Faster pulmonary oxygen uptake kinetics in trained versus untrained male adolescents

Simon Marwood¹, Denise Roche¹, Thomas Rowland², Max Garrard¹, Viswanath B. Unnithan¹ FACSM

¹Sport and Exercise Physiology Research Team, Liverpool Hope University, Liverpool, UK,
²Baystate Medical Center, Springfield, MA, USA

Corresponding author: Simon Marwood

Health & Applied Social Sciences
Liverpool Hope University
Liverpool
UK
L16 9JD
T: +44 (0)151 291 3629
F: +44 (0)151 291 3414
marwoos@hope.ac.uk

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Abstract

Exercise training results in a speeding of pulmonary oxygen uptake ($\dot{V}O_2$) kinetics at the onset of exercise in adults, however only limited research has been conducted with children and adolescents. **PURPOSE:** The aim of the present study was to examine $\dot{V}O_2$ and muscle deoxygenation kinetics in trained and untrained male adolescents. **METHODS:** 16 trained (15 ± 0.8 yrs, $\dot{V}O_2$ peak: 54.7 ± 6.2 mL.kg$^{-1}$.min$^{-1}$, Self-assessed Tanner stage range 2 – 4) and 9 untrained (15 ± 0.6 yrs, $\dot{V}O_2$ peak: 43.1 ± 5.2 mL.kg$^{-1}$.min$^{-1}$, Tanner stage range 2 – 4) male adolescents performed two 6-minute exercise transitions from a 3 minute baseline of 10W to a workload equivalent to 80% lactate threshold separated by a minimum of 1 hour passive rest. Oxygen uptake (breath-by-breath) and muscle deoxygenation (deoxyhaemoglobin signal from near infrared spectroscopy) were measured continuously throughout baseline and exercise transition. **RESULTS:** The time constant of the fundamental phase of $\dot{V}O_2$ kinetics was significantly faster in trained versus untrained subjects (Trained: 22.3 ± 7.2 s vs Untrained: 29.8 ± 8.4 s, p=0.03). In contrast, neither the time constant (Trained: 9.7 ± 2.9 s vs Untrained: 10.1 ± 3.4 s, p=0.78) or mean response time (Trained: 17.4 ± 2.5 s vs Untrained: 18.3 ± 2.3 s, p=0.39) of muscle deoxygenation kinetics differed with training status. **CONCLUSION:** The present data suggest that exercise training results in faster $\dot{V}O_2$ kinetics in male adolescents, though inherent capabilities cannot be ruled out. Since muscle deoxygenation kinetics were unchanged, it is likely that faster $\dot{V}O_2$ kinetics were due to adaptations to both the cardiovascular system and the peripheral musculature.

Key words: near infrared spectroscopy, oxidative metabolism, heart-rate kinetics, exercise
Introduction

**Paragraph 1** During the transition from rest to exercise at an intensity below the lactate threshold (i.e. moderate intensity exercise), pulmonary oxygen uptake ($\dot{V}O_2$) proceeds, following a short delay representing the muscle to lung blood transit time, along a time course that can be confidently modelled by a single exponential decay term (44). This “phase II” or “fundamental” response of $\dot{V}O_2$ kinetics is thought to closely represent the kinetics of muscle oxygen consumption, at least in healthy subjects (2,4,38). Perhaps the most potent stimulus to bring about a speeding of $\dot{V}O_2$ kinetics, and thus a faster attainment of a steady state of $\dot{V}O_2$, is exercise training. A number of both cross-sectional (10,12,27,28,30,37) and longitudinal (1,7,8,21,24,36,46) studies in adults have demonstrated training-induced reductions in the time constant of the fundamental phase of $\dot{V}O_2$ kinetics during both moderate and heavy intensity exercise. However, in contrast to this large body of evidence surrounding the impact of training on $\dot{V}O_2$ kinetics in adults, there is a relative dearth of data regarding the effects of training in children and adolescents.

**Paragraph 2** Since children and adolescents are characterised by higher oxidative enzyme activity (23) and faster oxygen uptake kinetics (19) compared to adults, the effects of training on oxygen uptake kinetics may be limited in this population. Alternatively, younger subjects respond to endurance training in a similar manner to adults, with improvements in aerobic capacity reflected by enhancements in blood volume and oxidative enzyme activity (16,17). Given the link between training status and oxygen uptake kinetics in adults, it therefore seems reasonable to also expect faster oxygen uptake kinetics in trained children and adolescents, compared to untrained controls. Conversely, in the only previous systematic study of this issue, Obert et al. (35) showed no difference in the fundamental phase of
\( \dot{V}O_2 \) kinetics in either moderate or heavy intensity exercise between trained and untrained children. However, there was a dichotomy between the training regime undertaken and the experimental exercise mode since this study utilised cycle ergometry as the mode of exercise, yet the experimental group were trained swimmers. Across a number of different sports, it has been shown that \( \dot{V}O_2 \) kinetics in trained subjects were markedly slower when the muscle group under investigation was not specific to the training (10). Hence it is perhaps unsurprising that Obert et al. (35) could show no difference in \( \dot{V}O_2 \) kinetics during cycle ergometry in trained swimmers versus untrained controls. It is therefore feasible that if the muscle group under investigation is that which is predominately exercised during training, faster \( \dot{V}O_2 \) kinetics will be demonstrable in trained subjects, versus untrained controls, in children and adolescents.

**Paragraph 3** Exercise training has the potential to augment both oxygen delivery and oxygen utilisation mechanisms, hence it is possible that improvements in oxygen delivery and utilisation mechanisms interact to result in the faster \( \dot{V}O_2 \) kinetics that are demonstrable in trained versus untrained subjects (42). The deoxyhaemoglobin signal from near infrared spectroscopy (NIRS) indicates the balance between oxygen supply and demand in the interrogated tissue; hence measurement of muscle deoxygenation kinetics in the exercising muscle can be used to examine the relative mismatch in oxygen delivery and oxygen utilisation at the onset of exercise. Slower (relative to a relevant control) muscle deoxygenation kinetics would indicate a tipping of the oxygen delivery/utilisation mismatch in favour of oxygen delivery, whereas faster muscle deoxygenation kinetics would indicate a tipping of this mismatch in favour of oxygen utilisation. Hence NIRS can be utilised to help determine mechanisms responsible for alterations in oxygen uptake dynamics (15).
Paragraph 4 Given the limitations of previous studies (13), the purpose of the present study was to examine pulmonary oxygen uptake kinetics in trained and untrained male adolescents. Based on data from adult populations, we hypothesised that the trained subjects would possess faster $\dot{V}O_2$, but unchanged muscle deoxygenation kinetics in response to exercise reflecting adaptations of both oxygen supply and oxygen utilisation mechanisms in response to chronic exercise training.
Methods

Subjects

Paragraph 5 Sixteen trained (15 ± 0.8 yrs) and nine untrained (15 ± 0.6 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 myocardial functional responses to exercise (39). 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.

Paragraph 6 Fourteen 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 have been 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 nine subjects who reported little regular physical activity and limited recreational sports participation.

Experimental protocol

Paragraph 7 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 ($V_{O2} \text{max}$) was defined as the mean of the two highest 30-s average values during the final stage of exercise. Additionally, the lactate threshold was estimated via the v-slope method and confirmatory inspection of the ventilatory equivalent and end-tidal pressure plots for oxygen uptake and carbon dioxide production (5). This process was conducted by two of the named authors with the data confirmed by an independent and appropriately experienced third party. At the second visit to the laboratory, subjects completed two 6-minute square-wave exercise transitions from a 3-minute baseline of 10 W to 80% of the workload which elicited the lactate threshold. Each exercise transition was separated by a minimum 1 hour of passive rest during which time subjects’ body composition was assessed via air-displacement plethysmography (BodPod, Life Measurement Inc., Concord, USA).

**Measurements**

**Paragraph 8** 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, thus enabling direct measurement of the scattering, and therefore absorption coefficient, of NIR-light hence producing absolute values (µM) of oxyhaemoglobin ($HbO_2$), deoxyhaemoglobin (Hb) and total haemoglobin (THb) concentration. Light source–detector separation distances of 1.50 – 3.04 cm for each wavelength were utilised with cell water concentration assumed constant at 70%. 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.

$\dot{V}O_2$ kinetic analysis

**Paragraph 9** Abnormal breaths due to coughs and swallows were first removed from the $\dot{V}O_2$ 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 two datasets were then ensemble averaged to produce a single response for each subject. The first 20 s of the ensemble dataset (cardiodynamic phase, (44)) were then removed (31). The remaining dataset (i.e. up until the end of exercise) was fitted to a mono-exponential curve (Origin, Microcal) with a delay relative to the onset of exercise of the form:

$$\dot{V}O_2(t) = \dot{V}O_2(b) + A_{\dot{V}O_2} \ast (1 - e^{-(t - TD_{\dot{V}O_2}) / \tau_{\dot{V}O_2}})$$
Paragraph 10 Where $\dot{V}O_{2(n)}$ is the mean $\dot{V}O_2$ measured during the final minute of baseline (10 W) exercise, $A_{\dot{V}O_2}$ is the asymptotic amplitude of the fundamental (phase II) response, $TD_{\dot{V}O_2}$ is a time delay relative to the onset of exercise and $\tau_{\dot{V}O_2}$ is the time constant for the fundamental component of the response. Steady state oxygen uptake ($\dot{V}O_{2(SS)}$) is therefore the sum of $\dot{V}O_{2(b)} + A_{\dot{V}O_2}$ and the mean response time ($MRT_{\dot{V}O_2}$) was defined as $\tau_{\dot{V}O_2} + TD_{\dot{V}O_2}$.

NIRS kinetic analysis

Paragraph 11 The Hb 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 Hb starts to increase following a short delay of $\sim 5 - 10$ s (15) was identified. This time-point was identified by fitting two linear regression curves to the first 20 s of the ensemble dataset and, using custom written software and the Solver function in Microsoft 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 increase in Hb, which given the short time frame appears reasonable. From this point up until the time at which the $\dot{V}O_2$ data achieved 98% of its final value, ($t = 4 \tau_{\dot{V}O_2} + TD_{\dot{V}O_2}$, 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:

$$Hb(t) = Hb_{(b)} + A_{Hb} \times (1 - e^{(t-TD_{Hb})/\tau_{Hb}})$$
Paragraph 12 Where $Hb_{(b)}$ is the mean $Hb$ measured during the final minute of baseline (10 W) exercise, $A_{Hb}$ is the asymptotic amplitude of the response, $TD_{Hb}$ is a time delay relative to the onset of exercise and $\tau_{Hb}$ is the time constant for the response. The absolute value of $Hb$ at $t = 4 \tau_{V_{O_2}} + TD_{V_{O_2}} (Hb_{(\phi_3)})$ is therefore the sum of $Hb_{(b)} + A_{Hb}$ and the mean response time ($MRT_{Hb}$) was defined as $\tau_{Hb} + TD_{Hb}$. Although it is not certain whether the processes underlying the $Hb$ response are exponential in nature, visual inspection of the data and reference to previous literature (15,22,33) suggests that a monoexponential decay model of the form below provides a reasonable estimate of the time course of muscle deoxygenation during this “primary” phase of the $Hb$ response. The average value during the final 30 s of exercise ($Hb_{(360-330b)}$) was also calculated, as was the difference between $Hb_{(360-330b)}$ and $Hb_{(\phi_3)} (A_{2Hb})$. Baseline THb and HbO$_2$ concentration were calculated as the average values during the final minute of 10W cycling.

Estimated capillary blood flow kinetics

Paragraph 13 Capillary blood flow ($\dot{Q}_{cap}$) kinetics were estimated by rearrangement of the Fick equation with the substitution of pulmonary oxygen uptake kinetics ($\dot{V}_{O_2p}$, fundamental phase) for muscle oxygen consumption kinetics ($\dot{V}_{O_2m}$) and Hb kinetics for the kinetics of oxygen extraction, ($a-v$)O$_2$, (20):

$$\dot{Q}_{cap}(t) = \frac{\dot{V}_{O_2m}(t)}{(a-v)O_2(t)} \frac{\alpha \dot{V}_{O_2p(fundamental)}(t)}{Hb(t)}$$
This method assumes that muscle oxygen uptake rises exponentially from time zero with the same time course as the fundamental phase of pulmonary oxygen uptake kinetics. To this end, the fundamental phase of pulmonary oxygen uptake kinetics was extrapolated backwards to the baseline value which was given as time zero. The resulting data set were modelled up to 360 s via a monoexponential term with delay of the form,

$$
\dot{Q}_{cap}(t) = \dot{Q}_{cap(b)} + A_{Qcap} \times (1 - e^{(t-TD_{Qcap})/\tau_{Qcap}})
$$

Where $\dot{Q}_{cap(b)}$ is equal to $\dot{V}O_2 / Hb$ during the last minute of unloaded pedalling, $A_{Qcap}$, $TD_{Qcap}$, and $\tau_{Qcap}$ are the amplitude, time delay and time constant of the “fundamental” response. The mean response time was calculated as $\tau + TD$. $\dot{Q}_{cap(b)}$ and $A_{Qcap}$ are expressed in arbitrary units, $TD_{Qcap}$, and $\tau_{Qcap}$ are expressed in seconds. To determine the optimum fitting window for the “fundamental” phase of $\dot{Q}_{cap}$ kinetics, all datasets from 0 – 360 s to 60 – 360 s were sequentially modelled and the best fit for each fitting window determined by nonlinear least squares fitting (see above). The fitting window chosen to represent each transition was that which produced minima in the chi-squared value concomitant with a plateau in the time constant of the response.

Heart-rate kinetics

Paragraph 14 Heart-rate kinetics were modelled via a similar monoexponential function as for $\dot{V}O_2$ kinetics but with the response constrained to start at the onset of exercise ($t=0$), i.e. no delay term (11). Baseline heart-rate ($HR_{(b)}$) was calculated as the average during the last
minute of 10W pedalling, with steady state heart-rate ($HR_{ss}$) being the sum of the amplitude ($A_{HR}$) and baseline heart-rate, and $\tau_{HR}$ defined as the time constant of the response.

Statistics

**Paragraph 15** Confidence intervals of $\dot{V}O_2$ kinetics were analysed by a paired t-test, (SPSS 16.0, SPSS Inc., Chicago, USA). All other comparisons between groups were made using an independent two-tailed t-test with homogeneity of variance checked via Levene’s test. Data are presented as mean ± standard deviation and statistical significance accepted at the $P \leq 0.05$ level.
Results

$\dot{V}O_2$ kinetics

**Paragraph 16** 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 $\tau_{\dot{V}O_2}$ lay within acceptable boundaries, (trained: 4.0 ± 1.1 vs untrained: 5.4 ± 1.9 s).

**Paragraph 17** The time constant and mean response time of $\dot{V}O_2$ kinetics was ~25% ($P=0.05$) and ~13% ($P=0.03$) faster in trained subjects compared to untrained, respectively (table 2). Due to the higher workload in the trained subjects compared to untrained (trained: 114 ± 22 vs untrained: 90 ± 23 W, $P=0.02$), the amplitude of the $\dot{V}O_2$ kinetics and steady state $\dot{V}O_2$ were higher in trained versus untrained subjects ($P<0.01$, table 2), with no difference in the exercise economy (gain) ($\Delta\dot{V}O_2/\Delta W$) of the response ($P=0.1$), though this latter result would have been reversed if the variances of the two groups for this parameter had not been unequal ($P=0.05$).

Hb kinetics

**Paragraph 18** Representative plots of Hb kinetics for an untrained and trained subject are presented in figure 2 with exponential curve fit and residuals shown. The 95% confidence interval for $\tau_{Hb}$ was 1.39 ± 0.58 s (trained) and 1.58 ± 0.48 s (untrained). Neither the time constant, time delay or mean response time of Hb kinetics differed between groups. $A_{Hb}$, $Hb_{(63)}$ and $Hb_{(360-330h)}$ were all higher in trained versus untrained subjects ($P=0.03$, $P=0.001$, $P=0.001$, respectively, table 3). This difference remained when the data was normalised for the increment in oxygen uptake, apart from $A_{Hb}$ in which case this difference was abolished ($P=0.1$, table 3). Representative plots of Hb kinetics are shown in figure 2.
Paragraph 19 Baseline THb (81 ± 24 vs 49 ± 19 μM, \(P<0.01\)) and HbO₂ (58 ± 18 vs 37 ± 13 μM, \(P<0.01\)) concentrations were significantly higher in the trained subjects compared to untrained.

\(\dot{Q}_{cap}\) kinetics

Paragraph 20 Representative plots of \(\dot{Q}_{cap}\) kinetics for an untrained and trained subject are presented in figure 3 with exponential curve fit and residuals shown. The 95% confidence interval for \(\tau_{\dot{Q}_{cap}}\) was 3.9 ± 1.8 s (trained) and 3.7 ± 1.2 s (untrained). The time delay of \(\dot{Q}_{cap}\) kinetics was not different between groups (trained: 20.3 ± 6.4 vs untrained: 20.7 ± 4.6 s). However, the time constant (trained: 19 ± 10 vs untrained: 30 ± 13 s, \(P=0.04\)) and mean response time (trained: 40 ± 11 vs untrained: 51 ± 10 s, \(P=0.03\)) were significantly faster in the trained subjects.

HR kinetics

Paragraph 21 Representative plots of HR kinetics for an untrained and trained subject are presented in figure 4 with exponential curve fit and residuals shown. The 95% confidence interval for \(\tau_{HR}\) was 3.8 ± 1.1 s (trained) and 5.9 ± 1.9 s (untrained). The baseline, (Trained: 90 ± 12 vs Untrained: 93 ± 12 bpm, \(P=0.47\)), amplitude (Trained: 43 ± 8 vs Untrained: 38 ± 6 bpm, \(P=0.17\)) and steady state (Trained: 133 ± 12 vs Untrained: 132 ± 12 bpm, \(P=0.84\)), of the heart-rate response to exercise was not different between groups. However, the time constant of heart-rate kinetics was significantly faster in the trained subjects (Trained: 37 ± 10 vs Untrained: 49 ± 14 s, \(P=0.03\)).
Discussion

Paragraph 22 The main finding of the present study was that pulmonary oxygen uptake kinetics were \(~25\%\) faster (dependent on the modelling strategy) in a group of trained male adolescents compared to their untrained counterparts during the transition to moderate intensity exercise. In addition to faster $\dot{V}O_2$ kinetics, heart-rate kinetics and estimated capillary blood flow kinetics were also faster in the trained subjects. In contrast muscle deoxygenation (Hb) kinetics, as measured by near infrared spectroscopy (NIRS), were unchanged between groups. Taken together the present data suggest that the faster $\dot{V}O_2$ kinetics were due to enhancements in both central (oxygen delivery) and peripheral (oxygen utilisation) mechanisms.

Paragraph 23 The $\dot{V}O_2$ kinetics data are in line with a large body of cross-sectional and longitudinal data in adults (1,7,8,10,12,21,24,27,28,30,36,37,46), but are in contrast to the only previous study to examine the effects of training status on oxygen uptake kinetics in children and adolescents (35). Whilst there was a difference in the physical maturity of the subjects between the two studies (subjects were pre-pubescent in the study by Obert et al. (35)), a major limitation of this previous study was the use of cycle ergometry with a group of trained swimmers. During the transition to moderate intensity exercise in healthy subjects, $\dot{V}O_2$ kinetics are thought to be primarily limited by factors affecting oxygen utilisation, (i.e. in the peripheral musculature) rather than oxygen delivery, (18,26,32,33,45). Hence it might be expected that the specific muscle group being trained and hence studied is important in establishing a difference between the trained and untrained state. Indeed, in a previous study (10), $\dot{V}O_2$ kinetics in trained swimmers and kayakers were markedly slower during leg compared to arm exercise, with the opposite being shown in trained runners. Hence the dichotomy between the muscles utilised during the training and experimental modes of
exercise is likely to have accounted for the lack of any effect of training status on $\dot{V}O_2$ kinetics in the study by Obert et al. (35). In contrast, the present study utilised cycle ergometry, with the trained group consisting of athletes whose training predominantly focuses on the leg musculature, hence there was a match between the muscle utilised during the training and experimental exercise.

Paragraph 24 The cross-sectional nature of the present study does not allow us to rule out the possibility that inherent physiological characteristics pre-disposed the trained subjects to have faster $\dot{V}O_2$ kinetics compared to the untrained subjects. Nevertheless, given the rapidity of physical changes that can occur in adolescents, cross-sectional designs such as the present study remain an appropriate experimental design to explore the effect of training status on physiological parameters such as $\dot{V}O_2$ kinetics. In the present study we were careful to select two samples that, as much as possible, differed only in their training status. In this regard a strength of the present study was the inclusion of 14 subjects from a professional soccer club (English Premiership) where it is possible to confirm the reported volume of training (see METHODS) completed in the months and years prior to the study. The untrained group, whilst healthy, reported very little and unstructured physical activity in their daily lives. Therefore we are confident that the significantly faster $\dot{V}O_2$ kinetics demonstrated by the trained group represent adaptations to long-term exercise training rather than differences in inherent physiological characteristics.

Paragraph 25 The training undertaken by the trained group in the present study involves the mobilisation of a large muscle mass and for 15 of the subjects, supporting the mass of the body on the muscle groups under investigation. Hence central and peripheral adaptations to training will both have the potential to determine the faster $\dot{V}O_2$ kinetic response to exercise
versus the untrained group. Certainly, non-invasive imaging techniques have demonstrated that trained adolescents have augmented stroke volume and thus cardiac output in response to maximal incremental exercise relative to their untrained peers (40), demonstrating a central cardiac adaptation to exercise training. However, ethical considerations preclude the analysis of peripheral adaptations to training in adolescents that would ordinarily require invasive techniques such as muscle biopsies. During the transition to exercise, the fundamental phase of $\dot{V}O_2$ kinetics are not thought to be limited by oxygen delivery (18,26,32,33,45), therefore one could surmise that these peripheral adaptations to training are more important in determining the faster $\dot{V}O_2$ kinetics relative to the untrained subjects. On the other hand, all of these previous studies were conducted with healthy adult populations and it is presently not clear whether the same would follow in children or adolescents. Indeed, though only indirect measures of muscle oxygen delivery, heart-rate kinetics, and therefore presumably cardiac output kinetics, (as has been shown previously in children and adolescents (13)) and estimated capillary blood flow kinetics were faster in the trained subjects in the present study. Hence in the present study the faster $\dot{V}O_2$ kinetics in trained subjects compared to untrained may have been due to enhanced oxygen delivery to the exercising muscle. Furthermore this may indicate that in adolescents $\dot{V}O_2$ kinetics are normally limited by oxygen delivery.

**Paragraph 26** The deoxyhaemoglobin (Hb) signal from NIRS represents the balance between oxygen supply and utilisation in the interrogated tissue and therefore represents an excellent means to help establish the mechanisms behind the faster $\dot{V}O_2$ kinetics in the trained subjects. Slower Hb kinetics following training would reflect adaptations in oxygen delivery at the onset of exercise that were in excess of enhancements in oxygen utilisation. In adolescents, training induces enhancements in ventricular preload and stroke volume (40), estimated capillary blood flow kinetics (present study) and may reduce heterogeneity of oxygen
delivery to the active muscle (15). Furthermore, aerobically-trained children and adolescents are also characterised by a higher proportion of type I muscle fibres in the trained musculature (14,34) and it has been shown that muscle composed predominantly of type I fibres possesses a higher oxygen delivery to oxygen consumption ratio during contractions as compared to muscle composed predominantly of type II fibres (6). Hence it might therefore be expected that Hb kinetics would be slower and have a reduced ΔHb / ΔVO₂ in the trained subjects compared to their untrained counterparts. However, the previous finding of no difference in the relative amplitude of the slow component of oxygen uptake kinetics during cycle exercise in trained and untrained children (35) suggests that muscle fibre type and recruitment patterns are unaffected by training in the presently studied population when the same relative exercise intensity is utilised (3). Faster Hb kinetics following training (in concert with faster VO₂ kinetics) would be indicative of enhancements in the rate of increase of oxygen utilisation at the onset of exercise in the face of relatively little improvement in oxygen delivery. Since enhancements in mitochondrial density and oxidative enzyme activity accompany endurance training in adults, children and adolescents (16,25,36,41) it might alternatively be expected that Hb kinetics would be faster in the trained group.

**Paragraph 27** The finding that Hb kinetics and ΔHb / ΔVO₂ were unchanged between the trained and untrained subjects in the present study suggests that enhancements in oxygen utilisation, oxygen supply and type I muscle fibre recruitment due to training status were equally important in determining the faster VO₂ kinetic response to exercise. Essentially, the unchanged dynamics of Hb between the trained and untrained subjects during the transition to exercise reflects an unchanged balance in the oxygen delivery to oxygen consumption ratio due to parallel enhancements in oxygen delivery (as indicated by faster heart-rate and Qcap kinetics) and oxygen utilisation in the trained subjects. The tendency for statistical
significance ($P=0.1$) in $\Delta\text{Hb} / \Delta\dot{V}o_2$ is likely to be due only to group differences in subcutaneous thigh thickness (as indicated by the significantly different percentage body fat) blunting the change in Hb with exercise in the untrained group (43) (see below). Furthermore, any correction for this effect would serve to bring the mean values for the two groups closer together, i.e. increase the mean value of the untrained group. Hence, taken together, the NIRS data strongly suggests that the faster $\dot{V}o_2$ kinetics in the trained, relative to the untrained group were due to adaptations in oxygen supply and oxygen utilisation mechanisms.

Methodological considerations

Paragraph 28 The NIRS data from the present study potentially highlight an important methodological consideration when comparing diverse groups as baseline THb, HbO$_2$ and Hb concentrations were significantly higher in the trained compared to the untrained subjects. Fat acts as a low-scattering constant absorber of near infrared light; therefore increased subcutaneous fat has the potential to blunt the interrogative depth of near infrared light into the exercising muscle (9,43) and reduce the detectable change in haemoglobin oxygenation in response to exercise (43). It is therefore possible that the significantly higher percentage whole-body fat in the untrained subjects could also have manifested itself as increased thigh subcutaneous fat at the site of NIRS interrogation. Hence some of the difference in the steady state NIRS measures between the two groups may be attributable to differences in subcutaneous fat, rather than (the Hb kinetic response should be relatively unaffected, though impacts upon the amplitude of the response will affect the confidence of parameter estimation of Hb kinetics), including those normalised for oxygen uptake (i.e. $\Delta\text{Hb} / \Delta\dot{V}o_2$). However, as discussed above, if the change in Hb with exercise is blunted in the untrained subjects, then this will lead to an artificial lowering of $\Delta\text{Hb} / \Delta\dot{V}o_2$ and correction for this effect will
serve to bring the $\Delta \text{Hb} / \Delta \dot{V}o_2$ data for the trained and untrained subjects closer together, demonstrating no effect of training status on this parameter. Unfortunately in the present study we did not collect skinfold / subcutaneous fat data and are therefore unable to determine the extent to which enhanced whole-body fat in the untrained subjects manifested as a relative increase in the subcutaneous fat of the thigh. Future studies should therefore consider the depth of subcutaneous fat at the site of interrogation before selecting source-detector distances of the NIRS apparatus, ensuring that subcutaneous fat thickness is well below 50% of the source-detector distance (9).

**Paragraph 29** The present discussion makes the implicit assumption that the region of muscle interrogated by the NIRS probe represents the entire exercising muscle group. However, a recent study showed spatial heterogeneity of muscle deoxygenation during exercise of the vastus lateralis (29). Therefore whilst we ensured that the NIRS probe was positioned at the same location for each subject (midway between the greater trochanter and the lateral condyle of the tibia), we cannot rule out the possibility that differences in whole-muscle Hb kinetics between groups were not detected due to inter-subject disparities in the site-specific Hb response to exercise.

**Paragraph 30** In conclusion, the present study has demonstrated for the first time faster $\dot{V}o_2$ kinetics in trained adolescents compared to untrained controls, adding to a large volume of similar data in adult populations (1,7,8,10,12,21,24,27,28,30,36,37,46). Though inherent capabilities of the trained subjects cannot be ruled out, we propose that the data represent adaptations to chronic training. Furthermore, muscle deoxygenation data, via near infrared spectroscopy, suggest that faster $\dot{V}o_2$ kinetics were due to enhancements in both central (oxygen delivery) and peripheral (oxygen utilisation) mechanisms.
Acknowledgements

The results of the present study do not constitute endorsement by ACSM.
References


Figure Captions

Figure 1. Representative plots of $\dot{V}o_2$ kinetics for trained (closed squares) and untrained subjects (open squares) with phase 2 exponential curve fit and residuals shown.

Figure 2. Representative plots of Hb kinetics for trained (closed squares) and untrained subjects (open 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. $t=4r_{\dot{V}o_2} + TD_{\dot{V}o_2}$.

Figure 3. Representative plots of $\dot{Q}_{cap}$ kinetics for trained (closed squares) and untrained subjects (open squares) with exponential curve fit and residuals shown.

Figure 4. Representative plots of heart-rate kinetics for trained (closed squares) and untrained subjects (open squares) with exponential curve fit and residuals shown.
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<th>Untrained</th>
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</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>15 ± 0.8</td>
<td>15 ± 0.6</td>
</tr>
<tr>
<td>% Tanner stage 2</td>
<td>6.7</td>
<td>11.1</td>
</tr>
<tr>
<td>% Tanner stage 3</td>
<td>33.3</td>
<td>33.3</td>
</tr>
<tr>
<td>% Tanner stage 4</td>
<td>60.0</td>
<td>55.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167 ± 7</td>
<td>171 ± 9</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>56.1 ± 9.8</td>
<td>59 ± 11</td>
</tr>
<tr>
<td>% Body Fat **</td>
<td>10.3 ± 5.6</td>
<td>17.1 ± 4.6</td>
</tr>
<tr>
<td>VO(_2) max (L.min(^{-1})) *</td>
<td>3.06 ± 0.61</td>
<td>2.55 ± 0.55</td>
</tr>
<tr>
<td>VO(_2) max (mL.kg(^{-1}).min(^{-1})) **</td>
<td>54.7 ± 6.2</td>
<td>43.1 ± 5.2</td>
</tr>
<tr>
<td>VO(_2) at LT (L.min(^{-1})) *</td>
<td>2.08 ± 0.51</td>
<td>1.59 ± 0.40</td>
</tr>
<tr>
<td>VO(_2) at LT (mL.kg(^{-1}).min(^{-1})) **</td>
<td>36.5 ± 6.0</td>
<td>27.8 ± 4.7</td>
</tr>
</tbody>
</table>

*Trained versus untrained, \( P<0.05 \); **Trained versus untrained, \( P<0.01 \).
Table 2. Kinetic characteristics of $\dot{V}O_2$ data in trained and untrained subjects for both methods of analysis

<table>
<thead>
<tr>
<th></th>
<th>Method 1</th>
<th>Method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trained</td>
<td>Untrained</td>
</tr>
<tr>
<td>$\dot{V}O_2(b)$ (l·min$^{-1}$)</td>
<td>0.64 ± 0.13</td>
<td>0.59 ± 0.10</td>
</tr>
<tr>
<td>$A_{\dot{V}O_2}$ (l·min$^{-1}$)**</td>
<td>1.11 ± 0.27</td>
<td>0.72 ± 0.25</td>
</tr>
<tr>
<td>$\dot{V}O_2(ss)$ (l·min$^{-1}$)**</td>
<td>1.75 ± 0.34</td>
<td>1.31 ± 0.34</td>
</tr>
<tr>
<td>$TD_{\dot{V}O_2}$ (s)</td>
<td>16.2 ± 3.3</td>
<td>14.6 ± 5.0</td>
</tr>
<tr>
<td>$\tau_{\dot{V}O_2}$ (s)*</td>
<td>22.3 ± 7.2</td>
<td>29.8 ± 8.4</td>
</tr>
<tr>
<td>$MRT_{\dot{V}O_2}$ (s)*</td>
<td>38.5 ± 6.2</td>
<td>44.4 ± 6.1</td>
</tr>
<tr>
<td>Gain (ml·min$^{-1}$·W$^{-1}$)</td>
<td>10.7 ± 1.2</td>
<td>9.1 ± 2.6</td>
</tr>
</tbody>
</table>

*Trained versus untrained, $P<0.05$; **Trained versus untrained, $P<0.01$. $\dot{V}O_2(b)$: mean $\dot{V}O_2$ during the final minute of unloaded pedalling; $A_{\dot{V}O_2}$: amplitude of fundamental component of $\dot{V}O_2$; $\dot{V}O_2(ss)$: steady state $\dot{V}O_2$ (derived from the fitting process); $TD_{\dot{V}O_2}$: timed delay of the fundamental component of $\dot{V}O_2$ (derived from the fitting process); $\tau_{\dot{V}O_2}$: time constant of the fundamental component; $MRT_{\dot{V}O_2}$: mean response time ($TD_{\dot{V}O_2} + \tau_{\dot{V}O_2}$); $TD_{\dot{V}O_2}$: Gain: $A_{\dot{V}O_2}/\Delta W$. 
Table 3. Kinetic characteristics of Hb data in trained and untrained subjects.

<table>
<thead>
<tr>
<th></th>
<th>Trained</th>
<th>Untrained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb(b) (μM)**</td>
<td>22.2 ± 7.1</td>
<td>12.0 ± 6.2</td>
</tr>
<tr>
<td>A_Hb (μM)*</td>
<td>11.0 ± 8.3</td>
<td>4.1 ± 4.9</td>
</tr>
<tr>
<td>Hb(φ) (μM)**</td>
<td>33.2 ± 3.7</td>
<td>16.0 ± 3.6</td>
</tr>
<tr>
<td>TD_Hb (s)</td>
<td>7.7 ± 2.4</td>
<td>8.2 ± 2.7</td>
</tr>
<tr>
<td>τ_Hb (s)</td>
<td>9.7 ± 2.9</td>
<td>10.1 ± 3.4</td>
</tr>
<tr>
<td>MRT_Hb (s)</td>
<td>17.4 ± 2.5</td>
<td>18.3 ± 2.3</td>
</tr>
<tr>
<td>Hb(360-330s) (μM)**</td>
<td>34 ± 15</td>
<td>16 ± 12</td>
</tr>
<tr>
<td>A_2Hb (μM)</td>
<td>0.6 ± 3.0</td>
<td>0.39 ± 0.87</td>
</tr>
<tr>
<td>ΔHb / ΔVO₂</td>
<td>10.4 ± 7.7</td>
<td>5.7 ± 5.2</td>
</tr>
</tbody>
</table>

*Trained versus untrained, P<0.05; **Trained versus untrained, P<0.01.  
Hb(b): average Hb during final minute of unloaded pedalling; A_Hb: amplitude of primary response of Hb; Hb(φ): absolute value of Hb at t = 4 τ_{VO₂} + TD_{VO₂}; TD_Hb: time delay relative to start of exercise of first increase in Hb; τ_Hb: time constant of primary Hb response; MRT_Hb: mean response time (TD_Hb+τ_Hb); Hb(360-330s): average Hb during final 30 s of exercise; A_2Hb: Hb(360-330s) - Hb(φ)