Relationship between changes in pulmonary $\dot{V}O_2$ kinetics and autonomic regulation of blood flow

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Abstract

Various regulatory mechanisms of pulmonary oxygen uptake ($\dot{V}O_2$) kinetics have been postulated. The purpose of this study was to investigate the relationship between vagal withdrawal, measured using RMSSDRR, the root mean square of successive differences in cardiac interval (RR) kinetics, a mediator of oxygen delivery, and $\dot{V}O_2$ kinetics.

49 healthy adults (23 ± 3 years; 72 ± 13 kg; 1.80 ± 0.08 m) performed multiple repeat transitions to moderate and heavy intensity exercise. ECG, impedance cardiography and pulmonary gas exchange parameters were measured throughout; time domain measures of heart rate variability were subsequently derived. The parameters describing the dynamic response of $\dot{V}O_2$, cardiac output ($\dot{Q}$) and RMSSDRR were determined using a mono-exponential model.

During heavy intensity exercise, the phase II τ of $\dot{V}O_2$ was significantly correlated with the τ of RR ($r=0.36$, $P<0.05$), Q ($r=0.67$, $P<0.05$) and RMSSDRR ($r=0.38$, $P<0.05$). The τ describing the rise in Q explained 47% of the variation in $\dot{V}O_2$ τ, with 30% of the rate of this rise in Q explained by the τ of RR and RMSSDRR. No relationship was evident between $\dot{V}O_2$ kinetics and those of Q, RR or RMSSDRR during moderate exercise.

Vagal withdrawal kinetics support the concept of a centrally mediated oxygen delivery limitation partly regulating $\dot{V}O_2$ kinetics during heavy, but not moderate, intensity exercise.

Introduction

It has long been acknowledged that pulmonary oxygen uptake ($\dot{V}O_2$) rises in an approximately exponential manner following an abrupt increase in external work rate (Whipp & Wasserman 1972), providing a surrogate measure of muscle oxygen consumption kinetics (Grassi et al. 1996). The mechanism(s) regulating the rate of this rise have been debated for many years: oxygen delivery (along the oxygen transport cascade from lungs to mitochondria) and oxygen utilisation (at the target cells) have both been proposed as putative mediators (Grassi 2000; Hughson et al. 2001; Grassi 2005; Hughson 2005). Although the balance of these factors appears to vary with age, health (or lack thereof), the exercise modality and experimental perturbations, it appears that in young, healthy participants during upright, moderate intensity cycling exercise the dynamic $\dot{V}O_2$ response is predominately regulated by O_2 utilisation. As exercise intensity increases (above the gas exchange threshold, GET), there is suggested to be a contributory role of oxygen delivery in determining the $\dot{V}O_2$ kinetics (Poole & Jones, 2011; Rossiter 2010).

An oxygen delivery mediation of $\dot{V}O_2$ kinetics may be manifest centrally (by the cardiac output ($\dot{Q}$) response) or peripherally (by local blood flow distribution). A central, $\dot{Q}$-related delivery limitation might be indirectly regulated by the autonomic nervous system (ANS) due to its chronotropic control of heart rate ($\dot{Q}$ = stroke volume (SV) * heart rate (HR)). Indeed, the potential importance of vagal and sympathetic tone in determining $\dot{V}O_2$ kinetics was acknowledged by Hughson (1990) on the basis of earlier studies illustrating that vagal withdrawal occurs more rapidly than the reciprocal increase in sympathetic tone in response to physical exercise (Hughson & Morrissey 1983; Inman et al. 1987). We might therefore hypothesize that the rate of vagal withdrawal at the onset of exercise is at least partly responsible for the dynamics of the simultaneous increase in $\dot{V}O_2$ that occurs. However, it is important to also consider the alternative hypothesis that $\dot{V}O_2$ kinetics are largely independent of oxygen delivery and are instead modulated by oxygen utilisation in the target cells. Proponents of this hypothesis suggest that the rate-limiting step is related to the concentration of cellular metabolic controllers and/or mitochondrial enzyme activity (for a thorough review, see: Grassi 2005). The observation that increasing oxygen delivery does not speed $\dot{V}O_2$ kinetics is a key tenet of this alternative hypothesis (Grassi et al. 1998a; Grassi et al. 1998b).

The influence of the ANS (through its control of HR) on $\dot{V}O_2$ kinetics at the onset of an increased external work rate has received little attention. Hayashi and colleagues (1998) investigated the hypothesis that a delayed vagal withdrawal, provoked by cold facial stimulation (CFS), would slow $\dot{V}O_2$ kinetics owing to slower HR and $\dot{Q}$ kinetics. Although the HR and $\dot{Q}$ responses were significantly slowed by this intervention, the $\dot{V}O_2$ kinetics remained unaffected. Endo et al. (2003) reported a similar influence of CFS during heavy intensity exercise: the dynamic HR response was slowed but the primary component $\dot{V}O_2$ kinetics were unaffected by CFS. These results were interpreted to suggest that oxygen delivery was not a limiting factor of $\dot{V}O_2$ kinetics. However, these small studies involved only a short period of CFS (dictated by participant discomfort) and cessation of CFS might have influenced the measured responses. Furthermore, neither of these studies assessed vagal withdrawal *per se*, precluding a direct assessment of ANS-mediation of $\dot{V}O_2$. To our knowledge, no previous study has quantified the dynamic responses of both vagal withdrawal and $\dot{V}O_2$ following exercise onset. We suggest that the putative role of vagal withdrawal kinetics in the determination of $\dot{V}O_2$ kinetics warrants further investigation.

The purpose of this cross-sectional study was therefore to assess the influence of vagal withdrawal dynamics on the central circulatory response and on pulmonary $\dot{V}O_2$ kinetics during moderate and heavy intensity exercise. This study therefore utilises a novel technique to extend and confirm the findings of earlier studies. We hypothesised that a faster vagal withdrawal would be associated with faster HR, $\dot{Q}$ and $\dot{V}O_2$ kinetics but that this association would only be evident during heavy intensity exercise.

Methods

Participants

Forty nine young adults volunteered for the study (mean ± S.D. age 23 ± 3 years; body mass 71.5 ± 13.1 kg; height 1.80 ± 0.08 m). The participants (28 male) were all recreationally active, but not highly trained. Prior to testing, participants were informed of the protocol and risks of the study and gave written consent to participate. All procedures were approved by the local ethics committee and were conducted in accordance with the Declaration of Helsinki. Participants were asked to arrive at the laboratory in a rested and fully hydrated

state, at least two hours postprandial and to avoid strenuous exercise in the 24 hours preceding each testing session. Participants were also asked to refrain from caffeine and alcohol for 6 h and 24 h before each test, respectively. All tests were performed at the same time of day ($\pm$ 2 h).

Experimental Design

Participants were required to visit the laboratory on four occasions, separated by at least 24 h recovery. Participants initially completed a ramp incremental exercise test for determination of $\dot{V}O_{2peak}$ and gas exchange threshold (GET). On each of the three subsequent visits, participants completed two bouts of moderate intensity exercise (at a work rate calculated to elicit 70% of the GET) followed by a bout of heavy intensity exercise (at a work rate calculated to elicit a $\dot{V}O_2$ equal to the GET plus 30% of the difference between the GET and peak $\dot{V}O_2$, i.e. Δ30%). All exercise testing was conducted using an electronically braked cycle ergometer (Lode Excalibur, Groningen, the Netherlands). Participants maintained constant pedal cadence of 70-80 rpm and this was strictly monitored to avoid the potential confounding influence of variations in pedal cadence on heart rate variability (HRV) parameters (Blain et al. 2009).

Incremental Test

Initially, participants completed 3 min of baseline cycling at no additional work rate to that inherent to the cycle ergometer., After this, the work rate was increased at a rate of 20-30 W·min^{-1} until the limit of tolerance. The participants were asked to maintain a cadence of 70–80 rpm. Breath-by-breath pulmonary gas exchange data were collected continuously during the incremental tests and averaged over consecutive 5-s periods (Oxycon Pro, Jaeger, Germany). The $\dot{V}O_{2peak}$ was taken as the highest 10-s average value attained before the subject's volitional exhaustion in the test. The GET was determined from a cluster of measurements, including *1*) the first disproportionate increase in CO_2 production ($\dot{V}CO_2$) from visual inspection of individual plots of $\dot{V}CO_2$ vs. $\dot{V}O_2$; *2*) an increase in expired ventilation $\dot{V}_E/\dot{V}O_2$ with no increase in $\dot{V}_E/\dot{V}CO_2$; and *3*) an increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension. The work rates that would require 70% of the GET (moderate exercise) and Δ30% were subsequently determined, accounting for the mean response time for $\dot{V}O_2$ during ramp exercise (i.e. two thirds of the ramp rate was deducted from the work rate at the GET and peak (Whipp et al. 1981)).

Step Exercise Tests

For the determination of $\dot{V}O_2$, HR, $\dot{Q}$ and HRV kinetics, participants completed a series of "step" tests. The protocol, which was repeated three times on separate days, comprised of two moderate intensity and one heavy intensity cycle transition, each of 6-min duration. Each transition was preceded by 6 min of baseline pedalling at 0 W followed by an abrupt transition to the target work rate. Therefore, all participants performed a total of six bouts of moderate-intensity exercise and three bouts of heavy intensity exercise.

Measurements

Throughout all exercise tests, participants wore a facemask and breathed through a low dead space (90 ml), low resistance (0.75 $mmHg \cdot l^{-1} \cdot s^{-1}$ at 15 $l \cdot s^{-1}$) impeller turbine assembly (Jaeger Triple V, Hoechberg, Germany). The inspired and expired gas volumes and gas concentration signals were continuously sampled at 100 Hz, the latter using paramagnetic (O_2) and infrared (CO_2) analysers (Jaeger Oxycon Pro, Hoechberg, Germany) via a capillary line connected to the mouthpiece. These analysers were calibrated before each test with gases of known concentrations, and the turbine volume transducer was calibrated using a 3 l syringe (Hans Rudolph, Kansas City, MO). The volume and concentration signals were time aligned by accounting for the delay in capillary gas transit and analyser rise time relative to the volume signal.

$\dot{Q}$ was determined noninvasively, on a beat-to-beat basis, throughout the exercise tests using a thoracic bioelectrical impedance device (TaskForce, CNSystems Medizintechnik GMBH, Austria) in a process known as impedance cardiography (ICG). This device has been validated under a variety of conditions against the gold standard thermodilution technique (Fortin et al. 2001; Fortin et al. 2006). A Reynolds Lifecard CF digital Holter recorder (Spacelabs Medical Ltd., Hertford, UK) was used to record a three-lead ECG continuously throughout the tests. The ECG leads were positioned in the modified V5, CC5, modified V5R electrode configuration. This system provided ECG data with a sample accuracy of 2.5 µV (magnitude of least significant bit; 12-bit resolution) and 1024 Hz sampling frequency.

ECG analysis

ECG recordings were analysed using a Reynolds Pathfinder digital analyser (Spacelabs Medical Ltd., UK). Beat-to-beat cardiac interval (RR) values were automatically measured for each sinus beat and exported for further analysis using the Reynolds Research Tools software (Spacelabs Medical Ltd., UK). All ECG data used for subsequent analysis in this study were free of any form of morphologically abnormal beat, and this was verified by both the Holter system and by visual inspection.

Heart rate variability derivation

HRV variables were quantified in the time domain (RMSSDRR: square root of the mean of the sum of the squares of differences between adjacent RR intervals; SDRR: standard deviation of all RR intervals) according to the Task Force guidelines on HRV (TaskForce 1996). RMSSDRR is a measure of short-term variation in RR, reflecting high frequency variations in cardiac interval that are mediated via parasympathetic (vagal) influence on the heart (TaskForce 1996). SDRR reflects all the cyclic components of heart rate and is thus synonymous with total HRV power. RMSDDRR was used as an indicator of vagal withdrawal in the present study because the non-stationarity of the RR intervals throughout the exercise transition precluded the use of frequency domain measures. In contrast, RMSSDRR is not influenced by RR interval non-stationarity (Pagani et al. 1988).

$\dot{V}O_2$ kinetics analysis

Initially, the breath-by-breath $\dot{V}O_2$ responses to each step transition were examined to remove any errant breaths caused by coughing, swallowing, sighing etc, using a 5 breath moving average to identify points lying in excess of 4 standard deviations from the local mean. Subsequently, each transition was interpolated to 1-s intervals, time aligned to the start of exercise and averaged.

To remove the influence of phase I on analysis of the subsequent $\dot{V}O_2$ response, the first 20-s of data were ignored. A mono-exponential model with a time delay (Eq.1) was then applied to the averaged response:

$$VO_{2\,(t)} = A_1 \cdot (1 - e^{-(t-\delta)/\tau}) \qquad \text{(Eq.1)}$$

where $\dot{V}O_2$ is the increase in $\dot{V}O_2$ at time t above the baseline value (calculated as the mean $\dot{V}O_2$ from the first 45-s of the last min of baseline pedalling), and A_1, δ and τ are the primary

component amplitude, time delay and time constant, respectively. Kinetic variables (A_1, δ and τ) and their 95% confidence intervals were determined by least squares non-linear regression analysis (Graphpad Prism, Graphpad Software, San Diego, CA).

For heavy intensity exercise, the fitting window was constrained to exclude all data the onset of the $\dot{V}O_2$ slow component. This approach avoids any possible influence of arbitrarily parameterising the slow component. The onset of the $\dot{V}O_2$ slow component was determined using purpose designed LabVIEW software which iteratively fits a monoexponential function to the $\dot{V}O_2$ data until the window encompasses the entire response. The resulting phase II time constants are plotted against time and the onset of the $\dot{V}O_2$ slow component identified as the point at which the phase II time constant consistently deviates from the previously "flat" profile (Rossiter et al. 2001). The amplitude of the $\dot{V}O_2$ slow component was subsequently determined by calculating the difference between the end exercise $\dot{V}O_2$ and the sum of the primary amplitude and baseline $\dot{V}O_2$. This was expressed both in absolute terms and relative to end exercise $\dot{V}O_2$. The functional gain of the primary $\dot{V}O_2$ response during both exercise intensities was also calculated by dividing the primary phase amplitude by the change in work rate.

RR and Q kinetic analysis

To provide information on the central circulatory response, we also modelled the RR and $\dot{Q}$ response to exercise. The RR response itself was used rather than the derived heart rate response as this precludes the potential confounding factor of subtle conversion inaccuracies. The time series representing the RR and $\dot{Q}$ responses to each exercise transition were interpolated to 1-s intervals, time aligned and averaged to produce a single data set. In accord with previous studies (Inman 1987; Barstow et al. 1990), the resulting responses were fitted with a mono-exponential. We omitted the time delay as the physiological rationale for such a time delay in the cardiovascular response to exercise is not clear (Eq. 2). For both moderate and heavy exercise, the fitting window commenced at t = 0; for heavy intensity exercise the window was constrained to the onset of the "slow component".

$$Y_{(t)} = A_1 \cdot (1 - e^{-(t/\tau)}) \qquad \text{(Eq.2)}$$

where Y is the decrease in RR or increase in $\dot{Q}$ at time t from the baseline (calculated as the mean from the first 45 s of the last min of baseline pedalling), and A_1 and τ are the primary component amplitude and time constant, respectively. The RR and $\dot{Q}$ "slow components" were calculated as the end exercise amplitude minus the primary component amplitude.

HRV kinetics analysis

The individual HRV responses to each transition were averaged over 20 s periods, time aligned to the start of exercise and ensemble averaged. Subsequently, to assess the autonomic nervous system response, the selected time domain HRV parameters were modelled using a mono-exponential without a time delay (Eq. 2), commencing at t = 0.

Statistical analysis

No gender differences were evident in the model-derived kinetic parameters according to independent t-tests; therefore, data were pooled for all subjects prior to further analysis. Following confirmation of a Gaussian distribution using the Shapiro Wilks test, the Pearson product moment correlation coefficient was used to quantify the relationship between HRV, central circulatory (RR and $\dot{Q}$) and $\dot{V}O_2$ kinetics. The ability of HRV and/or central circulatory kinetics to predict $\dot{V}O_2$ kinetics was assessed by stepwise multiple linear regression analysis after verification that the assumptions of multicollinearity and heteroscedasticity were not violated. The variance inflation factor and tolerance were used to assess multicollinearity whilst heteroscedasticity was verified using residual plots. All data are presented as means ± SD. Statistical significance was accepted when *P* < 0.05.

Results

The mean peak $\dot{V}O_2$ was 3.13 ± 0.97 l·min^{-1} (42.5 ± 12.2 ml·kg^{-1}·min^{-1}), the mean GET was 1.70 ± 0.61 (54 ± 10%) and peak power was 262 ± 87 W in the 49 participants. The relative exercise intensities equated to work rates of 38 ± 20 W and 159 ± 60 W for moderate and heavy intensity exercise, respectively.

Tables 1-3 present the $\dot{V}O_2$, central circulatory and HRV model-derived parameters, respectively, while Figure 1 shows the $\dot{V}O_2$, RR, $\dot{Q}$ and RMSSDRR responses for one representative participant. During moderate exercise, no relationships were evident between

the τ for the $\dot{V}O_2$ response and that for $\dot{Q}$ (Figure 2A), RR, or RMSSDRR. However, during heavy exercise, the $\dot{V}O_2$ τ was significantly related to the $\dot{Q}$ τ ($r = 0.67$, $P < 0.05$; Figure 2B), the RR τ ($r = 0.36$, $P < 0.05$), and the RMSSDRR τ ($r = 0.38$, $P < 0.05$). A significant correlation was also evident between the amplitude of the $\dot{V}O_2$ response to heavy exercise and the corresponding response amplitudes of RR ($r = 0.30$, $P < 0.05$), $\dot{Q}$ ($r = 0.45$, $P < 0.01$), RMSSDRR ($r = -0.44$, $P < 0.01$) and SDRR ($r = 0.40$, $P < 0.01$). The baseline HR, the inverse of the RR interval, was not significantly different prior to moderate or heavy intensity exercise (Moderate: 77 vs. Heavy: 80 b·min^{-1}). The magnitude of the $\dot{V}O_2$ slow component (whether expressed in absolute or relative terms) was not related to the amplitude of the $\dot{Q}$, RR or RMSSDRR "slow component".

Multiple linear regression analysis revealed that none of the derived kinetic parameters were able to predict the $\dot{V}O_2$ τ during moderate intensity exercise. During heavy intensity exercise the τ describing the simultaneous rise in $\dot{Q}$ explained a significant ($P < 0.01$) proportion (47%) of the $\dot{V}O_2$ τ, resulting in the regression equation: $\dot{V}O_2$ $\tau = 14.985 + (0.4 * \dot{Q}$ $\tau)$. Further regression analyses revealed that $\dot{Q}$ τ was determined by both the RR τ and RMSSDRR τ, which together explained a significant ($P < 0.01$) proportion (30%) of the variation in $\dot{Q}$ τ according to the equation: $\dot{Q}$ $\tau = 29.05 - (0.358*RMSSDRR$ $\tau) + (0.33*RR$ $\tau)$.

Discussion

The primary finding of this study is that the rate of vagal withdrawal influences $\dot{V}O_2$ kinetics in an exercise intensity dependent manner. During heavy intensity exercise the pulmonary $\dot{V}O_2$ response was significantly related to the rate of vagal withdrawal and to the kinetics of the HR and $\dot{Q}$ response. In contrast, during moderate intensity exercise the dynamics of these responses showed no association.

This is the first study to investigate the relationship between cardiac vagal activity, $\dot{V}O_2$ kinetics and $\dot{Q}$ kinetics within the same participants, the aim of which was to provide further insight into the mechanism(s) responsible for regulating $\dot{V}O_2$ kinetics (Grassi 2000; Hughson

2001; Grassi 2005; Hughson 2005). This work extends those previous studies that have assessed the relationship between $\dot{V}O_2$ kinetics and either $\dot{Q}$ or HR (De Cort et al. 1991; Fukuba et al. 2007) or only the relationship between vagal withdrawal and HR kinetics (Javorka et al. 2003; Ricardo et al. 2010).

Parasympathetic (vagal) withdrawal is associated with the rapid increase in heart rate at the onset of exercise (Fagraeus & Linnarsson 1976), delayed withdrawal being associated with slower responses in HR and $\dot{Q}$ (Hayashi 1998; Endo 2003). Although it might be anticipated that slower HR and $\dot{Q}$ kinetics would cause an oxygen delivery limitation and thus retard the dynamic $\dot{V}O_2$ response (Hughson 2001), previous studies have not observed such an effect during either moderate or heavy intensity exercise (Hayashi 1998; Endo 2003). However, a limitation of these small studies was that they used cold facial stimulation to reduce heart rate. Participant discomfort dictated that this was of short duration and the cessation of this procedure would probably have influenced the measured responses. We also note that we do not know the extent of the delayed vagal responses induced by CFS in these previous studies; it was not possible to verify this potential confounder since neither of those studies quantified vagal activity directly. Indeed, ours is the first study to consider ANS function (via HRV) *during* the transition to an increased metabolic rate, showing that 20-s averaged time domain indices are well characterised by a mono-exponential response. Furthermore, the model-derived parameters for these responses are associated with a high level of confidence (reflected by the narrow confidence intervals). It is pertinent to note that a mono-exponential model for HRV indices was chosen on the basis that it makes the fewest assumptions regarding underlying physiology. Further work is required to elucidate whether alternative (more complex) models might provide a superior description of the dynamic HRV response to exercise.

Although $\dot{V}O_2$ kinetics are conventionally considered to be independent of O_2 delivery, it remains controversial as to whether $\dot{V}O_2$ kinetics during moderate intensity exercise are modulated by O_2 delivery. In fact recent studies have shown that prior exercise that speeds the adjustment of local O_2 delivery is associated with faster $\dot{V}O_2$ kinetics during moderate intensity exercise (Gurd et al. 2005; Gurd et al. 2006; Murias et al. 2011), suggesting that O_2 delivery can mediate the dynamic $\dot{V}O_2$ response. The present results, which extend those of

Hayashi et al. (1998), show no relationship between the dynamic responses of $\dot{V}O_2$, vagal activity (RMSSDRR), $\dot{Q}$ or HR at the onset of moderate intensity exercise and therefore support the notion of a central O_2 delivery independence. These findings might be considered to contradict those of Lador et al. (2006) who found that the initial phase I increase in $\dot{V}O_2$ was entirely accounted for by the simultaneous increase in $\dot{Q}$. However, those results are not comparable to the present study as they refer to different phases of the dynamic $\dot{V}O_2$ response and thus different regulatory mechanisms are likely to be involved. It is important to highlight that whilst the present results suggest that central O_2 delivery is not a rate limiter for $\dot{V}O_2$ kinetics during moderate intensity exercise, this does not preclude a role of peripheral O_2 delivery in determining the dynamic $\dot{V}O_2$ response. Indeed Harper et al. (2006) found central and peripheral blood flow kinetics to be dissociated, with only peripheral blood flow kinetics being slower than those of $\dot{V}O_2$ and thus suggesting peripheral O_2 delivery as the sole potential rate-limiting factor. This finding of the potential importance of peripheral O_2 delivery is supported by recent studies showing significant influences of prior exercise (i.e. enhanced local O_2 delivery adjustment at the onset of exercise) on $\dot{V}O_2$ kinetics during moderate intensity exercise (Gurd 2005; Murias 2011). Alternatively, or additionally, if $\dot{V}O_2$ kinetics are not primarily modulated by peripheral O_2 delivery, intramuscular factors (such as the concentration of cellular metabolic controllers and/or the activity of mitochondrial enzymes (Grassi 2005) may represent the dominant mediators of the dynamic $\dot{V}O_2$ response during moderate intensity exercise. Although resolution of the relative importance of peripheral O_2 delivery and intramuscular factors in determining the $\dot{V}O_2$ kinetic response is beyond the scope of the present study, it is interesting to note that when the influence of prior exercise on a subsequent bout of moderate intensity exercise was investigated in a study population with a phase II $\dot{V}O_2$ τ similar to that in our study, no effect of prior exercise was found (Burnley et al. 2000).

During heavy intensity exercise, Endo et al. (2003) did not observe any effect of cold facial stimulation on $\dot{V}O_2$ kinetics. This contrasts with the present findings where the rate of vagal withdrawal was significantly and positively related to the $\dot{V}O_2$ τ: that is, slower vagal withdrawal kinetics are associated with slower $\dot{V}O_2$ kinetics. This agrees with the hypothesis of Hughson (2001), as well as the slower $\dot{V}O_2$ kinetics observed following beta-adrenergic

blockade (Petersen et al. 1983; Hughson 1984). The present study is the first to 'directly' assess the relationship between $\dot{V}O_2$ kinetics and vagal withdrawal and, as hypothesized, the influence of vagal withdrawal was reflected in the $\dot{Q}$ response. The rate of vagal withdrawal (and RR reduction) accounted for 30% of the rise in $\dot{Q}$, which in turn accounted for 47% of the simultaneous rise in $\dot{V}O_2$. Fukuba et al. (2007) reported a similar contribution of $\dot{Q}$ to the $\dot{V}O_2$ response, accounting for 100% of the initial rise in $\dot{V}O_2$ 10 seconds after the step transition and 56% of the rise in $\dot{V}O_2$ 30 seconds after the step transition. Despite these values being similar to our findings, caution is required when interpreting the findings of Fukuba et al. (2007): the use of 10-s averaged $\dot{V}O_2$ and $\dot{Q}$ values, the absence of model-based analyses, the limited confidence associated with these data (because of small response amplitudes that were attributable to the small muscle mass engaged in knee extension exercise), and the limited number of transitions used. The present findings therefore support a role of both oxygen delivery and oxygen utilisation in the regulation of $\dot{V}O_2$ kinetics during heavy intensity exercise, as recently concluded elsewhere (Gurd et al. 2006; DeLorey et al. 2007).

The mechanistic basis of the rise in $\dot{Q}$ at the onset of step exercise has not previously been investigated. Therefore, a novel finding of the current study is that at the onset of heavy intensity exercise 30% of the rise in $\dot{Q}$ was explained by reductions in RR and RMSSDRR. This suggests that chronotropic changes in HR (caused by vagal withdrawal) are partly responsible for determining central oxygen delivery during heavy intensity exercise. The factors explaining the remaining 70% of the variation in $\dot{Q}$ are not clear from this work but it would be expected that both stroke volume (SV) and the reciprocal increase in sympathetic tone (via its influence on HR and inotropy) would be prominent factors in this process. A dominant role of SV in determining the rise in $\dot{Q}$ at the onset of an increased metabolic demand has previously been reported and is suggested to be indicative of a greater reliance of $\dot{Q}$ on sympathetic activation than on vagal withdrawal (Faisal et al. 2009). This dominance of sympathetic activation might be anticipated owing to the wider regulatory influences it exerts compared to those of vagal withdrawal (Stubenitsky et al. 1998). Specifically, through its influence on β- and α-adrenoceptors, sympathetic activity is at least partly responsible for increases in HR and myocardial contractility as well as alterations in systemic and pulmonary circulatory vasodilation. Whether HRV indices might be able to clarify the changes in

sympathetic tone during an exercise transition remains to be determined; to date this has been hindered by the continued ambiguity regarding the interpretation of HRV indices with respect to sympathetic tone (TaskForce 1996).

Although the aetiology of the slow component remains to be conclusively elucidated (Jones et al. 2011), around 90% of this component has been shown to arise from within the exercising muscle (Poole et al. 1991). The recruitment of additional, less efficient type II muscle fibres has been widely purported to account for this (Rossiter et al. 2002; Krustrup 2004) but Cannon et al. (2011) have recently suggested that the $\dot{V}O_2$ slow component is instead related to an increased ATP and/or O_2 cost of power production in the fatigued type I muscle fibres. In the present study the magnitude of the $\dot{V}O_2$ slow component was independent of the simultaneous changes in $\dot{Q}$, RR or RMSSDRR. To the best of our knowledge, this is the first study to investigate the relationship between ANS regulation of oxygen delivery and the $\dot{V}O_2$ slow component, and our results contradict suggestions of an oxygen delivery limitation as a putative mechanism for the $\dot{V}O_2$ slow component.

At rest, HRV has been shown to be sensitive to ageing, disease and exercise training (Pagani 1988; Albinet et al. 2010). The present findings of a strong association between peak $\dot{V}O_2$ and the amplitude of HRV indices at the onset of exercise further exemplifies the important relationship between the overall level of HRV and fitness. Given the clinical importance of resting HRV as a predictor of increased risk of mortality and morbidity (Kleiger et al. 1987; TaskForce 1996), it is possible that a diminished HRV response to a sudden change in external work rate might provide an even more sensitive predictor of such risk. Indeed, it could reveal influential derangements not manifested at rest, thereby facilitating earlier intervention.

A fundamental limitation of the current study is the potential dissociation between cardiac output (or limb blood flow) and the blood flow within the microcirculation, as previously suggested during moderate intensity knee-extension exercise (Harper 2006). While the applicability of that previous work (small muscle mass exercise) to the exercise regime in the present study needs further investigation, this potential confounder on the current results

cannot be overlooked. Irrespective of this limitation, the actual relationship between the rate of vagal withdrawal and that of $\dot{V}O_2$ kinetics during heavy intensity exercise is not altered.

Another limitation of the present study design was the reliance on correlation analyses to examine the relationship between the dynamic responses of $\dot{V}O_2$, vagal activity, $\dot{Q}$ and HR. It is important to note that correlation does not imply causation. Future studies might usefully incorporate the novelties of this paper (assessing vagal withdrawal kinetics using HRV) with the experimental manipulation of vagal withdrawal as reported elsewhere (Hayashi 1998; Endo 2003). The use of unloaded cycling as the baseline condition was necessary to avoid issues associated with flywheel acceleration at the onset of the step transition which could have distorted the physiological responses. However, it might also have been associated with a degree of vagal withdrawal before the step transition. No resting measures of HR are available to elucidate the likelihood of this potential confounding factor but given the relatively low baseline HR observed before both moderate and heavy intensity exercise (77 and 80 b·min^{-1}, respectively) we do not believe it represents a significant issue. It is also pertinent to note the relationship between respiratory activity and vagal modulation, which might contribute to our exercise intensity-dependent findings. However, whilst the change in tidal volume associated with heavy intensity exercise was significantly greater than that during moderate exercise (Moderate: 0.3 vs. Heavy: 1.2 l; $P < 0.001$), no differences were evident in respiratory frequency between exercise intensities. Furthermore, no correlations were evident between the dynamics describing the changes in respiratory, HRV or cardiovascular parameters, suggesting that this did not play a major role in the present findings. Finally, the 20s averaging period for the HRV parameters is not ideal for investigating the dynamic response to a sudden transition in metabolic demand but until superior analysis methods are available it is the optimal solution.

In conclusion, in this first study to simultaneously assess vagal withdrawal, $\dot{Q}$ and $\dot{V}O_2$ kinetics, the findings support a prominent role of oxygen delivery in determining the dynamics of $\dot{V}O_2$ at the onset of heavy, but not moderate, intensity exercise. The lack of association between the parameters describing the $\dot{V}O_2$ slow component and those of $\dot{Q}$ and vagal withdrawal over the same time period provide further evidence against an oxygen delivery-related explanation for this reduction in muscle efficiency. These findings (and their associated confidence levels) support the use of vagal withdrawal kinetics to investigate the

neural basis of oxygen delivery regulation. We suggest that the HRV response to constant workload transitions could also provide a sensitive measure for determining the risk associated with various cardiovascular pathologies.

Perspectives

The rate-limiting determinant of the $\dot{V}O_2$ kinetic response at the onset of exercise continues to be surrounded by lively controversy (Poole & Jones, 2012), with oxygen delivery and oxygen utilisation both proposed as putative mediators. A central, $\dot{Q}$-related delivery limitation might be indirectly regulated by the autonomic nervous system (ANS) due to its chronotropic control of heart rate. Although the potential importance of vagal withdrawal in determining $\dot{V}O_2$ kinetics has previously been acknowledged, this is the first study to assess $\dot{V}O_2$, $\dot{Q}$ and vagal withdrawal simultaneously during exercise. The present study provides additional data using a novel technique to inform the debate surrounding the rate-limiting influences on $\dot{V}O_2$ kinetics, demonstrating in agreement with previous studies that central O_2 delivery is a major determinant of $\dot{V}O_2$ during heavy (but not moderate) intensity exercise. Further empirical evidence of these initial findings will be important if the influence of exercise training on $\dot{V}O_2$ kinetics is to be fully understood, thereby enabling optimisation of training programmes.

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Table 1. Oxygen uptake kinetics during moderate and heavy intensity exercise

	Moderate	Heavy
Baseline $\dot{V}$ O$_2$ (l·min^{-1})	0.65 ± 0.12	0.64 ± 0.12
Primary time delay (s)	8 ± 6	11 ± 7
Primary τ (s) [CI]	22 ± 10 [3 ± 1]	29 ± 10 [3 ± 1]
Primary amplitude (l·min^{-1})	0.43 ± 0.29	1.53 ± 0.61
Gain (ml O$_2$·min^{-1}·W^{-1})	9.24 ± 6.4	9.63 ± 1.3
Slow component amplitude (l·min^{-1})	*N/A*	0.25 ± 0.24
Slow component amplitude (% end exercise $\dot{V}$ O$_2$)	*N/A*	13.9 ± 11.3

Values are mean ± SD. $\dot{V}$ O$_2$, oxygen uptake; τ, time constant; CI, 95% confidence interval associated with the phase II τ. N = 49

Table 2. RR and Q̇ kinetics during moderate and heavy intensity exercise

	Moderate	Heavy
Baseline RR (ms)	775 ± 101	748 ± 103
Baseline HR (beats·min^{-1})	77 ± 10	80 ± 11
RR primary τ (s) [CI]	26 ± 17 [3 ± 2]	31 ± 15 [2 ± 1]
RR primary amplitude (ms)	-86 ± 61	-240 ± 89
HR primary amplitude (beats·min^{-1})	10 ± 3	38 ± 6
Baseline Q̇ (l·min^{-1})	9.7 ± 2.0	10.7 ± 2.1
Q̇ Primary τ (s) [CI]	22 ± 15 [7 ± 3]	27 ± 15 [6 ± 2]
Q̇ Primary amplitude (l·min^{-1})	1.9 ± 1.3	5.0 ± 1.9

Values are mean ± SD. RR, RR interval; τ, time constant; Q̇, cardiac output; CI, 95% confidence interval in the mean. N = 49

Table 3. RMSSDRR and SDRR HRV kinetics during moderate and heavy intensity exercise

	Moderate	**Heavy**
RMSSDRR primary τ (s) [CI]	30 ± 19 [11 ± 4]	26 ± 15 [9 ± 6]
RMSSDRR primary amplitude (ms)	-10.0 ± 8.5	-15.6 ± 10.9
SDRR primary τ (s) [CI]	30 ± 19 [10 ± 4]	44 ± 16 [8 ± 5]
SDRR primary amplitude (l·min⁻¹)	-13.2 ± 7.8	-26.1 ± 14.9

Values are mean ± S.D. RMSSDRR, square root of the mean of the sum of the squares of differences between adjacent RR intervals; SDRR, standard deviation of all RR intervals; τ, time constant; CI, confidence interval associated with the τ.

Figure 1. $\dot{V}O_2$, $\dot{Q}$, RR and RMSSDRR responses to a step increment in work rate from an unloaded baseline to a heavy intensity work rate (30%Δ) in a representative participant are shown in panels A-D, respectively. The solid lines show the mono-exponential model fit to the data; the vertical dotted lines indicate the onset of the increased external work rate. For clarity, the $\dot{V}O_2$, RR and $\dot{Q}$ data are displayed as 5-s bin averages

Figure 2. Relationship between $\dot{V}O_2$ and $\dot{Q}$ τ during a) moderate and b) heavy intensity exercise. Note the marked lack of association during moderate compared to the positive correlation during heavy intensity exercise.

A

B

$VO_2 = (0.50*Q) + 13.41$

$R^2 = 0.47$