ORIGINAL ARTICLE

Central circulatory and peripheral O_2 extraction changes as interactive facilitators of pulmonary O_2 uptake during a repeated high-intensity exercise protocol in humans

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Abstract It has frequently been demonstrated that prior high-intensity exercise facilitates pulmonary oxygen uptake ($\dot{V}O_2$) response at the onset of subsequent identical exercise. To clarify the roles of central O_2 delivery and/or peripheral O_2 extraction in determining this phenomenon, we investigated the relative contributions of cardiac output (CO) and arteriovenous O_2 content difference (a-$\bar{v}DO_2$) to the $\dot{V}O_2$ transient during repeated bouts of high-intensity knee extension (KE) exercise. Nine healthy subjects volunteered to participate in this study. The protocol consisted of two consecutive 6-min KE exercise bouts in a supine position (work rate 70–75% of peak power) separated by 6 min of rest. Throughout the protocol, continuous-wave Doppler ultrasound was used to measure beat-by-beat CO (i.e., via simultaneous measurement of stroke volume and the diameter of the arterial aorta). The phase II $\dot{V}O_2$ response was significantly faster and the slow component (phase III) was significantly attenuated during the second KE bout compared to the first. This was a result of increased CO during the first 30 s of exercise: CO contributing to 100 and 56% of the $\dot{V}O_2$ speeding at 10 and 30 s, respectively. After this, the contribution of a-$\bar{v}DO_2$ became increasingly more predominant: being responsible to an estimated 64% of the $\dot{V}O_2$ speeding at 90 s, which rose to 100% by 180 s. This suggests that, while both CO and a-$\bar{v}DO_2$ clearly interact to determine the $\dot{V}O_2$ response, the speeding of $\dot{V}O_2$ kinetics by prior high-intensity KE exercise is predominantly attributable to increases in a-$\bar{v}DO_2$.

Keywords High-intensity exercise · Cardiac output · Arteriovenous O_2 content difference

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Introduction

It has been consistently demonstrated that the pulmonary oxygen uptake ($\dot{V}O_2$) response to a bout of high-intensity [i.e., supra-lactate threshold (LT)] exercise is faster throughout the transient when recently preceded by a similar high-intensity bout, i.e., a "double-transition" protocol (e.g., Gerbino et al. 1996; MacDonald et al. 1997; Burnley et al. 2000; Fukuba et al. 2002). The majority of these studies (using cycle ergometer exercise) point to an attenuation of the $\dot{V}O_2$ slow component as the main mediator of this phenomenon (e.g., Burnley et al. 2000; Gerbino et al. 1996; Koppo and Bouckaert 2000; MacDonald et al. 1997). However,

even for a single high-intensity transition, there is still debate regarding the relative contributions of O_2 delivery to the exercising muscles (i.e., vascular limitation) and O_2 extraction (i.e., metabolic "inertia") consequent to intramuscular enzyme-linked control mechanisms (e.g., Grassi 2001; Hughson et al. 2001; Poole et al. 1994; Whipp et al. 2002) in determining the $\dot{V}O_2$ kinetic response. The characteristics of the $\dot{V}O_2$ kinetic response to the double-transition protocol has been widely addressed (see Jones et al. 2003 for review), but the details of the potential cardiovascular determinants still remain to be elucidated. For example, to what extent does the magnitude and time course of the cardiac output (CO; reflective of the central component) and the arteriovenous O_2 content difference (a-$\bar{v}DO_2$; indicative of the peripheral O_2 extraction component) contribute to the differences in $\dot{V}O_2$ between the first and second bouts of the double-transition protocol?

There are, to our knowledge, no studies that have tracked central cardiovascular changes throughout the double-transition protocol to establish their potential proportional contributions. Recent advances in continuous-wave Doppler ultra-sonography provide the opportunity for CO to be assessed continuously throughout the transients from measurements at the ascending aorta. We have, therefore, determined the magnitude and time course of the CO response simultaneously with that of $\dot{V}O_2$ throughout a high-intensity knee-extension double-transition exercise protocol in humans, thereby also allowing the dynamic features of the a-$\bar{v}DO_2$ response to be calculated via the Fick equation.

Methods

Subjects

Nine healthy Japanese subjects (5 women and 4 men: age = 29.1 ± 9.1 years; height = 165.8 ± 7.6 cm; body weight = 58.9 ± 14.0 kg, mean ± SD) were selected for the study on the basis of being able to provide high-quality Doppler signals from the ascending aorta. The subjects were all volunteers and were aware of all the testing procedures, having given informed consent to participate as approved by the ethics committee of the local institution (in accordance with the Declaration of Helsinki).

Exercise protocols

During preliminary investigations using high-intensity cycle ergometry, we found that the continuous mea-

surement of loud, high-pitched audio signals and bright visual signals (required for accurate determination of the ascending aortic flow) was technically challenging due to the cardiac movement and interference from respiratory and body movements. In this study, we therefore adopted knee extension (KE) exercise with the subject strapped to the table by belts placed across the iliac spines and shoulder that allowed stable measurement of blood flow in the ascending aorta from continuous echo-Doppler applied via the supra-sternal notch. This both minimized the effects of body (especially thoracic) movement and allowed the subject to perform bilateral KE exercise in the supine position with the hips flexed and stabilized at an angle of approximately 150°: the lower leg being free to move over the required range of motion. The bilateral KE exercise involved lifting and lowering a weight at 1-s intervals (i.e., 60 cycles per min) for each leg in an alternating pattern. The weight was connected to the ankle by a wire-and-pulley mechanism. Timed audio signals provided the subjects with a constant rhythm to cue exercise cadence. Soft rubber was used to cushion the heel during knee flexion and to minimize eccentric muscle activation and maximize concentric muscle activation. A bar (that the subjects were required to touch with their toes on each leg excursion) was used to set the range of motion during the KE exercise, which was continuously monitored (and verbal feedback provided) to ensure a consistent lifting distance. The average lifting distance for this KE exercise protocol was 16.5 cm.

Initially, the subjects each performed a stepwise incremental KE exercise test (0.5 kg each 30 s, from a baseline of 0.5 kg) to the limit of tolerance, which occurred at a peak work rate of 18.3 ± 3.3 W for each leg. The main components of the protocol (each performed on different days) consisted of an initial 3-min resting control phase immediately followed by two consecutive 6-min KE exercise bouts separated by a 6-min resting recovery phase (a double-transition protocol). This was followed by a 6-min resting recovery. The work rate selected was 70–75% of the peak power achieved on the incremental test that consistently resulted in the development of a $\dot{V}O_2$ slow component but also ensured that each subject was able to sustain the 2×6-min exercise durations required by the double-transition protocol. Each subject performed the protocol on two occasions at the same time on different days.

Measurements

Ventilatory and gas exchange responses were determined breath-by-breath using a computerized meta-

bolic measuring system (RM-300, Minato Medical Co., Japan). Prior to each exercise test, a hot-wire flow-sensor and gas analyzers were calibrated by inputting a known volume of air (at several mean flow rates) and gas mixtures of known concentrations, respectively.

The time-serial CO was obtained using continuous-wave Doppler, a two-dimensional and M-mode echocardiography apparatus (SSD-2000, Aloka, Japan) to measure the mean blood velocity (V_{mean}) and diameter of the ascending aorta (just above the aortic valve), i.e., similar to standard, previously described, methods (Christie et al. 1987; Miyachi et al. 1998; Rowland et al. 1998; Nottin et al. 2002; Sugawara et al. 2003). Briefly, the ascending aorta was assumed to be circular, and its cross-sectional area (CSA) was calculated using the diameter measured by two-dimensional and M-mode echocardiography during supine rest. The insertion point of the aortic valve tips at end-diastole was set using two-dimensional imaging in the parasternal long axis view. The subsequent M-mode echocardiogram was recorded at the same level. The aortic diameter was measured at mid-systole and end-diastole from the mean of 3–5 consecutive cardiac cycles by the leading edge to leading edge method. Continuous-wave Doppler echocardiographic recordings of the ascending aortic blood velocity were obtained with a small-dedicated 2.0 MHz non-imaging transducer (SSD-870, Aloka, Japan) held in the supra-sternal notch. The ascending aortic flow was identified by a loud, high-pitched audio signal and a bright well-defined video display. The Doppler and simultaneous ECG signals were stored on S-VHS video during the protocol. They were subsequently digitally converted and analyzed using image analysis software (NIH image). The V_{mean} throughout a cardiac cycle was determined between consecutive R spikes by planimetry. The velocity integral was calculated as the product of V_{mean} and ejection time during a cardiac cycle. The stroke volume (SV) was calculated as the product of the velocity integral and aortic CSA. The CO was, therefore, calculated as the product of the SV and the simultaneous heart rate (HR). The arteriovenous O_2 content difference (a-$\bar{v}DO_2$) was calculated from the Fick equation by dividing $\dot{V}O_2$ by CO. A second-by-second time course was calculated for each variable by interpolation and then stored on disk for further analysis.

Data analysis

The temporal profiles of variables at the onset of both bouts of high-intensity KE exercise were displayed by 10-s averaged data. We did not, however, perform further model-based analyses of the response transients (such as time constants, gains or amplitudes) due to the limited confidence of the estimation resulting from the small-step increment of work rate and limited number of repetitions (Lamarra et al. 1987). Instead, the summarized data from all subjects were displayed at distinct time points: 0, 10, 30, 50, 90, 180 and 360 s after the onset of each bout (where time = 0 is the baseline value averaged from the 30 s immediately preceding the exercise onset). The value at each representative time was determined as average for a 10-s bin placed equidistant around each corresponding time point. Then, the difference between the $\dot{V}O_2$ values in bout 1 and bout 2 were determined [$\dot{V}O_2$ (second)/$\dot{V}O_2$ (first)] and used to calculate the relative contributions of CO and a-$\bar{v}DO_2$ to this difference in each subject i.e., similar to the method previously used by De Cort et al. (1991) and MacDonald et al. (2001). Briefly, the relative contribution of a-$\bar{v}DO_2$ to the speeding of the $\dot{V}O_2$ kinetics observed in bout 2 can be evaluated by assuming that the CO kinetic profile is unchanged between bouts. Consequently, the relative contribution of a-$\bar{v}DO_2$ can be calculated from a-$\bar{v}DO_2$ (second)/a-$\bar{v}DO_2$ (first). The same process can be applied to a-$\bar{v}DO_2$ (i.e., assuming both bouts follow the measured bout 1 profile) in order to calculate the relative contribution of CO [CO(second)/CO(first)] to the speeding of the $\dot{V}O_2$ kinetics observed in bout 2. The difference between the $\dot{V}O_2$ kinetic profiles during the double-transition protocol (i.e., the degree of speeding of the $\dot{V}O_2$ kinetics) can thereby be attributed to the quantitative contributions of CO and/or a-$\bar{v}DO_2$.

The increment in $\dot{V}O_2$ between 180 and 360 s of each exercise bout ($\Delta\dot{V}O_{2(6-3)}$) was used to estimate the magnitude of the $\dot{V}O_2$ slow component. In addition, the increment in $\dot{V}O_2$ between 120 and 360 s of each exercise bout ($\Delta\dot{V}O_{2(6-2)}$) was also calculated to aid direct comparison with other recently published data (e.g., Koppo et al. 2002).

The values are expressed as mean ± SD. The time-serial changes in the variables were tested with respect to the differences between first and second bouts by repeated-measures ANOVA with time. When a significant difference was detected, this was further examined by Tukey's post-hoc test. All statistical analyses were performed with SPSS for Windows (SPSS Inc.). The statistical significance was accepted at $P < 0.05$.

Results

Examples of the 10-s averaged $\dot{V}O_2$ and cardiac responses (i.e., HR, SV and CO) for a representative

subject during the double-transition KE protocol are shown in Fig. 1. The group mean on-transient responses are shown in Fig. 2 at 0, 10, 30, 50, 90, 180 and 360 s of each bout (where $t = 0$ represents the baseline preceding exercise onset). The temporal profile of $\dot{V}O_2$ after the onset of bout 2 was consistently and significantly higher than that in bout 1 for all values from 10 to 180 s, consistent with speeded $\dot{V}O_2$ kinetics. Note also that $\dot{V}O_2$ had recovered to its prior control value
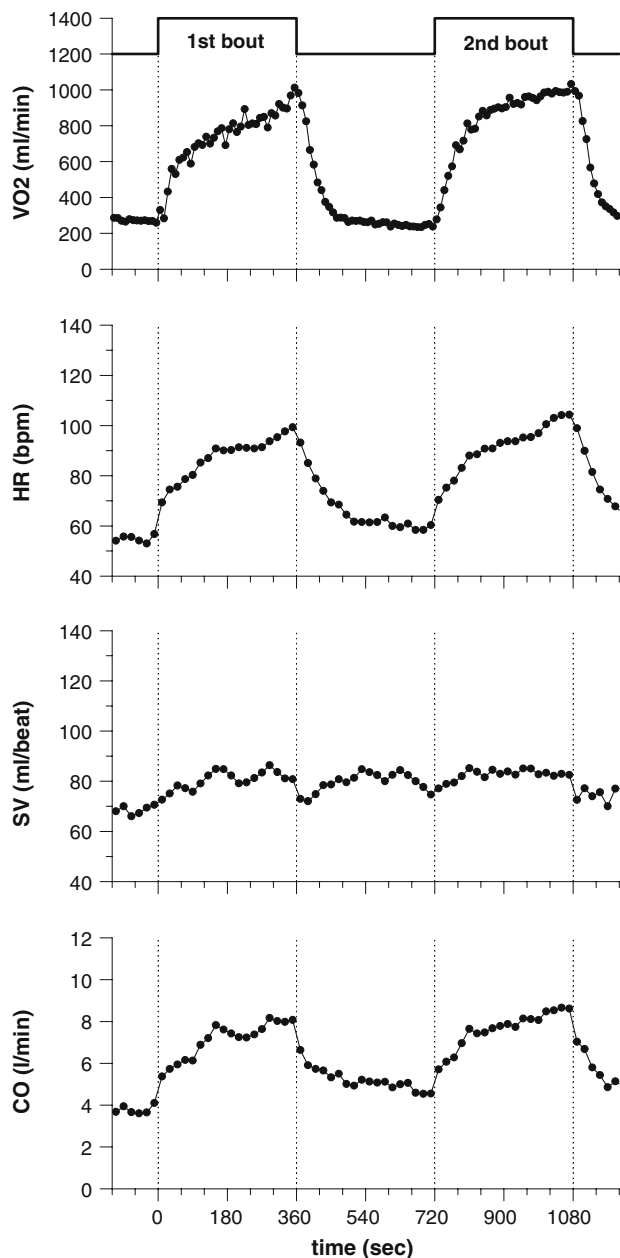

Fig. 1 The pulmonary $\dot{V}O_2$, heart rate (*HR*), stroke volume (*SV*) and cardiac output (*CO*) responses (averaged every 20 s) during repeated bouts of high-intensity knee extension exercise in a representative subject

by the start of the second exercise bout ($\dot{V}O_2$ values at $t = 0$ were the same for both bouts). The $\Delta\dot{V}O_{2(6-3)}$ was significantly higher in bout 1 (78 ± 44 ml min^{-1}) compared to bout 2 (57 ± 36 ml min^{-1}). This was also the case for the $\Delta\dot{V}O_{2(6-2)}$ index (bout 1, 132 ± 51 ml min^{-1} vs. bout 2, 94 ± 42 ml min^{-1}).

Following the initial 20 s, CO showed a very similar temporal response during both exercise bouts, with the CO only being greater ($P < 0.05$) at 0 and 10 s of bout 2. In contrast, the HR remained significantly elevated throughout the bout 2, including the baseline prior to the exercise onset (Fig. 2). The a-$\bar{v}DO_2$ was significantly lower at the onset of the bout 2 as a consequence of the high CO residual from the prior exercise (Fig. 2). The difference of a-$\bar{v}DO_2$ between the bouts gradually widened after phase I and was significantly higher in bout 2 (compared to bout 1) by 180 s (Fig. 2). It is salient to note that the qualitative difference between the bouts in the $\dot{V}O_2$ profiles was closer to that of a-$\bar{v}DO_2$ than CO. We could not discern a slow component-like phase in the CO response in either bout: $\Delta CO_{(6-3)}$ bout 1, 0.21 ± 0.46 vs. bout 2, 0.40 ± 0.31 l min^{-1}. However, Δa-$\bar{v}DO_{2(6-3)}$ was significantly lower in bout 2 (-0.071 ± 0.401 ml 100 ml) compared with bout 1 (0.635 ± 0.388 ml 100 ml).

The relative contributions (expressed in %) of CO and a-$\bar{v}DO_2$ to the speeding of $\dot{V}O_2$ observed in bout 2 between 0 and 180 s (i.e., the region encompassing phase II) are displayed in Fig. 3. Using the 90 s time point as an example, the absolute mean values for $\dot{V}O_2$ increased from 617 ml min^{-1} in bout 1 to 656 ml min^{-1} in bout 2: an increase of 6.3%. The corresponding values for CO and a-$\bar{v}DO_2$ were 8.41 l min^{-1} and 73.34 ml l^{-1}, respectively, during bout 1, and 8.60 l min^{-1} and 76.31 ml l^{-1}, respectively, during bout 2. If it is assumed that CO did not change between bouts (i.e., was 8.41 l min^{-1} at 90 s in both bouts), then the expected increase in $\dot{V}O_2$ would be 8.41 l min^{-1} multiplied by 76.31 ml l^{-1}, or 642 ml min^{-1}. This can then be used to estimate the relative contribution of a-$\bar{v}DO_2$ to the speeded $\dot{V}O_2$ kinetics observed in bout 2, i.e., 642/617 ml min^{-1}, or 4%. A similar calculation for CO (assuming unchanged a-$\bar{v}DO_2$) yields an expected $\dot{V}O_2$ of 631 ml min^{-1} (i.e., 8.60 l min^{-1} multiplied by 73.34 ml l^{-1}). The relative contribution of CO was therefore estimated to be 631/617, or 2.3%.

These calculations were repeated for each individual at each time point. The increase in the $\dot{V}O_2$ of bout 2 was approximately 6–7% throughout the transient phase (i.e., until 180 s; Fig. 3). Using the approach described above, approximately 2–3% was attributable to a-$\bar{v}DO_2$ during the first 30 s, after which this con-

Fig. 2 The superimposition of pulmonary $\dot{V}O_2$, cardiac output (*CO*), arteriovenous O_2 content difference (a-v̄DO_2) and hear rate (*HR*) responses determined at district time points during bout 1 (*filled circle*) and bout 2 (*open circle*) of repeated high-intensity KE exercise (group mean and SD). The values at time 0 represent the average of the 30 s baseline just prior to the exercise onset. *Asterisks* represent significant difference between first and second bouts ($P < 0.05$)
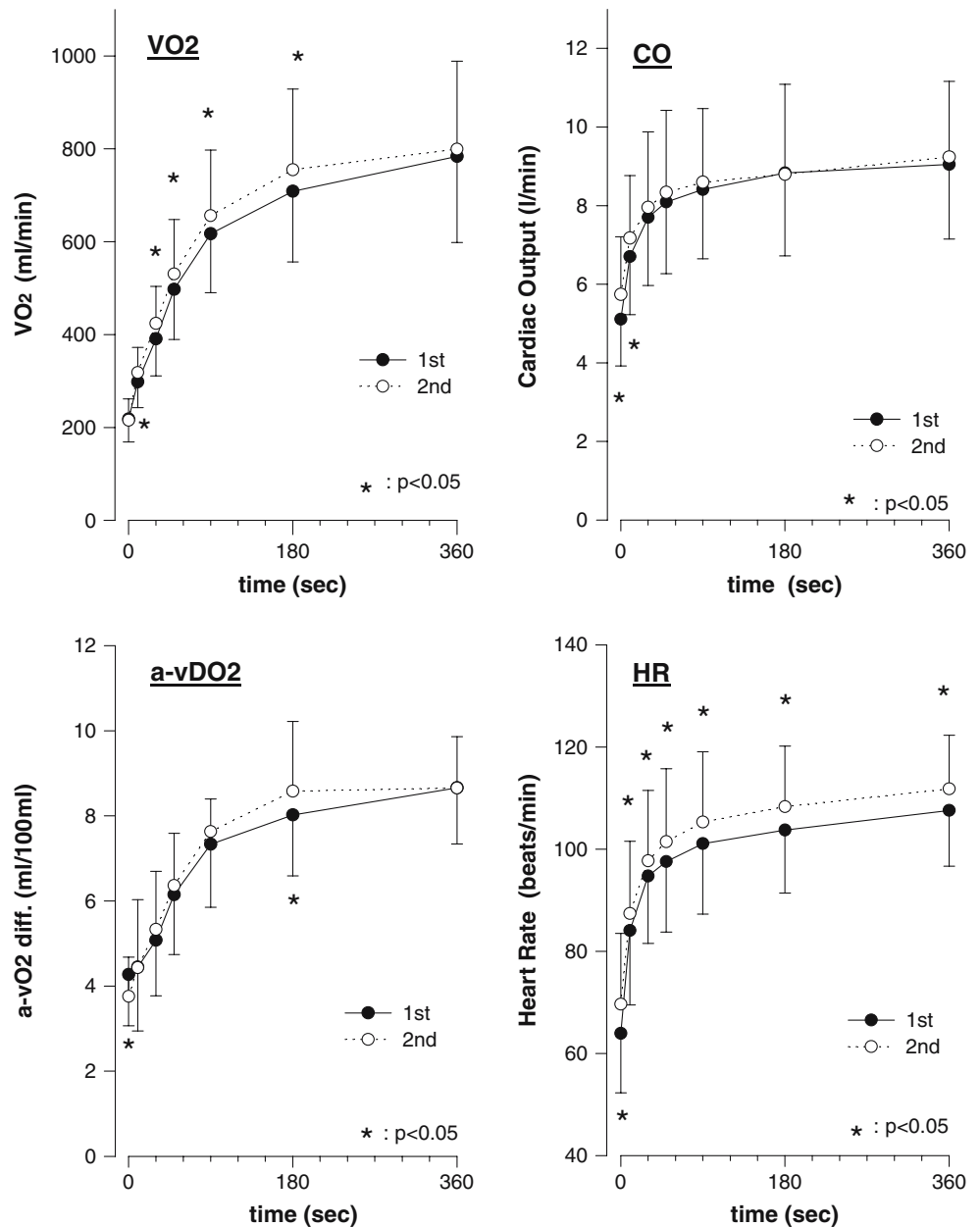
tribution continuously increased reaching 7% by 180 s. The higher $\dot{V}O_2$ measured at 180 s in bout 2 (compared to bout 1) could, therefore, be entirely attributed to a proportional increase in a-v̄DO_2 by the end of phase II. The proportional contribution of CO showed the opposite trend. At 10 s, the higher $\dot{V}O_2$ in bout 2 could be entirely attributed to a proportionally higher CO. However, this proportional contribution fell rapidly over the initial 30 s and subsequently continued to decrease, such that by 180 s, none of the $\dot{V}O_2$ speeding could be attributed to CO (Fig. 3). There were statistical differences between contributions of both CO and a-v̄DO_2 at 10 and 180 s (Fig. 3). Overall, following high-intensity KE exercise, the $\dot{V}O_2$ response to a

second identical exercise bout 2 was ~7% greater at each time point considered from 0 to 180 s. This was consequent to interactive contributions by both O_2 delivery and extraction, with the predominant proportion attributable to a progressively increasing extraction (greater a-v̄DO_2) following the initial 30 s of exercise (i.e., rising from 43 to 107% between 30 and 180 s of exercise).

Discussion

This investigation provides, we believe, the first description of the relative contributions of CO and
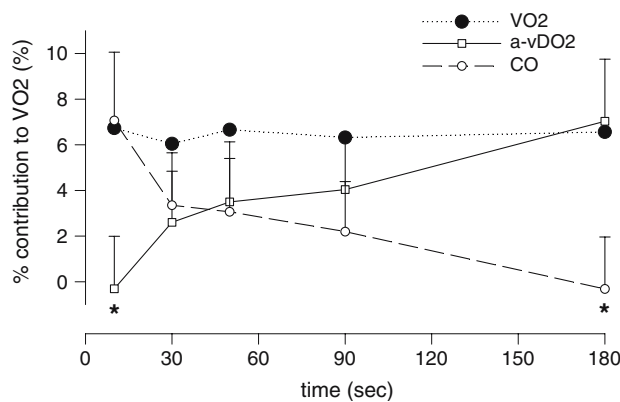
Fig. 3 The percentage contribution of arteriovenous O_2 content difference (a-$\bar{v}DO_2$) (*square*) and cardiac output (*CO*) (*circle*) throughout the transient, to the difference in $\dot{V}O_2$ (*closed circle*) between the first and second exercise bouts. See the detailed explanations in the text. *Asterisks* represent significant difference between CO and a-$\bar{v}DO_2$ contributions ($P < 0.05$)

a-$\bar{v}DO_2$ to the faster $\dot{V}O_2$ response manifest during the transient phase of the second bout of a repeated high-intensity exercise protocol. In order to determine both $\dot{V}O_2$ and CO in concert (and calculate a-$\bar{v}DO_2$) we used KE exercise, not cycle ergometry as is more typical. However, through solving the Fick equation using these measurements during KE exercise, we have elucidated the relative and interactive contributions to $\dot{V}O_2$ from central circulatory and peripheral O_2 extractive components. The findings suggest that the ~7% speeding of the $\dot{V}O_2$ kinetics in the second bout of the double-transition protocol are mainly derived from a gradually widening a-$\bar{v}DO_2$ (relative to that of the first bout). However, during a very early stage of the transition (the first 50 s after onset) the CO contributes to a greater degree to the speeding of $\dot{V}O_2$ in repeated bouts.

In the present study, we chose a conservative strategy to analyze the time-serial data without identifying kinetic parameters (see section Limitations). However, it should be noted that the relationships among CO, $\dot{V}O_2$ and a-$\bar{v}DO_2$ in the present study are compatible to those previously reported for a single transition of cycling exercise (e.g., Cummin et al. 1986; De Cort et al. 1991). Even without estimation of the kinetic parameters, it is clear from the profiles shown in Fig. 2 that the time course of CO is relatively faster than that of $\dot{V}O_2$ in both exercise transitions. Despite these faster kinetics, the magnitude of the CO increase (relative to that of $\dot{V}O_2$) is not adequate to prevent slow increases in a-$\bar{v}DO_2$ throughout the transient. By comparing the $\dot{V}O_2$ kinetics of two bouts of repeated high-intensity exercise, we were able to calculate the relative contributions of CO and a-$\bar{v}DO_2$ to the $\dot{V}O_2$

speeding in the second bout. To do this, we assumed that either of the two variables contributing to $\dot{V}O_2$ (i.e., CO or a-$\bar{v}DO_2$) would have the same absolute value throughout the bout 2 transition as that measured during bout 1. That is, with a-$\bar{v}DO_2$ assumed to respond in bout 2 precisely as it did during bout 1, we can calculate the degree to which an augmented CO may have contributed to the greater $\dot{V}O_2$ measured. Comparison of these calculated and measured $\dot{V}O_2$ values allows a relative weighting to be placed on either of the two components, CO or a-$\bar{v}DO_2$. These data suggest that CO had a relatively important contribution to the increment in $\dot{V}O_2$ during phase I and the following few seconds of bout 2 (the first ~50 s; Fig. 3). Consequently, mechanism(s) related to the peripheral extraction and utilization of oxygen seem to be relatively more important than central circulatory factors in determining the speeding of the phase II and III (i.e., 50–180 s after the exercise onset) pulmonary $\dot{V}O_2$ kinetics during the high-intensity double-transition protocol; although, of course, the interaction of both factors ultimately determine the $\dot{V}O_2$ kinetics.

We are aware of only one study with respect to "double-transition" protocol that has experimentally addressed the potential contribution of circulatory factors in determining the faster $\dot{V}O_2$ kinetics during bout 2. MacDonald et al. (2001) used a similar strategy to the present study to explore the relative contributions of peripheral "blood flow" (BF) and a-vDO_2 (where *v* means antecubital venous blood) to exercising limb $\dot{V}O_2$ (not pulmonary $\dot{V}O_2$) during repeated bouts of forearm exercise (lateral handgrip). In that study, the forearm $\dot{V}O_2$ was raised by ~30% at 30 s after exercise onset in bout 2 compared to bout 1. They calculated (using the same methods as those used here) that the increase in $\dot{V}O_2$ was consequent to a 25.1% increase in forearm BF and 3.7% increase in a-vDO_2. MacDonald et al. (2001) concluded that this relative contribution indicated that the major factor influencing exercising-limb $\dot{V}O_2$ was the increase in BF.

The main discrepancy between the study of MacDonald et al. (2001) and the present findings is the exercising muscle mass and the intensity of the exercise bouts. The limb $\dot{V}O_2$ in the study of MacDanolad et al. (2001) reached a plateau within 2 min and there was no evidence of a slow component, suggesting a light or moderate intensity (see Fig. 2 in MacDonald et al. 2001). During larger muscle mass exercise (such as cycling or KE), a speeded $\dot{V}O_2$ response is typically only manifest if the preceding exercise is above LT and $\dot{V}O_2$ consequently manifests a slow component. The discrepancy between the studies may, therefore, be derived from the difference in the relative intensity of

the exercise for the involved muscle mass adopted (e.g., Shephard et al. 1988). We suggest that when a larger, locomotory muscle mass is engaged in exercise, the predominant cause of speeded $\dot{V}O_2$ kinetics is an increase in a-vDO$_2$. In support of this notion, Endo et al. (2003) recently demonstrated that attenuation of central circulatory dynamics (reducing HR by cold face stimulation; CFS) applied at the onset of bout 2 during high-intensity cycling had no effect on the pulmonary $\dot{V}O_2$ response. This supports the notion that the speeded $\dot{V}O_2$ response during the double-transition protocol is not dominated by central factors.

Attenuation of the magnitude of the $\dot{V}O_2$ slow component by prior exercise was a characteristic of the present study. This is similar to previous observations during a double-transition protocol using cycle ergometry (e.g., Burnley et al. 2000; Gerbino et al. 1996; Koppo and Bouckaert 2000; MacDonald et al. 1997). However, in the present study the reduced $\dot{V}O_2$ slow component was not associated with an alteration of CO during KE exercise. Here, CO did not show evidence of a slow component-like phase, with $\Delta CO_{(6-3)}$ being essentially zero (bout 1: 0.21 ± 0.46 vs. bout 2: 0.40 ± 0.31 l min^{-1}). Rather the attenuation of the $\dot{V}O_2$ slow component was more closely associated with the profile of a-$\bar{v}$DO$_2$. This also indicates that O$_2$-extractive factor(s) appear to be more important in speeding the overall $\dot{V}O_2$ response dynamic using a double-transition protocol.

The present result revealed a relatively dominant contribution of a-$\bar{v}$DO$_2$ to the augmentation of the $\dot{V}O_2$ response during the high-intensity double-transition protocol. While it was, of course, determined by the effective interaction of both central circulatory and peripheral extractive factors, we calculated that a-$\bar{v}$DO$_2$ contributed to over 50% of the $\dot{V}O_2$ speeding (i.e., from 50 s onwards, or the majority of phase II). In line with previous suggestions, these findings indicate that the predominant determinants for the speeding of $\dot{V}O_2$ kinetics by prior exercise (and the consequent reduction of the $\dot{V}O_2$ slow component) is likely to be attributable to factors more proximal to the exercising limb (the lower limb in this case). One candidate was within the peripheral bulk circulation; that is a more efficient distribution of CO to the exercising limb. However, a recent study from our laboratory utilizing kinetic analyses (Endo et al. 2005) indicated that the optimization of femoral artery blood flow could not explain the faster pulmonary $\dot{V}O_2$ kinetics during the second bout of repeated high-intensity KE exercise. For blood flow to be responsible for a greater proportion of the speeded $\dot{V}O_2$ response, a flow optimization that is more peripheral than the femoral artery

would be required. Such a mechanism is yet to be elucidated.

Intramuscular mechanisms, that are thought to determine $\dot{V}O_2$ kinetics, have been investigated during the double-transition protocol by Rossiter et al. (2001). These authors suggested that attenuation of the $\dot{V}O_2$ slow component following prior exercise in humans was consequent to an intramuscular "sparing" of [PCr] degradation. This suggestion that the $\dot{V}O_2$ slow component is determined by intramuscular mechanisms was in accordance with the findings of Poole et al. (1991), who showed that ~80–90% of the pulmonary $\dot{V}O_2$ slow component could be accounted for by an increase in the leg $\dot{V}O_2$. Consequently, the control of the $\dot{V}O_2$ slow component is typically ascribed to intramuscular factors in the exercising limb, rather than to the rest of the body. Therefore, the attenuation of the slow component by prior exercise (and perhaps the speeding of phase II kinetics) ought also to be determined by intramuscular events, such as the pattern of motor unit recruitment and/or fatigue (Barstow et al. 1996; Rossiter et al. 2002), intracellular factors other than O$_2$ availability (Hogan 2001; Behnke et al. 2002), which may arise from either activation of the pyruvate dehydrogenase complex (Timmons et al. 1998; Howlett and Hogan 2003; Rossiter et al. 2003), and/or be related to the attenuation of the blood lactate increase (Gerbino et al. 1996), or altered phosphate-mediated feedback control (Rossiter et al. 2001). Identifying the intramuscular source of the faster $\dot{V}O_2$ kinetics is, however, beyond the scope of this study.

Limitations

The development of the Doppler ultrasound technique for the measurement of time-resolved CO has made it possible to observe the kinetics CO throughout an exercise transition in greater detail than has previously been possible. Traditional non-invasive methods, such as CO$_2$ or acetylene rebreathing or prolonged exhalations (e.g., Sackner 1987), have poor time resolution and are therefore not ideal for kinetic observations. However, the continuous Doppler wave method is itself sensitive to signal "noise" derived from several technical and spontaneous sources. We attempted to minimize these by averaging two identical repetitions for each subject with simultaneous $\dot{V}O_2$ measurement. The $\dot{V}O_2$ kinetic responses were further enhanced by additional repetitions, with particular care not to induce significant training effects. Despite this, the CO responses were not sufficiently noise-free to confidently estimate kinetic parameters. We, therefore, chose a conservative strategy to analyze the time-serial

data without estimation of kinetic parameters in any of the measured or calculated variables. However, it should be noted that the relationships among CO, $\dot{V}O_2$ and a-$\bar{v}DO_2$ in the present study were very compatible to those previously reported for a single transition of leg dynamic cycling exercise (e.g., Cummin et al. 1986; De Cort et al. 1991).

In general, the KE exercise modality has frequently led to a reduction in the time constant of $\dot{V}O_2$ primary component (τ_p) as well as the magnitude of the $\dot{V}O_2$ slow component (e.g., Hughson et al. 2003; Fukuba et al. 2004; Rossiter et al. 2001). However, investigations using cycle ergometry typically find that prior exercise-induced changes are limited to the slow component region; in other words, the phase II $\dot{V}O_2$ τ_p is unaltered (e.g., Burnley et al. 2000; Endo et al. 2003; Gerbino et al. 1996; Koppo and Bouckaert 2000; Wilkerson et al. 2004). In addition, KE exercise can result in longer $\dot{V}O_2$ τ_p (~50 s) and higher fundamental gain ($\Delta\dot{V}O_2/\Delta W$; ~20 ml min^{-1} W^{-1}), compared to those seen during cycle erogmetry in healthy subjects (e.g., ~25–35 s; ~10 ± 1 ml min^{-1} W^{-1}) (e.g., Endo et al. 2005). The distinction between KE and cycle erogometry, therefore, may be of importance in this regard. Furthermore, because the KE exercise requires substantially high intramuscular force, there are presumably differences in circulatory adjustments (including CO) at the onset of KE exercise compared to cycle ergometry. Steady-state relationships among CO, $\dot{V}O_2$ and a-$\bar{v}DO_2$ in the present study (i.e., KE exercise) were, however, very compatible to those previously reported for a single transition of leg cycling ergometer exercise (e.g., Cummin et al. 1986; De Cort et al. 1991). Therefore, caution should be used in extrapolating the results of the present study to other modes of exercise, such as conventional cycling exercise.

In summary, this study demonstrated that: (1) the pulmonary $\dot{V}O_2$ was significantly higher between 10 and 180 s (and slow component reduced) after the onset of high-intensity KE exercise when preceded by an identical first bout of KE; (2) cardiac output manifests a faster on-transient time course than pulmonary $\dot{V}O_2$ throughout, but was unchanged between the phase II transient of the first and second exercise bouts; and (3) the apparent speeding of the $\dot{V}O_2$ response during the phase II region of bout 2 was initially greatly determined by a large contribution of CO, but later (and more predominantly), as a result of increased O_2 extraction. This suggests that the mechanism(s) modulating the speeding of the $\dot{V}O_2$ response during bout 2 of the double-transition protocol should be sought for in event(s) within the exercising muscles themselves.

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