}$Off- versus on-kinetics, $P \leq 0.01$;

Figure legends

Figure 1. Representative plots of $\dot{V}o_2$ kinetics for trained (circles) and untrained subjects (squares) with phase 2 exponential curve fit and residuals shown. Off-transition commenced at t = 0 s as indicated by the vertical line.

Figure 2. Representative plots of [*HHb*] kinetics for trained (circles) and untrained subjects (squares) with exponential curve fit and residuals shown. Data is modelled from TD_{Hb} up to the point at which a steady state of $\dot{V}o_2$ was effectively achieved, i.e. t=4 τ_{Vo_2} + TD_{Vo_2}. Off-transition commenced at t = 0 s as indicated by the vertical line.

Figure 3. Group mean responses of *THb* for trained (circles) and untrained subjects (squares). Off-transition commenced at t = 0 s as indicated by the vertical line. Error bars removed for clarity.