O_2 UPTAKE KINETICS AS A DETERMINANT OF EXERCISE TOLERANCE

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

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Abstract

Oxygen uptake ($\dot{V}O_2$) kinetics determine the magnitude of the O_2 deficit and the degree of metabolic perturbation and is considered to be an important determinant of exercise tolerance; however, there is limited empirical evidence to demonstrate that $\dot{V}O_2$ kinetics is a direct determinant of exercise tolerance. The purpose of this thesis was to investigate $\dot{V}O_2$ kinetics as a determinant of exercise tolerance and to consider its potential interaction with the maximum $\dot{V}O_2$ ($\dot{V}O_{2max}$) and the W′ (the curvature constant of the hyperbolic power-duration relationship) in setting the tolerable duration of exercise. Recreationally-active adult humans volunteered to participate in the investigations presented in this thesis. Pulmonary $\dot{V}O_2$ kinetics was assessed on a breath-by-breath basis and exercise tolerance was assessed by a time-to-exhaustion trial, with exhaustion taken as the inability to maintain the required cadence. A period of repeated sprint training (RST) resulted in faster phase II $\dot{V}O_2$ kinetics (Pre: 29 ± 5, Post: 23 ± 5 s), a reduced $\dot{V}O_2$ slow component (Pre: 0.52 ± 0.19, Post: 0.40 ± 0.17 L·min^{-1}), an increased $\dot{V}O_{2max}$ (Pre: 3.06 ± 0.62, Post: 3.29 ± 0.77 L·min^{-1}) and a 53% improvement in severe exercise tolerance. A reduced $\dot{V}O_2$ slow component and enhanced exercise tolerance was also observed following inspiratory muscle training (Pre: 0.60 ± 0.20, Post: 0.53 ± 0.24 L·min^{-1}; Pre: 765 ± 249, Post: 1061 ± 304 s, respectively), L-arginine (ARG) administration (Placebo: 0.76 ± 0.29 L·min^{-1} *vs.* ARG: 0.58 ± 0.23; Placebo: 562 ± 145 s *vs.* ARG: 707 ± 232 s, respectively) and dietary nitrate supplementation administered as nitrate-rich beetroot juice (BR) (Placebo: 0.74 ± 0.24 *vs.* BR: 0.57 ± 0.20 L·min^{-1}; Placebo: 583 ± 145 s vs. BR: 675 ± 203, respectively). However, compared to a control condition without prior exercise, the completion of a prior exercise bout at 70% Δ (70% of the difference between the work rate at the gas exchange threshold [GET] and the work rate at the $\dot{V}O_{2max}$ + the work rate at the GET) with 3 minutes recovery (70-3-80) speeded overall $\dot{V}O_2$ kinetics by 41% (Control: 88 ± 22 s, 70-3-80: 52 ±

13 s), but impaired exercise tolerance by 16% (Control: 437 ± 79 s, 70-3-80: 368 ± 48 s) during a subsequent exercise bout. When the recovery duration was extended to 20 minutes (70-20-80) to allow a more complete replenishment of the W′, overall kinetics was speeded to a lesser extent (by 23%; 70-20-80: 68 ± 19 s) whereas exercise performance was enhanced by 15% (70-20-80: 567 ± 125 s) compared to the control condition. In addition, the faster $\dot{V}_{O_2}$ kinetics observed when exercise was initiated with a fast start (FS; 35 ± 6 s), compared to an even start (ES; 41 ± 10 s) and slow start (SS; 55 ± 14 s) pacing strategy, allowed the achievement of $\dot{V}_{O_{2max}}$ in a 3 minute trial and exercise performance was enhanced. Exercise performance was unaffected in a 6 minute trial with a FS, despite faster $\dot{V}_{O_2}$ kinetics, as the $\dot{V}_{O_{2max}}$ was attained in all the variously paced trials. Therefore, the results of this thesis demonstrate that changes in exercise performance cannot be accounted for, purely, by changes in $\dot{V}_{O_2}$ kinetics. Instead, enhanced exercise performance appears to be contingent on the interaction between the factors underpinning $\dot{V}_{O_2}$ kinetics, the $\dot{V}_{O_{2max}}$ and the W′, in support of the proposed ‘triad model’ of exercise performance.

Table of contents

Chapter 3 General methods

EXPERIMENTAL CHAPTERS

List of Tables

List of Figures

Chapter 9 Dietary nitrate supplementation reduces the O_2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans

Chapter 10 Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans

Symbols and abbreviations

[ ]	concentration
Δ	difference
$\Delta[HHb]/\Delta\dot{V}_{O_2}$	index of O_2 extraction per $\dot{V}_{O_2}$ increment
$\Delta iEMG_{6\text{-}2}$	change in iEMG between 2 and 6 minutes of exercise
%Δ	% difference between GET and $\dot{V}_{O_{2max}}$
^{31}P-MRS	^{31}phosphorous nuclear magnetic resonance spectroscopy
A	exponential response amplitude
ADP	adenosine diphosphate
ARG	L-arginine
ATP	adenosine triphosphate
ATP_{Gly}	estimated ATP turnover rate from anaerobic glycolysis
ATP_{Ox}	estimated ATP turnover rate from oxidative phosphorylation
ATP_{PCr}	estimated ATP turnover rate from phosphocreatine hydrolysis
ATP_{Total}	estimated total ATP turnover rate
BR	beetroot
Ca^{2+}	calcium
CI	confidence interval (e.g., 95% CI; CI_{95})
CK	creatine kinase
CO_2	carbon dioxide
CP	critical power (i.e., asymptote of the power/time hyperbola)
EMG	electromyogram
eNOS	endothelial nitric oxide synthase
ES	even start

ET	endurance training
FS	fast start
GET	gas exchange threshold
H^+	hydrogen ion/proton
Hb	haemoglobin
HbO_2	oxygenated haemoglobin
Hb_{tot}	total haemoglobin
HHb	deoxygenated haemoglobin
HR	heart rate
iEMG	integrated electromyogram (μV·s)
IMT	inspiratory muscle training
iNOS	inducible nitric oxide synthase
K^+	potassium ion
L-NAME	N^G-nitro-L-arginine methyl ester
Mb	myoglobin
MIP	maximal inspiratory pressure
MRT	mean response time (approximated by τ + TD of an exponential)
MVC	maximal voluntary contraction
NAC	N-acetylcysteine
$NaNO_3^-$	sodium nitrate
NIRS	near-infrared spectroscopy
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NO_2^-	nitrite
NO_3^-	nitrate

NOS	nitric oxide synthase
O_2	oxygen
P	power output
PL and PLA	placebo
P/O	oxygen cost of ATP resynthesis
PCr	phosphocreatine (or creatine phosphate)
P_i	inorganic phosphate
ROS	reactive oxygen species
RST	repeated sprint training
SR	sarcoplasmic reticulum
SS	slow start
τ	time constant (time to reach 63% of an exponential response)
TD	exponential response time delay
T_E	time-to-exhaustion
T_{lim}	limit of tolerance
UCP3	uncoupling protein 3
VCl_3	vanadium chloride
$\dot{V}c_{O_2}$	carbon dioxide output
$\dot{V}_E$	pulmonary ventilation (expired)
$\dot{V}_{O_2}$	pulmonary oxygen uptake
$\dot{V}_{O_{2max}}$	maximum oxygen uptake
$\dot{V}_{O_{2peak}}$	peak oxygen uptake
W	watt
W′	curvature constant of the hyperbolic power-duration relationship
WR	work rate

Declaration

The material contained within this thesis is original work conducted and written by the author. The following communications and publications are a direct consequence of this work.

Publications

Bailey SJ, Vanhatalo A, Wilkerson DP, DiMenna FJ, Jones AM (2011). A fast-start pacing strategy speeds O_2 uptake kinetics and improves performance during high-intensity exercise. *Medicine and Science in Sports and Exercise*, **43**, 457-467.

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“There is no such thing as public opinion. There is only published opinion.”

-Winston Churchill

“The noblest pleasure is the joy of understanding.”

-Leonardo da Vinci

“If you want to win anything - a race, your self, your life - you have to go a little berserk.”

-William Shakespeare

Chapter 1 Introduction

Skeletal Muscle Bioenergetics

The energy requirements of the contracting locomotor muscles are met, in entirety, through liberating the energy stored in the adenosine tri-phosphate (ATP) molecule. However, the intramuscular [ATP] (square brackets denote concentration) in human skeletal muscle is limited and is exhausted within a few seconds of muscle contraction. In order to prevent a precipitous fall in muscle [ATP] following the onset of exercise, an immediate and continued ATP resynthesis is obligatory.

In synchrony with the immediate increase in muscle ATP turnover rate, there is an instantaneous decline in muscle [phosphocreatine] ([PCr]) at exercise onset. This reaction provides the requisite chemical energy for ATP resynthesis in the first few seconds (~ 10 s) of intense exercise. During this period, the breakdown metabolites of ATP and PCr accumulate and provide the stimuli for activating the second energy system, termed anaerobic glycolysis. This pathway metabolises glucose into lactate and a hydrogen ion (H^+) with a net yield of 2 ATP molecules per glucose molecule. These anaerobic energy pathways are fuelled by the finite PCr and glycogen reserves and yield metabolites that have been implicated in the process of muscle fatigue (e.g., P_i [inorganic phosphate], H^+; Allen *et al*., 2008).

The aerobic metabolic pathway, on the other hand, can use both carbohydrate and fat as metabolic substrates. This is important given that: 1) the aerobic catabolism of a glucose molecule generates a total of 38 ATP molecules (a 19-fold increase compared to anaerobic glycolysis); and 2) fats are far more energetically rich than are carbohydrates, e.g., metabolism of the free fatty acid, palmitate, yields 129 ATP molecules. Moreover, the by-

products of aerobic metabolism (water and carbon dioxide [CO_2]) are well regulated, which limits the metabolic perturbation associated with this energy pathway.

Oxygen Uptake Kinetics

In order to capitalize on these metabolic advantages, muscle O_2 consumption must increase with rapid response dynamics once exercise has been initiated. However, O_2 uptake ($\dot{V}_{O_2}$) rises in a mono-exponential fashion and does not attain a 'steady-state' until 2-3 min following the onset of moderate-intensity exercise (performed below the gas exchange threshold [GET]; see Figure 1.1). Until the steady-state is attained, there is a difference between the energy requirements of the contracting muscles and the energy provision by oxidative phosphorylation, which is termed the O_2 deficit. This exponential increase in oxidative metabolism not only compromises the potential energy yield within the muscles, but mandates elevated rates of ATP resynthesis from anaerobic metabolism.

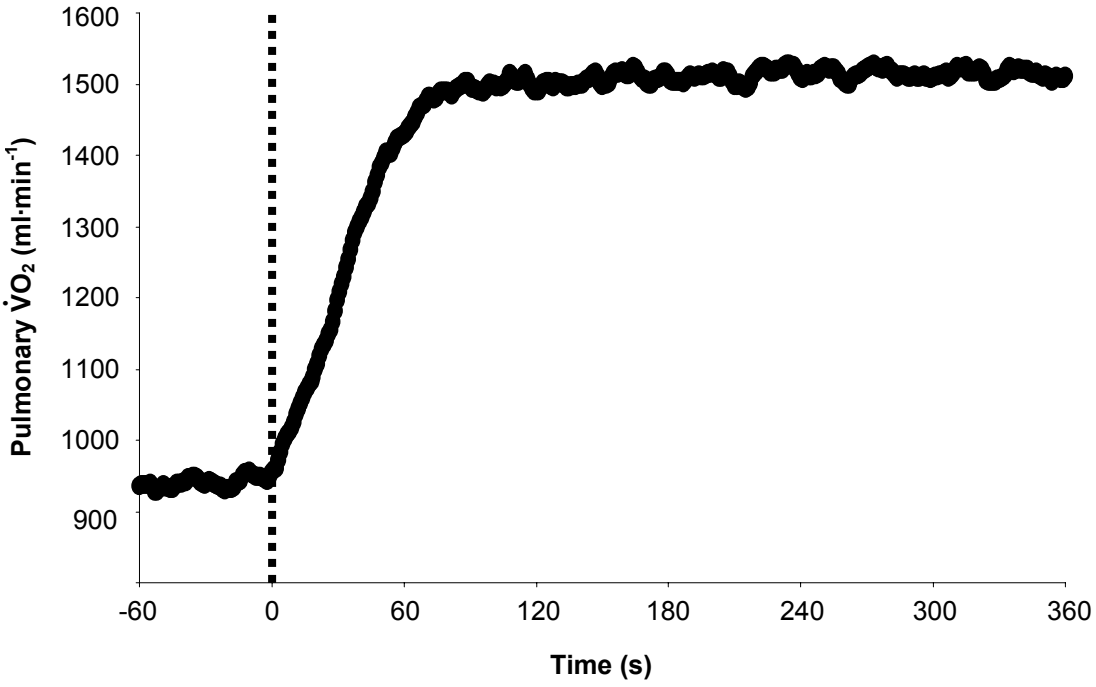

Figure 1.1: A typical pulmonary $\dot{V}O_2$ response during a step increment from an unloaded baseline to a moderate-intensity work rate during cycle ergometry. The dashed vertical line represents the point of work rate imposition and the filled black circles represent the pulmonary $\dot{V}O_2$ data. Note that $\dot{V}O_2$ increases with 'exponential response' dynamics and attains a steady state within approximately 120s.

During exercise performed above the GET, an additional 'slow component' rise in $\dot{V}O_2$ is superimposed upon the fundamental $\dot{V}O_2$ response (the $\dot{V}O_2$ attained upon completion of the phase II $\dot{V}O_2$ response- see '*Human $\dot{V}O_2$ in the Non-Steady State'* in the critical review of literature), which can stabilise during heavy-intensity exercise (performed below the critical power [CP]), or set the $\dot{V}O_2$ on a trajectory leading to the attainment of the maximum $\dot{V}O_2$ ($\dot{V}O_{2max}$) during severe-intensity exercise (performed above CP; see Figure 1.2). Importantly, this $\dot{V}O_2$ slow component is accompanied by a commensurate fall in muscle [PCr] and greater glycogen utilisation and metabolite accumulation (Rossiter *et al*., 2001, 2002; Krustrup *et al*., 2004).

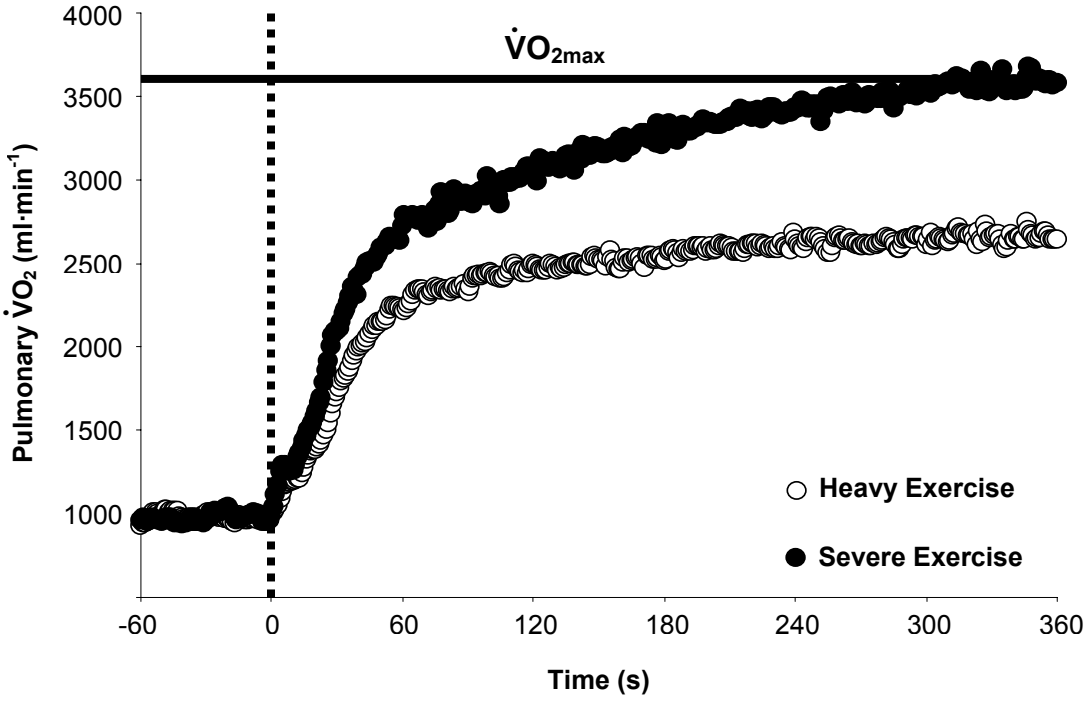

Figure 1.2: A typical pulmonary $\dot{V}_{O_2}$ response during step increments from an unloaded baseline to heavy- and severe-intensity work rates during cycle ergometry. The dashed vertical line represents the point of work rate imposition, the solid horizontal line represents the $\dot{V}_{O_{2max}}$ and the pulmonary $\dot{V}_{O_2}$ data during the heavy and severe work rates are presented as clear circles and filled black circles, respectively. Note that the fundamental $\dot{V}_{O_2}$ response is supplemented by a $\dot{V}_{O_2}$ slow component that emerges after ~ 90-180 s. It is also noteworthy that this $\dot{V}_{O_2}$ slow component asymptotes at a submaximal $\dot{V}_{O_2}$ during heavy exercise but projects to the $\dot{V}_{O_{2max}}$ during severe exercise.

Therefore, faster $\dot{V}_{O_2}$ kinetics and/or a reduced $\dot{V}_{O_2}$ slow component kinetics would be expected to spare the utilisation of the muscles' finite anaerobic reserves and reduce the accumulation of fatigue related metabolites. These changes in $\dot{V}_{O_2}$ kinetics are summarised in Figure 1.3 and have been hypothesised to enhance severe-intensity exercise performance (Burnley and Jones, 2007; Jones and Burnley, 2009). However, the extent to which improved $\dot{V}_{O_2}$ kinetics is performance enhancing appears to be dependent on the interaction of $\dot{V}_{O_2}$ kinetics with the W′ (the curvature constant of the hyperbolic power duration relationship that represents a fixed amount of work that can be performed above the CP) and the $\dot{V}_{O_{2max}}$ (Burnley and Jones, 2007; Jones and Burnley, 2009).

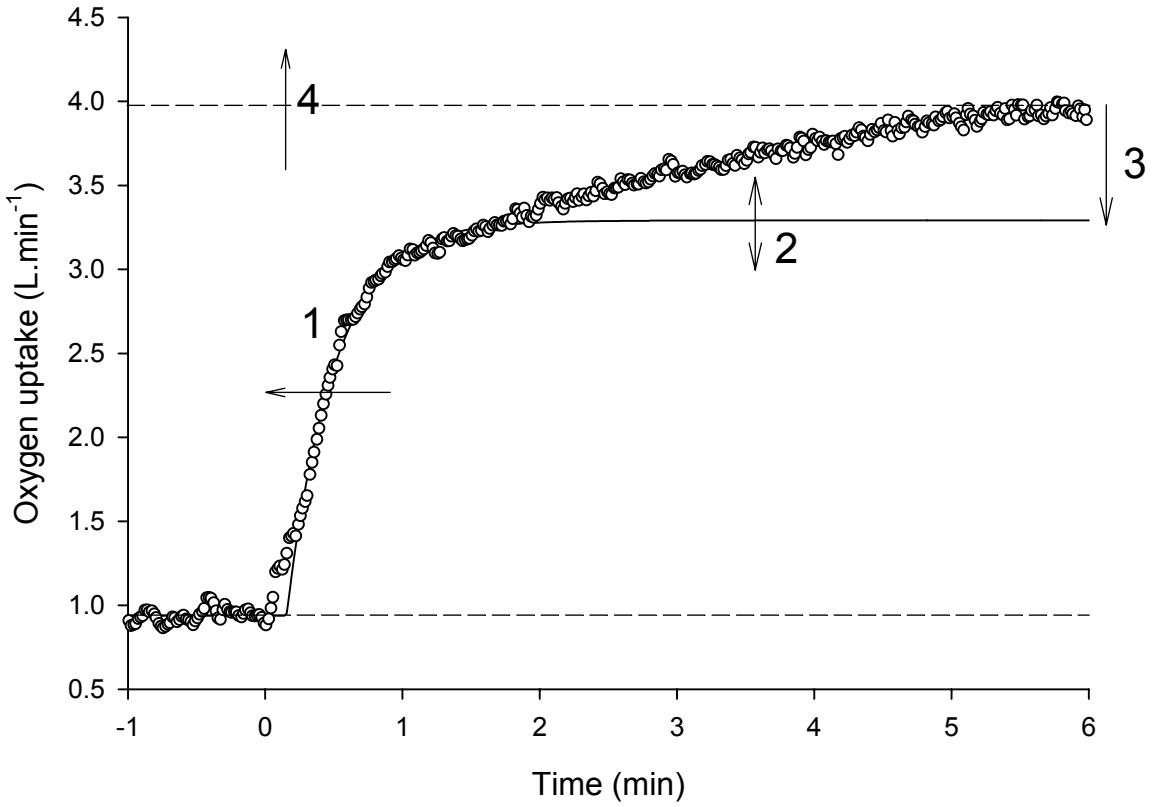

Figure 1.3: A typical pulmonary $\dot{V}_{O_2}$ response during a step increment from an unloaded baseline to a severe-intensity work rate during cycle ergometry. **1** indicates a speeding in phase II $\dot{V}_{O_2}$ kinetics; **2** indicates a change in the fundamental $\dot{V}_{O_2}$ amplitude; **3** indicates a reduction in the $\dot{V}_{O_2}$ slow component; and **4** indicates an increase in the $\dot{V}_{O_{2max}}$. Note that these changes have been hypothesised to improve severe exercise tolerance. Redrawn from Jones and Burnley (2009).

The purpose of this thesis is therefore to use a variety of both physical and nutritional/pharmacological interventions to modulate aspects of $\dot{V}_{O_2}$ kinetics and to investigate how this translates to the ability to tolerate intense exercise. These data will be used to test the theoretical model of Burnley and Jones (2007) and the notion that $\dot{V}_{O_2}$ kinetics is an important determinant of exercise tolerance.

Chapter 2 Review of Literature

Human $\dot{V}O_2$ in the Non-Steady State

Almost a century has elapsed since the first assessment of gas exchange dynamics in the exercising human. In this landmark study, a step increment in work rate was administered and the adjustment of $\dot{V}O_2$ was investigated using the Douglas bag technique (Krogh and Lindhard, 1913). Despite the immediate increase in work rate and presumably, ATP turnover rate, $\dot{V}O_2$ increased with comparatively slow response dynamics (Krogh and Lindhard, 1913). While the Douglas bag method is regarded as the gold standard technique for assessing gas exchange in the steady state, an accurate determination of $\dot{V}O_2$ kinetics in the non- steady state (i.e., following the onset of exercise) requires $\dot{V}O_2$ to be measured with a greater temporal resolution.

When assessed on a breath-by-breath basis, pulmonary $\dot{V}O_2$ displays three distinct phases following the onset of moderate-intensity exercise: phase I, an initial response phase of ~ 19 s consequent to an increase in pulmonary blood flow; phase II, in which $\dot{V}O_2$ increases with approximately exponential response dynamics; and phase III, the steady state (Whipp *et al*., 1982; Figure 1.1). When subjected to the appropriate data analysis procedures, phase II pulmonary $\dot{V}O_2$ kinetics provides an accurate reflection of muscle $\dot{V}O_2$ kinetics (Barstow *et al*., 1990; Grassi *et al*., 1996; Krustrup *et al*., 2009). This is important as the determination of pulmonary $\dot{V}O_2$ kinetics offers a non-invasive alternative for the assessment of muscle oxidative metabolism.

Intensity dependent $\dot{V}O_2$ kinetics

The $\dot{V}O_2$ profile during exercise is dependent on the work rate that is imposed and has been used to categorize the various 'exercise-intensity domains'.

Moderate-Intensity Exercise

During moderate-intensity exercise, which comprises all work rates below the GET, pulmonary $\dot{V}O_2$ rises in a mono-exponential fashion to attain a steady state within 2-3 minutes (Whipp and Wasserman, 1972; Whipp and Mahler, 1980; Figure 1.1). The functional 'gain' of the fundamental amplitude approximates a 10 ml·min^{-1} increase in $\dot{V}O_2$ per Watt increment in work rate (Mallory *et al*., 2002) and upon attainment of the steady state, the rate of ATP resynthesis within the contracting myocytes is in equilibrium with the rate of ATP resynthesis from oxidative phosphorylation. Consequently, there is no significant elevation in blood [lactate] and as such moderate exercise can be tolerated for several hours.

Heavy-Intensity Exercise

The fundamental $\dot{V}O_2$ response is supplemented by an additional $\dot{V}O_2$ slow component at work rates above the GET. This $\dot{V}O_2$ slow component emerges ~ 100-180s into the exercise bout (Figure 1.2) and is accompanied by an elevated blood [lactate]. When the supra-GET work rate is below the CP, this is classified as heavy-intensity exercise. In this intensity domain, the $\dot{V}O_2$ slow component and blood [lactate] will eventually stabilise at submaximal values (Whipp and Wasserman, 1972; Linnarsson, 1974; Whipp and Mahler, 1980; Barstow and Molé, 1991; Paterson and Whipp, 1991; Figure 1.2) and the tolerable duration of exercise is in the range of 2-4 hours.

Severe-Intensity Exercise

Exercise performed above the CP is classified as severe-intensity exercise and in this intensity domain, a steady state in $\dot{V}_{O_2}$ and blood [lactate] is not possible. Indeed, both $\dot{V}_{O_2}$ and blood [lactate] increase inexorably until the maximum values are attained (Åstrand and Saltin, 1961; Wasserman and Whipp, 1975; Poole *et al*., 1988; Gaesser and Poole, 1996; Figure 1.2). As a result, the tolerable duration of severe exercise is < 20 minutes (Poole *et al*., 1988).

Extreme-Intensity Exercise

Whereas severe exercise leads to fatigue owing to the attainment of the $\dot{V}_{O_{2max}}$, extreme-intensity exercise is characterised by fatigue ensuing prior to the attainment of the $\dot{V}_{O_{2max}}$ (Hill *et al*., 2002; Figure 2.1). The tolerable duration of exercise within this intensity domain is typically restricted to <140 s and blood [lactate] may be lower than that observed immediately post severe exercise consequent to the reduced exercise duration (Hill *et al*., 2002).

Therefore, the tolerable duration of exercise appears to be linked to the $\dot{V}_{O_2}$ profile that is exhibited during exercise.

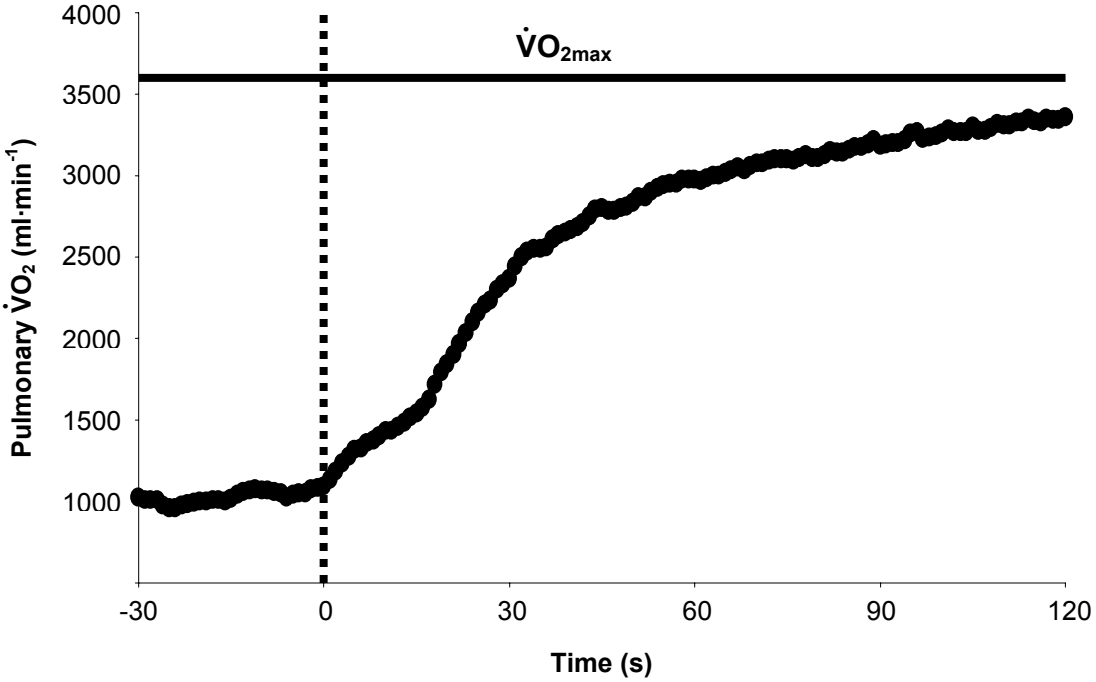

Figure 2.1: A typical pulmonary $\dot{V}_{O_2}$ response during a step increment from an unloaded baseline to an extreme work rate during cycle ergometry. The dashed vertical line represents the point of work rate imposition, the solid horizontal line represents the $\dot{V}_{O_{2max}}$ and the pulmonary $\dot{V}_{O_2}$ data during the extreme work rate is presented as filled black circles. Note that achievement of the $\dot{V}_{O_{2max}}$ is precluded by the rapid development of fatigue.

$\dot{V}_{O_2}$ Kinetics as Determinant of Exercise Tolerance

Investigations into the potential for $\dot{V}_{O_2}$ kinetics to directly influence exercise tolerance are sparse. This section of the literature review will describe the current body of evidence that has investigated the link between $\dot{V}_{O_2}$ kinetics and exercise tolerance and will highlight interventions that have, or would be expected to, modulate $\dot{V}_{O_2}$ kinetics and exercise tolerance.

$\dot{V}O_2$ Kinetics and the O_2 Deficit

Whereas the rate of ATP turnover presumably increases instantaneously at exercise onset, $\dot{V}O_2$ increases with exponential response dynamics (Whipp and Wasserman, 1972; Whipp and Mahler, 1980; Whipp *et al*., 1982). The resulting deficit between the ATP requirements in the contracting myocytes and the ATP derived from oxidative phosphorylation is known as O_2 deficit (Krogh and Lindhard, 1913; Figure 2.2). In order to satisfy the energy demands of the contracting muscles, the energy equivalent of this O_2 deficit must be compensated through an increased rate of ATP turnover through PCr degradation and anaerobic glycolysis, with a small contribution from the muscle O_2 stores (note that only PCr hydrolysis and muscle O_2 stores contribute appreciably during moderate exercise). Therefore reducing the magnitude of the O_2 deficit, by speeding $\dot{V}O_2$ kinetics, would reduce the extent of PCr and glycogen utilisation and the accumulation of fatigue-related metabolites. In turn, this reduced metabolic perturbation would be expected to improve the tolerable duration of exercise (Burnley and Jones, 2007; Jones and Burnley, 2009).

Faster $\dot{V}O_2$ kinetics may be particularly beneficial during severe exercise, where exhaustion ensues shortly after the attainment of the $\dot{V}O_{2max}$. Indeed, faster $\dot{V}O_2$ kinetics may delay the point at which the finite anaerobic substrates and fatigue-inducing metabolites reach 'critical' levels following the attainment of the $\dot{V}O_{2max}$. Similarly, speeding the initial rate of $\dot{V}O_2$ would increase the proportional energy contribution from oxidative phosphorylation during extreme exercise. The associated reduction in metabolic perturbation may allow exercise to be prolonged allowing $\dot{V}O_2$ to continue to increase to or towards the $\dot{V}O_{2max}$, such that the total oxidative energy yield is increased.

It is clear that $\dot{V}O_2$ kinetics is faster in elite athletes compared to club level athletes (Ingham *et al*., 2007; Jones and Koppo, 2005), in moderately trained athletes compared to untrained subjects (Koppo *et al*., 2004) and that $\dot{V}O_2$ kinetics is slowed with ageing (Barstow and Scheuerman, 2005) and in various disease states (Poole *et al*., 2005; Grassi *et al*., 2009). These findings indicate that improved physical fitness is accompanied by faster $\dot{V}O_2$ kinetics, while ageing and pathology are accompanied by a slowing of $\dot{V}O_2$ kinetics and a pronounced reduction in exercise tolerance. Collectively, these findings support the notion that the τ (time constant; time taken to achieve 63% of the end amplitude) for $\dot{V}O_2$ kinetics is an important determinant of exercise tolerance. However, in a recent review, Grassi and colleagues (2011) argue that the τ is not a direct determinant, but a marker of exercise tolerance, at least during moderate-intensity exercise. This supports the notion that $\dot{V}O_2$ kinetics must interact with other physiological parameters to determine exercise tolerance (Burnley and Jones, 2007).

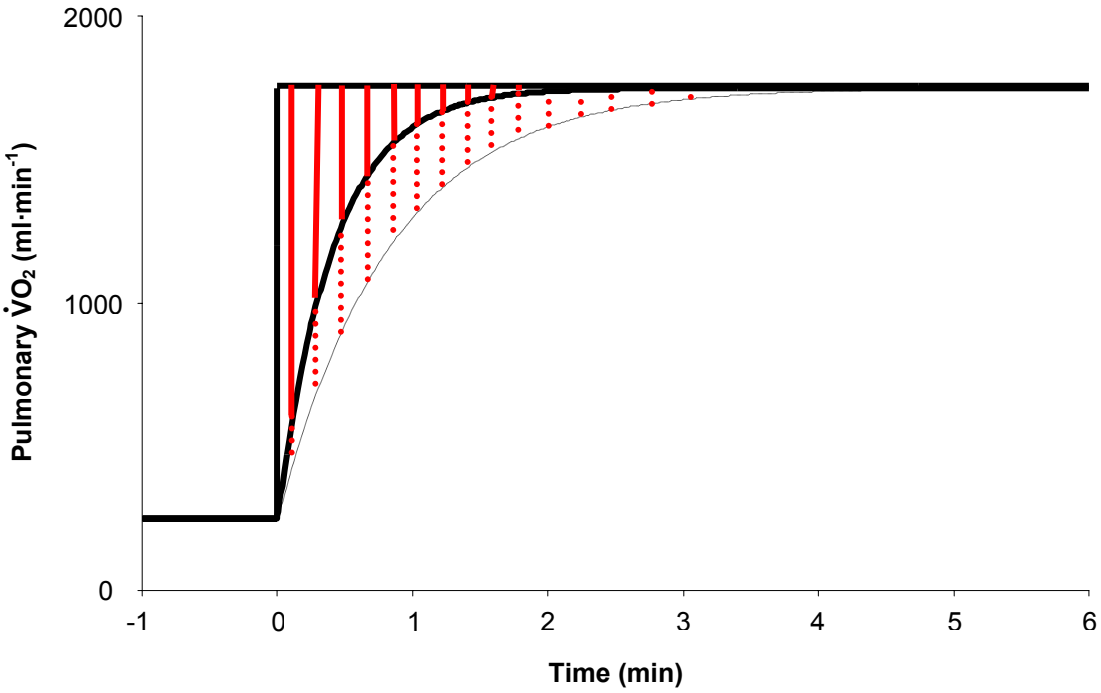

Figure 2.2: Effect of faster $\dot{V}O_2$ kinetics on the magnitude of the O_2 deficit. A slower exponential response (the dashed exponential function) results in a larger O_2 deficit (both the solid and dashed red segments). A faster exponential response (the solid exponential function) results in a smaller O_2 deficit (only the solid red segment).

Influence of the $\dot{V}O_2$ Slow Component

The importance of the $\dot{V}O_2$ slow component in determining the tolerable duration of exercise was established by David Poole and colleagues in 1988. It was demonstrated that when cycle exercise was performed at the CP, the $\dot{V}O_2$ slow component stabilised at a submaximal $\dot{V}O_2$ and all subjects completed the target exercise duration of 24 min, whereas when the imposed work rate was 5% above the CP, the $\dot{V}O_2$ slow component developed with time such that the $\dot{V}O_{2max}$ was attained and the tolerable duration was reduced to ~ 18 min (Poole *et al*., 1988). Further research has established that the $\dot{V}O_2$ slow component is accompanied by a commensurate fall in muscle [PCr] (Rossiter *et al*., 2002) and greater glycogen utilisation (Krustrup *et al*., 2004). In addition, the $\dot{V}O_2$ slow component has important implications for exercise tolerance by determining if/when the $\dot{V}O_{2max}$ will be attained (and therefore the onset of rapid 'anaerobic' substrate depletion and metabolite accumulation) and the speed of the overall $\dot{V}O_2$ kinetics (and therefore the overall O_2 deficit; Burnley and Jones, 2007; Jones and Burnley, 2009; Figure 2.3).

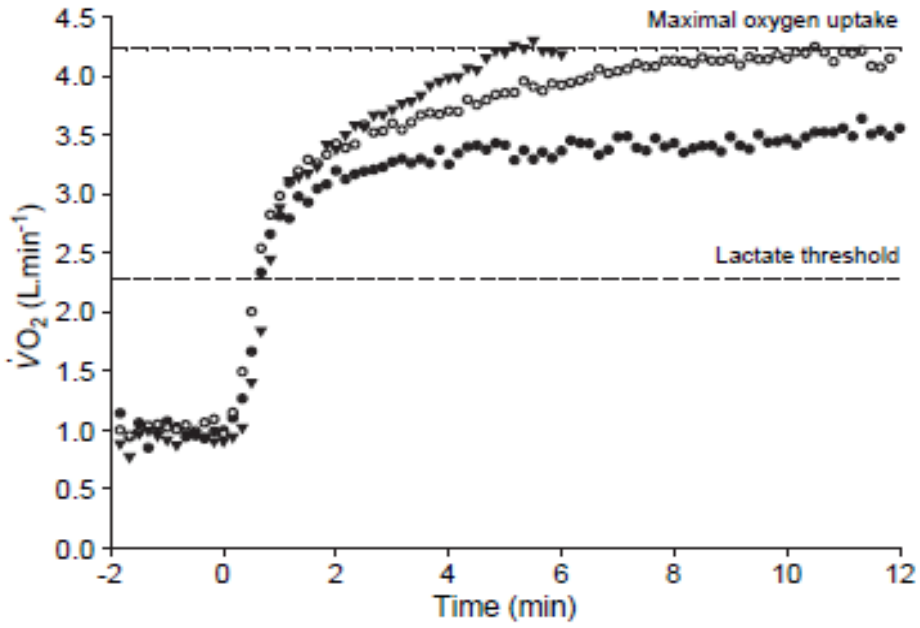

Figure 2.3: The $\dot{V}O_2$ profile during heavy (filled circles) and severe (open circles, filled triangles) exercise. Note that the $\dot{V}O_{2max}$ is not attained during heavy-intensity exercise, but the rapidity with which the $\dot{V}O_{2max}$ is achieved during severe exercise is determined by the trajectory of the $\dot{V}O_2$ slow component. Redrawn from Burnley and Jones (2007).

Influence of the $\dot{V}O_2$ Fundamental Amplitude

Another important component of $\dot{V}O_2$ dynamics that has implications for severe exercise tolerance is the fundamental $\dot{V}O_2$ amplitude. Given that the $\dot{V}O_2$ slow component is constrained by the $\dot{V}O_{2max}$ during severe exercise (Whipp and Wasserman, 1972; Poole *et al*., 1988), increasing the fundamental $\dot{V}O_2$ amplitude would reduce the proportion of the overall $\dot{V}O_2$ response commandeered by the $\dot{V}O_2$ slow component, provided that the $\dot{V}O_{2max}$ is unchanged (Figure 2.4). Moreover, if the proportion of the $\dot{V}O_2$ response consumed by the 'fast' fundamental response is increased, overall $\dot{V}O_2$ kinetics will be faster resulting in an increased energy contribution from oxidative phosphorylation and a corresponding reduction in the O_2 deficit. An increase in the fundamental $\dot{V}O_2$ amplitude therefore has clear implications for severe exercise tolerance.

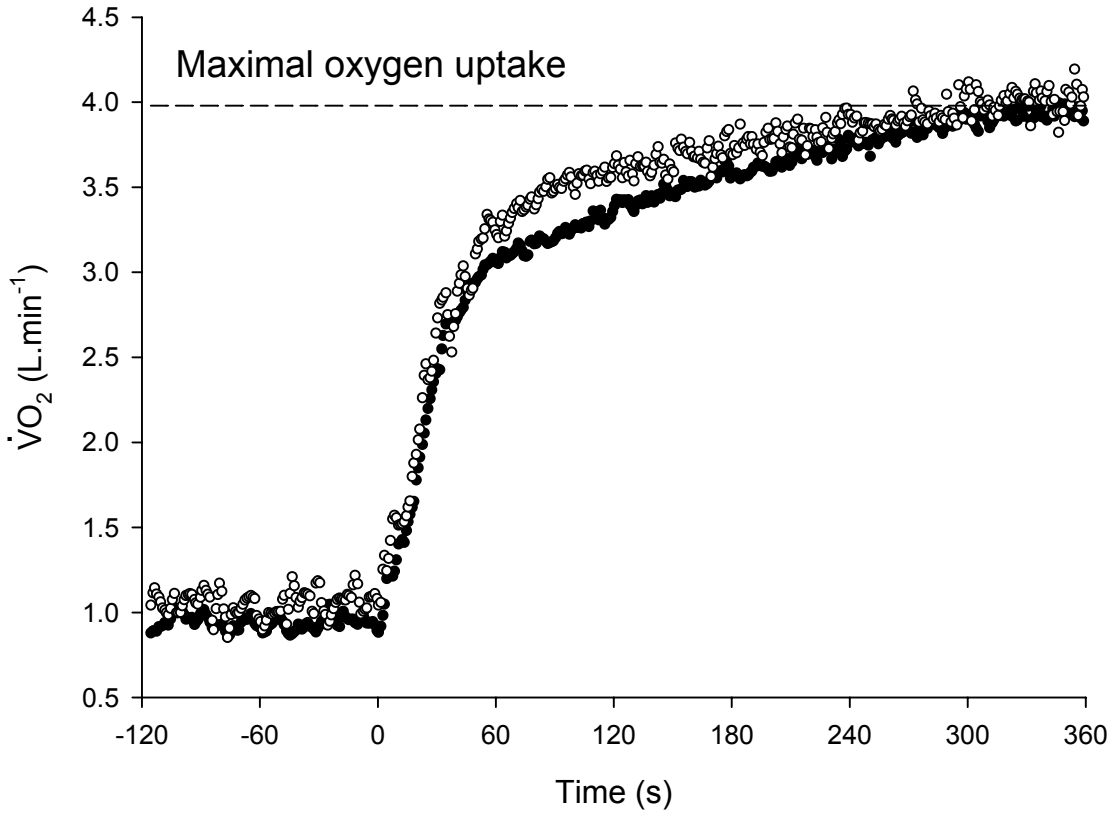

Figure 2.4: The $\dot{V}_{O_2}$ profile during two bouts of severe exercise at the same absolute work rate separated by 20 minutes of passive recovery. Note that compared to the first exercise bout (shown as filled circles), the $\dot{V}_{O_2}$ fundamental amplitude is increased reducing the $\dot{V}_{O_2}$ slow component in the second bout (shown as open circles). Redrawn from Burnley and Jones (2007).

Interaction with other Parameters

These aforementioned improvements in $\dot{V}_{O_2}$ kinetics (summarized in Figure 1.3) would be expected to elicit an improved exercise tolerance. However, in the theoretical model proposed by Burnley and Jones (2007) it was hypothesized that improved $\dot{V}_{O_2}$ kinetics may not determine exercise tolerance in isolation, but by interacting with other physiological parameters (Burnley and Jones, 2007). These parameters, along with their interactive role with $\dot{V}_{O_2}$ kinetics, will now be discussed to create a holistic summary of $\dot{V}_{O_2}$ kinetics as a determinant of exercise tolerance.

Parameters of the Hyperbolic Power-Duration Relationship

The relationship between exercise tolerance and power output takes the form of a rectangular hyperbola (Monod and Scherrer, 1965; Poole *et al*., 1988; Jones *et al*., 2010; Figure 2.5). The CP (the power asymptote of this hyperobla) has been demonstrated to be the highest constant work rate at which the profiles of $\dot{V}O_2$, blood and muscle lactate and H^+ and muscle P_i can stabilise (Poole *et al*., 1988; Jones *et al*., 2008). At work rates above the CP, however, $\dot{V}O_2$, lactate, H^+ and P_i increase to maximum values and the tolerable duration of exercise is < 20 minutes (Poole *et al*., 1988; Jones *et al*., 2008). Unsurprisingly, therefore, the CP has been identified as an important correlate of endurance exercise performance (Kolbe *et al*., 1995). The curvature constant of this power-duration relationship, W′, represents a finite amount of work that can be performed above CP and is related to the potential for ATP yield from substrate-level phosphorylation and/or the accumulation of fatigue-related metabolites (e.g., H^+, $P_{i,}$, extra-cellular potassium [K^+]; Monod and Scherrer, 1965; Moritani *et al*., 1981 Poole *et al*., 1988; Fukuba *et al*., 2003; Jones *et al*., 2008; Figure 2.5).

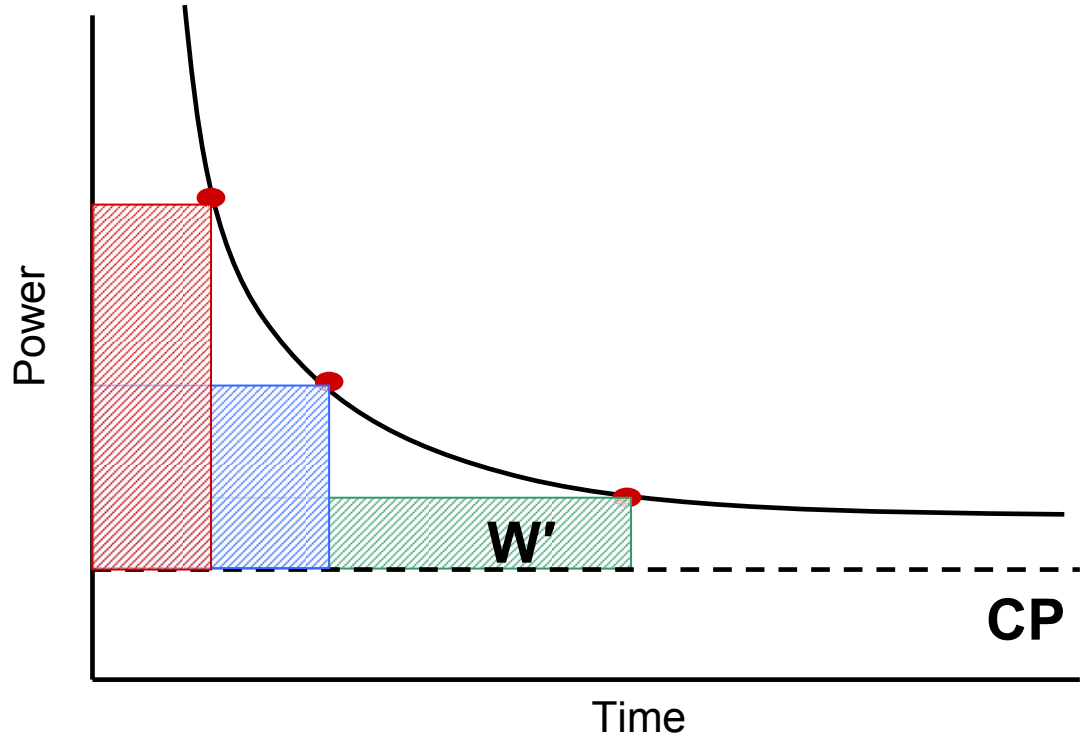

Figure 2.5: Illustration of the hyperbolic power-duration relationship. When time-to-exhaustion during exercise performed above the critical power (CP; severe exercise) is plotted as a function of the imposed power output, time-to-exhaustion increases hyperobolically. The CP is shown as the dashed horizontal line, the filled red circles indicate the time-to-exhaustion at a given power output and the solid line hyperbola characterises the hyperbolic association between these two parameters. The W′ is shown in the dashed green and red rectangles and the blue square. Note that, while its rate of utilisation differs based on the magnitude of the imposed power output, the magnitude of the W′ is identical for all three power outputs and the full expression of the W′ coincides with the termination of exercise.

To improve the tolerance of severe/extreme exercise it is therefore necessary to either increase the W′ or retard the rate at which the W′ is depleted. Speeding phase II or overall $\dot{V}O_2$ kinetics (by increasing the fundamental $\dot{V}O_2$ amplitude and/or reducing the $\dot{V}O_2$ slow component) would delay the depletion of the W′ and increase the tolerable duration of exercise. Any intervention that reduces W′, on the other hand, would be expected to compromise exercise tolerance, unless changes in $\dot{V}O_2$ kinetics could compensate for the

depleted W′. This indicates that the interaction between $\dot{V}_{O_2}$ kinetics and the W′ has clear potential to influence the tolerable duration of exercise (Burnley and Jones, 2007).

Maximum Oxygen Uptake

During severe exercise, the $\dot{V}_{O_{2max}}$ is attained due to the continued slow component rise in $\dot{V}_{O_2}$ (Poole *et al*., 1988). Attainment of the $\dot{V}_{O_{2max}}$ (i.e., the maximal rate of ATP synthesis through oxidative phosphorylation) coincides with a rapid utilisation of the finite anaerobic stores and an inexorable accumulation of fatiguing metabolites. Reducing the $\dot{V}_{O_2}$ slow component will increase the tolerable duration of exercise, in part, through delaying the point at which the $\dot{V}_{O_{2max}}$ is attained (Figure 2.3). Alternatively, modulating the $\dot{V}_{O_{2max}}$ will have implications for exercise tolerance by determining the scope for development of the $\dot{V}_{O_2}$ slow component (Berger *et al*., 2006a; Burnley *et al*., 2006; Burnley and Jones, 2007; Figure 1.3). Thus, the interaction between the $\dot{V}_{O_{2max}}$ and the $\dot{V}_{O_2}$ slow component represents an important determinant of exercise tolerance.

Therefore, a vigorous investigation of $\dot{V}_{O_2}$ kinetics as a determinant of exercise tolerance requires an appreciation of the interaction between $\dot{V}_{O_2}$ kinetics, the W′ and the $\dot{V}_{O_{2max}}$. In this regard, administering interventions to variously alter these parameters would provide an appropriate experimental paradigm to investigate this issue. These interventions and their influence on the physiological ($\dot{V}_{O_2}$ kinetics, W′ and $\dot{V}_{O_{2max}}$) and performance (exercise tolerance) parameters will now be discussed.

Whole Body Exercise Training

Of all the interventions investigated, the greatest adjustments in $\dot{V}O_2$ kinetics have been observed following a period of endurance exercise training (Hickson *et al*., 1978; Hagberg *et al*., 1980; Phillips *et al*., 1995; Womack *et al*., 1995; Carter *et al*., 2000; Saunders *et al*., 2003; Jones and Koppo, 2005; McKay *et al*., 2009). Some studies have observed similar adaptations in $\dot{V}O_2$ kinetics irrespective of whether the training stimulus has been administered as continuous or interval training (Berger *et al*., 2006b; McKay *et al*., 2009). However, Daussin *et al*. (2008) reported that interval training was more effective in accelerating $\dot{V}O_2$ kinetics and enhancing exercise tolerance than was work-matched continuous training.

It has been reported that all-out repeated sprint training (RST) can provoke similar physiological and performance adaptations to those observed following conventional endurance training (Gibala *et* al., 2006; Burgomaster *et al*., 2008). RST has also been demonstrated to increase the W′ (Jenkins and Quigley, 1993) and the $\dot{V}O_{2max}$ (Burgomaster *et al*., 2008), but the influence of RST on $\dot{V}O_2$ kinetics of has yet to be investigated. Therefore, it is unclear if the improved exercise tolerance reported following this training intervention (Burgomaster *et al*., 2005) can be attributed to improved $\dot{V}O_2$ kinetics and its subsequent interaction with the elevated W′ and $\dot{V}O_{2max}$.

Inspiratory Muscle Training

As high-intensity exercise proceeds, the increased ventilatory rate dictates that the respiratory muscles consume ~10-15% of the total $\dot{V}O_2$ (Aaron *et al*., 1992) and up to 14-16% of the cardiac output (Harms *et al*., 1998). Increasing the work of breathing is accompanied by an increased $\dot{V}O_2$ slow component (Carra *et al*., 2003), whereas partially

unloading the respiratory muscles has been reported to reduce the $\dot{V}O_2$ slow component (Cross *et al*., 2010). These data indicate that the extent of respiratory muscle fatigue can have ramifications for aspects of $\dot{V}O_2$ kinetics.

An intervention that has been demonstrated to reduce inspiratory muscle fatigue is a period of inspiratory muscle training (IMT; reviewed in McConnell and Romer, 2004). IMT has been shown to attenuate peripheral fatigue following prior inspiratory muscle fatigue (McConnell and Lomax, 2006) and has often been associated with improved exercise performance (Voliantis *et al*., 2001; Romer *et al*., 2002; McConnell and Romer, 2004; Griffiths and McConnell, 2007). However, to what extent IMT influences $\dot{V}O_2$ kinetics, and the extent to which this IMT-induced improvement in exercise tolerance can be attributed to improved $\dot{V}O_2$ kinetics, is presently unknown. While the overwhelming majority of studies indicate that IMT does not elicit an increase in $\dot{V}O_{2max}$ (reviewed in McConnell and Romer, 2004), the W′ has been reported to increase with IMT (Johnson *et al*., 2007). A physiological corollary of IMT could therefore be enhanced $\dot{V}O_2$ kinetics that interacts with an elevated W′ to determine the tolerable duration of intense exercise.

Prior 'Warm Up' Exercise

In the landmark study of Gerbino *et al*. (1996), it was shown that prior heavy exercise resulted in faster overall $\dot{V}O_2$ kinetics and a reduced O_2 deficit during a heavy-intensity criterion exercise bout. Subsequent research established that prior heavy exercise did not speed phase II $\dot{V}O_2$ kinetics but rather, increased absolute $\dot{V}O_2$ fundamental amplitude (baseline + amplitude) and reduced the $\dot{V}O_2$ slow component (Burnley *et al*., 2000). Therefore, the performance of a supra-GET prior exercise bout profoundly alters the $\dot{V}O_2$ dynamics during a subsequent supra-GET exercise bout.

The extent to which prior exercise facilitates exercise performance is ambiguous (Jones *et al*., 2003a; Wilkerson *et al*., 2004b; Burnley *et al*., 2005; Carter *et al*., 2005; Ferguson *et al*., 2007). This may be due, in the large part, to the diverse combination of prior exercise intensities and recovery durations that have preceded the criterion exercise bouts in these investigations. It appears that supra-CP priming elicits greater adaptations to $\dot{V}_{O_2}$ kinetics than sub-CP priming (Burnley *et al*., 2005; Carter *et al*., 2005) and that the ergogenic efficacy of supra-CP priming is dependent on the recovery duration separating the priming and criterion exercise bouts (Wilkerson *et al*., 2004b; Burnley *et al*., 2005; Carter *et al*., 2005; Ferguson *et al*., 2007; Vanhatalo and Jones, 2009). However, the precise prior exercise regime that is required to optimize $\dot{V}_{O_2}$ kinetics, W′ and exercise tolerance has yet to be elucidated.

Pacing Strategy

It has been appreciated for some time that the total oxidative energy yield is increased when utilising all-out or fast start (FS) pacing strategies (Ariyoshi *et al*., 1979; Bishop *et al*., 2002; Aisbett *et al*., 2009a, b). Faster $\dot{V}_{O_2}$ kinetics, a reduced O_2 deficit and an increased O_2 consumption have also been reported when using a FS strategy compared to an even start (ES) and slow start (SS) strategy (Jones *et al*., 2008). Therefore, the rate at which work is performed at exercise onset has an important regulatory influence on the dynamics of oxidative metabolism.

During continuous athletic events lasting less than 2 min, optimal performance may be achieved by adopting an all-out/FS pacing strategy (e.g., Foster *et al*., 1993, 1994; De Koning *et al*., 1999; Bishop *et al*., 2002; Jones *et al*., 2008). Jones *et al*. (2008) showed

that the percentage improvement in exercise tolerance was correlated with the % speeding in $\dot{V}O_2$ kinetics when FS was compared to ES. These data suggested that the improved exercise performance with a FS strategy may be linked to improved $\dot{V}O_2$ kinetics. However, a concern with this methodology is that the work rate continued to fall with time as the exercise bout proceeded beyond 120 s in the FS condition. Moreover, time-to-exhaustion trials do not adequately reflect the physiological demands or pacing strategy adopted in athletic competition (e.g., St Clair Gibson *et al*., 2001). To what extent the improved $\dot{V}O_2$ kinetics with a FS strategy translates to improved exercise performance during an ecologically valid performance test is presently unclear.

L-arginine

It is well documented that nitric oxide (NO) is synthesised by the NOS family of enzymes. There are three major NOS isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) (Stamler and Meissner, 2001). Both the eNOS and nNOS isoforms are constitutively expressed in skeletal muscle (Kobzik *et al*., 1994) and these enzymes synthesise NO by catalysing the five electron oxidation of L-arginine (Bredt *et al*., 1991). It is clear that the activities of eNOS and nNOS are increased during repeated muscle contractions resulting in a significant increase in NO synthesis during exercise (Balon and Nadler, 1994).

Pharmacological inhibition of the NOS enzymes via L-NAME administration, which would alleviate the inhibitory effect of NO on mitochondrial respiration (Brown and Cooper, 1994), has been shown to result in faster phase II $\dot{V}O_2$ kinetics during moderate (Jones *et al*., 2003b) heavy (Jones *et al*., 2004b) and supra-maximal (Wilkerson *et al*., 2004a) exercise in humans. In addition, the $\dot{V}O_2$ slow component during heavy exercise was increased (Jones

et al., 2004b) and the $\dot{V}_{O2max}$ during ramp incremental exercise was reduced (Jones *et al*., 2004a) following L-NAME administration. These data indicate that NO synthesis has a profound and complex influence on oxidative metabolism.

While the effect of NOS inhibition on the kinetics of $\dot{V}_{O2}$ has been comprehensively assessed following L-NAME administration, the influence of increasing the NOS substrate, L-arginine, on $\dot{V}_{O2}$ kinetics is unclear. Surprisingly, Koppo *et al*. (2009) reported a small (< 2 s), yet significant, speeding in phase II $\dot{V}_{O2}$ kinetics during moderate exercise with L-arginine, a finding which conflicts with the speeding in phase II $\dot{V}_{O2}$ kinetics observed following L-NAME administration (Jones *et al*., 2003b). The influence of L-arginine supplementation on the $\dot{V}_{O2}$ slow component has yet to be examined.

The effects of L-arginine supplementation on exercise performance in healthy humans is controversial (Colombani *et al*., 1999; Stevens *et al*., 2000; Buford *et al*., 2004; Campbell *et al*., 2006; Liu *et al*., 2009). Indeed, improvements in short duration supramaximal exercise tasks following L-arginine supplementation have been reported on some (Buford *et al*., 2004; Campbell *et al*., 2006; Stevens *et al*., 2000), but not other (Liu *et al*., 2009) occasions, while marathon performance is unaffected by L-arginine supplementation (Colombani *et al*., 1999). In young healthy mice, on the other hand, L-arginine supplementation has been shown to increase the $\dot{V}_{O2max}$ with a tendency to increase total treadmill distance covered during an incremental test (Maxwell *et al*., 2001). Therefore, to what extent $\dot{V}_{O2}$ dynamics and exercise tolerance may be influenced by supplementation with the NOS substrate, L-arginine, in humans is unclear.

Dietary Nitrate

Conventionally, NO was considered to be derived, universally, from the NOS enzymes; however, more recent research has identified an alternative NO generating pathway whereby nitrite (NO_2^-) can be reduced to NO (Benjamin *et al*., 1994; Lundberg *et al*., 1994). One intervention that has been employed to stimulate this NO generating pathway is dietary supplementation with pharmacological sodium nitrate ($NaNO_3^-$; Larsen *et al*., 2007, 2010). However, $NaNO_3^-$ is not ethically approved for human consumption in the UK and so an alternative source of nitrate (NO_3^-) is required for experimental testing with humans in this country.

The diet constitutes the main source of NO_3^- in humans, with vegetables accounting for 60-80% of the daily NO_3^- intake in a Western diet (Ysart *et al*., 1999). Therefore, dietary supplementation with NO_3^- -rich vegetables may provide a practical and effective solution to this problem. In a recent investigation, Webb *et al*. (2008) administered NO_3^- -rich beetroot juice and observed an increase in plasma [NO_2^-] and a reduction in blood pressure, which is indicative of an enhanced NO production (Gruetter *et al*., 1979; Ignarro *et al*., 1993; Siani *et al*., 2000; Lauer *et al*., 2001; Kleinbongard *et al*., 2003; Rassaf *et al*., 2007). These findings are consistent with those reported by Larsen *et al*. (2007, 2010) following $NaNO_3^-$ and indicate that supplementation with NO_3^- -rich vegetables can also predispose to an increased NO synthesis. However, the influence of dietary NO_3^- supplementation on the dynamics of $\dot{V}_{O_2}$ and exercise tolerance is unclear.

It was recently reported that 6 days of dietary supplementation with pharmacological $NaNO_3^-$ reduced the O_2 cost of sub-maximal cycling at work rates expected to require 45-80% $\dot{V}_{O_{2max}}$ (Larsen *et al*., 2007). In a follow up investigation, these authors demonstrated

that the $\dot{V}_{O2max}$ was reduced during concurrent arm and leg cycle ergometry with a trend towards an improved exercise tolerance with NO_3^- supplementation (Larsen *et al*., 2010). In both these investigations, $\dot{V}_{O2}$ was determined using the Douglas bag method. As such, it is unclear if this reduced O_2 cost of exercise is consequent to a reduction in the fundamental or slow component $\dot{V}_{O2}$ amplitudes, or both. Also it is not known whether the reduced O_2 cost of exercise reported by Larsen *et al*. (2007) may translate into an enhanced exercise tolerance.

***N*-acetylcysteine**

It has been reported that administration of the pharmacological antioxidant, N-acetylcysteine (NAC), which is capable of scavenging a number of reactive oxygen species (ROS; Aruoma *et al*., 1989; Benrahmoune *et al*., 2000), can improve the tolerable duration of high-intensity exercise (Medved *et al*., 2004; McKenna *et al*., 2006). Moreover, NAC elicits a vasodilatory response (Andrews *et al*., 2001) and may attenuate the deleterious effects of ROS on mitochondrial metabolism (Pruijn *et al*., 1992). The accumulation of ROS can also disrupt sarcoplasmic reticulum (SR) calcium (Ca^{2+}) release channels (Anzai *et al*., 2000), SR Ca^{2+}-ATPase (Klebl *et al*., 1998), actin (Liu et al., 1990) and myosin (Bailey and Perry, 1989). Therefore, NAC has the potential to influence Ca^{2+}-ATPase and actomyosin-ATPase, which are the predominant determinants of myocyte ATP consumption (Barclay *et al*., 2007). Accordingly, inhibiting these ROS-induced metabolic perturbations may modulate aspects of the $\dot{V}_{O2}$ kinetics. It is therefore possible that the improved exercise tolerance that has been noted with NAC could be consequent, at least in part, to improved $\dot{V}_{O2}$ kinetics.

Summary

The $\dot{V}O_2$ response during various exercise intensities has been thoroughly investigated. It is also clear that the kinetics of $\dot{V}O_2$ determines the proportional energetic contribution from aerobic and anaerobic metabolism, and the rate of metabolite accumulation. Enhancing aspects of the dynamic $\dot{V}O_2$ response (i.e., faster phase II $\dot{V}O_2$ kinetics, increased fundamental $\dot{V}O_2$ amplitude, reduced $\dot{V}O_2$ slow component or some combination thereof) may positively influence the utilisation of the muscles' finite anaerobic reserves and the accumulation of fatigue-related metabolites, and subsequently improve exercise tolerance. Despite this strong theoretical basis for linking improved $\dot{V}O_2$ kinetics with an improved exercise tolerance, supportive experimental evidence is lacking. Moreover, the extent to which $\dot{V}O_2$ kinetics influences exercise tolerance appears to be critically depend upon the interaction of the $\dot{V}O_2$ kinetics with other physiological parameters, including the $\dot{V}O_{2max}$ and the W′.

Aims

The overall aim of this thesis is therefore to ascertain if $\dot{V}O_2$ kinetics is indeed an important determinant of exercise tolerance and to investigate how $\dot{V}O_2$ kinetics interacts with the $\dot{V}O_{2max}$ and the W′ to influence exercise tolerance. The specific aims of this thesis are as follows:

1) Compare the influence of worked-matched RST and continuous endurance training on $\dot{V}O_2$ kinetics and severe exercise tolerance.

2) Determine whether the improved exercise tolerance observed following inspiratory muscle training is accompanied by improved $\dot{V}O_2$ kinetics.

3) Establish the optimal combination of prior exercise intensity and recovery duration required to enhance $\dot{V}O_2$ kinetics and severe exercise tolerance.

4) Establish the optimal pacing strategy to enhance $\dot{V}O_2$ kinetics and exercise performance.

5) Investigate the influence of L-arginine supplementation on NO synthesis, $\dot{V}O_2$ kinetics, exercise efficiency and severe exercise tolerance.

6) Investigate the influence of dietary nitrate supplementation on NO synthesis, $\dot{V}O_2$ kinetics, exercise efficiency and severe exercise tolerance.

7) Determine the influence of dietary nitrate supplementation on NO synthesis, muscle metabolism and exercise tolerance.

8) Investigate the influence of NAC supplementation on NO synthesis, $\dot{V}O_2$ kinetics and exercise tolerance.

Hypotheses

This thesis will address the following hypotheses:

1) RST will result in faster phase II $\dot{V}O_2$ kinetics, a greater reduction in the $\dot{V}O_2$ slow component and a greater improvement in exercise tolerance than work-matched continuous endurance training.

2) IMT will improve exercise tolerance during severe and maximal-intensity exercise through reducing the $\dot{V}O_2$ slow component amplitude, whereas $\dot{V}O_2$ kinetics and exercise tolerance will be unaffected by a SHAM training intervention.

3) Exercise tolerance will be optimised following severe-intensity prior exercise with a 20 minute recovery period and this will be accompanied by an increased $\dot{V}O_2$ fundamental amplitude and a reduced $\dot{V}O_2$ slow component, whereas exercise tolerance will be impaired following severe-intensity prior exercise with a 3 minute recovery period in spite of faster overall $\dot{V}O_2$ kinetics.

4) Overall $\dot{V}O_2$ kinetics will be faster and exercise performance will be enhanced during a 3 minute exercise test initiated with a FS pacing strategy compared to ES and SS strategies, whereas exercise performance will be unaffected during a 6 minute exercise test initiated with a fast-start pacing strategy, in spite of faster overall $\dot{V}O_2$ kinetics.

5) Acute L-arginine supplementation will increase indices of NO production, reduce the O_2 cost of moderate-intensity exercise and improve severe-intensity exercise tolerance by reducing the $\dot{V}O_2$ slow component amplitude.

6) Dietary nitrate supplementation will increase indices of NO production, reduce the O_2 cost of moderate-intensity exercise and improve severe-intensity exercise tolerance by reducing the $\dot{V}O_2$ slow component amplitude.

7) Dietary nitrate supplementation will reduce the O_2 cost of low- and high-intensity exercise and improve high-intensity exercise tolerance by reducing the ATP cost of force production and the degree of metabolic perturbation.

8) NAC administration will increase indices of NO production, reduce the O_2 cost of moderate-intensity exercise and improve severe-intensity exercise tolerance by reducing the $\dot{V}_{O_2}$ slow component amplitude.

Chapter 3 General Methods

General Experimental Procedures

The eight experimental Chapters (Chapters 4-11) that comprise this thesis required 680 exercise tests to be conducted along with 72 exercise training sessions and 640 inspiratory muscle training sessions. All of the exercise tests and training sessions were conducted in an air conditioned exercise physiology laboratory at sea level with an ambient temperature of 18-22°C. The procedures employed in each of these experimental Chapters were approved by the University Ethics Committee prior to the commencement of data collection.

Subjects

The subjects who volunteered to participate in these investigations were recruited from the student and staff University community. Subjects were non-smokers who were free from disease and were not currently using dietary supplements. The subjects were recreationally active at the point of recruitment and participated in regular structured exercise and/or competitive sport, although none were elite-level athletes. Subjects were instructed to report to the laboratory at least 3 hours postprandial in a rested state, having completed no strenuous exercise within the previous 24 hours. Subjects were also instructed to avoid alcohol and caffeine for 24 and 6 hours, preceding each exercise test, respectively. Each subject underwent testing at the same time of day (±2 hours) and all subjects were familiarised with the mode(s) of exercise and experimental procedures prior to the initiation of the experimental testing.

Informed Consent

Before agreeing to participate in these investigations, subjects were given an information sheet that provided a detailed description of the experimental procedures they would be subjected to. The potential risks and benefits of participating in each of these investigations was also clarified in the information sheet and subjects were informed that, while their anonymity would be preserved and their data safely stored, the data of the group of subjects investigated may be published in academic journals or presented at national/international conferences. It was also made clear to the participants that they were free to withdraw from the investigation at any point with no disadvantage to themselves. Any additional questions or concerns the subjects had were answered and, provided the subjects were clear and happy with all aspects of the study, they gave their written informed consent to participate.

Health and Safety

All testing procedures adhered to the health and safety guidelines established by the School of Sport and Health Sciences and great care was taken to ensure that the laboratory provided a clean and safe environment that was appropriate for exercise testing of human subjects. Ergometers, trolleys and work surfaces were cleaned using dilute Virkon disinfectant and all respiratory apparatus was similarly disinfected according to manufacturers' recommendations. Experimenters wore disposable latex gloves during blood sampling and all sharps and biohazard materials were disposed of appropriately. A proper 'cool-down' was provided upon completion of the requisite exercise challenge and subjects were allowed and encouraged to drink water *ad librium* upon completion of testing.

Measurement Procedures

Descriptive Data

For all investigations, each subject's stature and mass were measured and these parameters along with age were recorded prior to the initiation of testing. In all experiments that employed cycle ergometry, the peak power output and $\dot{V}_{O_{2max}}$ as well as the power output and $\dot{V}_{O_2}$ at the GET were also determined during the preliminary exercise testing session (as described below).

Cycle Ergometry

In Chapters 4-9 and 11 cycle ergometry was the exercise modality employed to investigate the physiological and performance parameters of interest. All these cycle tests were performed on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) which can administer work rate in various functions. The ergometer functions that were used in the series of experiments that comprise this thesis include the step, proportional and linear work rate forcing functions. The step function allows work rate to be increased or decreased, rapidly ($1000 W \cdot s^{-1}$), from one constant work rate to another in a stepwise manner for a predetermined duration. This work rate forcing function was employed during all the step exercise tests of various exercise intensities. The proportional function allows work rate to increase or decrease linearly as a function of time and this work rate forcing function was employed during the ramp incremental exercise tests and the pacing trials (Chapter 7). Both the step and proportional work rate functions administer the external power output independent of pedal cadence by instantaneously adjusting flywheel resistance via electrical braking. The linear work rate function, on the other hand, is a cadence dependent method of work rate imposition and is given by the following equation:

$$\text{Linear factor} = \text{Power output} \div \text{Cadence}^2 \quad \text{(Eqn. 1)}$$

In this mode the ergometer imposes a fixed work rate such that the attainment of a particular cadence will elicit a known power output. This work rate forcing function was employed during the all-out sprint exercise tests described in Chapter 7. The ergometer was calibrated regularly by a laboratory technician in accordance with the manufacturer's guidelines.

Two-Legged Knee-Extension Ergometer

The exercise tests described in Chapter 10 were conducted in the prone position, with subjects secured to the ergometer bed via Velcro straps at the thigh, buttocks, lower back and middle back to minimise extraneous movement. The custom-designed ergometer consisted of a nylon frame that fitted onto the bed in alignment with the subject's feet and a base unit that was positioned behind the bed. Cuffs with Velcro straps were secured to the subject's feet and ropes were attached to the cuffs. These ropes passed around pulleys housed within the frame to points of attachment on chains that meshed with a cassette of sprockets on the base unit. The sprocket arrangement was such that when a bucket containing non-magnetic weights was attached to each chain it provided a concentric-only resistive load for each leg. This allowed for the performance of rhythmic two-legged knee-extension exercise in a contra-lateral alternating manner over a distance of ~0.22 m. Subjects lifted the weight in accordance with a visual cue at a frequency of $40 \cdot \text{min}^{-1}$.

Pulmonary Gas Exchange

With the exception of the exercise tests that were conducted within the bore of the magnetic resonance scanner, pulmonary gas exchange and ventilation were measured

breath-by-breath during all laboratory exercise tests. For the exercise tests conducted in Chapters 4-6 and 8-9, this analysis was performed using a portable system that consisted of a bidirectional digital transducer that measured inspired and expired airflow and electro-chemical cell and nondispersive (ND) infrared analysers that measured expired O_2 and CO_2 concentrations, respectively (MetaMax 3B, Cortex Biophysik, Leipzig, Germany). However, in Chapters 7, 10 and 11, this analysis was performed using a metabolic cart system that comprised of a bidirectional "TripleV" digital transducer and differential paramagnetic (O_2) and infrared absorption (CO_2) analysers (Jaeger Oxycon Pro, Hoechberg, Germany). Irrespective of the gas analysis system utilised, the gas analysers were calibrated before each test with gases of known concentration and the volume sensor was calibrated using a 3-liter syringe (Hans Rudolph, Kansas City, MO). During all tests subjects wore a nose clip and breathed through a low-dead-space, low-resistance mouthpiece that was connected securely to the transducer. Gas was sampled continuously via a capillary line and $\dot{V}_{O_2}$, carbon dioxide output ($\dot{V}_{CO_2}$) and minute ventilation ($\dot{V}_E$) were displayed breath-by-breath on-line following correction of the delay between the volume and concentration signals for each breath. Following the completion of each test, raw breath-by-breath gas exchange and ventilation data were exported for later analysis.

Inspiratory Muscle Strength

In Chapter 5 it was necessary to determine the strength/force generating capacity of the inspiratory muscles. This was assessed by the performance of maximum inspiratory pressure (MIP) manoeuvres in a standing position using a hand-held mouth pressure meter (MPM, Micro Medical Ltd, Kent, UK). Each MIP was initiated at residual volume and subjects wore a nose clip during the inspiratory manoeuvre. A minimum of five well

executed MIP measurements were conducted and the highest of three measurements within 5 cmH_2O difference was defined as maximum (Volianitis *et al*., 2001).

Heart Rate

During all exercise tests except for those conducted within the magnetic resonance scanner, heart rate (HR) was measured using short-range telemetry (Polar S610, Polar Electro Oy, Kempele, Finland). For the tests in Chapters 4-6 and 8-9, HR was recorded during the duration of each breath via the portable gas exchange analysis system that was used (MetaMax 3B, Cortex Biophysik, Leipzig, Germany). For the tests in Chapters 7, 10 and 11, 5-s average value for HR were recorded via a HR monitor (Polar Electro Oy, Kempele, Finland). After all tests, raw HR data were exported for later analysis.

Blood Pressure

In Chapters 8-10 the blood pressure of the brachial artery was measured with subjects in a rested, seated position prior to each exercise bout using an automated sphygmomanometer (Dinamap Pro, GE Medical Systems, Tampa, USA). Following 10 min of rest, four measurements were taken with the mean of the measurements being recorded.

Electromyography

During the exercise tests conducted in Chapters 6 and 10, the surface electromyography (EMG) of the *m. vastus lateralis* of the left and right leg, respectively, was measured to assess the gross neuromuscular activity and infer muscle activation during exercise. For these measurements, the leg was initially shaved and cleaned with alcohol around the belly of the muscle and graphite snap electrodes (Unilect 40713, Unomedical, Stonehouse, Great Britain) were adhered to the prepared area in a bipolar arrangement (interelectrode

distance, 40 mm) with ground electrodes positioned on nearby tissue. Elastic bandages were used to secure electrodes and wires in place and pen marks were made around electrodes to enable precise placement reproduction on subsequent tests. The EMG signal was recorded at 1000 Hz using a ME3000PB Muscle Tester (Mega Electronics Ltd, Finland), the bipolar signal was amplified (amplifier input impedance > 1 M Ω) and data were collected online in raw form and stored on a computer using MegaWin software (Mega Electronics Ltd, Finland).

Near-infrared Spectroscopy

During the exercise tests conducted in Chapters 4 and 6-8, a commercially-available near-infrared spectroscopy (NIRS) system (model NIRO 300, Hamamatsu Photonics KK, Hiugashi-ku, Japan) was used to assess the oxygenation status of the *m. vastus lateralis* of the right leg. NIRS provides a non-invasive method by which the concentration changes in oxygenated and deoxygenated haemoglobin [Hb+Mb] can be assessed based upon changes in near-infrared light absorption by the tissue under interrogation. The system consists of an emission probe that irradiates laser beams and a detection probe positioned several centimetres from the emission probe in an optically-dense rubber holder. Four different wavelength laser diodes provide the light source (776, 826, 845 and 905 nm) and the light returning from the tissue is detected by a photomultiplier tube in the spectrometer. For these measurements, the leg was initially shaved and cleaned with alcohol around the belly of the muscle and the probes were placed in the holder, which was secured to the skin with adhesive. Elastic bandages were used to secure the holder and wires in place and pen marks were made around the holder to enable precise placement reproduction in subsequent tests. Prior to the commencement of exercise, baseline values were established with the subject at rest with leg extended at downstroke in a seated position for upright

cycling. All subsequent concentration changes of oxygenated and deoxygenated [Hb+Mb] relative to this baseline were estimated via the intensity of incident and transmitted light (recorded continuously at 2 Hz throughout exercise) and these values were averaged into one-second bins. Following each test, raw NIRS data were exported for later analysis.

Blood Lactate Concentration

During one series of step tests (see below) for each experimental condition, a fingertip blood sample was obtained to determine the whole blood [lactate]. Prior to drawing the initial sample for the exercise bout, the sampling site was cleaned thoroughly with alcohol and a disposable safety lancet (Safety-Lanzette, Sarstedt) was used to puncture the skin. For all samples that were subsequently drawn from this puncture, initial drops of blood were wiped away and ~ 20-25 μL of free-flowing arterialised blood was collected into a heparinised microvette (Microvette CB 300, Sarstedt) and analysed using an automated blood lactate analyser (YSI 1500, Yellow Springs Instruments, Yellow Springs, OH, United States). The analyser was calibrated regularly by a laboratory technician in accordance with the manufacturer's guidelines. In all cases, blood was sampled ~20 seconds prior to a step increment in work rate (for determination of the baseline blood [lactate]), six minutes into the constant work rate bouts (Chapters 4-6, 8 and 10-11) and at the cessation of exercise during the exhaustive exercise bouts (for determination of the end-exercise blood [lactate]. Blood lactate accumulation (Δblood [lactate]) over the first six minutes of the exercise bout and up to exhaustion was determined by subtracting the respective blood [lactate] from the baseline blood [lactate]. Blood [lactate] was determined at different time points to those described above in Chapters 7 and 9, but the data were collected and analysed using the same procedures described above (see the specific Chapter for further information of the blood sampling times).

Plasma Potassium Concentration

During the exercise protocol employed in Chapter 11 venous blood samples were obtained through a 20 gauge intravenous cannula (BD Venflon, Becton Dickinson, Helsingborg, Sweden), which was inserted into an antecubital forearm vein under local anaesthesia (Marcain 0.5%, Astrazeneca, Cheshire). The cannula was kept patent with an infusion of 0.9% saline at 10 $ml \cdot h^{-1}$ using a syringe driver (Graseby 3200 Syringe Pump, Graseby Medical, Watford). Blood was drawn into 2ml lithium-heparin tubes and was centrifuged, within 30 s, at 4000 rpm and 4°C for 10 min. A 250μL plasma aliquot was immediately analysed for determination of the plasma $[K^+]$ using an automated ion-selective electrode analyzer (9180 Electrolyte Analyzer, Roche Diagnostics, Mannheim, Germany). The analyser was calibrated regularly by a laboratory technician in accordance with the manufacturer's guidelines. In Chapter 11, blood was sampled at rest, following the loading infusion phase, 20 minutes into the maintenance infusion phase, ~20 s prior to the step increment in work rate, six minutes into the constant work rate bouts, and at the cessation of exercise during the exhaustive exercise bouts for determination of the plasma $[K^+]$. Plasma $[K^+]$ accumulation (Δ plasma $[K^+]$) over the first six minutes of the exercise bout and up to exhaustion was determined by subtracting the respective plasma $[K^+]$ from the baseline plasma $[K^+]$.

Plasma Nitrite Concentration

In Chapters 8-10 venous blood samples were obtained from the antecubital fossa by venupuncture, while in Chapter 11 venous blood samples were obtained from an antecubital forearm vein through a 20 gauge intravenous cannula. These venous blood samples were drawn into lithium-heparin tubes at the designated sample point and centrifuged at 4000 rpm and 4°C for 10 min, within 3 min of collection. Plasma was

subsequently extracted and immediately frozen at -80°C, for later analysis of [NO_2^-] via chemiluminescence. Prior to analysis, all glass wear, utensils and surfaces were rinsed with deionized water to remove residual NO_2^-. After thawing at room temperature, plasma samples were deproteinised prior to analysis. Initially, 100 µl of sample was placed in a microcentrifuge tube along with 200 µl of deionised H_2O and 300 µl 0.3 N NaOH, and left to stand at room temperature for 5 min. Then, 300 µl of 5% by weight aqueous $ZnSO_4$ was added to the mixture, after which the sample was vortexed and left to stand at room temperature for a further 10 min. Thereafter, samples were centrifuged at 4000 rpm for 15 min and the supernatant was removed for subsequent analysis. The [NO_2^-] of the deproteinised plasma samples was determined by its reduction to NO in the presence of 5ml glacial acetic acid and 1% NaI under nitrogen at room temperature in a gas-sealed purging vessel. Samples were introduced to the vessel via injection into the septum at the top of the vessel.

The NO content was quantified by a chemiluminescence nitric oxide analyzer (Sievers, 280i nitric oxide analyzer, CO, USA). The reaction of NO with ozone in the chemiluminescent reaction chamber yielded electronically excited NO_2 (nitrogen dioxide) which emits light at the infra-red region of the electromagnetic spectrum. Ozone was generated from an O_2 supply via an electrostatic ozone generator and high voltage transformer. To minimise the interference of the chemiluminescent reactions with sulfur-containing compounds an optical filter transmitted only red wavelengths (>600 nm), since the light emitted by sulfur-containing compounds is of shorter wavelengths. The intensity of the filtered infra-red light was quantified by a red-sensitive photomultiplier tube and amplified producing an analog mV output signal. The [NO_2^-] was derived from the integral

of the NO-generated mV signal over time compared to those obtained for $NaNO_2^-$ standards.

Nitrate Determination

In Chapters 8 and 9 the NO_3^- content of the administered beetroot juice was determined using ozone based chemiluminescence. For this analysis, 100 µl of beetroot juice was diluted (1:1000) with deionized water and the concentration of NO_3^- was determined by its reduction to NO in the presence of vanadium chloride (VCl_3) at 90° C in a chemiluminescence NO analyzer.

Free Plasma Sulfhydryl Groups

Plasma free sulfhydryl groups were determined in Chapter 11 using the method of Ellman (1959) as later modified by Hu *et al*. (1993). Briefly, 1mL of buffer containing $0.1 mol \cdot L^{-1}$ Tris, $10 mmol \cdot L^{-1}$ EDTA, pH 8.2 and 50µL plasma were added to cuvettes, followed by 50µL $10 mmol \cdot L^{-1}$ DTNB in methanol. Sample absorbance was measured at 412 nm using a spectrophotometer (Jenway 6310 visible spectrophotometer, Jenway, Essex, UK) following a 15 min incubation period at room temperature. Blanks were run for each sample, with no DTNB in the methanol, and for the reagents and these absorbencies were subtracted from the absorbance of each sample. The concentration of free sulfhydryl groups was subsequently determined using the TNB molar extinction coefficient of 14,100 M^{-1} cm^{-1}, yielding results in µM (Riddles *et al*., 1979). Total plasma free sulfhydryl groups are presented herein as micromolar per gram protein per litre ($\mu M \cdot g^{-1}$).

Total Plasma Protein

In Chapter 11 the total plasma solubilised [protein] was determined using a microplate protein assay kit (Bio-Rad, München, Germany) using the method of Bradford (1976). Briefly, 160μL of each sample was added to separate micotiter plate wells followed by 40μL of dye reagent concentrate. The sample and reagent were subsequently mixed for 10 min using a microplate mixer and incubated at room temperature for a further 10 min prior to analysis. Thereafter, sample absorbance at 595 nm was measured using a microplate reader (Dynex MRX microplate reader, Dynex Technologies, Chantilly, VA, USA).

Exercise Tolerance/Performance

In Chapters 4-6, 8-9 and 11 exercise tolerance was assessed by the time to the limit of tolerance (T_{lim}) during constant work rate cycle exercise at intensities ranging from 60% Δ (the difference between the power output at the $\dot{V}O_{2max}$ and the power output at the GET) to the $\dot{V}O_{2max}$. During these tests T_{lim} was defined as the point at which the participant's cadence (a self selected value between 70-90 rev·min^{-1} that was to be held constant during the exercise) dropped by more than 10 rpm despite strong verbal encouragement to continue. Exercise tolerance was determined during two-legged knee- extension exercise in Chapter 10 at an intensity corresponding to 35% of the maximum voluntary contraction (MVC). In this investigation T_{lim} was taken as the time at which the participants were no longer able to keep pace with the required contraction frequency (40 repetitions/min). However, in Chapter 7 exercise performance was measured by assessing the peak and average power attained during an all-out sprint during the last minute of the exercise protocol. Again strong verbal encouragement was provided throughout the test.

^{31}Phosphorous Magnetic Resonance Spectroscopy

Intramuscular metabolic responses to exercise were measured *in vivo* by calibrated ^{31}Phosphorous Magnetic Resonance Spectroscopy (^{31}P-MRS) in Chapter 10. During these tests, subjects were positioned in the prone body position within the bore of a 1.5-T superconducting MR scanner. Before the exercise protocol commenced, absolute baseline concentrations of metabolites were established via a technique similar to that described by Kemp *et al.* (2007) using a 6-cm ^{31}P transmit/receive surface coil. Firstly, spatially localised spectroscopy was undertaken to determine the relative signal intensities obtained from a phosphoric acid source within the scanner bed and inorganic phosphate (Pi) from the subject's right quadriceps muscle which was centred over the coil. A subsequent scan was obtained comparing the signals obtained from the phosphoric acid standard and an external Pi solution, where the localised voxel sampled within the external solution were of the same dimensions and distance from the coil as the muscle, allowing the calculation of muscle Pi concentration following corrections for relative coil loading. Absolute values of PCr and ATP concentrations were subsequently calculated via the ratio of Pi:PCr and Pi:ATP.

For the exercise protocol, the knee-extension rate was set in unison with the magnetic pulse sequence to ensure the quadriceps muscles were positioned in approximately the same phase of contraction during each MR pulse acquisition. Initially, fast field echo images were acquired to determine whether the muscle was positioned correctly relative to the coil. This was aided by placing cod liver oil capsules, which yield high-intensity signal points within the image, adjacent to the coil, allowing its orientation relative to the muscle volume under examination to be assessed. A number of pre-acquisition steps were carried out to optimise the signal from the muscle under investigation. Matching and tuning of the coil

was performed and an automatic shimming protocol was then undertaken within a volume that defined the quadriceps muscle. Before and during exercise, data were acquired every 1.5 s, with a spectral width of 1,500 Hz and 1K data points. Phase cycling with four phase cycles was employed, leading to a spectrum being acquired every 6 s. These spectra were analysed via peak fitting at a later date.

Testing Procedures

Preliminary Exercise Testing

In all studies, the first visit to the laboratory was used for the completion of an incremental test. For investigations that assessed cycle ergometer exercise, preliminary testing involved the performance of 'ramp' incremental cycling test to the limit of tolerance. These incremental tests consisted of a three minute period of baseline pedalling at 0 W, followed by a continuous linear (ramp) increase in work rate of 25 $W \cdot min^{-1}$ (Chapter 4 and for females only in Chapter 5) or 30 $W \cdot min^{-1}$ (Chapters 6-8, 9 and 11 and for males only in Chapter 5) until the subject was unable to continue. The ramp test was terminated when a drop of > 10 $rev \cdot min^{-1}$ below the prescribed cadence was observed and strong verbal encouragement was provided throughout the test. Saddle and handlebar heights were recorded following the ramp test and the same settings were reproduced on all subsequent tests. Pulmonary gas exchange and heart rate were measured throughout these incremental tests.

In Chapter 10, where two-legged knee-extension exercise was performed, the participants performed a step incremental test during their initial laboratory visit. This incremental test consisted of two-minute stages with load progressed according to subject perceived exertion such that six stages would be completed prior to exhaustion. The termination

criterion was the inability to maintain the prescribed cadence. During these incremental tests the surface EMG of the *m. vastus lateralis* of the right leg was measured to infer muscle activation during exercise.

In Chapter 7 an additional preliminary test was conducted to establish the hyperbolic power-duration relationship. The W′ and CP parameters that characterise this relationship were quantified using a 3 min all-out critical power test (Vanhatalo *et al*., 2007). Prior to the test, subjects performed a 5 min warm-up at 90% GET, followed by 5 min of rest. The test then began with 3 min of unloaded baseline pedalling, followed by a 3 min all-out effort against a fixed resistance. Subjects were asked to accelerate to 110-120 rpm over the last 5 s of the baseline period. The resistance on the pedals during the 3 min all-out effort was set for each individual using the linear mode of the Lode ergometer so that the subject would attain the power output calculated to be 50%Δ (the work rate that typically coincides with the CP; Smith and Jones, 2001; Pringle and Jones, 2002) on reaching their preferred cadence (linear factor = power/preferred cadence^2). Strong verbal encouragement was provided throughout the test, but subjects were not informed of the elapsed time in order to prevent pacing. To ensure an all-out effort, subjects were instructed to attain their peak power output as quickly as possible from the start of the test and to maintain the cadence as high as possible at all times throughout the 3 min. The CP was estimated as the mean power output over the final 30 s of the test and W′ as the power-time integral above CP.

Determination of $\dot{V}_{O2peak}$ and Gas Exchange Threshold

From the preliminary ramp incremental cycling tests breath-by-breath $\dot{V}_{O2}$ data were averaged into 10-s bins and $\dot{V}_{O2peak}$ was defined as the highest 30-s rolling average value. GET was estimated from the 10 s average gas exchange data using a cluster of measures

including: 1) the first disproportionate increase in $\dot{V}_{CO_2}$ from visual inspection of individual plots of $\dot{V}_{CO_2}$ *v.* $\dot{V}_{O_2}$ (Beaver *et al.*, 1986); 2) an increase in $\dot{V}_E/\dot{V}_{O_2}$ with no increase in $\dot{V}_E/\dot{V}_{CO_2}$; 3) an increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension.

Experimental Exercise Testing

For the investigations conducted in Chapters 4-6 and 8-11, constant-load exercise tests were used to assess pulmonary $\dot{V}_{O_2}$ on-kinetics. These tests involved an abrupt transition from a lower to higher work rate on the ergometer used for the study (cycle or knee extension; see above). For Chapters 4-6, 8-9 and 11, constant-load tests were performed at work rates calculated with respect to the GET and peak work rates attained in the ramp incremental tests. However, an important consideration when prescribing work rate based on pulmonary gas exchange data from an incremental test is that the $\dot{V}_{O_2}$ mean response time must be corrected for, which was assumed to approximate two-thirds of the ramp rate during incremental exercise (Whipp *et al.*, 1981). Accordingly, the GET and peak work rates used to calculate exercise intensity in Chapters 4-6, 8-9, and 11 reflect work rates that are 20 W less than the work rates that coincided with the appearance of GET and peak $\dot{V}_{O_2}$ during the preliminary incremental test. For Chapter 10, constant-load tests were initiated from a resting baseline and were performed at work rates calculated according to the average iEMG measured during the stages of the preliminary step incremental test.

The experimental testing administered in Chapter 7 subjected the participants to a variety of work rate forcing functions in the same exercise test (Figure 7.1). This involved an initial step to a work rate that then either declined or inclined linearly over the first half of the trial followed by a step increment or decrement, respectively, to a work rate that was held constant up to the final minute of the test. On another occasion, participants completed

a step increment to a constant work rate that was held constant up to the final minute of the test. In all cases participants completed an all-out sprint in the final minute of the test with the ergometer in linear mode. The work rates that were imposed in these tests were determined by using the parameters derived from the 3 min all-out test (as described below).

Calculation of Work Rates for Constant-Load Tests

The moderate-intensity work rates imposed in Chapters 4-6, 8-9 and 11, were calculated as a percentage of the work rate at GET (90% for the testing in Chapter 4; 80% for the testing in Chapters 5-6, 8-9 and 11). For Chapters 4-6, 8-9 and 11, severe-intensity work rates were calculated as a percentage of 'delta' (Δ) (80% Δ for the testing in Chapter 6; 70% Δ for the testing in Chapters 4, 8 and 9; 65%Δ for the testing in Chapter 11; 60% Δ for the testing in Chapter 5) where Δ represents the difference between the work rate at GET and the work rate at $\dot{V}_{O2peak}$, which is subsequently added to the work rate at the GET to derive the required work rate.

Exercise intensity was prescribed using the procedures described by DiMenna *et al*. (2010) during the step tests performed in Chapter 10. Firstly, participants completed three MVCs prior to the onset of the incremental test in order to establish the peak iEMG attained during the three MVCs. Thereafter, the iEMG response for each of the two-minute stages of the preliminary incremental test, which was defined as the average from 15-75 s, was normalised to the iEMG observed during an MVC. Work rates corresponding to ~15 and ~35% of the iEMG MVC were imposed as the low- and high-intensity knee-extension work rates, respectively, as these procedures have been shown to elicit $\dot{V}_{O2}$ responses that

are characteristic of low- and high-intensity knee-extension exercise (DiMenna *et al*., 2010).

Calculation of Work Rates for Variable Work Rate Tests

In Chapter 7, the work rate which would be expected to lead to exhaustion in 3 min (3-tlim-WR) and 6 min (6-tlim-WR) was calculated from the parameters determined in the 3 min all-out test using the equation:

$$P = (W'/T_E) + CP \qquad \text{(Eqn. 2)}$$

Where P is the target work rate, T_E is the time-to-exhaustion, CP is the critical power and the W′ is the finite work capacity >CP in Joules. For example, the work rate estimated to elicit a time-to-exhaustion of 180 s in a subject with W′ of 20000 J and CP of 250 W would be: (20000/180) + 250 = 361 W.

Data Analysis Procedures

Gas Exchange Data

Enhancing Signal-to-Noise Ratio

Upon inspection of the 'raw' pulmonary $\dot{V}_{O_2}$ response, it is clear that there is appreciable 'noise' in the $\dot{V}_{O_2}$ signal; that is pulmonary $\dot{V}_{O_2}$ displays a sporadic breath-by-breath variability which can make the underlying response characteristics difficult to discern. However, despite considerable noise, the pulmonary $\dot{V}_{O_2}$ response during exercise is used to draw mechanistic inferences from the $\dot{V}_{O_2}$ response within the contracting skeletal muscle. The important question is, therefore, whether this is a valid practise. Importantly, it has been demonstrated that, provided the pulmonary $\dot{V}_{O_2}$ data undergoes a number of

preliminary data editing procedures prior to analysis, the dynamics of pulmonary $\dot{V}O_2$ provide are closely matched to the dynamics of $\dot{V}O_2$ in the contracting skeletal muscles, to within 10% (Barstow *et al*., 1990; Poole *et al*., 1991 Grassi *et al*., 1996; Krustrup *et al*., 2009). It is therefore imperative that these preliminary data editing procedures are imposed prior to analysis to improve the signal-to-noise ratio of the pulmonary $\dot{V}O_2$ response.

In this regard, the first step that was undertaken was to perform multiple step tests, at the same exercise intensity, so that the pulmonary $\dot{V}O_2$ responses from these like- transitions could be averaged prior to analysis. This procedure has been shown to decrease the noise in the pulmonary $\dot{V}O_2$ response by (Lamarra *et al.*, 1987). Essentially, the averaging of repeated transitions serves to average–out the variation inherent in pulmonary $\dot{V}O_2$ data. This, in turn, increases the signal-to-noise ratio which increases the confidence in the results obtained from the exponential analysis (Whipp and Rossiter, 2005). Secondly, each of these individual transitions was inspected prior to analysis to exclude raw data points that were deemed inappropriately placed to be indicative of the underlying physiology response. These 'outliers' may be caused by coughing, swallowing, premature ending of a breath, etc. Data points lying more than four standard deviations from the five-breath local rolling average were removed from the raw pulmonary $\dot{V}O_2$ response for each individual transition prior to analysis (Lamarra *et al*., 1987). Thereafter, data were linearly interpolated using a dedicated algorithm to provide second-by-second values.

Given that the breath-by-breath noise in the $\dot{V}O_2$ response is attenuated with an increased $\dot{V}O_2$ response amplitude (Lamarra *et al.*, 1987), the number of like transitions required to attain the requisite confidence in the parameter estimates will vary depending on the magnitude of the work rate imposed. As such, a total of four moderate-intensity exercise

bouts were completed in Chapters 4-5 and 8-10, and a total of two severe-intensity exercise bouts were completed in Chapters 4-6 and 8-10. The subsequent averaging of these filtered, like-transitions yielded an appropriate signal-to-noise ratio (Figures 3.1 and 3.2). In Chapters 4-5 and 8-11, a moderate exercise transition often preceded a subsequent moderate or severe exercise transition following a passive recovery period. This was done to reduce the total number of laboratory visits. Importantly, the performance of moderate exercise prior to a subsequent moderate or severe exercise bout does not influence the $\dot{V}_{O_2}$ response during the subsequent exercise bout (Gerbino *et al*., 1996; Burnley *et al*., 2000).

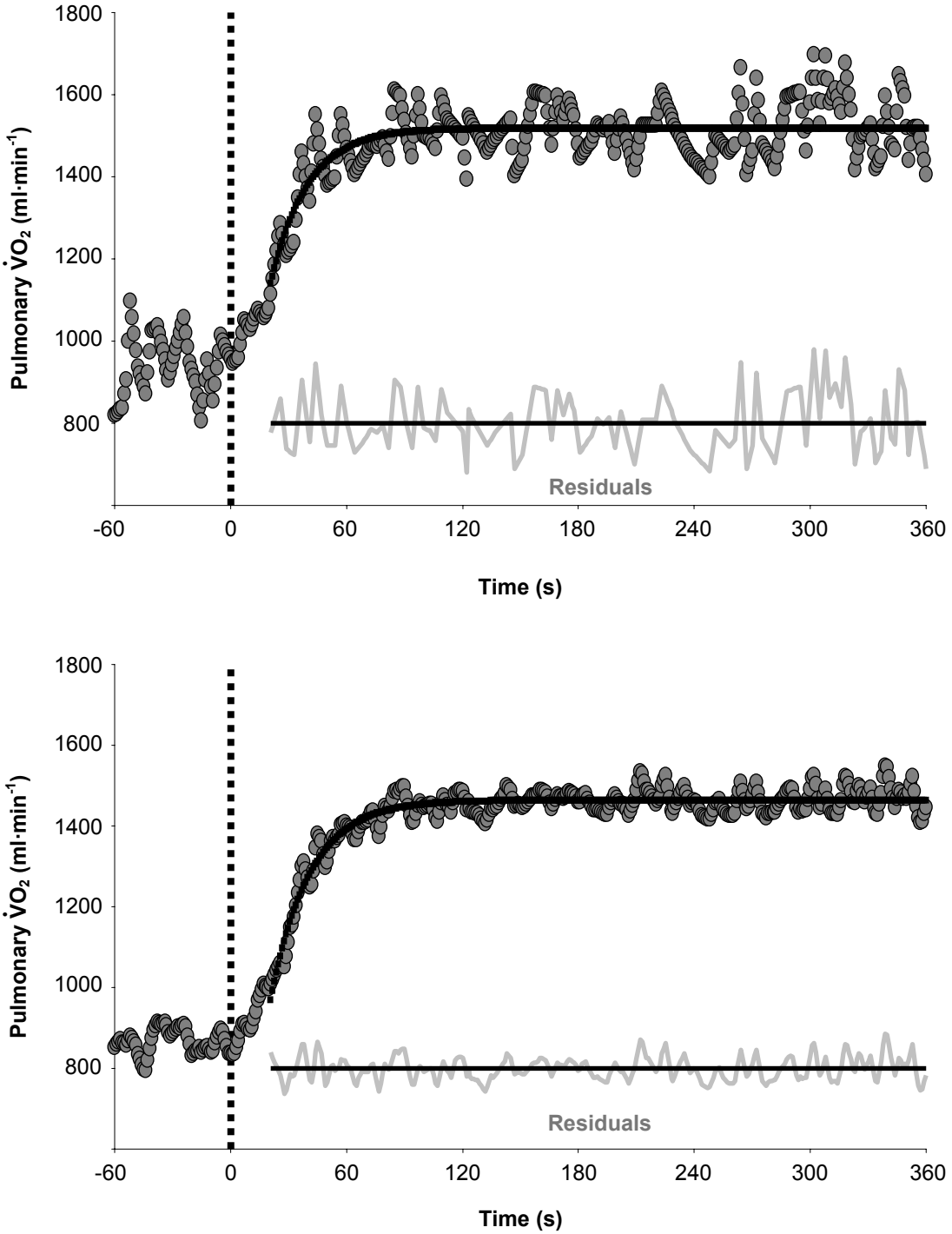

Figure 3.1: Pulmonary $\dot{V}_{O_2}$ response during moderate exercise prior to and following preliminary data analysis procedures. An untreated pulmonary $\dot{V}_{O_2}$ response during one bout of moderate-intensity cycle exercise is shown in the upper panel. The amalgamated response of four individual moderate $\dot{V}_{O_2}$ responses that have been filtered and ensemble-averaged is shown in the lower panel. The dashed vertical lines indicate the point of work rate imposition, the grey filled circles indicate pulmonary $\dot{V}_{O_2}$ responses, the black lines indicates the mono-exponential fit of the data and the grey lines indicate the residuals associated with the exponential fit. Note that in the

lower panel, where the data have been subjected to preliminary data analysis procedures, the signal-to-noise ratio is enhanced such that the residuals deviate less from the exponential response and the confidence in the parameters derived from the mathematical modelling is correspondingly enhanced.

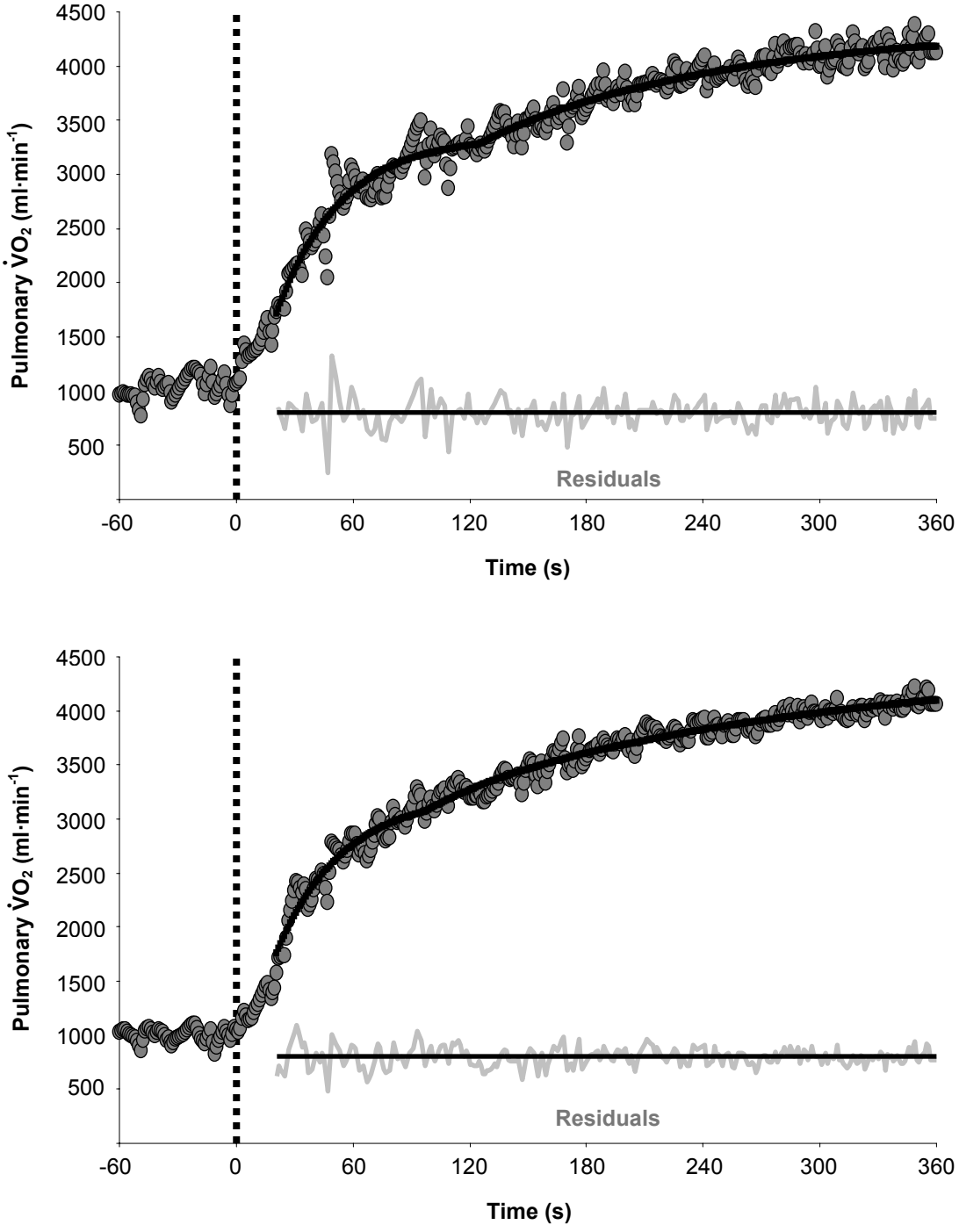

Figure 3.2: Pulmonary $\dot{V}_{O_2}$ response during severe exercise prior to and following preliminary data analysis procedures. An untreated pulmonary $\dot{V}_{O_2}$ response during one bout of severe-intensity cycle exercise is shown in the upper

panel. The amalgamated response of two individual severe $\dot{V}_{O_2}$ responses that have been filtered and ensemble-averaged is shown in the lower panel. The dashed vertical lines indicate the point of work rate imposition, the grey filled circles indicate pulmonary $\dot{V}_{O_2}$ responses, the black lines indicates the bi-exponential fit of the data and the grey lines indicate the residuals associated with the exponential fit. Note that in the lower panel, where the data have been subjected to preliminary data analysis procedures, the signal-to-noise ratio is enhanced such that the residuals deviate less from the exponential response and the confidence in the parameters derived from the mathematical modelling is correspondingly enhanced.

Mathematical Modelling of $\dot{V}_{O_2}$ Data

Following filtering, linear interpolation and averaging of the breath-by-breath data for each individual transition, the composite second-by-second $\dot{V}_{O_2}$ response was imported into a purpose-written modelling program that characterised the $\dot{V}_{O_2}$ response dynamics by way of a nonlinear least-square regression algorithm. This program employs an iterative process that minimises the sum of the squared error between the fitted function and the observed data. However, prior to this curve fitting, a final pre analysis step was taken whereby the first 20 s of data after the onset of exercise were deleted. This was necessary to ensure that the cardiodynamic phase (phase I) of the $\dot{V}_{O_2}$ response did not contaminate the phase II fit. For moderate-intensity exercise, a single-exponential model was used to fit the $\dot{V}_{O_2}$ response, whereas for high-intensity exercise both a single- and bi-exponential model were used and the better fitting model was determined by comparing residual sum of squared error values associated with the fit. In all cases, the bi-exponential model provided a better representation of the $\dot{V}_{O_2}$ response to high-intensity exercise. The single- (equation 8) and bi- (equation 9) exponential models are described in the following equations:

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2\ baseline} + A_p(1-e^{-(t-TDp)/\tau_p}) \quad \text{(Eqn. 3)}$$

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2\ baseline} + A_p(1-e^{-(t-TDp)/\tau_p}) + A_s(1-e^{-(t-TDs)/\tau_s}) \quad \text{(Eqn. 4)}$$

where $\dot{V}O_2$ (t) represents the absolute $\dot{V}O_2$ at any given time t; $\dot{V}O_{2baseline}$ represents the mean $\dot{V}O_2$ in the baseline (i.e., pre-transition) period; A_p, TD_p, and τ_p represent the amplitude, time delay, and time constant, respectively, describing the phase II increase in $\dot{V}O_2$ above baseline; and A_s, TD_s, and τ_s represent the amplitude of, time delay before the onset of, and time constant describing the development of, the $\dot{V}O_2$ slow component, respectively. However, because the asymptotic value of the exponential term describing the $\dot{V}O_2$ slow component may represent a higher value than is actually reached at the end of the exercise, the actual amplitude of the slow component at the end of exercise was defined as A_s'. The functional gains of the fundamental and overall $\dot{V}O_2$ response were computed by dividing response-phase amplitudes by the increase in work rate. The amplitude of the slow component was also described relative to the entire $\dot{V}O_2$ response and, in some studies, the trajectory of the $\dot{V}O_2$ slow component was also calculated. Furthermore, the $\dot{V}O_2$ MRT was determined in some studies by fitting a single exponential without time delay to all data from t = 0. This parameter provides information on the 'overall' $\dot{V}O_2$ kinetics with no distinction made for various phases of the response and is also used to estimate the O_2 deficit during exercise. The O_2 deficit was calculated using the following equation:

$$O_2 \text{ Deficit} = MRT \times \Delta \dot{V}O_2 \qquad \text{(Eqn. 5)}$$

Electromyographic Data

In Chapters 6 and 10, the raw EMG data were filtered with a 20 Hz high-pass second-order Butterworth filter to remove contamination from movement artefacts. The signal was then rectified and low-pass filtered at a frequency of 50 Hz to produce a linear envelope. The derived iEMG values were then averaged over 10 s intervals throughout the exercise bouts.

In Chapter 6 the iEMG signal was expressed as a percentage of the iEMG signal during the baseline cycling period, whereas in Chapter 10 the iEMG signal was expressed as a percentage of the iEMG signal attained during a maximal voluntary contraction (MVC). These normalised average iEMG values were then time aligned and ensemble averaged across repeat trials of identical exercise bouts and from these averaged files, iEMG at discrete time points (e.g., minute 2 or minute 6) and the change in iEMG between time points (e.g., $\Delta iEMG_{6-2}$) was determined.

Mathematical Modelling of Heart Rate Data

The HR response to exercise was modelled to provide indirect information on cardiac output dynamics. For this analysis, HR data collected during the duration of each breath were linearly interpolated to provide second-by-second values such that data from like-transitions could be time-aligned to the start of exercise and ensemble-averaged. In Chapter 4, a single-exponential model similar to the one used to fit the $\dot{V}O_2$ data (see equation 8 above) was also employed to characterise the HR response to both moderate and severe exercise; however, this model commenced at exercise onset (i.e., TD was fixed at t = 0). To avoid contamination by any HR 'slow component' (i.e., a HR response accompanying the $\dot{V}O_2$ slow component), the fitting window for high-intensity exercise was constrained at the time delay before the onset of the $\dot{V}O_2$ slow component (see above). In Chapters 5-6, the HR response was characterised by a single-exponential model commencing at exercise onset (i.e., TD was fixed at t = 0) to derive the HR MRT. In Chapter 7, HR was not assessed with mathematical modelling but was assessed through comparing the HR designated time points throughout the exercise. In Chapters 8-9 and 11 the HR response was characterised with a single-exponential model during moderate-

intensity exercise and a bi-exponential model during severe-intensity exercise with both models commenced at exercise onset (i.e., TD was fixed at $t = 0$).

^{31}Phosphorous Magnetic Resonance Spectroscopy Data

Peak fitting of ^{31}Phosphorous magnetic resonance spectra

In Chapter 10, the spectra acquired via ^{31}P-MRS were quantified by peak fitting, with assumption of prior knowledge, using jMRUI (version 2) software and the AMARES fitting algorithm. Spectra were fitted according to the assumption that Pi, PCr, α-ATP (two peaks, amplitude ratio 1:1), γ-ATP (two peaks, amplitude ratio 1:1), β-ATP (three peaks, amplitude ratio 1:2:1) and phosphodiester peaks were present. In all cases, relative amplitudes were corrected for partial saturation due to the repetition time relative to T1 via an unsaturated spectra acquired prior to exercise.

Mathematical Modelling of Phosphocreatine Concentration Data

The [PCr] data was modelled according to the following equation:

$$\Delta PCr\ (t) = [PCr]ss\ (1 - e^{-t/\tau}) \qquad \text{(Eqn. 6)}$$

However, only one set of data was collected for this analysis. During high-intensity exercise, a secondary component response was evident. Therefore, to characterise the [PCr] dynamics during high-intensity exercise, the fitting window was constrained to an initial start point of 60 s and increased iteratively thereafter until there was a clear departure of the measured data from the model fit, as judged from visual inspection of a plot of the residuals. In this way, the best-fit exponential for the fundamental component of the response was established. The magnitude of any possible [PCr] slow component was then

calculated as the difference between the asymptotic amplitude of the fundamental response and the mean value measured over the last 30 s of exercise for that condition.

Calculation of Intramuscular Metabolites

Intracellular pH was calculated using the chemical shift of the P_i spectral peak relative to the PCr peak (Taylor *et al*., 1983). ADP was calculated via knowledge of P_i, PCr, and pH values, as described by Kemp *et al.* (2001) taking into account the dependency of rate constants on pH. Resting and end-exercise values of [PCr], [P_i], and pH were calculated over the last 30 s of the rest or exercise period.

Calculation of ATP Turnover Rates

The total ATP turnover rate (ATP_{total}) was calculated as the sum of the total ATP turnover deriving from PCr hydrolysis, glycolysis and oxidative phosphorylation (ATP_{PCr}, ATP_{Gly} and ATP_{Ox}, respectively) using the methods of Lanza *et al*. (2006) and Layec *et al*. (2009). ATP_{PCr} was obtained from the rate of change in [PCr] from the modelling of [PCr] values acquired at each time point during the exercise protocol. ATP_{Gly} was determined by proton flux, assuming that the production of 1 mol of H^+ yields 1.5 mol of ATP. Proton flux calculations were based on determining the protons consumed by the creatine kinase (CK) reaction and buffering capacity and those produced via oxidative phosphorylation and expelled by cellular efflux. ATP_{Ox} was determined based on the hyperbolic relationship between ATP production rate and free cytosolic ADP concentration, requiring the calculation of the first-order PCr recovery rate-constant determined from fitting PCr to a single exponential function.

Mathematical Modelling of Deoxyhaemoglobin Concentration Data

In Chapters 4, 6, 7 and 8, [HHb] data (a proxy for muscle O_2 extraction; Delorey *et al*., 2007; Grassi *et al*., 2003) was modelled in a similar way to $\dot{V}_{O_2}$ as described above. Specifically, the single- and bi-exponential models (Equations 8 and 9) were used to fit the [HHb] data for moderate- and high intensity exercise, respectively. However, in this case, the fitting window commenced from the first datum that was one standard deviation above the baseline mean after initiation of the transition. Information pertaining to the dynamics of the fundamental phase of the [HHb] response was derived from the sum of the fundamental τ and TD ([HHb] τ + TD). The ratio of [HHb] amplitude to $\dot{V}_{O_2}$ amplitude (Δ[HHb]/$\Delta\dot{V}_{O_2}$) was used as an index of O_2 extraction during various phases of the response in Chapter 4.

Analysis of Oxyhaemoglobin Concentration Data

Prior research indicates that [HbO_2] responses do not approximate an exponential (DeLorey *et al.*, 2007). Consequently, NIRS-derived [HbO_2] data collected during the tests performed in Chapters 4, 6, 7 and 8 were not modelled. Rather, baseline and end-exercise [HbO_2] were determined and the sum of [HbO_2] and [HHb] at baseline and at 60-second intervals throughout exercise was used to provide an estimate of changes in total haemoglobin ([Hb_{tot}]).

Statistical Methods

All statistical analyses within the experimental chapters of this thesis were conducted with the Statistical Package for Social Sciences. Specific information regarding the particular statistical tests that were employed for the different investigations is provided within each of the experimental chapters. Before any statistical tests were carried out, the data were

screened for normal distribution using standard procedures. Statistical significance was accepted at $P < 0.05$. All data are presented as means ± SD unless otherwise indicated in the individual experimental chapters.

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Influence of repeated sprint training on pulmonary O_2 uptake and muscle deoxygenation kinetics in humans

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Bailey SJ, Wilkerson DP, DiMenna FJ, Jones AM. Influence of repeated sprint training on pulmonary O_2 uptake and muscle deoxygenation kinetics in humans. *J Appl Physiol* 106: 1875–1887, 2009. First published April 2, 2009; doi:10.1152/japplphysiol.00144.2009.—We hypothesized that a short-term training program involving repeated all-out sprint training (RST) would be more effective than work-matched, low-intensity endurance training (ET) in enhancing the kinetics of oxygen uptake ($\dot{V}O_2$) and muscle deoxygenation {deoxyhemoglobin concentration ([HHb])} following the onset of exercise. Twenty-four recreationally active subjects (15 men, mean ± SD: age 21 ± 4 yr, height 173 ± 9 cm, body mass 71 ± 11 kg) were allocated to one of three groups: RST, which completed six sessions of four to seven 30-s RSTs; ET, which completed six sessions of work-matched, moderate-intensity cycling; and a control group (CON). All subjects completed moderate-intensity and severe-intensity "step" exercise transitions before (Pre) and after the 2-wk intervention period (Post). Following RST, [HHb] kinetics were speeded, and the amplitude of the [HHb] response was increased during both moderate and severe exercise ($P < 0.05$); the phase II $\dot{V}O_2$ kinetics were accelerated for both moderate (Pre: 28 ± 8, Post: 21 ± 8 s; $P < 0.01$) and severe (Pre: 29 ± 5, Post: 23 ± 5 s; $P < 0.05$) exercise; the amplitude of the $\dot{V}O_2$ slow component was reduced (Pre: 0.52 ± 0.19, Post: 0.40 ± 0.17 l/min; $P < 0.01$); and exercise tolerance during severe exercise was improved by 53% (Pre: 700 ± 234, Post: 1,074 ± 431 s; $P < 0.01$). None of these parameters was significantly altered in the ET and CON groups. Six sessions of RST, but not ET, resulted in changes in [HHb] kinetics consistent with enhanced fractional muscle O_2 extraction, faster $\dot{V}O_2$ kinetics, and an increased tolerance to high-intensity exercise.

oxygen uptake kinetics; exercise performance; near-infrared spectroscopy

THE COMMENCEMENT OF MUSCULAR exercise mandates an immediate increase in ATP turnover in the recruited myocytes. Transiently, the bulk of this additional energy requirement is met through intramuscular phosphocreatine (PCr) degradation and the anaerobic catabolism of glycogen, and, as such, an "oxygen deficit" is incurred (42). Simultaneously, oxygen uptake ($\dot{V}O_2$) rises in an exponential fashion, such that oxidative phosphorylation makes a progressively greater contribution to ATP resynthesis as exercise proceeds. Pulmonary $\dot{V}O_2$ reaches a "steady-state" within 2–3 min of the onset of moderate-intensity exercise [below the gas exchange threshold (GET); Refs. 67, 68]; during >GET exercise, however, a "steady state" is either delayed (for heavy exercise performed below the critical power) or is unattainable (for severe exercise performed above the critical power) due to the emergence of an additional $\dot{V}O_2$ "slow component" (68). This slow component rise in $\dot{V}O_2$ is associated with a commensurate fall in muscle PCr concentration ([PCr]) (57, 58) and greater glycogen utilization (43). Thus features of the dynamic adjustment of $\dot{V}O_2$ following the onset of exercise have been suggested to be related to the process of muscular fatigue (9, 37, 66). Interventions that accelerate the phase II $\dot{V}O_2$ kinetics and/or reduce the amplitude of the $\dot{V}O_2$ slow component should theoretically result in enhanced exercise tolerance (36, 66).

A potent stimulus to enhance the dynamics of $\dot{V}O_2$ during exercise is endurance training (ET). Indeed, a period of ET has been shown to accelerate phase II $\dot{V}O_2$ kinetics (4, 18, 31, 43) and reduce the amplitude of the $\dot{V}O_2$ slow component (4, 10, 11, 59), adaptations that can be discerned after only a few days of training (54, 71). These adaptations might, therefore, account, at least in part, for the enhanced exercise tolerance typically observed after a period of ET (e.g., Refs. 13, 18, 35). However, whether there is an "optimal" training strategy to elicit improvements in $\dot{V}O_2$ kinetics is presently unclear. Berger et al. (4) reported that adaptations in $\dot{V}O_2$ kinetics were similar when the ET consisted of continuous, low-intensity exercise or as a series of repeated high-intensity exercise bouts interspersed with a short recovery period. In contrast, the data of Daussin et al. (13) suggest that interval training may be a more efficacious intervention for accelerating phase II $\dot{V}O_2$ kinetics compared with continuous lower intensity ET.

A novel, and time-efficient, approach to elicit the adaptations associated with ET has recently been introduced (6–8, 22). Specifically, six sessions of repeated sprint training (RST) comprising four to six repeats of an all-out 30-s Wingate cycle test, separated by 4-min recovery, induced strikingly similar increases in muscle oxidative enzyme activity, buffering capacity, glycogen content, and exercise tolerance to those observed following six sessions of 90–120 min of continuous cycling at ~65% peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$) (22). Earlier work revealed that this RST protocol resulted in increases in cycle endurance capacity (time to exhaustion at ~80% $\dot{V}O_{2peak}$), citrate synthase activity (8), and reductions in glycogenolysis and lactate accumulation (6), compared with a control group (CON), who remained sedentary. Moreover, RST and ET (40- to 60-min cycling continuously at 65% $\dot{V}O_{2peak}$) resulted in similar reductions in glycogen and PCr degradation during 1 h of exercise at 65% $\dot{V}O_{2peak}$ (7, 23). Collectively, these findings indicate that RST is as effective as more prolonged ET for increasing the capacity for oxidative metabolism, sparing the contribution of substrate level phosphorylation to energy turnover, and enhancing exercise tolerance. However, to what extent RST enhances $\dot{V}O_2$ kinetics (by speeding the phase II $\dot{V}O_2$ response and/or reducing the $\dot{V}O_2$ slow component) relative to traditional ET, and to what extent any improvements in $\dot{V}O_2$ kinetics are related to enhanced exercise tolerance following RST, are presently unclear.

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While it has been known for some time that ET subjects exhibit rapid $\dot{V}O_2$ response dynamics (3, 28, 40, 56), the factors that regulate the dynamic $\dot{V}O_2$ response during exercise continue to be debated (37, 55, 62). Depending on the circumstances, $\dot{V}O_2$ kinetics might be principally limited by muscle O_2 delivery or its distribution, or by an intrinsic inertia of the muscle metabolic machinery (37, 55, 62). Exercise training results in rapid adaptations in muscle blood flow (46, 61) and mitochondrial enzyme activity (6–8, 22, 43, 54), but information on the impact of these changes on the faster $\dot{V}O_2$ kinetics following training is presently limited. The available evidence indicates that, while training results in enhanced muscle blood flow and muscle O_2 extraction, the latter might be particularly important in the speeding of $\dot{V}O_2$ kinetics observed following training during high-intensity exercise (43). The deoxyhemoglobin/myoglobin concentration ([HHb]) signal derived from near-infrared spectroscopy (NIRS) measurements reflects the balance between O_2 delivery and O_2 utilization in the field of interrogation and has been used to provide a noninvasive estimate of fractional O_2 extraction in the microcirculation during exercise (16, 17, 21, 27, 34, 41). The assessment of [HHb] kinetics (to reflect muscle fractional O_2 extraction; Refs. 26, 27) and heart rate (HR) kinetics (as a crude estimate of muscle blood flow kinetics; Ref. 50) might, therefore, facilitate investigation of the mechanisms by which $\dot{V}O_2$ kinetics are altered with different types of training. Unaltered muscle [HHb] dynamics [as assessed by changes in the time delay (TD), time constant (τ), and amplitude of the response] following training, despite evidence of faster $\dot{V}O_2$ kinetics, would be interpreted to indicate that local muscle blood flow remained well matched to the increased muscle O_2 utilization following training. A longer TD and/or τ or a smaller [HHb] amplitude following training would indicate that microvascular blood flow increased more than muscle O_2 extraction, whereas a shorter TD and/or τ or a greater [HHb] amplitude would indicate that O_2 extraction had increased more than local blood flow. The direction of any changes in [HHb] kinetics as a consequence of training, therefore, has implications for the mechanism(s) by which $\dot{V}O_2$ kinetics are accelerated.

The purpose of the present study was, therefore, to assess the effect of work-matched RST and ET on the kinetics of $\dot{V}O_2$, HR, and muscle deoxygenation during moderate- and severe-intensity exercise in recreationally active subjects, as well as the impact of these training methods on severe-intensity exercise tolerance. We reasoned that simultaneous assessment of the kinetics of $\dot{V}O_2$, HR, and [HHb] might provide insight into the mechanistic bases (i.e., increased muscle blood flow and/or metabolic adjustments) for the enhanced $\dot{V}O_2$ kinetics observed following exercise training. We hypothesized that both the phase II $\dot{V}O_2$ τ and the $\dot{V}O_2$ slow-component amplitude would be reduced, and exercise tolerance enhanced, by training in both groups. We also hypothesized that the magnitude of these changes would be greater for the RST group, particularly during severe-intensity exercise, consequent to a greater improvement in muscle O_2 extraction (as estimated by changes in [HHb] kinetics).

METHODS

Subjects

Twenty-four healthy subjects (15 male, mean ± SD: age 21 ± 4 yr, height 173 ± 9 cm, body mass 71 ± 11 kg) volunteered to participate in this study. The subjects participated in exercise at a recreational level, but were not highly trained, and were familiar with laboratory exercise testing procedures, having previously participated in studies employing cycle ergometry in our laboratory. The procedures employed in this study were approved by the University of Exeter Research Ethics Committee, and all subjects were required to give their written, informed consent before the commencement of the study, once the experimental procedures, associated risks, and potential benefits of participation had been explained. Subjects were instructed to arrive at the laboratory in a rested and fully hydrated state, at least 3 h postprandial, and to avoid strenuous exercise in the 24 h preceding each testing session. Each subject was also asked to refrain from caffeine and alcohol 6 and 24 h before each test, respectively. All tests were performed at the same time of day (±2 h).

Experimental Design

The subjects were required to report to the laboratory on 12 occasions over a 4- to 5-wk period (six occasions for subjects assigned to the control condition), and all tests were interspersed with at least 24-h recovery. Subjects underwent a number of preliminary tests for the determination of $\dot{V}O_{2peak}$, GET, $\dot{V}O_2$ kinetics, and exercise tolerance. Upon completion of the preliminary tests, subjects were randomly assigned to one of the following training interventions: 30-s RST, continuous ET, or CON. Following completion of the training protocols, subjects repeated all of the baseline tests at the same absolute work rates to determine the effect of the respective training interventions on the physiological and performance parameters.

Incremental Test

Both before and after the intervention period, the subjects completed a ramp incremental exercise test for determination of the $\dot{V}O_{2peak}$ and GET. All cycle tests were performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). Initially, subjects performed 3 min of baseline cycling at 0 W, after which the work rate was increased at a rate of 25 W/min until the limit of tolerance. The subjects cycled at a self-selected pedal rate (70–90 rpm), and this pedal rate, along with saddle and handle bar height and configuration, was recorded and reproduced in subsequent tests. Breath-by-breath pulmonary gas-exchange data were collected continuously during the incremental tests and averaged over consecutive 10-s periods. The $\dot{V}O_{2peak}$ was taken as the highest 30-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 90% of the GET (moderate exercise) and 70% Δ (GET plus 70% of the difference between the work rate at the GET and $\dot{V}O_{2peak}$; severe exercise) were subsequently calculated, with account taken of the mean response time (MRT) for $\dot{V}O_2$ during ramp exercise (i.e., two-thirds of the ramp rate was deducted from the work rate at GET and peak; Ref. 64).

Step Exercise Tests

On two occasions, both before and after the intervention period, the subjects also completed three "step" tests for the determination of $\dot{V}O_2$ kinetics. The protocol comprised two moderate-intensity and one severe-intensity cycle transition, each of 6-min duration. Each transition began with 3 min of baseline pedaling at 0 W before an abrupt transition to the target work rate. A passive recovery of 8 min separated the transitions. On one occasion, both before and after the intervention period, the severe-intensity transition was continued until task failure as a measure of exercise tolerance. The time to task failure was recorded when the pedal rate fell by >10 rpm below the required

pedal rate. Therefore, all subjects performed a total of four bouts of moderate-intensity exercise and two bouts of severe-intensity exercise, both before and after the intervention period. The $\dot{V}O_2$ responses from these like transitions were averaged before any analysis to enhance the signal-to-noise ratio and improve confidence in the parameters derived from the model fits (45, 65).

Training Interventions

After completing the initial stage of experimental testing, the male and female subjects were randomly assigned to either a RST group (mean ± SD: age 21 ± 5 yr, height 172 ± 8 cm, body mass 74 ± 9 kg), an ET group (age 20 ± 4 yr, height 1.76 ± 12 cm, body mass 71 ± 11 kg), or a CON group (age 20 ± 1 yr, height 171 ± 7 cm, body mass 66 ± 9 kg). All three groups contained five male and three female subjects. Both training groups performed a total of six training sessions over a 2-wk period, with at least 24-h recovery separating sessions, while the CON group maintained their habitual levels of physical activity.

The RST group performed a number of "all-out" 30-s cycle sprints (Wingate test) against a resistance equivalent to 0.075 kg/kg body mass on a mechanically braked cycle ergometer (model 814E bicycle ergometer, Monark, Stockholm, Sweden; Refs. 6–8, 22). The subjects completed a total of four repetitions of the 30-s sprint in the first training session, five repetitions in the second session, six repetitions in the third and fourth sessions, and seven repetitions in the fifth and sixth sessions. Subjects were instructed to pedal maximally against the ergometer's inertial resistance ~2 s before the appropriate load was applied. Strong verbal encouragement was provided to the subjects during all sprints to ensure a maximal effort was achieved. All sprints were separated by 4-min recovery, during which subjects were permitted to cycle at a low cadence (<50 rpm) against a light resistance (<30 W) to reduce sensations of nausea. The online data-acquisition system determined peak power, mean power, and rate of fatigue for each test, and these data were stored on a personal computer for subsequent analysis.

The ET required subjects to cycle continuously on a cycle ergometer (model 814E bicycle ergometer; Monark, Stockholm, Sweden) at 80 rpm for a predetermined duration at an intensity corresponding to 90% of the GET. The exercise duration was calculated so that the total work performed per training session for each subject was matched to the mean work completed by the RST group as a whole in the corresponding session. The mean work done and the duration of exercise completed by the RST and ET groups are presented in Table 1 for each of the six training sessions.

Measurements

During all tests, pulmonary gas exchange and ventilation were measured continuously using a portable metabolic cart (MetaMax 3B, Cortex Biophysik, Leipzig, Germany), as described previously (19). Pulmonary gas exchange and ventilation were calculated and displayed breath by breath. HR was measured during all tests using short-range radiotelemetry (Polar S610, Polar Electro Oy, Kempele, Finland).

Table 1. *Total work done and duration of exercise completed by the RST and ET groups for each training session*

Session No.	RST Total Work Done, kJ	RST Exercise Duration, min	ET Exercise Duration, min
1	63±15	2	14.9±2.8
2	76±17	2.5	18.0±3.3
3	92±20	3	21.6±4.0
4	93±21	3	21.8±4.0
5	107±23	3.5	25.1±4.6
6	106±22	3.5	25.0±4.6

Values are means ± SD. The subjects in the endurance training (ET) group completed cycle exercise at 90% gas exchange threshold (GET) until they had completed the same amount of work as the repeated sprint training (RST) group for the equivalent session.

During one of the transitions to moderate and severe exercise, pre- and posttraining, a blood sample was collected from a fingertip into a capillary tube over the 20 s preceding the step transition in work rate and within the last 20 s of exercise. A capillary blood sample was also collected at the limit of tolerance for the severe-intensity bout. These whole blood samples were subsequently analyzed to determine blood lactate concentration ([lactate]) (YSI 1500, Yellow Springs Instruments, Yellow Springs, OH) within 30 s of collection. Blood lactate accumulation (Δblood [lactate]) was calculated as the difference between blood [lactate] at end exercise and blood [lactate] at baseline.

The oxygenation status of the *m. vastus lateralis* of the right leg was monitored using a commercially available NIRS system (model NIRO 300, Hamamatsu Photonics KK, Hiugashi-ku, Japan). The system consisted of an emission probe that irradiates laser beams and a detection probe. Four different wavelength laser diodes provided the light source (776, 826, 845, and 905 nm), and the light returning from the tissue was detected by a photomultiplier tube in the spectrometer. The intensity of incident and transmitted light was recorded continuously at 2 Hz and used to estimate concentration changes from the resting baseline for oxygenated, deoxygenated, and total tissue hemoglobin/myoglobin. Therefore, the NIRS data represent a relative change based on the optical density measured in the first datum collected. The [HHb] signal can be regarded as being essentially blood-volume insensitive during exercise (14, 27, 41) and was, therefore, assumed to provide an estimate of changes in O_2 extraction in the field of interrogation (17, 21, 27). It should be noted here that the contribution of deoxygenated myoglobin to the NIRS signal is presently unclear, and, as such, the terms [Hb_{tot}], [HbO_2], and [HHb] used in this paper should be considered to refer to the combined concentrations of total, oxygenated, and deoxygenated hemoglobin and myoglobin, respectively.

The leg was initially cleaned and shaved around the belly of the muscle, and the probes were placed in the holder, which was secured to the skin with adhesive at 20 cm above the fibular head. To secure the holder and wires in place, an elastic bandage was wrapped around the subject's leg. The wrap helped to minimize the possibility that extraneous light could influence the signal and also ensured that the optodes did not move during exercise. Pen marks were made around the probes to enable precise reproduction of the placement in subsequent tests. The probe gain was set with the subject at rest in a seated position with leg extended at down stroke on the cycle ergometer before the first exercise bout, and NIRS data were collected continuously throughout the exercise protocols. The data were subsequently downloaded onto a personal computer, and the resulting text files were stored on disk for later analysis.

Data Analysis Procedures

The breath-by-breath $\dot{V}O_2$ data from each test were initially examined to exclude errant breaths caused by coughing, swallowing, sighing, etc., and those values lying more than 4 SDs from the local mean were removed. The breath-by-breath data were subsequently linearly interpolated to provide second-by-second values, and, for each individual, identical repetitions were time aligned to the start of exercise and ensemble averaged. The first 20 s of data after the onset of exercise (i.e., the phase I response) were deleted (65), and a nonlinear least squares algorithm was used to fit the data thereafter. A single-exponential model was used to characterize the $\dot{V}O_2$ responses to moderate exercise, and a biexponential model was used for severe exercise, as described in the following equations:

$$\dot{V}O_2(t) = \dot{V}O_{2baseline} + A_p[1 - e^{-(t-TD_p)/\tau_p}] \quad \text{(moderate)} \qquad (1)$$

$$\dot{V}O_2(t) = \dot{V}O_{2baseline} + A_p[1 - e^{-(t-TD_p)/\tau_p}] + A_s[1 - e^{-(t-TD_s)/\tau_s}] \quad \text{(severe)} \quad (2)$$

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time t; $\dot{V}O_{2baseline}$ represents the mean $\dot{V}O_2$ in the baseline period; A_p, TD_p, and τ_p represent the amplitude, TD, and τ, respectively, describing the phase II increase in $\dot{V}O_2$ above baseline; and A_s, TD_s, and τ_s represent the amplitude of, TD before the onset of, and τ describing the development of the $\dot{V}O_2$ slow component, respectively.

An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. $\dot{V}O_{2baseline}$ was defined as the mean $\dot{V}O_2$ measured over the final 90 s of baseline pedaling. The end-exercise $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 30 s of exercise. The absolute fundamental component amplitude (absolute A_p) was defined as the sum of $\dot{V}O_{2baseline}$ and A_p. Because the asymptotic value (A_s) of the exponential term describing the $\dot{V}O_2$ slow component may represent a higher value than is actually reached at the end of the exercise, the actual amplitude of the $\dot{V}O_2$ slow component at the end of exercise was defined as A_s'. The A_s parameter was compared at the same iso-time (360 s) pre- and posttraining. The amplitude of the slow component was also described relative to the entire $\dot{V}O_2$ response. The rate at which the $\dot{V}O_2$ slow component developed (i.e., in ml/min^2) was also calculated. In addition, the functional "gain" of the fundamental $\dot{V}O_2$ response was computed by dividing A_p by the Δwork rate. The functional gain of the entire response (i.e., end-exercise gain) was calculated in a similar manner.

To provide information on muscle oxygenation, we also modeled the [HHb] response to exercise. Mono- and biexponential models, similar to those described above, were applied to the ensemble-averaged data with the exception that the fitting window commenced at the time at which the [HHb] signal increased 1 SD above the baseline mean. Moderate-intensity [HHb] kinetics were determined by constraining the fitting window to the point at which monoexponentiality became distorted, consequent to a gradual fall in [HHb] (41), as determined by visual inspection of the residual plots. Severe-intensity [HHb] kinetics were determined by fitting a biexponential model from the first data point, which was 1 SD above the baseline mean through the entire response. It should be noted that, although the [HHb] response can be conveniently described by an exponential function, it has not been established that an exponential function best reflects the underlying physiological response. The [HHb] TD and τ values were summed to provide information on the overall [HHb] response dynamics in the fundamental phase of the response. In addition to the [HHb] τ and TD derived from the model fits for both moderate and severe exercise, we also used the fundamental [HHb] amplitude to determine the Δ[HHb]/$\Delta\dot{V}O_2$ during this phase of the response. We chose to express the [HHb] slow component, observed during severe-intensity exercise, both relative to the entire [HHb] response and as the Δ[HHb]/$\Delta\dot{V}O_2$. The [HbO_2] and [Hb_{tot}] responses do not approximate an exponential (15) and were, therefore, not modeled. Rather, we assessed training-induced changes in these parameters by determining the [HbO_2] and [Hb_{tot}] at baseline (90-s preceding step transition), 120 s, and [HHb] TD_s (for severe-intensity exercise), and end exercise (average response over the final 30 s).

We also modeled the HR response to exercise in each condition. For this analysis, HR data were linearly interpolated to provide second-by-second values, and, for each individual, identical repetitions from like transitions were time aligned to the start of exercise and ensemble averaged. A nonlinear least squares monoexponential model without TD was used to fit the data to moderate-intensity exercise, with the fitting window commencing at $t = 0$ s. The fitting window was constrained to the $\dot{V}O_2$ TD_s in the case of severe-intensity exercise (see above). The HR τ_p so derived provides information on the overall HR response dynamics in the absence of any HR "slow component." The HR slow component was computed as the difference between the HR attained at the $\dot{V}O_2$ TD_s and the HR attained at end exercise. In addition to this analysis, we also modeled the HR response with a biexponential model from the onset of exercise without constraining the fit to the $t < \dot{V}O_2$ TD_s region.

Statistical Analysis

A 2 × 3 (time by group) ANOVA with repeated measures for time was employed to determine the effects on the relevant physiological variables elicited by the differing training protocols. Where the analysis revealed a significant difference, individual paired *t*-tests were employed with a Bonferroni correction to determine the origin of such effects. Pearson's product-moment correlation was employed to examine the interrelationships between the parameters of $\dot{V}O_2$ kinetics and [HHb] and exercise tolerance. All data are presented as means ± SD. Statistical significance was accepted when $P < 0.05$.

RESULTS

All subjects were recreationally active on recruitment to the study and the physiological parameters of interest (i.e., $\dot{V}O_{2peak}$, $\dot{V}O_2$ at GET, $\dot{V}O_2$ τ_p, $\dot{V}O_2$ slow-component amplitude) were similar between the three groups before the commencement of the study (Tables 2 and 3). Each subject completed 100% of the training sessions required by the specific training groups and self-reported that they did not alter their activity levels outside of training for the duration of the study. The ET group completed ~126 min of low-intensity exercise, and the RST group completed 17.5 min of high-intensity exercise over the 2-wk training period.

Incremental Test

The $\dot{V}O_2$ and work rate attained at the GET and at the limit of tolerance before (Pre) and following (Post) the 2-wk intervention period are presented in Table 2. The RST group demonstrated a significant ($P < 0.05$) increase in both absolute (Pre: 3.06 ± 0.60, Post: 3.29 ± 0.65 5 l/min) and relative (Pre: 42 ± 6, Post: 45 ± 6 ml·kg^{-1}·min^{-1}) $\dot{V}O_{2peak}$ following

Table 2. *Work rate and oxygen uptake at the GET and at peak during ramp incremental exercise in the RST, ET, and control groups pre- and postintervention*

	RST Pre	RST Post	ET Pre	ET Post	CON Pre	CON Post
$\dot{V}O_2$ at GET, l/min	1.45±0.64	1.47±0.37	1.41±0.34	1.44±0.45	1.38±0.33	1.39±0.41
WR at GET, W	103±18	129±16	82±16	90±16	88±11	98±12
$\dot{V}O_{2peak}$, l/min	3.06±0.62	3.29±0.77*	3.16±0.73	3.14±0.74	3.09±0.81	3.00±0.85
$\dot{V}O_{2peak}$, ml·kg^{-1}·min^{-1}	42±6	45±6*	43±5	43±5	47±8	46±8
Peak WR, W	299±63	313±58†	284±49	288±52	294±64	289±61

Values are means ± SD. Pre, preintervention; Post, postintervention; CON, control; $\dot{V}O_2$, oxygen uptake; $\dot{V}O_{2peak}$, peak $\dot{V}O_2$; WR, work rate. *Significantly different from RST Pre ($P < 0.05$); †significantly different from RST Pre ($P < 0.01$).

Table 3. *Oxygen uptake kinetics and blood [lactate] during moderate-intensity and severe-intensity exercise in the RST, ET, and control groups pre- and postintervention*

	RST Pre	RST Post	ET Pre	ET Post	CON Pre	CON Post
			Moderate-intensity exercise			
Baseline $\dot{V}O_2$, l/min	0.81±0.09	0.78±0.05	0.84±0.15	0.79±0.13	0.77±0.17	0.71±0.10
Primary time delay, s	16±5	16±5	16±2	14±5	15±2	13±5
Primary time constant, s	28±8	21±8†	26±4	24±7	24±3	23±3
95% Confidence interval, s	2±1	2±1	3±2	4±2	3±1	4±2
Primary amplitude, l/min	0.64±0.09	0.63±0.10	0.41±0.14	0.41±0.15	0.48±0.09	0.49±0.13
Gain, ml·min^{-1}·W^{-1}	10.1±1.2	10.0±1.1	10.0±0.9	10.4±1.6	10.5±0.7	10.8±1.3
Mean response time, s	45±7	35±6*	40±7	35±7	37±4	35±8
Oxygen deficit, liter	0.46±0.10	0.36±0.10*	0.29±0.10	0.26±0.01	0.29±0.01	0.28±0.01
End-exercise blood [lactate], mM	1.2±0.3	1.0±0.2	0.9±0.1	0.8±0.2	1.1±0.5	1.0±0.3
ΔBlood [lactate], mM	0.2±0.2	−0.1±0.2*	−0.1±0.3	0.0±0.2	0.1±0.3	0.0±0.3
			Severe-intensity exercise			
Baseline $\dot{V}O_2$, l/min	0.81±0.07	0.80±0.06	0.86±0.17	0.80±0.11	0.79±0.18	0.76±0.17
Primary time delay, s	12±3	16±2	12±3	13±4	10±5	11±5
Primary time constant, s	29±5	23±5*	26±6	25±8	25±7	24±5
95% Confidence interval, s	2±2	3±1	3±2	3±2	5±3	4±1
Primary amplitude, l/min	1.78±0.53	1.85±0.49	1.65±0.42	1.57±0.46	1.67±0.46	1.69±0.46
Gain, ml·min^{-1}·W^{-1}	9.6±0.9	10.0±0.9	9.9±0.9	9.7±0.8	9.5±0.9	9.7±0.7
Slow-component amplitude, l/min	0.52±0.19	0.40±0.17†	0.47±0.19	0.47±0.19	0.51±0.15	0.47±0.15
Slow-component development, l/min^2	0.12±0.04	0.10±0.04*	0.12±0.04	0.11±0.03	0.11±0.03	0.10±0.03
Slow-component amplitude, %end	23±8	18±7†	23±6	22±6	24±6	23±7
Mean response time, s	62±11	54±12*	62±11	61±10	58±8	57±10
End 6-min exercise blood [lactate], mM	6.1±1.4	5.2±1.2*	6.3±0.8	6.0±0.9	6.1±2.0	6.1±2.4
ΔBlood [lactate], mM	5.3±1.6	4.3±1.6*	5.4±1.0	5.2±1.3	5.3±1.7	5.4±2.4

Values are means ± SD. [Lactate], lactate concentration; Δ, difference. *Significantly different from RST Pre ($P < 0.05$); †significantly different from RST Pre ($P < 0.01$).

training, while these parameters were not significantly altered in the ET and CON groups (Table 2). Similarly, peak work rate was only improved significantly following RST (Pre: 299 ± 63, Post: 313 ± 58 W; $P < 0.01$). The work rate at the GET demonstrated a significant main effect for time ($P < 0.05$); however, further analysis revealed no effect in any individual group. The $\dot{V}O_2$ associated with the GET was not altered significantly following any of the interventions.

$\dot{V}O_2$ Kinetics

The parameters derived from the monoexponential model fits, including the 95% confidence intervals surrounding the τ_p estimate, are presented in Table 3. The $\dot{V}O_2$ response of a representative subject from each training group is illustrated in Fig. 1 for moderate-intensity exercise and in Fig. 2 for severe-intensity exercise.

Moderate-intensity exercise. Neither the baseline nor the amplitude of the $\dot{V}O_2$ response was affected by any of the interventions. For τ_p, however, there was a significant main effect for time ($P < 0.01$). Further analyses revealed that only the RST τ_p was significantly reduced following training (Pre: 28 ± 8, Post: 21 ± 8 s; $P < 0.01$), whereas τ_p was not significantly altered following ET and CON (Table 3). Likewise, significant reductions in the MRT (Pre: 45 ± 7, Post: 36 ± 6 s) and O_2 deficit (Pre: 0.45 ± 0.10, Post: 0.36 ± 0.10 liter) were observed following RST (both $P < 0.05$), but not ET or CON (Table 3). There was a strong correlation between the initial τ_p value and the reduction in τ_p observed with RST ($r = 0.80$; $P < 0.05$). The Δblood [lactate] was significantly reduced in the RST group, but not in the ET or CON groups (Table 3).

Severe-intensity exercise. The baseline $\dot{V}O_2$ and primary $\dot{V}O_2$ amplitude were not altered in RST, ET, or CON. Only the RST intervention elicited a significant speeding of the τ_p (Pre: 29 ± 5, Post: 23 ± 5 s; $P < 0.05$) and MRT (Pre: 62 ± 11, Post: 54 ± 12 s; $P < 0.05$). There was a strong correlation between the initial τ_p value and the reduction in τ_p observed with RST ($r = 0.71$; $P = 0.05$). The A_s' (Pre: 0.52 ± 0.19, Post: 0.40 ± 0.17 l/min; $P < 0.01$) and the rate at which the slow component developed were significantly reduced following RST, but were not altered in ET or CON (Table 3). The end-exercise and Δblood [lactate] values were significantly reduced in the RST group, but not in the ET or CON groups (Table 3).

NIRS Measurements

[HbO_2] and [Hb_{tot}] were not significantly different before or during exercise in the three groups before the intervention, and the responses were not altered by the training or control periods. The parameters derived from modeling the [HHb] response are shown for each group pre- and posttraining in Table 4. The [HHb] dynamics of a representative subject from each training group are illustrated in Fig. 3 for moderate-intensity exercise and in Fig. 4 for severe-intensity exercise.

Moderate-intensity exercise. A significant interaction effect was observed for the TD of the [HHb] response ($P < 0.05$). Follow-up analyses revealed that the [HHb] TD was significantly reduced in RST (Pre: 14 ± 4, Post: 10 ± 2 s; $P < 0.05$), but not in ET or CON (Table 4). The [HHb] τ did not differ significantly between groups and was not altered following the intervention period. However, the [HHb] TD + τ was significantly reduced following RST only (Pre: 25 ± 8, Post: 20 ± 3 s; $P < 0.05$). The amplitude of the [HHb] response and the

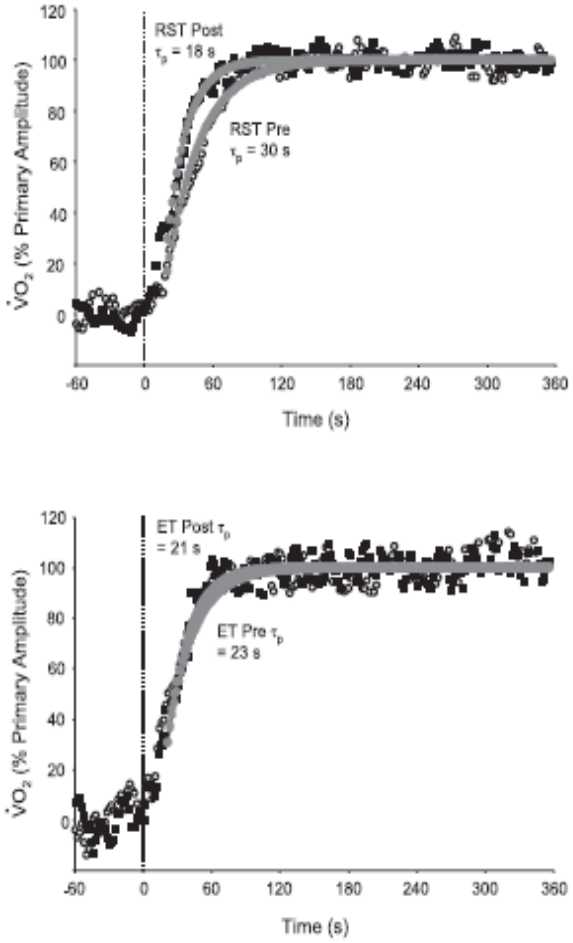

Fig. 1. Pulmonary oxygen uptake ($\dot{V}O_2$) response to a step increment from an unloaded baseline to a moderate-intensity work rate in a representative subject from the repeated sprint training (RST) group (*top*) and the endurance training (ET) group (*bottom*). These data are expressed as a percentage of the overall response. The preintervention (Pre) responses are shown as open circles, and the postintervention (Post) responses are shown as solid squares. The vertical line represents the abrupt transition to the higher work rate. The solid lines represent the monoexponential model fits to the data. Note the significantly faster phase II $\dot{V}O_2$ kinetics following RST. τ_p, phase II time constant.

$\Delta[HHb]/\Delta\dot{V}O_2$ were also significantly increased in RST, but not in ET or CON (Table 4). The changes in $\dot{V}O_2$ τ_p with RST were significantly correlated with changes in the [HHb] TD + τ ($r = 0.71$; $P < 0.05$) and the [HHb] amplitude ($r = -0.81$; $P < 0.05$; Fig. 5); correlations with the [HHb] TD and the [HHb] τ also approached statistical significance (both $r = 0.66$; $P = 0.07$).

Severe-intensity exercise. The TD of the [HHb] response was not different before or after the intervention period in any group. However, a significant interaction effect was observed for the τ of the [HHb] response ($P < 0.05$). Follow-up analyses revealed that the [HHb] τ was significantly reduced in RST (Pre: 12 ± 3, Post: 9 ± 3 s; $P < 0.05$), but not in ET or CON (Table 4). The [HHb] TD + τ also tended to be faster following RST (Pre: 18 ± 6, Post: 15 ± 3 s; $P = 0.12$). The amplitude of the [HHb] response and the $\Delta HHb/\Delta\dot{V}O_2$ for the fundamental phase were significantly increased only in the RST group (Table 4). The [HHb] A_s' and the $\Delta[HHb]/\Delta\dot{V}O_2$ in the slow phase of the response were not altered in any condition (Table 4). The changes in the $\dot{V}O_2$ τ_p following RST were significantly correlated with changes in the [HHb] τ ($r = 0.78$; $P < 0.05$), the [HHb] TD + τ ($r = 0.78$; $P < 0.05$), and the [HHb] amplitude ($r = -0.81$; $P < 0.05$; Fig. 5). The reduction in the $\dot{V}O_2$ slow component with RST was not correlated with changes in [HHb] kinetics.

HR Kinetics

HR kinetics were not altered in the RST, ET, or CON groups for either moderate- or severe-intensity exercise (Table 5). The only significant difference observed was in the end-exercise HR, which was reduced in the RST condition (Table 5). When the fitting window for severe exercise was not constrained (see METHODS), the τ values were slightly shorter (~25 s), and the amplitude of the slow component was slightly larger (~25 beats/min), but again there was no difference between groups before or after the intervention period.

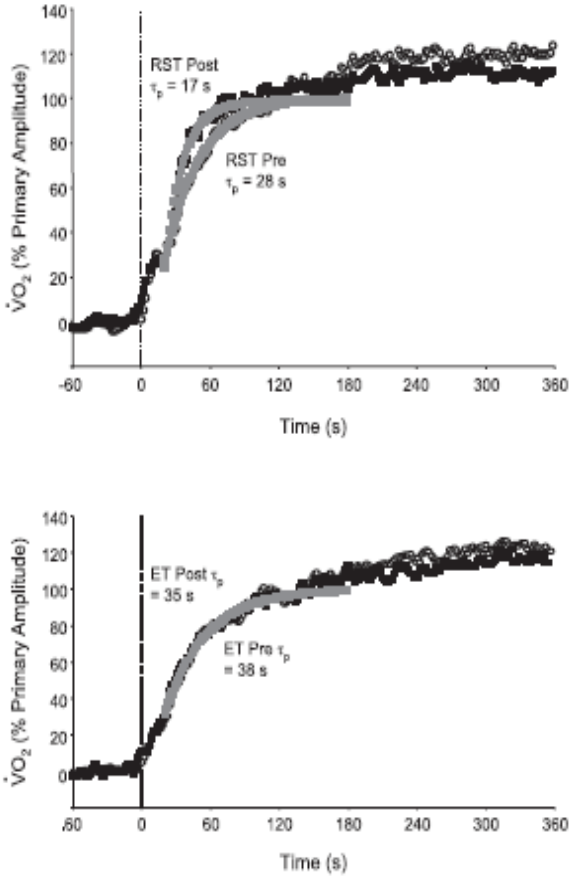

Fig. 2. Pulmonary $\dot{V}O_2$ response to a step increment from an unloaded baseline to a severe-intensity work rate in a representative subject from the RST group (*top*) and the ET group (*bottom*). These data are expressed as a percentage of the overall response. The Pre responses are shown as open circles, and the Post responses are shown as solid squares. The vertical line represents the abrupt transition to the higher work rate. The solid lines represent the monoexponential model fits to the data. Note that RST resulted in a speeding of the phase II $\dot{V}O_2$ kinetics and a reduction of the $\dot{V}O_2$ slow component.

Table 4. *Deoxyhemoglobin/myoglobin kinetics during moderate-intensity and severe-intensity exercise in the RST, ET, and control groups pre- and postintervention*

	RST Pre	RST Post	ET Pre	ET Post	CON Pre	CON Post
Moderate-intensity exercise						
[HHb] baseline, AU	−81±48	−95±41	−81±57	−82±62	−77±52	−83±50
[HHb] time delay, s	14±4	10±2*	14±2	14±4	13±2	12±4
[HHb] time constant, s	11±4	10±2	12±3	10±4	11±4	11±5
[HHb] time delay + time constant, s	25±8	20±3*	26±3	24±6	24±5	23±5
[HHb] amplitude, AU	60±46	73±41*	42±28	41±29	44±33	45±26
Phase II Δ[HHb]/$\Delta\dot{V}O_2$, AU·l^{-1}·min^{-1}	92±69	114±60*	93±39	92±43	85±53	87±42
Severe-intensity exercise						
[HHb] baseline, AU	−127±54	−112±40	−90±47	−117±62	−106±60	−104±60
[HHb] primary time delay, s	6±2	6±2	6±1	7±3	7±1	7±2
[HHb] primary time constant, s	12±3	9±3*	10±3	12±4	10±5	12±6
[HHb] time delay + time constant, s	18±6	15±3	16±7	19±6	17±5	19±6
[HHb] primary-phase amplitude, AU	165±108	220±137†	127±76	138±92	145±92	139±89
Primary-phase Δ[HHb]/$\Delta\dot{V}O_2$, AU·l^{-1}·min^{-1}	91±46	113±52*	89±46	99±42	84±40	80±38
[HHb] slow-phase amplitude, AU	32±20	37±24	24±16	29±18	26±17	32±13
Slow-phase Δ[HHb]/$\Delta\dot{V}O_2$, AU·l^{-1}·min^{-1}	63±54	94±46	52±34	63±42	52±38	68±39

Values are means ± SD. [HHb], deoxyhemoglobin/myoglobin concentration. *Significantly different from RST Pre ($P < 0.05$); †significantly different from RST Pre ($P < 0.01$).

Exercise Tolerance

The alterations in exercise tolerance are illustrated in Fig. 6. A significant time and interaction effect was indicated ($P < 0.01$), and further analyses revealed that, of the three groups, only the RST group demonstrated a significant increase in the time to exhaustion during severe-intensity exercise (Pre: 700 ± 284, Post: 1,074 ± 331 s; $P < 0.01$; Fig. 6). Before RST, exercise tolerance was correlated with A_s' ($r = -0.83$; $P < 0.05$), but not τ_p ($r = -0.40$; $P > 0.05$). After RST, exercise tolerance was correlated with τ_p ($r = -0.81$; $P < 0.05$), but not A_s' ($r = -0.70$; $P = 0.06$). However, the improvement in exercise tolerance with RST was not significantly correlated with either the $\Delta\tau_p$ ($r = -0.58$; $P > 0.05$) or the $\Delta A_s'$ ($r = -0.44$; $P > 0.05$). The change in exercise tolerance was not significantly correlated with the changes in $\dot{V}O_{2peak}$ ($r = 0.13$) or GET ($r = 0.68$).

DISCUSSION

To our knowledge, this investigation is the first to have compared the influence of six sessions of RST (6–8, 22) and work-matched, continuous, moderate-intensity ET on exercise tolerance and the kinetics of $\dot{V}O_2$, [HHb], and HR in young adults. In contrast to our first hypothesis, $\dot{V}O_2$ kinetics were speeded, and exercise tolerance was enhanced, only following RST. However, in support of our second hypothesis, the enhanced $\dot{V}O_2$ kinetics following RST were associated with a greater muscle O_2 extraction (as estimated by changes in [HHb] kinetics; Refs. 17, 26, 27). Indeed, the faster phase II $\dot{V}O_2$ kinetics following RST were significantly correlated with changes in [HHb] kinetics, suggesting that the training intervention impacted $\dot{V}O_2$ kinetics by enhancing muscle fractional O_2 extraction.

Improvements in $\dot{V}O_{2peak}$ (8%) and peak work rate (5%) in the incremental test were only observed following the RST intervention in the present study. Likewise, previous investigators have reported a 7–8% increases in $\dot{V}O_{2peak}$ following 30-s RST (2, 7, 49), although others have reported no effect (6, 8, 33). The $\dot{V}O_2$/work rate slope during incremental exercise and the gain of the fundamental phase of the $\dot{V}O_2$ response to moderate and severe exercise (~10 ml·min^{-1}·W^{-1}) were not altered by training, consistent with other studies that have shown that short-term training interventions do not alter cycling efficiency (4, 35, 53). The GET was not altered by either RST or ET, suggesting that longer term training might be required to impact measurably on this parameter of aerobic function (4, 31, 35, 53).

$\dot{V}O_2$ Kinetics

A novel finding of the present investigation was that just six sessions of RST resulted in 20–25% reductions in both the $\dot{V}O_2$ τ_p and the $\dot{V}O_2$ slow-component amplitude, whereas $\dot{V}O_2$ kinetics were not altered by six sessions of work-matched ET and were also unchanged in the CON group (Fig. 6). A more rapid approach toward, or attainment of, the requisite $\dot{V}O_2$ "steady state" would reduce the O_2 deficit and result in corresponding reductions in energy supply from substrate-level phosphorylation (9, 18, 38, 66) and the accumulation of fatigue-related metabolites, such as H^+ and P_i (1). Moreover, a reduction in the $\dot{V}O_2$ slow component should attenuate the depletion of the muscles' finite PCr and glycogen stores (39, 43, 57, 58). Our results contrast with those of Berger et al. (4), who reported that 6 wk of interval training (3–4 sessions/wk of 20 × 1-min exercise bouts at 90% $\dot{V}O_{2peak}$ separated by 1-min rest) and ET (3–4 sessions/wk of 30-min continuous exercise at 60% $\dot{V}O_{2peak}$) were equally effective in speeding $\dot{V}O_2$ kinetics and reducing the $\dot{V}O_2$ slow component. These authors concluded that the type of exercise training performed (i.e., high-intensity intermittent or low-intensity continuous) was less important than the total volume of training completed. The present study demonstrates that just six sessions of RST (amounting to a total of 17.5 min of exercise over 2 wk) has a greater effect on $\dot{V}O_2$ kinetics than six sessions of work-matched ET (present study), but a similar effect as 6 wk of conventional ET (4). RST, therefore, clearly repre-

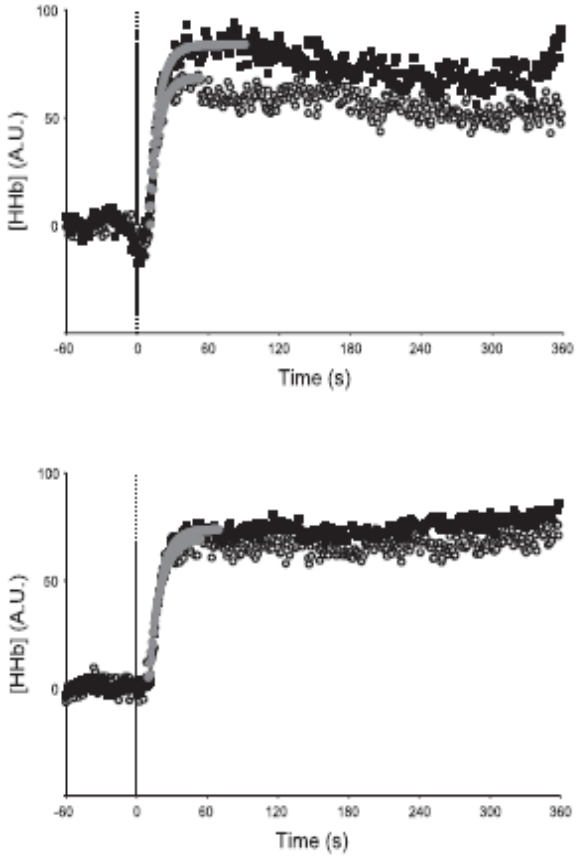

Fig. 3. Muscle deoxyhemoglobin/myoglobin (HHb) response to a step increment from an unloaded baseline to a moderate-intensity work rate in a representative subject from the RST group (*top*) and the ET group (*bottom*). The Pre responses are shown as open circles, and the Post responses are shown as solid squares. The vertical line represents the abrupt transition to the higher work rate. The solid lines represent the monoexponential model fits. [HHb], HHb concentration; AU, arbitrary units.

sents a potent and time-effective strategy for enhancing $\dot{V}O_2$ kinetics, at least in the recreationally active young subjects who participated in the present study.

In contrast to RST, six sessions of ET had no significant effect on the kinetics of $\dot{V}O_2$, HR, or [HHb], or exercise tolerance. Whether or not ET impacts upon $\dot{V}O_2$ kinetics might be dependent on the precise nature of the training program performed (intensity, volume, frequency), as well as its duration (35). Several earlier studies demonstrated that ET, which typically involves three to four sessions per week of continuous exercise at ~60–80% $\dot{V}O_{2peak}$ for 4–8 wk, resulted in significant reductions in the τ_p and/or the $\dot{V}O_2$ slow component (4, 10, 11, 18, 31, 52, 54, 59, 71). One explanation for the lack of effect might be that our subjects were recreationally active in various sports upon their recruitment to the present study. It has been suggested that, once a given level of aerobic fitness has been attained, high-intensity intermittent training, rather than continued ET, is required to elicit further performance gains (47). Moreover, since the RST and ET were work matched in the present study, the combination of a limited number of training sessions ($n = 6$), short session duration (10–30 min), and low training intensity (90% GET, equivalent to 40–45% $\dot{V}O_{2peak}$) may have represented an insufficient stimulus to elicit improvements in the parameters of aerobic fitness and exercise tolerance. The lack of effect of ET on parameters of aerobic fitness and exercise performance in the present study was striking, and it is clear that the program of training completed did not exceed the minimum "threshold" stimulus (i.e., intensity and/or volume and/or frequency and/or duration) necessary to enable $\dot{V}O_2$ kinetics to be altered (4).

Muscle Oxygenation and Estimated O_2 Extraction

We used NIRS to assess the effects of RST and ET on muscle oxygenation during exercise. The [HbO_2] and [Hb_{tot}] values were not different in the three groups before or during moderate or severe intensity, and they were not altered by the intervention period. The observed training adaptations might, therefore, be considered to be independent of changes in blood volume and O_2 content in the area of interrogation. HR kinetics were also not altered by RST or ET in the present study. If HR kinetics broadly reflect leg blood flow kinetics during cycling exercise, as has been suggested for knee-extension exercise (50), these data suggest that bulk O_2 delivery to muscle was not appreciably altered by the training interventions. Longer dura-

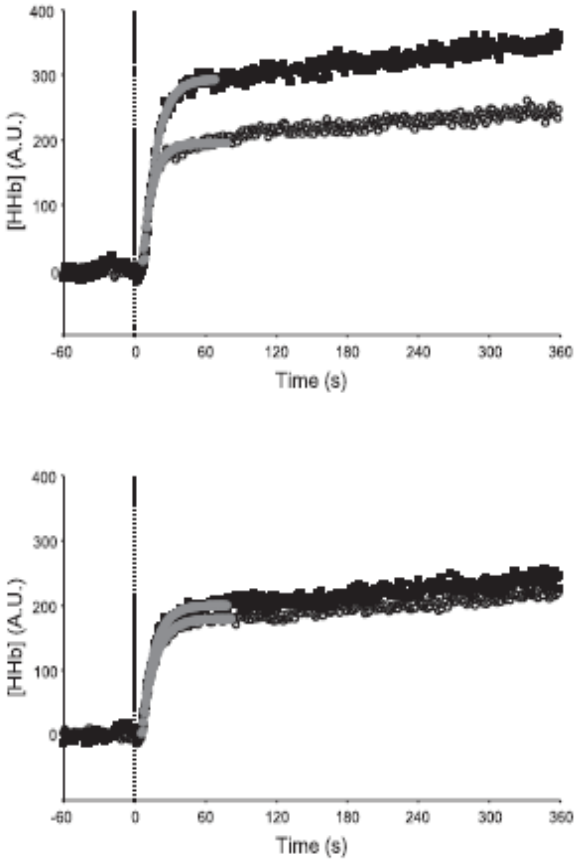

Fig. 4. Muscle HHb response to a step increment from an unloaded baseline to a severe-intensity work rate in a representative subject from the RST group (*top*) and the ET group (*bottom*). The Pre responses are shown as open circles, and the Post responses are shown as solid squares. The vertical line represents the abrupt transition to the higher work rate. The solid lines represent the monoexponential model fits.

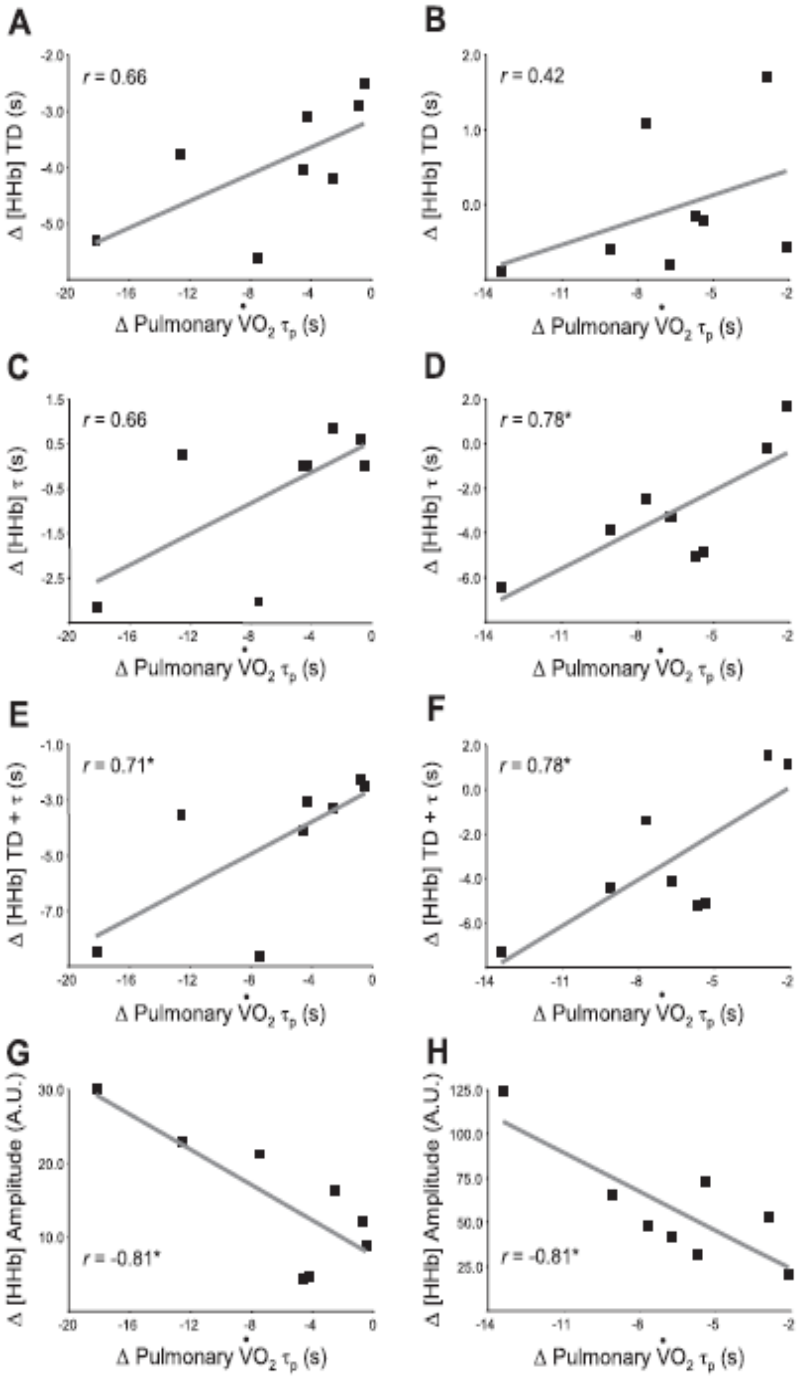

Fig. 5. Relationship between RST-induced changes in phase II $\dot{V}O_2$ kinetics and [HHb] dynamics. Relationships are shown between difference (Δ) in τ_P and Δ[HHb] time delay (TD) during moderate- (*A*) and severe-intensity exercise (*B*); between $\Delta\tau_P$ and Δ[HHb] τ during moderate- (*C*) and severe-intensity exercise (*D*); between $\Delta\tau_P$ and Δ[HHb] TD + τ during moderate- (*E*) and severe-intensity exercise (*F*); and between $\Delta\tau_P$ and Δ[HHb] amplitude during moderate- (*G*) and severe-intensity exercise (*H*). The relationships illustrated in *D*–*H* were statistically significant ($P < 0.05$).

tions of continuous ET (61) and intense interval training (43) have been reported to result in faster conduit artery blood flow kinetics and greater vascular conductance. The HR kinetics and [HbO_2] data in the present study collectively suggest that alterations in bulk O_2 delivery to muscle were not responsible for the effects of RST on $\dot{V}O_2$ kinetics. This might imply that $\dot{V}O_2$ kinetics were not limited by muscle O_2 availability in our subjects, a position that has support in the literature (16, 26, 34, 55, 69, 70). However, it should be acknowledged that our measurements were indirect, and we are, therefore, unable to rule out the possibility that faster muscle blood flow kinetics contributed, in part, to the faster $\dot{V}O_2$ kinetics observed following RST. Indeed, even if bulk muscle blood flow was not altered, it is possible that microvascular adaptations with training enabled a better matching of perfusion to local metabolic rate, perhaps especially in the type II fiber population, which would have received particular stimulus with RST (25, 43, 49). Preferential alterations in blood flow to fibers that were relatively underperfused before training might facilitate greater O_2 extraction and faster $\dot{V}O_2$ kinetics after training (46).

Table 5. *Heart rate kinetics during moderate-intensity and severe-intensity exercise in the RST, ET, and control groups pre- and postintervention*

	RST Pre	RST Post	ET Pre	ET Post	CON Pre	CON Post
Moderate-intensity exercise						
Baseline HR, beats/min	95±9	95±11	93±10	98±11	89±10	92±10
End-exercise HR, beats/min	115±13	115±14	110±11	109±14	110±11	109±10
Primary time constant, s	39±17	35±14	33±24	34±16	37±15	30±17
HR primary amplitude, beats/min	21±6	20±5	16±6	17±5	20±4	20±6
Severe-intensity exercise						
Baseline HR, beats/min	103±15	100±13	90±27	98±29	92±14	90±12
End-exercise HR, beats/min	178±10	171±12*	175±8	172±9	173±9	174±9
Primary time constant, s	35±11	38±6	34±88	31±11	34±12	32±8
HR primary amplitude, beats/min	56±12	58±9	63±8	58±11	65±9	67±9
HR slow component amplitude, beats/min	20±8	15±7	23±9	19±7	19±7	19±5

Values are means ± SD. HR, heart rate. *Significantly different from RST Pre ($P < 0.01$).

The rate and magnitude of [HHb] changes during exercise provide information on the dynamic balance between O_2 delivery and O_2 utilization within the area of interrogation (15–17, 21, 27, 34). To our knowledge, the present study is the first to document the influence of RST and ET on [HHb] kinetics following the onset of exercise. The [HHb] TD and the [HHb] TD + τ were significantly reduced during moderate exercise, the [HHb] τ was significantly reduced during severe exercise, and the [HHb] primary amplitude and Δ[HHb]/$\Delta\dot{V}O_2$ were significantly increased for both moderate and severe exercise in the RST group (but not the ET or CON groups). Assuming that changes in [HHb] dynamics accurately reflect changes in muscle fractional O_2 extraction (17, 21, 27), these data indicate that RST enabled O_2 extraction to be initiated more rapidly, and to a greater extent, following the onset of exercise. Although complicated by possible changes in blood volume following the onset of contractions, the [HHb] TD has been interpreted to represent a period of time during which O_2 delivery to the muscle fibers in the area of interrogation is adequate to meet the augmented O_2 demand following the initiation of contractions (17, 27). A shorter [HHb] TD during moderate exercise following RST, therefore, implies that the active muscle fibers extracted O_2 more rapidly in the metabolic transient. Similarly, the reduced [HHb] τ during severe exercise following RST indicates that, following the initial TD, muscle O_2 extraction increased more rapidly towards the "steady-state" requirement for the metabolic rate. Assuming similar muscle O_2 availability (see earlier discussion), the reduced [HHb] TD and/or τ, along with the greater [HHb] primary amplitude following RST, would be expected to enable muscle O_2 consumption to rise more rapidly following the onset of exercise. Consistent with this interpretation, the faster phase II $\dot{V}O_2$ kinetics observed following RST was significantly correlated with changes in both the amplitude and some temporal aspects of the kinetics of the [HHb] response during both moderate-intensity and severe-intensity exercise (Fig. 5).

It was of interest that RST had a somewhat different effect on [HHb] kinetics during moderate exercise (reduced TD, unchanged τ) and severe exercise (unchanged TD, reduced τ). These data might imply that training resulted in intensity-dependent adaptations in factors that influence the balance between muscle O_2 availability (e.g., muscle pump vs. regulation of vasodilatation; Ref. 63) and O_2 utilization. However, the net result of RST was for [HHb] kinetics to be generally faster and for the amplitude of the response (reflecting the magnitude of O_2 extraction) to be greater. Collectively, the results indicate that the adaptation of muscle O_2 extraction was greater than the adaptation of local muscle blood flow, such that the former contributed more than the latter to the faster $\dot{V}O_2$ kinetics following RST.

Models of respiratory control predict that a speeding of $\dot{V}O_2$ kinetics is contingent upon increased mitochondrial volume (e.g., Ref. 51), and, at least in young, physically active subjects, the phase II $\dot{V}O_2$ response appears to be limited by an inertia of the intracellular oxidative metabolic processes (26, 34, 55, 69, 70). It has been well documented that RST results in increased mitochondrial enzyme activity, including cytochrome-*c* oxidase (22), citrate synthase (8), and pyruvate dehydrogenase (6). Increases in succinate dehydrogenase, malate dehydrogenase, and hexokinase activity have also been reported using a similar training intervention (49). Similar enzymatic adaptations in the present study would be expected to increase the capacity for muscle fractional O_2 extraction, which, in turn, might have facilitated the acceleration of $\dot{V}O_2$ kinetics that we observed.

The increased [HHb] amplitude (and Δ[HHb]/$\Delta\dot{V}O_2$) in the primary response phase observed following RST corroborates the findings of Krustrup et al. (43) during severe-intensity exercise. Specifically, those authors reported that supramaximal, intermittent, one-legged, knee-extensor exercise training resulted in an increased thigh O_2 extraction between 20 and 70 s of exercise, accompanied by an elevated thigh $\dot{V}O_2$ between 20 and 110 s of exercise. Importantly, during the first 75 s of exercise, thigh blood flow was not significantly enhanced posttraining, indicating that the increased $\dot{V}O_2$ was consequent to an increased muscle O_2 extraction. Our results are also consistent with Daussin et al. (13), who recently reported that $\dot{V}O_2$ kinetics were accelerated by interval training (which resulted in increased muscle oxidative capacity), but not by continuous ET (which did not alter oxidative capacity). That $\dot{V}O_2$ kinetics were only accelerated when skeletal muscle oxidative capacity was increased, while improvements in systemic vascular conductance and muscle capillary density were greater following continuous training, also indicates that the acceleration of $\dot{V}O_2$ kinetics was related to an improved capacity for muscle O_2 extraction. It should be cautioned, however,

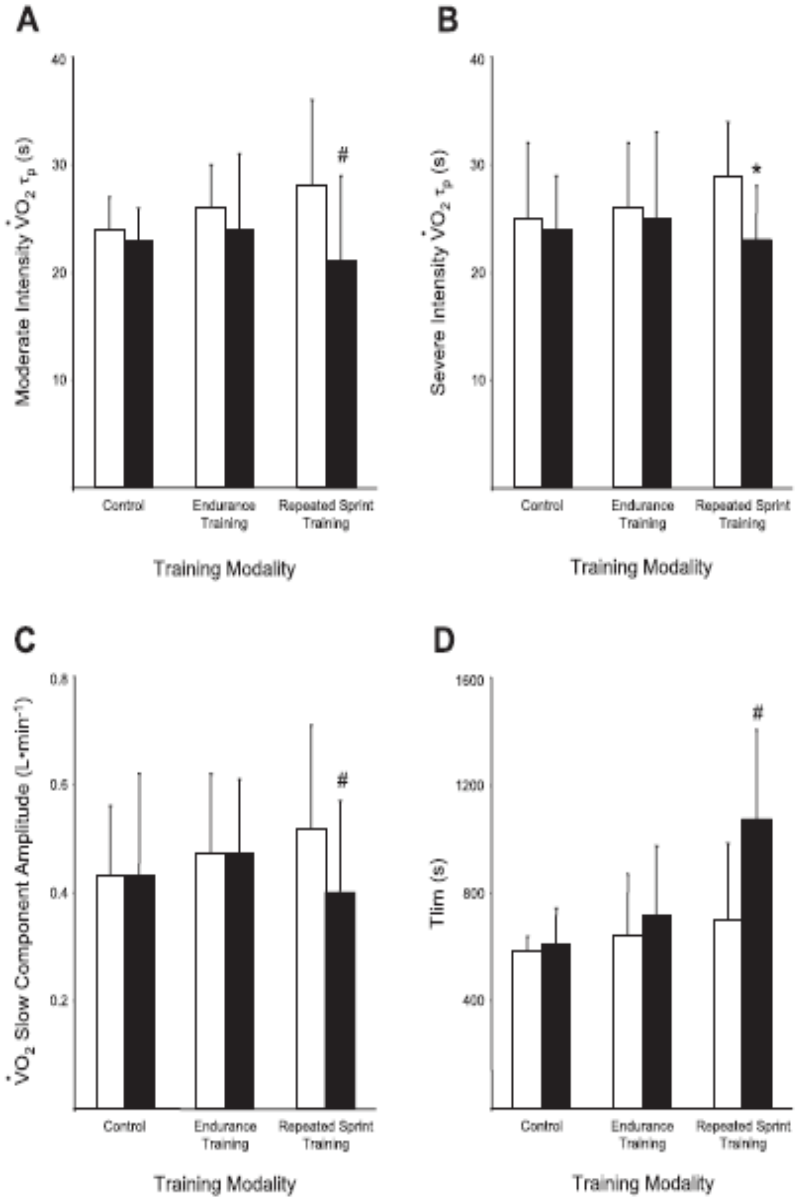

Fig. 6. Group mean ± SD alterations in the parameters of $\dot{V}O_2$ kinetics and exercise tolerance following the control, ET, and RST intervention periods. *A*: changes in moderate exercise phase II $\dot{V}O_2$ kinetics. *B*: changes in severe exercise phase II $\dot{V}O_2$ kinetics. *C*: changes in the $\dot{V}O_2$ slow component amplitude. *D*: changes in exercise tolerance. The Pre responses are shown as open bars, and the Post responses are shown as solid bars. Note that only RST resulted in improvements in $\dot{V}O_2$ kinetics and exercise tolerance. *$P < 0.05$; #$P < 0.01$.

that the τ_p values reported by Daussin et al. (13) were surprisingly long (>60 s) for healthy subjects (e.g., Ref. 16).

Exercise Tolerance

Six sessions of RST resulted in a 53% improvement in exercise tolerance (determined as the time to "exhaustion") during severe-intensity exercise, whereas six sessions of ET resulted in a nonsignificant 13% increase. It has previously been reported that RST resulted in a doubling of exercise time at 80% $\dot{V}O_{2peak}$ (8) and ~10% improvements in time trial performance (6, 22). The available evidence indicates that the ergogenic effect of RST may be attributed, in part, to alterations in muscle oxidative capacity and substrate metabolism (6–8, 22, 23). For example, the rates of glycogen and PCr degradation were reduced, and the rates of carbohydrate and fat utilization were reduced and increased, respectively, during 60 min of cycle exercise at 65% $\dot{V}O_{2peak}$ following RST (7). A greater energetic contribution from oxidative phosphorylation and a reduced reliance on substrate-level phosphorylation, with an attendant reduction in muscle PCr depletion and H^+ and P_i accumulation, are consistent with the faster phase II $\dot{V}O_2$ kinetics and reduced $\dot{V}O_2$ slow-component amplitude observed in the present study. That $\dot{V}O_2$ kinetics have the potential to impact upon exercise tolerance is demonstrated by the significant correlations observed between the $\dot{V}O_2$ τ_p and the time to exhaustion and the $\dot{V}O_2$ slow component and the time to exhaustion, both before and after the intervention period. Although changes in these parameters were not correlated with the improvement in exercise tolerance in the RST group, this might be explained by the relatively small sample size ($n = 8$). Other factors that might have contributed to the enhanced exercise tolerance following RST include the following: *1*) the

small elevation in $\dot{V}O_{2peak}$, which, when combined with the reduced $\dot{V}O_2$ slow-component amplitude, would have delayed the attainment of $\dot{V}O_{2peak}$ and thus the point of rapid substrate depletion and fatiguing metabolite accumulation (9); and 2) a possible increased anaerobic energy yield, resulting from an improved muscle buffering capacity (22).

The features of RST that promote the profound improvements in parameters of aerobic fitness and exercise tolerance documented in this and previous studies (2, 6–8, 22, 49) are not entirely clear. However, in addition to providing near-maximal stress to the energy pathways during the repeated 30-s sprints, the perturbations in substrate availability and metabolite accumulation would require substantial oxidative energy transfer to restore homeostasis following each sprint (5). Moreover, the severe fluctuations in work rate and ATP turnover with RST, and the associated profound perturbations to cellular homeostasis, including changes in oxygen tension and the redox and phosphorylation potentials, might be a potent stimulus to the signaling pathways, resulting in mitochondrial biogenesis (32). One adaptation associated with RST is an increase in the protein content of peroxisome proliferator-activated receptor-γ coactivator-1α (7). A corollary of peroxisome proliferator-activated receptor-γ coactivator-1α expression is the modulation of the skeletal muscle phenotype via a fast- to slow-twitch fiber conversion (48). However, whether this could occur to any appreciable extent following just six training sessions is unclear. Alternatively, given the profound differences in the cadence and force generation requirements of continuous low-intensity ET and RST, it is probable that fiber recruitment differed considerably in the two training groups (25, 60). Specifically, the all-out nature of the RST would have required the recruitment of a large proportion of type II fibers, whereas ET would require the recruitment of predominantly type I fibers (29). It has been shown that interval training induces greater oxidative enzyme adaptations in type II fibers than continuous training (24, 30), and that type IIb fibers manifest greater training-induced elevations in oxidative capacity as training intensity increases above $\dot{V}O_{2peak}$ (20). These fiber-type-specific adaptations would be expected to result in faster $\dot{V}O_2$ kinetics and enhanced tolerance to high-intensity exercise (12, 18, 38, 44).

Conclusion

Six sessions of RST resulted in an acceleration of $\dot{V}O_2$ kinetics during step transitions to both moderate-intensity and severe-intensity exercise and enhanced exercise tolerance. In contrast, six sessions of work-matched ET did not alter $\dot{V}O_2$ kinetics or exercise tolerance. HR kinetics were not altered by either training intervention. However, the acceleration of $\dot{V}O_2$ kinetics with RST was associated with an augmented change in the NIRS-derived muscle deoxygenation signal. These data indicate that RST provokes rapid adaptations of estimated muscle O_2 extraction that facilitated an acceleration of $\dot{V}O_2$ kinetics and improved exercise performance.

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Inspiratory muscle training enhances pulmonary O_2 uptake kinetics and high-intensity exercise tolerance in humans

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Bailey SJ, Romer LM, Kelly J, Wilkerson DP, DiMenna FJ, Jones AM. Inspiratory muscle training enhances pulmonary O_2 uptake kinetics and high-intensity exercise tolerance in humans. *J Appl Physiol* 109: 457–468, 2010. First published May 27, 2010; doi:10.1152/japplphysiol.00077.2010.—Fatigue of the respiratory muscles during intense exercise might compromise leg blood flow, thereby constraining oxygen uptake ($\dot{V}O_2$) and limiting exercise tolerance. We tested the hypothesis that inspiratory muscle training (IMT) would reduce inspiratory muscle fatigue, speed $\dot{V}O_2$ kinetics and enhance exercise tolerance. Sixteen recreationally active subjects (mean ± SD, age 22 ± 4 yr) were randomly assigned to receive 4 wk of either pressure threshold IMT [30 breaths twice daily at ~50% of maximum inspiratory pressure (MIP)] or sham treatment (60 breaths once daily at ~15% of MIP). The subjects completed moderate-, severe- and maximal-intensity "step" exercise transitions on a cycle ergometer before (Pre) and after (Post) the 4-wk intervention period for determination of $\dot{V}O_2$ kinetics and exercise tolerance. There were no significant changes in the physiological variables of interest after Sham. After IMT, baseline MIP was significantly increased (Pre vs. Post: 155 ± 22 vs. 181 ± 21 cmH_2O; $P < 0.001$), and the degree of inspiratory muscle fatigue was reduced after severe- and maximal-intensity exercise. During severe exercise, the $\dot{V}O_2$ slow component was reduced (Pre vs. Post: 0.60 ± 0.20 vs. 0.53 ± 0.24 l/min; $P < 0.05$) and exercise tolerance was enhanced (Pre vs. Post: 765 ± 249 vs. 1,061 ± 304 s; $P < 0.01$). Similarly, during maximal exercise, the $\dot{V}O_2$ slow component was reduced (Pre vs. Post: 0.28 ± 0.14 vs. 0.18 ± 0.07 l/min; $P < 0.05$) and exercise tolerance was enhanced (Pre vs. Post: 177 ± 24 vs. 208 ± 37 s; $P < 0.01$). Four weeks of IMT, which reduced inspiratory muscle fatigue, resulted in a reduced $\dot{V}O_2$ slow-component amplitude and an improved exercise tolerance during severe- and maximal-intensity exercise. The results indicate that the enhanced exercise tolerance observed after IMT might be related, at least in part, to improved $\dot{V}O_2$ dynamics, presumably as a consequence of increased blood flow to the exercising limbs.

inspiratory muscle fatigue; $\dot{V}O_2$ slow component; pulmonary ventilation; dyspnea; exercise performance

THE INCREASED ACTIVATION OF the muscle contractile machinery at the onset of exercise results in an immediate increase in the rate of ATP turnover. However, oxygen uptake ($\dot{V}O_2$) rises exponentially following the start of exercise, with the energy equivalent of the "O_2 deficit" compensated through an increased energy yield from phosphocreatine (PCr) degradation and "anaerobic" glycolysis (36, 45). After the onset of moderate-intensity exercise below the gas-exchange threshold (GET), pulmonary $\dot{V}O_2$ reaches a "steady state" within 2–3 min (68, 69). In contrast, during supra-GET exercise, the development of the so-called $\dot{V}O_2$ slow component delays the attainment of steady state during heavy-intensity exercise (below the critical power) or results in a continued increase in $\dot{V}O_2$ until the maximal O_2 uptake ($\dot{V}O_{2max}$) is reached during severe-intensity exercise (above the critical power) (47, 69, 71). The $\dot{V}O_2$ slow component reflects an increased muscle energy turnover as constant-work-rate exercise proceeds (8, 57) and is associated with a concomitant depletion of the finite PCr stores (57) and greater glycogen utilization (38). Therefore, interventions that speed the overall $\dot{V}O_2$ kinetics, reduce the $\dot{V}O_2$ slow-component amplitude, and/or increase the $\dot{V}O_{2max}$ would be expected to enhance exercise tolerance by reducing the utilization of the finite anaerobic reserves and the accumulation of metabolites associated with the fatigue process (4, 5, 14, 30).

Classically, the respiratory system has been considered to be overbuilt relative to the other components of the O_2 delivery system and was therefore not deemed as rate limiting to limb O_2 delivery or utilization (18, 58, 66). During high-intensity exercise, however, the respiratory muscles consume ~10–15% of the total $\dot{V}O_2$ (1), require up to 14–16% of the cardiac output (25), and are susceptible to fatigue (3, 28). These data suggest that the respiratory system has the potential to limit leg O_2 delivery and thus $\dot{V}O_2$. Fatigue of the inspiratory muscles results in a sympathetically mediated metaboreflex that increases sympathetic efferent discharge (63), thereby reducing limb blood flow both at rest (60, 61) and during exercise (24, 51). Alleviating respiratory muscle work during severe exercise via a proportional assist ventilator prevents diaphragmatic fatigue (2) and increases leg blood flow and the proportional contribution of leg $\dot{V}O_2$ to pulmonary $\dot{V}O_2$ (24). Moreover, reducing the work of breathing with HeO_2 results in a reduction of the $\dot{V}O_2$ slow-component amplitude during severe exercise (17). Conversely, increasing the work of breathing reduces leg blood flow and the proportional contribution of leg $\dot{V}O_2$ to pulmonary $\dot{V}O_2$ (24) and also increases the amplitude of the $\dot{V}O_2$ slow component (16). Importantly, unloading the respiratory muscles increases the tolerance to severe exercise (26, 27, 48), consequent to reductions in peripheral limb muscle fatigue (53) and the sensations of limb discomfort and dyspnea (26, 53). In contrast, loading the respiratory muscles reduces severe exercise tolerance (26, 53), exacerbates limb muscle fatigue (53), and heightens the sensations of limb discomfort and dyspnea (26, 53). Collectively, these data suggest that fatigue of the inspiratory muscles, which is consistently observed in response to high-intensity exercise, compromises leg blood flow, $\dot{V}O_2$, and exercise tolerance, whereas interventions that reduce inspiratory muscle fatigue have the opposite effects.

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One practical intervention that may reduce exercise-induced inspiratory muscle fatigue is inspiratory muscle training (IMT) (44, 54, 65). IMT has been hypothesized to increase leg blood flow and O_2 delivery (43, 56, 74) and has often been associated with improved exercise performance (e.g., 23, 40, 44, 54, 65). A period of IMT has also been shown to reduce peripheral limb muscle fatigue after prior inspiratory muscle fatigue (43), blood lactate accumulation (11, 12, 40, 62), dyspnea (23, 54, 65), limb discomfort (54), and occasionally minute ventilation ($\dot{V}_E$) (11, 40). Reducing $\dot{V}_E$ at a given work rate post-IMT would be expected to reduce the blood flow requirements of the respiratory muscles to such that a greater proportion of cardiac output would be available to distribute to the exercising limbs. Moreover, IMT has the potential to reduce lactate accumulation in the contracting inspiratory muscles (12), which might reduce the stimulation of diaphragm metaboreceptors (51, 61) and vasoconstrictor outflow (63) and thereby increase limb blood flow and O_2 delivery.

It is known that interventions that might increase muscle O_2 delivery, including the performance of priming exercise (4, 13, 32) and the inspiration of a hyperoxic gas mixture (41, 49, 52, 70), result in a reduced $\dot{V}O_2$ slow-component amplitude, faster "overall" $\dot{V}O_2$ kinetics, and an increased tolerance of high-intensity exercise (4, 70). It is therefore possible that IMT might reduce the metabolic requirements of the inspiratory muscles during intense exercise, delaying inspiratory muscle fatigue, facilitating increased limb O_2 availability, and resulting in a reduced $\dot{V}O_2$ slow-component amplitude and improved exercise tolerance. The purpose of the present study was therefore to assess the influence of a 4-wk period of either IMT or a placebo intervention (Sham) on pulmonary $\dot{V}O_2$ kinetics, $\dot{V}_E$, blood lactate accumulation, and exercise tolerance during moderate-, severe-, and maximal-intensity exercise. We hypothesized that IMT, but not Sham, would be expected to reduce the $\dot{V}O_2$ slow-component amplitude and improve exercise tolerance during severe- and maximal-intensity exercise. We also hypothesized that these adaptations in the IMT group would be accompanied by reductions in blood lactate accumulation, $\dot{V}_E$, and the sensations of limb and respiratory discomfort at the same absolute work rates posttraining.

METHODS

Subjects. Sixteen healthy subjects (12 men, mean ± SD age = 22 ± 4 yr, stature = 1.79 ± 0.06 m, body mass = 77 ± 8 kg; and 4 women, age = 20 ± 1 yr, stature = 1.63 ± 0.05 m, body mass = 65 ± 6 kg) volunteered to participate in this study. All subjects were nonsmokers, free from asthma and other respiratory impairments, and had normal pulmonary function (forced vital capacity = 5.21 ± 0.99 liters; forced expiratory volume in 1 s = 4.44 ± 0.86 liters; forced expiratory volume in 1 s/forced vital capacity = 85 ± 7%). The subjects participated in exercise at a recreational level but were not highly trained and were familiar with laboratory exercise testing procedures, having previously participated in studies employing cycle ergometry in our laboratory. The procedures employed in this study were approved by the University of Exeter Research Ethics Committee, and all subjects were required to give their written informed consent before the commencement of the study after the experimental procedures, associated risks, and potential benefits of participation had been explained. Subjects were instructed to arrive at the laboratory in a rested and fully hydrated state, at least 3 h postprandial, and to avoid strenuous exercise in the 24 h preceding each testing session. Each subject was also asked to refrain from caffeine and alcohol 6 and 24 h before each test, respectively. All tests were performed at the same time of day (±2 h).

Experimental design. The subjects were required to report to the laboratory on five occasions over a 2-wk period prior to the intervention period, with all tests separated by at least 24 h. During these five laboratory visits, subjects underwent a number of preliminary tests for the determination of maximum inspiratory pressure (MIP), $\dot{V}O_{2max}$ and GET, $\dot{V}O_2$ kinetics, and exercise tolerance. After completion of the preliminary tests, subjects were randomly assigned to either the IMT or Sham group. After completion of the training protocols, subjects returned to the laboratory on five occasions and repeated all the baseline tests at the same absolute work rates to determine the effect of the respective training interventions on the physiological and performance parameters.

Incremental test. Both before and after the intervention period, the subjects completed a ramp incremental exercise test for determination of the $\dot{V}O_{2max}$ and GET (67). All exercise tests were performed on an electrically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). Initially, subjects performed 3 min of baseline cycling at 0 W, after which the work rate was increased by 30 W/min for male subjects and by 25 W/min for female subjects until the limit of tolerance. The subjects cycled at a self-selected pedal rate (70–90 rpm), and this pedal rate, along with saddle and handle bar height and configuration, was recorded and reproduced in subsequent tests. Breath-by-breath pulmonary gas-exchange data were collected continuously during the incremental tests and averaged over consecutive 10-s periods. The $\dot{V}O_{2max}$ was taken as the highest 30 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 data collected during the incremental test were used to calculate the work rates that were employed during the subsequent step tests. Specifically, the work rates that would require 80% of the GET (termed "moderate" exercise), 60% of the difference between the work rate at the GET and $\dot{V}O_{2max}$ (60%Δ; termed "severe" exercise), and 100% $\dot{V}O_{2max}$ (termed "maximal" exercise) were subsequently calculated, with account taken of 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 GET and peak) (67).

Step tests. Both before and after the intervention period, the subjects completed two step tests on four occasions for the determination of pulmonary $\dot{V}O_2$ kinetics. The protocol comprised one moderate-intensity followed by either one severe-intensity or one maximal-intensity cycle transition. Therefore, all subjects performed a total of four bouts of moderate-intensity exercise, two bouts of severe-intensity exercise, and two bouts of maximal-intensity exercise both before and after the intervention period. The order of the administered step test combinations was randomly assigned and counterbalanced, and each subject completed the same order of step tests following the intervention period. Each transition began with 3 min of baseline cycling at 20 W before an abrupt transition to the target work rate. A passive recovery of 15 min separated the transitions. The four moderate-intensity steps were each of 6-min duration both before and after the intervention period. Of the two severe-intensity transitions performed before and after the intervention period, one was of 6-min duration, whereas the other was continued to the limit of tolerance. The two maximal-intensity transitions performed before the intervention period were both continued to the limit of tolerance. After the intervention period, one of the maximal transitions was continued to the limit of tolerance, whereas the remaining transition was terminated at the preintervention task failure iso-time (defined as the mean limit of tolerance recorded in the two maximal transitions completed before the intervention period). The time to task failure was used as a

measure of exercise tolerance and was recorded when the pedal rate fell more than 10 rpm below the required pedal rate.

Training interventions. After the initial stage of experimental testing, the male and female subjects were ranked separately by baseline MIP and then assigned to either the IMT group (age 20 ± 2 yr, stature 1.75 ± 0.10 m, body mass 74 ± 10 kg) or the Sham group (age 22 ± 4 yr, stature 1.75 ± 0.09 m, body mass 74 ± 9 kg). Both groups contained six male and two female subjects. Each subject performed a total of 1,680 inspiratory maneuvers over a 4-wk period. The IMT group completed 30 dynamic inspiratory efforts twice daily for a 4-wk period against a pressure-threshold load equivalent to ~50% of the MIP, as employed previously (e.g., Refs. 64, 65). The Sham group completed 60 slow protracted breaths once daily for 4 wk at ~15% of the MIP, a protocol known to elicit negligible changes in inspiratory muscle function (54, 65). Subjects were instructed to initiate each breath from residual volume and to continue the inspiratory effort until the maximal lung volume was attained. A nose clip was worn during all breaths, and, to avoid hyperventilation, subjects were instructed to maintain a low breathing frequency. The initial training loads were set by the investigators, and all of the inspiratory efforts were performed using a pressure-threshold device (Powerbreathe, HaB International, Southam, UK). The subjects in the IMT group were instructed to periodically increase the resistive load, such that the completion of 30 breaths approximated the limit of inspiratory muscle tolerance, whereas the subjects in the placebo group were instructed to retain the resistive load set by the investigators for the duration of the training intervention. To ensure that subjects adhered to the specific training requirements of the respective training interventions, subjects reported to the laboratory on a twice weekly basis to ensure that the pressure-threshold load was increasing as expected for the IMT group and was unchanged for the Sham group and to observe that the correct breathing technique was upheld. To ensure that the subjects were naive to the purpose and hypotheses of the investigation, the IMT group was told they were undertaking an inspiratory strength training intervention, and the Sham group was informed that they were undertaking an inspiratory endurance training intervention. All subjects ceased training 48 h before the posttraining exercise tests.

Measurements. During all exercise tests, pulmonary gas exchange and ventilation were measured continuously using a portable metabolic cart (MetaMax 3B, Cortex Biophysik, Leipzig, Germany), as described previously (4–6). A DVT turbine digital transducer measured inspired and expired airflow, and an electrochemical cell O_2 analyzer and infrared CO_2 analyzer simultaneously measured expired gases. Subjects wore nose clips and breathed through a low-dead-space, low-resistance mouthpiece that was securely attached to the volume transducer. The inspired and expired gas volume and gas concentration signals were continuously sampled via a capillary line connected to the mouthpiece. The gas analyzers were calibrated before each test with gases of known concentration, and the turbine volume transducer was calibrated using a 3-liter syringe (Hans Rudolph, Kansas City, MO). Pulmonary gas exchange and ventilation were calculated and displayed breath-by-breath. Heart rate (HR) was measured during all tests using short-range radiotelemetry (Polar S610, Polar Electro Oy, Kempele, Finland).

During one of the transitions to moderate-, severe-, and maximal-intensity exercise, pre- and posttraining, a blood sample was collected from a fingertip into a capillary tube over the 20 s preceding the step transition in work rate and within the last 20 s of exercise. A capillary blood sample was also collected at the limit of tolerance for the severe- and maximal-intensity bouts. These whole blood samples were subsequently analyzed to determine blood lactate concentration (YSI 1500, Yellow Springs Instruments, Yellow Springs, OH) within 30 s of collection. Blood lactate accumulation was calculated as the difference between blood lactate concentration at the end of exercise and blood lactate concentration at baseline. Ratings of perceived exertion (RPE; respiratory and leg discomfort) were also obtained every 2 min using Borg's 6–20 scale.

MIP was assessed in a standing position using a handheld mouth pressure meter (Micro Medical, Kent, UK) as described previously (22). Each MIP was initiated at residual volume, and subjects wore nose clips during the inspiratory maneuvers. A minimum of five well-executed MIP measurements were conducted, and the highest of three measurements within 5 cmH_2O difference was defined as the maximum (22). MIP was assessed pre- and postintervention in the IMT and Sham groups at baseline and at 2 and 10 min following the completion of all exercise bouts. In addition, MIP was assessed after the preintervention task failure iso-time during maximal exercise following the intervention period, where appropriate. The baseline MIP was taken as the mean of the five measurements made at baseline on the testing day in question.

Data analysis procedures. The breath-by-breath $\dot{V}O_2$ data from each test were initially examined to exclude errant breaths caused by coughing, swallowing, sighing, and so forth, and those values that were more than four standard deviations from the local mean were removed. The breath-by-breath data were subsequently linearly interpolated to provide second-by-second values, and, for each individual, like transitions were time aligned to the start of exercise and ensemble averaged to enhance the signal-to-noise ratio and improve confidence in the parameters derived from the model fits (39). The first 20 s of data after the onset of exercise (i.e., the phase I response) were deleted (68), and a nonlinear least-squares algorithm was used to fit the data thereafter. A single-exponential model was used to characterize the $\dot{V}O_2$ responses to moderate exercise, and a biexponential model was used for severe exercise as described in *Eqs. 1* and 2, respectively.

$$\dot{V}O_2(t) = \dot{V}O_{2baseline} + A_p[1 - e^{-(t-TD_p/\tau_p)}] \quad (1)$$

$$\dot{V}O_2(t) = \dot{V}O_{2baseline} + A_p[1 - e^{-(t-TD_p/\tau_p)}] + A_s[1 - e^{-(t-TD_s/\tau_s)}] \quad (2)$$

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time t; $\dot{V}O_{2baseline}$ represents the mean $\dot{V}O_2$ in the baseline period; A_p, TD_p, and τ_p represent the amplitude, time delay, and time constant, respectively, describing the phase II increase in $\dot{V}O_2$ above baseline; and A_s, TD_s, and τ_s represent the amplitude of, time delay before the onset of, and time constant describing the development of the $\dot{V}O_2$ slow component, respectively. We also fit the severe exercise data with a monoexponential model to characterize the overall response dynamics. To quantify the $\dot{V}O_2$ response dynamics during maximal exercise, we first fit the data with a mono- and biexponential model with the fitting window constrained to 120 s to determine the goodness of fit, as determined by the mean squared error. Once the appropriate model fit was identified, we compared the pre- and postintervention maximal exercise $\dot{V}O_2$ response dynamics by *1*) fitting both the pre- and postintervention data to 120 s (*fit 1*); 2) fitting the preintervention data to task failure and the postintervention data to the preintervention task failure iso-time (*fit 2*); and *3*) fitting the pre- and postintervention data both to task failure (*fit 3*).

An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. $\dot{V}O_{2baseline}$ was defined as the mean $\dot{V}O_2$ measured over the final 90 s of baseline pedaling. The end-exercise $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 30 s of exercise. Because the asymptotic value (A_s) of the exponential term describing the $\dot{V}O_2$ slow component may represent a higher value than is actually reached at the end of the exercise, the amplitude of the $\dot{V}O_2$ slow component at the end of exercise was defined as A'_s. The A'_s parameter was compared at the same iso-time (360 s) pre- and posttraining for severe exercise. During maximal exercise, A'_s was compared according to *fits 1–3* as described above. The amplitude of the $\dot{V}O_2$ slow component was also described relative to the entire $\dot{V}O_2$ response.

Table 1. *MIP at baseline and following moderate-intensity, severe-intensity, and maximal-intensity exercise in the IMT and Sham groups pre- and postintervention*

	MIP, cmH_2O			
	IMT Pre	IMT Post	Sham Pre	Sham Post
Baseline MIP	155 ± 22	181 ± 21†	153 ± 34	159 ± 30
Moderate-intensity exercise				
MIP at 2 min	151 ± 26	175 ± 27†	149 ± 26	155 ± 24
MIP at 10 min	150 ± 27	177 ± 24†	148 ± 27	157 ± 26
Severe-intensity exercise				
MIP at 2 min	124 ± 28*	168 ± 29*†	143 ± 31*	142 ± 37*
MIP at 10 min	120 ± 23*	173 ± 28†	140 ± 33*	146 ± 27*
MIP at 2 min Tlim	120 ± 28*	165 ± 33*†	138 ± 32*	143 ± 28*
MIP at 10 min Tlim	118 ± 16*	167 ± 31*†	138 ± 36*	141 ± 29*
Maximal-intensity exercise				
MIP at 2 min ISO	128 ± 21*	160 ± 33*†	140 ± 34*	143 ± 28*
MIP at 10 min ISO	132 ± 20*	164 ± 33*†	145 ± 32*	144 ± 28*
MIP at 2 min Tlim	128 ± 21*	167 ± 36†	140 ± 34*	142 ± 29*
MIP at 10 min Tlim	132 ± 20*	171 ± 35†	145 ± 32*	143 ± 25*

Values are means ± SD. IMT, inspiratory muscle training; ISO, preintervention task failure iso-time; MIP, maximum inspiratory pressure; Pre, preintervention; Post, postintervention; Sham, placebo intervention; Tlim, limit of tolerance. *Significantly different from baseline ($P < 0.05$); †significantly different from Pre ($P < 0.05$).

We also modeled the HR response to exercise in each condition. For this analysis, HR data were linearly interpolated to provide second-by-second values, and, for each individual, identical repetitions from like transitions were time aligned to the start of exercise and ensemble averaged. A nonlinear least-square monoexponential model without time delay was used to fit the data to moderate-, severe-, and maximal-intensity exercise, with the fitting window commencing at $t = 0$ s. The derived HR mean response time provides an insight into the overall rate of adjustment of HR dynamics. To determine whether $\dot{V}_E$ was influenced by the IMT or Sham intervention, we compared $\dot{V}_E$ at baseline (90 s preceding step transition) and at 120 s, at 360 s, and at exhaustion (average response over the final 30 s) during moderate-, severe-, and maximal-intensity exercise before and after the intervention period, as appropriate.

Statistics. A two-way (time-by-group) ANOVA with repeated measures for time was employed to determine the effects on the relevant physiological variables elicited by the different interventions. Where the analysis revealed a significant main or interaction effect, simple follow-up contrasts were employed to determine the origin of such effects. The relationship between changes in MIP and changes in $\dot{V}O_2$ kinetics and exercise tolerance were assessed with Pearson product moment correlation coefficients. All data are presented as means ± SD unless otherwise indicated. Statistical significance was accepted at $P < 0.05$.

RESULTS

All subjects were recreationally active on recruitment to the study, and the physiological parameters of interest (i.e., MIP, $\dot{V}O_{2max}$, $\dot{V}O_2$ at GET, $\dot{V}O_2$ phase II τ_p, $\dot{V}O_2$ slow-component amplitude) were not significantly different between the IMT and Sham groups before the commencement of the study (Tables 1–3). Each subject self reported 100% compliance to the prescribed training and also self-reported that they did not alter their physical activity for the duration of the study.

Incremental test. The $\dot{V}O_{2max}$ preintervention was 47 ± 5 $ml \cdot kg^{-1} \cdot min^{-1}$ in the IMT group and 48 ± 8 $ml \cdot kg^{-1} \cdot min^{-1}$ in the Sham group, with the GET occurring at ~50% $\dot{V}O_{2max}$ in both groups. There were no significant changes in the $\dot{V}O_2$, $\dot{V}_E$, or work rate attained at the GET or at peak exercise during the incremental test following either the IMT or Sham interventions.

Maximum inspiratory pressure. The MIP at baseline and after moderate-, severe-, and maximal-intensity exercise for the IMT and Sham groups, pre- (Pre) and postintervention (Post), are presented in Table 1. There was a significant interaction effect between the IMT and Sham groups ($P < 0.01$) such that baseline MIP was significantly increased (17%) after IMT (155 ± 22 Pre vs. 181 ± 21 cmH_2O Post; $P < 0.01$) but not changed with Sham (153 ± 34 Pre vs. 159 ± 30 cmH_2O Post).

Compared to baseline, the MIP was not significantly reduced at 2 or 10 min after the completion of moderate exercise either before or after the intervention in either group (Table 1). However, a significant interaction effect was observed for the absolute MIP at both time points after moderate exercise ($P < 0.05$), with MIP being significantly increased in the IMT group ($P < 0.05$) but not in the Sham group ($P > 0.05$). After 6 min of severe exercise and severe exercise to exhaustion, MIP was reduced compared with baseline in the IMT and Sham groups both before and after the intervention period (Table 1). However, after the intervention period, the absolute MIP after severe exercise manifested a significant interaction effect ($P < 0.01$), with the MIP increased in the IMT group ($P < 0.05$) but not in the Sham group ($P > 0.05$; Table 1). Ten minutes after the completion of 6 min of severe exercise, MIP was not significantly different from the baseline value in the IMT group but remained significantly below the baseline value at all time points after Sham (Table 1). Similar to results with severe exercise, MIP was significantly reduced below the baseline following maximal exercise in both the IMT and Sham groups prior to the intervention period (Table 1). After the intervention period, there was a significant interaction effect between the IMT and Sham groups for the MIP after maximal exercise ($P < 0.05$), with the absolute MIP being significantly increased at the preintervention exhaustion time (iso-time) and also after

Table 2. *$\dot{V}_E$ during moderate-intensity, severe-intensity, and maximal-intensity exercise in the IMT and Sham groups pre- and postintervention*

	$\dot{V}_E$, l/min			
	IMT Pre	IMT Post	SHAM Pre	SHAM Post
Moderate-intensity exercise				
Baseline $\dot{V}_E$	23 ± 3	24 ± 4	21 ± 2	22 ± 2
$\dot{V}_E$ at 120 s	33 ± 7	33 ± 7	29 ± 3	29 ± 2
$\dot{V}_E$ at 360 s	34 ± 7	35 ± 7	30 ± 3	30 ± 2
Severe-intensity exercise				
Baseline $\dot{V}_E$	23 ± 4	24 ± 5	23 ± 3	22 ± 2
$\dot{V}_E$ at 120 s	78 ± 17	78 ± 17	73 ± 14	73 ± 14
$\dot{V}_E$ at 360 s	106 ± 35	102 ± 29	88 ± 15	73 ± 14
$\dot{V}_E$ at exhaustion	135 ± 47	138 ± 45	120 ± 26	118 ± 13
Maximal-intensity exercise				
Baseline $\dot{V}_E$	25 ± 5	25 ± 4	23 ± 2	24 ± 3
$\dot{V}_E$ at 120 s	126 ± 33	119 ± 32*	124 ± 29	120 ± 29
$\dot{V}_E$ at exhaustion	148 ± 46	148 ± 45	139 ± 27	140 ± 22

Values are means ± SD. $\dot{V}_E$, minute ventilation. *Significantly different from Pre ($P < 0.05$).

maximal exercise to exhaustion in the IMT ($P < 0.05$) group but not in the Sham group ($P > 0.05$). Moreover, the MIP was not significantly different from the baseline after exhaustive maximal exercise post-IMT, whereas it remained significantly below baseline following Sham (Table 1).

Pulmonary ventilation. The $\dot{V}E$ results at baseline and during moderate-, severe-, and maximal-intensity exercise in the IMT and Sham groups for Pre and Post are presented in Table 2. There was no significant difference in the absolute $\dot{V}E$ during moderate and severe exercise after either the IMT or Sham interventions. During maximal exercise, however, there was a significant main effect for time for the $\dot{V}E$ at 120 s ($P < 0.05$). Follow-up analyses revealed that the $\dot{V}E$ at 120-s was significantly reduced after IMT ($P < 0.05$) but was not significantly different after Sham ($P > 0.05$; Table 2).

$\dot{V}O_2$ kinetics. The parameters of $\dot{V}O_2$ dynamics before and after the IMT and Sham interventions during moderate-, severe-, and maximal-intensity exercise are presented in Table 3 and illustrated in Figs. 1, 2, and 3, respectively. The dynamics of $\dot{V}O_2$ during moderate exercise were unaffected by the IMT and Sham interventions (Table 3, Fig. 1).

There was a significant interaction effect between the IMT and Sham groups for the overall $\dot{V}O_2$ kinetics during severe exercise ($P < 0.01$; as assessed with a monoexponential function), with follow-up analyses revealing that the overall $\dot{V}O_2$ kinetics was 21% faster after IMT (99 ± 18 s Pre vs. 78 ± 13 s Post; $P < 0.01$) but was not different after Sham. The phase II $\dot{V}O_2$ time constant was not significantly altered by IMT or Sham. However, there was a significant main effect for time for the fundamental and slow-component $\dot{V}O_2$ amplitudes ($P < 0.05$). Subsequent analyses revealed that the fundamental component $\dot{V}O_2$ amplitude was significantly increased (1.77 ± 0.41 l/min Pre vs. 1.92 ± 0.49 l/min Post; $P < 0.05$) and the $\dot{V}O_2$ slow-component amplitude was significantly reduced (0.60 ± 0.20 l/min Pre vs. 0.53 ± 0.24 l/min Post; $P < 0.05$) during severe exercise after IMT, whereas these parameters were not significantly different after Sham (Table 3, Fig. 2). The $\dot{V}O_2$ at exhaustion was not significantly different from the $\dot{V}O_{2max}$ measured in the initial incremental test either before or after the interventions.

During maximal exercise, there was a significant interaction effect between the IMT and Sham groups for the $\dot{V}O_2$ at 120 s

Table 3. *Pulmonary $\dot{V}O_2$ dynamics during moderate-intensity, severe-intensity, and maximal-intensity exercise in the IMT and Sham groups pre- and postintervention*

	IMT Pre	IMT Post	SHAM Pre	SHAM Post
		Moderate-intensity exercise		
Baseline $\dot{V}O_2$, l/min	0.93 ± 0.11	0.94 ± 0.11	0.93 ± 0.08	0.92 ± 0.09
End-exercise $\dot{V}O_2$, l/min	1.50 ± 0.27	1.56 ± 0.28	1.44 ± 0.20	1.43 ± 0.16
Phase II time constant, s	22 ± 7	23 ± 7	21 ± 5	21 ± 8
Fundamental amplitude, l/min	0.58 ± 0.19	0.60 ± 0.20	0.51 ± 0.18	0.51 ± 0.17
Mean response time, s	37 ± 5	38 ± 7	38 ± 5	40 ± 11
		Severe-intensity exercise		
Baseline $\dot{V}O_2$, l/min	0.96 ± 0.12	0.94 ± 0.11	0.95 ± 0.08	0.97 ± 0.09
$\dot{V}O_2$ at 360 s, l/min	3.32 ± 0.69	3.37 ± 0.74	3.17 ± 0.49	3.20 ± 0.44
$\dot{V}O_2$ at exhaustion, l/min	3.42 ± 0.73	3.47 ± 0.74	3.25 ± 0.42	3.28 ± 0.41
Phase II time constant, s	26 ± 7	27 ± 6	27 ± 7	29 ± 7
Fundamental amplitude, l/min	1.77 ± 0.41	1.92 ± 0.49*	1.77 ± 0.26	1.81 ± 0.33
Slow-component amplitude, l/min	0.60 ± 0.20	0.53 ± 0.24*	0.46 ± 0.20	0.43 ± 0.16
Slow-component amplitude, %end	25 ± 3	21 ± 4†	20 ± 6	19 ± 6
Overall time constant, s	99 ± 18	78 ± 13†	74 ± 19	74 ± 12
		Maximal-intensity exercise		
Baseline $\dot{V}O_2$, l/min	0.98 ± 0.12	1.03 ± 0.17	1.01 ± 0.10	0.99 ± 0.12
$\dot{V}O_2$ at 120 s, l/min	3.31 ± 0.63	3.41 ± 0.64†	3.34 ± 0.43	3.35 ± 0.48
$\dot{V}O_2$ at exhaustion, l/min	3.42 ± 0.65	3.61 ± 0.68†	3.44 ± 0.44	3.48 ± 0.48
Biexponential *fit 1* (120 s vs. 120 s)				
Phase II time constant, s	20 ± 6	23 ± 7	20 ± 6	19 ± 5
Fundamental amplitude, l/min	2.07 ± 0.45	2.23 ± 0.52*	2.16 ± 0.43	2.19 ± 0.45
Mean response time, s	30 ± 5	32 ± 5	30 ± 3	29 ± 3
Slow-component amplitude, l/min	0.28 ± 0.14	0.18 ± 0.07*	0.19 ± 0.10	0.18 ± 0.14
Slow-component amplitude, %end	12 ± 4	7 ± 2*	9 ± 5	8 ± 7
Biexponential *fit 2* (Tlim vs. ISO)				
Phase II time constant, s	20 ± 6	24 ± 7	21 ± 6	19 ± 6
Fundamental amplitude, l/min	2.08 ± 0.46	2.23 ± 0.50	2.17 ± 0.41	2.19 ± 0.44
Mean response time, s	31 ± 5	32 ± 4	30 ± 5	29 ± 3
Slow-component amplitude, l/min	0.41 ± 0.21	0.35 ± 0.18	0.28 ± 0.14	0.30 ± 0.18
Slow-component amplitude, %end	16 ± 6	13 ± 6†	12 ± 6	12 ± 8
Biexponential *fit 3* (Tlim vs. Tlim)				
Phase II time constant, s	20 ± 6	23 ± 7	21 ± 6	19 ± 6
Fundamental amplitude, l/min	2.08 ± 0.46	2.21 ± 0.51	2.17 ± 0.41	2.19 ± 0.44
Mean response time, s	31 ± 5	32 ± 5	30 ± 5	29 ± 3
Slow-component amplitude, l/min	0.41 ± 0.21	0.41 ± 0.21	0.28 ± 0.14	0.33 ± 0.17
Slow-component amplitude, %end	16 ± 6	16 ± 7	12 ± 6	13 ± 7

Values are means ± SD. $\dot{V}O_2$, oxygen uptake. *Significantly different from Pre ($P < 0.05$); †significantly different from Pre ($P < 0.01$).

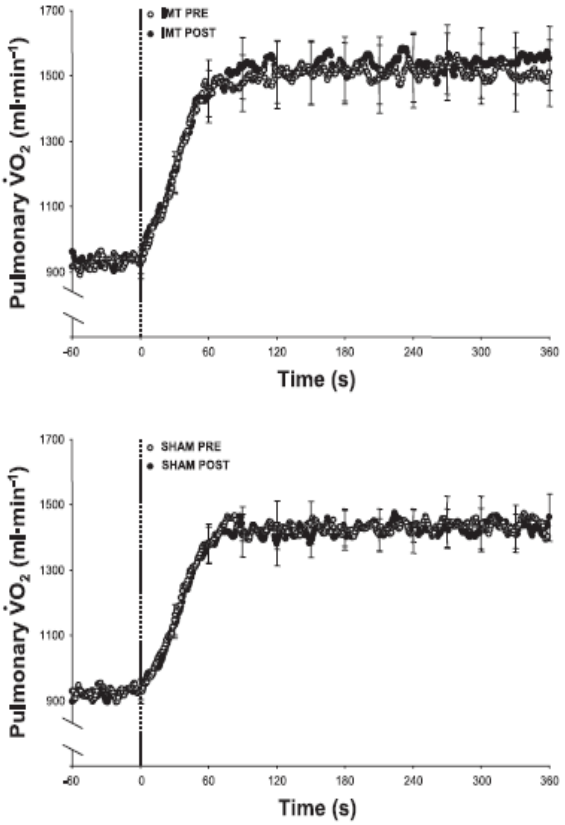

Fig. 1. Group mean ± SE pulmonary oxygen uptake ($\dot{V}O_2$) responses to a step increment from an unloaded baseline to a moderate-intensity work rate in the inspiratory muscle training (IMT) group (*top*) and the sham intervention (Sham) group (*bottom*). ○, Preintervention (Pre) responses; ●, postintervention (Post) responses. The vertical line represents the abrupt transition to the higher work rate. Note that pulmonary $\dot{V}O_2$ kinetics was unaffected by both IMT and Sham.

($P < 0.05$), with a significantly greater $\dot{V}O_2$ at 120 s after IMT (3.31 ± 0.63 l/min Pre vs. 3.41 ± 0.64 l/min Post; $P < 0.01$) but not after Sham. During maximal exercise, the $\dot{V}O_2$ phase II time constant and the mean response time were unaffected after the IMT and Sham interventions regardless of the fitting procedure employed (Table 3). However, using *fit 1*, the $\dot{V}O_2$ fundamental component amplitude was significantly increased (2.07 ± 0.45 l/min Pre vs. 2.23 ± 0.52 l/min Post; $P < 0.05$), and the $\dot{V}O_2$ slow-component amplitude was significantly reduced after IMT (0.28 ± 0.14 l/min Pre vs. 0.18 ± 0.07 l/min Post; $P < 0.05$), whereas these parameters were not significantly different after Sham (Fig. 3). There was a significant main effect for time ($P < 0.05$) with no interaction effect ($P > 0.05$) for the peak value of $\dot{V}O_2$ attained during maximal exercise. The follow-up *t*-test revealed that $\dot{V}O_2$ at exhaustion was significantly greater after IMT than before IMT ($P < 0.01$). The $\dot{V}O_2$ at exhaustion was significantly lower ($P < 0.05$) than the $\dot{V}O_{2max}$ measured in the initial incremental test before, but not after, the IMT intervention.

HR dynamics and blood lactate concentration. HR was not significantly different during moderate-, severe-, and maximal-intensity exercise after the IMT and Sham intervention periods (Table 4). Blood lactate accumulation was unaffected after the IMT and Sham interventions during moderate exercise. However, there was a significant interaction effect for the blood lactate accumulation over the first 360 s of severe exercise ($P < 0.05$), whereby blood lactate accumulation was significantly reduced after IMT (6.0 ± 1.2 mM Pre vs. 5.0 ± 1.0 mM Post; $P < 0.01$) but was not significantly altered after Sham (Table 4). During maximal exercise, the blood lactate concentration at exhaustion was significantly greater after IMT (7.2 ± 1.8 mM Pre vs. 9.0 ± 1.2 mM Post; $P < 0.01$) but was not significantly different after Sham.

Ratings of dyspnea and limb discomfort. Ratings of dyspnea and limb discomfort were not significantly different during moderate exercise following either the IMT or Sham interventions (Table 5). During severe exercise, however, ratings of dyspnea ($P < 0.01$) and limb discomfort ($P < 0.05$) were significantly reduced at 2, 4, and 6 min of exercise following IMT, whereas only limb RPE at 2 min was reduced after the sham intervention ($P < 0.05$). During maximal exercise, dyspnea, but not limb discomfort, was significantly reduced at 2 min of exercise after IMT, but neither dyspnea nor limb discomfort was affected by the Sham intervention.

Exercise tolerance. A significant main effect for time ($P < 0.01$) was observed for severe-intensity exercise tolerance. Four weeks of IMT resulted in a 39% improvement in severe-

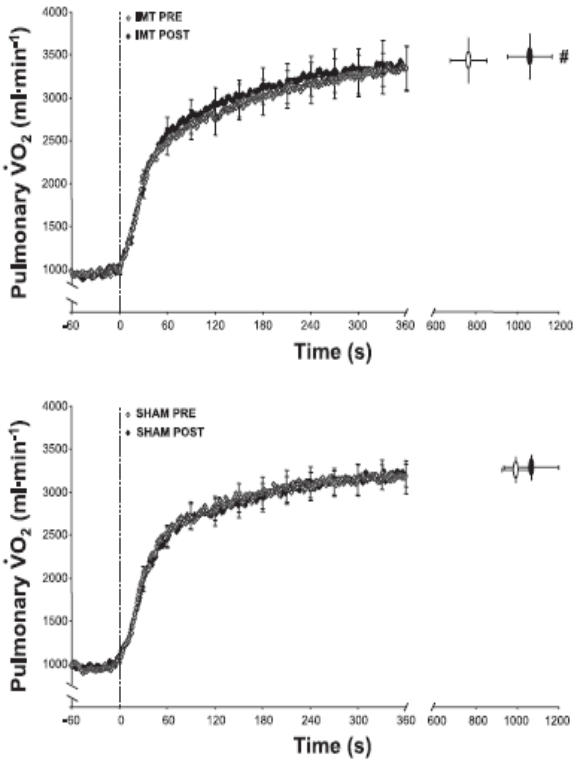

Fig. 2. Group mean ± SE pulmonary $\dot{V}O_2$ responses to a step increment from an unloaded baseline to a severe-intensity work rate in the IMT group (*top*) and Sham group (*bottom*). ○, Pre responses; ●, Post responses. The vertical line represents the abrupt transition to the higher work rate. Note that the overall pulmonary $\dot{V}O_2$ kinetics was significantly faster post-IMT consequent to an increased fundamental and reduced slow-component amplitude, whereas these parameters were not affected by Sham intervention. Exercise tolerance was significantly enhanced post-IMT but not post-Sham. #Significantly different from Pre ($P < 0.01$).

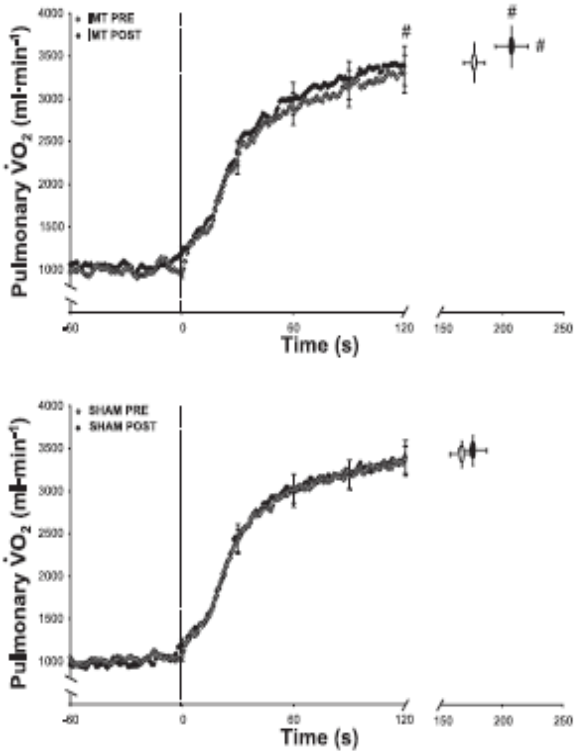

Fig. 3. Group mean ± SE pulmonary $\dot{V}O_2$ response to a step increment from an unloaded baseline to a maximal work rate in the IMT group (*top*) and Sham group (*bottom*). ○, Pre responses; ●, Post responses. The vertical line represents the abrupt transition to the higher work rate. The pulmonary $\dot{V}O_2$ was significantly greater at 120 s and at exhaustion post-IMT but not post-Sham. Exercise tolerance was significantly enhanced post-IMT but not post-Sham. #Significantly different from Pre ($P < 0.01$).

intensity exercise tolerance (765 ± 249 s Pre vs. 1,061 ± 304 s Post; $P < 0.01$), whereas the 8% increase after Sham was not significant (992 ± 188 s Pre vs. 1,068 ± 374 s Post; Fig. 4). A significant interaction effect between groups was observed for maximal-intensity exercise tolerance ($P < 0.01$), with maximal exercise tolerance being significantly enhanced (by 18%) after IMT (177 ± 24 s Pre vs. 208 ± 37 s Post; $P < 0.01$) but not significantly different (5%) after Sham (167 ± 28 s Pre vs. 176 ± 32 s Post; Fig. 4).

Relationships between changes in MIP and changes in $\dot{V}O_2$ kinetics and exercise tolerance. Although IMT resulted in improvements in baseline MIP and in $\dot{V}O_2$ kinetics and exercise tolerance during severe- and maximal-intensity exercise, the changes in these variables were not significantly correlated. For example, changes in baseline MIP were not significantly correlated with changes in overall $\dot{V}O_2$ kinetics (severe: $r = 0.20$, $P = 0.63$; maximal: $r = -0.62$, $P = 0.10$) or the $\dot{V}O_2$ slow-component amplitude (severe: $r = 0.20$, $P = 0.63$; maximal: $r = -0.62$, $P = 0.10$). Changes in baseline MIP were also not significantly correlated with changes in exercise tolerance during severe-intensity ($r = 0.60$, $P = 0.12$) or maximal-intensity ($r = 0.29$, $P = 0.48$) exercise.

DISCUSSION

This is the first investigation to comprehensively assess the influence of IMT on $\dot{V}O_2$ kinetics and exercise tolerance across a range of exercise intensities. The principal original finding was that, compared with Sham training, 4 wk of pressure threshold IMT resulted in significant changes in several features of the dynamic $\dot{V}O_2$ response to severe- and maximal-intensity (but not moderate-intensity) exercise in young healthy adults. For severe exercise, the faster overall $\dot{V}O_2$ dynamics was accompanied by reductions in blood lactate accumulation and the perceptions of dyspnea and limb discomfort at the same absolute work rate posttraining and improved exercise tolerance. Similarly, during maximal exercise, the $\dot{V}O_2$ slow-component amplitude was reduced, and end-exercise $\dot{V}O_2$ and exercise tolerance were increased after IMT. These results demonstrate that IMT, presumably through reducing the extent of fatigue and therefore the metabolic requirements of the inspiratory muscles during high-intensity exercise, enhanced $\dot{V}O_2$ dynamics and exercise tolerance. The improvements in exercise tolerance after IMT that have been reported previously (e.g., 23, 40, 44, 54, 65) might therefore be linked, at least in part, to changes in $\dot{V}O_2$ kinetics.

MIP and pulmonary ventilation. We observed a 17% increase in baseline MIP following IMT, indicating that the maximum force-generating capacity of the inspiratory muscles was substantially increased post-IMT, consistent with earlier studies (44). Moderate exercise did not cause significant changes in MIP. However, after severe exercise before the intervention period, the MIP was significantly reduced (by ~23%) below the baseline value, suggestive of the development of inspiratory muscle fatigue. After IMT, the extent of this fatigue was reduced, with the MIP being within 9% of the baseline, consistent with previous studies (54, 65). Furthermore, 10 min after 6 min of severe-intensity exercise, the MIP was not significantly different from the baseline value, indicating that the inspiratory muscles recovered more rapidly after IMT than after Sham. Short-duration maximal-intensity exercise also resulted in a significant reduction (by ~18%) of MIP below the baseline value. The extent of this reduction was reduced after IMT (MIP was 8% lower than baseline).

The absolute $\dot{V}E$ during moderate- and severe-intensity exercise was not different after IMT or Sham. However, $\dot{V}E$ was slightly but significantly reduced at 2 min during maximal-intensity exercise after IMT. Some previous studies have also reported reductions in $\dot{V}E$ after IMT (10, 11, 40), although others have found no change (62, 64, 73). This reduction in $\dot{V}E$ during maximal-intensity exercise might have contributed to the reduced degree of inspiratory muscle fatigue that was measured and the reduced perception of dyspnea. A reduction in $\dot{V}E$ for the same work rate after IMT might also reduce the metabolic requirements of, and thus blood flow commandeered by, the respiratory muscles.

Fatigue of the inspiratory muscles stimulates diaphragm innervating metaboreceptors (7), invoking sympathetically mediated vasoconstrictor outflow (63) and a reduction in limb blood flow (60). It is known that the metaboreflex is activated once a threshold of fatiguing inspiratory work has been exceeded (61) and in response to the accumulation of fatigue-related metabolites, including lactic acid (51). We observed a reduced blood lactate accumulation during the first 6 min of severe exercise after IMT, which may be, in part, due to the reduced inspiratory muscle fatigue (12). Therefore, the reduced blood lactate accumulation and inspiratory muscle fatigue might have reduced the magnitude of the metaboreflex (74)

Table 4. *Heart rate and blood lactate concentration dynamics during moderate-intensity, severe-intensity, and maximal-intensity exercise in the IMT and Sham groups pre- and postintervention*

	IMT Pre	IMT Post	SHAM Pre	SHAM Post
		Moderate-intensity exercise		
Baseline HR, beats/min	90 ± 8	91 ± 6	100 ± 13	99 ± 12
End-exercise HR, beats/min	109 ± 8	110 ± 7	113 ± 14	111 ± 13
HR Fundamental time constant, s	32 ± 10	29 ± 9	27 ± 16	28 ± 15
Baseline blood [lactate], mM	1.0 ± 0.1	1.1 ± 0.3	0.9 ± 0.4	0.9 ± 0.3
End-exercise blood [lactate], mM	1.2 ± 0.4	1.3 ± 0.6	0.9 ± 0.4	1.0 ± 0.3
ΔBlood [lactate], mM	0.2 ± 0.5	0.3 ± 0.4	0.0 ± 0.3	0.1 ± 0.5
		Severe-intensity exercise		
Baseline HR, beats/min	93 ± 7	92 ± 7	101 ± 15	99 ± 14
HR at 120 s, beats/min	151 ± 9	151 ± 8	155 ± 13	153 ± 11
HR at 360 s, beats/min	168 ± 8	168 ± 9	170 ± 11	169 ± 9
HR mean response time, s	63 ± 18	58 ± 18	58 ± 17	61 ± 18
Baseline blood [lactate], mM	0.9 ± 0.2	1.0 ± 0.2	0.8 ± 0.3	0.9 ± 0.2
Blood [lactate] at 360 s, mM	6.9 ± 1.3	6.0 ± 1.1*	5.0 ± 1.0	4.8 ± 1.0
Blood [lactate] at exhaustion, mM	8.8 ± 2.2	8.5 ± 1.8	6.9 ± 1.2	6.5 ± 1.3
ΔBlood [lactate] 360 s-baseline, mM	6.0 ± 1.2	5.0 ± 1.0†	4.1 ± 0.8	3.9 ± 1.0
ΔBlood [lactate] exhaustion-baseline, mM	7.9 ± 2.1	7.5 ± 1.7	6.1 ± 1.1	5.7 ± 1.4
		Maximal-intensity exercise		
Baseline HR, beats/min	91 ± 6	92 ± 6	102 ± 10	102 ± 14
HR at 120 s, beats/min	164 ± 7	164 ± 8	170 ± 9	169 ± 9
HR mean response time, s	38 ± 20	34 ± 10	27 ± 7	26 ± 4
Baseline blood [lactate], mM	0.9 ± 0.2	1.0 ± 0.2	0.9 ± 0.2	0.9 ± 0.1
Blood [lactate] at ISO, mM	7.2 ± 1.8	7.4 ± 1.5	7.5 ± 1.0	7.8 ± 1.5
Blood [lactate] at exhaustion, mM	7.2 ± 1.8	9.0 ± 1.2†	7.5 ± 1.0	8.6 ± 1.6
Δ blood [lactate] ISO-baseline, mM	6.3 ± 1.7	6.4 ± 1.6	6.6 ± 1.1	6.9 ± 1.4
Δ blood [lactate] exhaustion-baseline, mM	6.3 ± 1.7	8.0 ± 1.2†	6.6 ± 1.1	7.7 ± 1.5

Values are means ± SD. HR, heart rate; [lactate], lactate concentration; Δ, difference. *Significantly different from Pre ($P < 0.05$); †significantly different from Pre ($P < 0.01$).

and, subsequently, increased blood flow to the exercising limbs. The reduced $\dot{V}E$ during maximal-intensity exercise would also reduce the $\dot{V}O_2$ requirements of the respiratory muscles (1) such that leg blood flow might be greater. Although empirical evidence that IMT increases leg blood flow during whole-body exercise is currently lacking, it is clear that reducing the work of breathing via proportional assist ventilator prevents diaphragm fatigue (2) and increases leg blood flow (24), whereas increasing the work of breathing reduces leg blood flow (24).

$\dot{V}O_2$ kinetics. The phase II $\dot{V}O_2$ time constant, which reflects the rate at which $\dot{V}O_2$ in the contracting muscles increases toward the required metabolic rate (21, 37), was not altered by IMT during moderate-, severe-, or maximal-intensity exercise. This is consistent with previous reports that indicate that the phase II $\dot{V}O_2$ time constant is insensitive to interventions which might enhance muscle O_2 delivery, at least in young healthy subjects performing large muscle group exercise (4, 13, 41, 45, 70, 72). The results for moderate-intensity exercise are also consistent with a previous report that IMT did not alter $\dot{V}O_2$ kinetics (20). However, the amplitudes of the fundamental and slow components of $\dot{V}O_2$ were increased and decreased, respectively, during both severe- and maximal-intensity exercise. It has been suggested that the fundamental and slow-component $\dot{V}O_2$ response amplitudes might be sensitive to muscle O_2 availability (32, 35). Interventions that likely result in an acute increase in muscle O_2 delivery during exercise, such as the performance of prior exercise and the inspiration of hyperoxic gas, result in an increased $\dot{V}O_2$ fundamental component amplitude and/or a reduced $\dot{V}O_2$ slow-component amplitude (4, 13, 41, 70), whereas interventions that might compromise muscle O_2 delivery have the opposite effects (34, 35). That IMT altered $\dot{V}O_2$ dynamics during severe- and maximal-intensity exercise in which inspiratory muscle fatigue was reduced, but not during moderate-intensity exercise, which did not fatigue the inspiratory muscles, suggests that a redistribution of blood flow from the respiratory muscles to the exercising limbs might have facilitated the altered $\dot{V}O_2$ response. However, it is noteworthy that the effects of IMT on $\dot{V}O_2$ kinetics during severe exercise (i.e., ~12% reduction in the $\dot{V}O_2$ slow-component amplitude and ~21% speeding of the overall $\dot{V}O_2$ dynamics) are somewhat less impressive than the effects of interventions such as training and prior exercise, both of which can result in 40–50% reductions in the $\dot{V}O_2$ slow-component amplitude and $\dot{V}O_2$ mean response time. This is likely because, in addition to enhancing muscle O_2 delivery, these other interventions also alter factors such as motor unit recruitment patterns and muscle oxidative enzyme activity (and thus O_2 utilization) (4, 5, 31, 32).

Measurements of pulmonary $\dot{V}O_2$ predominantly reflect leg muscle $\dot{V}O_2$ over both the fundamental (21, 37) and slow phases (37, 46) of the $\dot{V}O_2$ response to exercise. It has been reported that loading the inspiratory muscles during intense cycle exercise reduces leg blood flow and leg $\dot{V}O_2$ as a percentage of pulmonary $\dot{V}O_2$, whereas unloading the inspiratory muscles increases leg blood flow and leg $\dot{V}O_2$ as a percentage of pulmonary $\dot{V}O_2$ (24). In the present study, IMT increased the $\dot{V}O_2$ fundamental component amplitude during severe- and maximal-intensity exercise, presumably by en-

Table 5. *Ratings of perceived exertion for limb and respiratory discomfort during moderate-intensity, severe-intensity, and maximal-intensity exercise in the IMT and Sham groups pre- and postintervention*

	IMT Pre	IMT Post	SHAM Pre	SHAM Post
Moderate-intensity exercise				
Limb RPE at baseline	7 ± 1	7 ± 1	7 ± 1	7 ± 2
Limb RPE at 2 min	9 ± 1	9 ± 1	9 ± 1	8 ± 1
Limb RPE at 4 min	9 ± 1	9 ± 2	10 ± 1	9 ± 1
Limb RPE at 6 min	10 ± 1	10 ± 2	10 ± 1	9 ± 1
Respiratory RPE at baseline	7 ± 1	6 ± 1	7 ± 1	7 ± 2
Respiratory RPE at 2 min	9 ± 1	9 ± 1	10 ± 1	9 ± 2
Respiratory RPE at 4 min	9 ± 1	9 ± 2	10 ± 1	9 ± 2
Respiratory RPE at 6 min	10 ± 1	10 ± 2	10 ± 1	9 ± 2
Severe-intensity exercise				
Limb RPE at baseline	8 ± 2	7 ± 1	8 ± 2	7 ± 2
Limb RPE at 2 min	13 ± 2	12 ± 2*	15 ± 2	13 ± 2*
Limb RPE at 4 min	15 ± 2	13 ± 1*	16 ± 2	15 ± 2
Limb RPE at 6 min	16 ± 2	14 ± 2*	16 ± 2	15 ± 1
Respiratory RPE at baseline	7 ± 2	7 ± 1	8 ± 2	8 ± 2
Respiratory RPE at 2 min	13 ± 1	11 ± 1†	13 ± 1	12 ± 1
Respiratory RPE at 4 min	14 ± 2	12 ± 1†	14 ± 1	13 ± 2
Respiratory RPE at 6 min	15 ± 2	13 ± 1†	15 ± 1	14 ± 2
Maximal-intensity exercise				
Limb RPE at baseline	8 ± 1	7 ± 1	8 ± 1	7 ± 1
Limb RPE at 2 min	16 ± 1	16 ± 2	18 ± 1	17 ± 2
Respiratory RPE at baseline	7 ± 1	7 ± 1	8 ± 1	7 ± 1
Respiratory RPE at 2 min	16 ± 1	15 ± 1†	17 ± 2	16 ± 2

Values are means ± SD. RPE, rating of perceived exertion. *Significantly different from Pre ($P < 0.05$); †significantly different from Pre ($P < 0.01$).

abling a greater leg blood flow. This suggests that muscle O_2 delivery might play an important role in setting the $\dot{V}O_2$ fundamental component amplitude during high-intensity exercise and that IMT, through reducing inspiratory muscle fatigue and thus the onset of the metaboreflex, increased muscle O_2 delivery and thus $\dot{V}O_2$ in the early minutes of such exercise.

The increased $\dot{V}E$ during high-intensity exercise has been estimated to account for up to 24% of the $\dot{V}O_2$ slow-component amplitude (15). In keeping with this, loading the inspiratory muscles increases the $\dot{V}O_2$ slow-component amplitude (16), whereas changing lung mechanics by breathing HeO_2 reduces the $\dot{V}O_2$ slow-component amplitude (17). A reduction in absolute $\dot{V}E$ (as was observed at 2 min into maximal-intensity exercise) or in the $\dot{V}O_2$ requirement for a given $\dot{V}E$ (i.e., improved efficiency) might therefore contribute to the reduced $\dot{V}O_2$ slow component that we observed after IMT. Specific training of the respiratory muscles has been reported to increase the proportion of type I fibers in the external intercostal muscles (50), which would be expected to result in improved contractile efficiency (9). Another explanation for the reduced $\dot{V}O_2$ slow component after IMT is that an enhanced leg blood flow across the initial exercise transient reduced the rate of limb muscle fatigue development (24, 53) and thus the requirement to recruit lower-efficiency type II muscle fibers to support power production as exercise continued. This delayed-onset recruitment of type II muscle fibers has been proposed to be a key determinant of the $\dot{V}O_2$ slow component (9, 38, 46, 57).

The $\dot{V}O_2$ at exhaustion during maximal-intensity exercise was significantly greater after IMT. This observation is somewhat surprising given that previous studies have shown no effect of IMT on $\dot{V}O_{2max}$, at least during incremental exercise (e.g., 42, 44, 59, 64). It is possible that high-intensity exercise is terminated when the energy available from substrate-level phosphorylation has been exhausted and/or fatigue-related muscle metabolites have accumulated to intolerable levels (33, 47). During maximal-intensity (or "extreme") exercise (71), the duration of exercise might be so short (≤3 min) that the limit of tolerance is reached before the $\dot{V}O_{2max}$ is attained. Consistent with this, the $\dot{V}O_2$ attained at the termination of maximal-intensity exercise in the present study was slightly but significantly lower than the $\dot{V}O_{2max}$ measured in the initial incremental test before, but not after, the IMT intervention. It is possible that the higher $\dot{V}O_2$ (and thus oxidative contribution to energy turnover) over the first 2 min of maximal-intensity exercise after IMT spared the energetic contribution from substrate-level phosphorylation, thereby extending the duration of exercise and permitting the $\dot{V}O_{2max}$ to be attained.

Exercise tolerance. After 4 wk of IMT, which significantly increased MIP and reduced the extent of inspiratory muscle fatigue, exercise tolerance was improved by a mean of 39% and 18% during severe- and maximal-intensity exercise, respectively. Improved exercise performance has also been observed previously during constant work-rate tests to exhaustion (10, 11, 20, 42), as well as during time trials (29, 54, 65) and fixed duration exercise (23) after training of the respiratory muscles. Increasing the work of breathing exacerbates peripheral leg fatigue (53), heightens dyspnea and sensations of leg discomfort, and compromises leg blood flow and whole body exercise tolerance (26, 53). However, when the work of breathing is reduced, the extent of peripheral limb muscle fatigue (53), diaphragm fatigue (2), dyspnea, and leg discomfort (53) are reduced, and leg blood flow (24) and exercise tolerance (26, 27, 48) are improved. Collectively, these findings indicate that the increased inspiratory muscle fatigue resistance that we observed post-IMT might have reduced the fraction of total cardiac output required by the respiratory muscles during exercise and blunted the accumulation of fatigue-related metabolites. In this way, any potential reflex vasoconstrictive effects on the locomotor muscle vasculature would be attenuated (61, 74). Leading to an increased limb blood flow (24) and enhanced exercise tolerance (26).

Given that certain aspects of $\dot{V}O_2$ dynamics are sensitive to changes in muscle O_2 availability (41, 45, 70) and IMT has the potential to increase leg O_2 supply, the improved exercise performance often observed after IMT might be ascribed, in part, to an enhanced dynamic $\dot{V}O_2$ response to exercise. A faster adjustment of oxidative phosphorylation in response to the same absolute work rate after IMT would reduce the magnitude of the O_2 deficit and thus the energy contribution from substrate-level phosphorylation (14, 45). Likewise, a reduction in the $\dot{V}O_2$ slow-component amplitude would reduce PCr breakdown (57) and glycogen depletion (38). Interventions that elicit such alterations in the $\dot{V}O_2$ response would therefore spare the finite anaerobic reserves, reduce the accumulation of fatiguing metabolites, and improve exercise tolerance (4–6, 14, 30). Consistent with earlier studies, blood lactate accumulation (10, 11, 40, 62) and the perceptions of dyspnea and limb discomfort (23, 54, 65) were reduced after IMT during severe exercise. The improved $\dot{V}O_2$ dynamics after IMT would be expected to reduce blood lactate accumulation

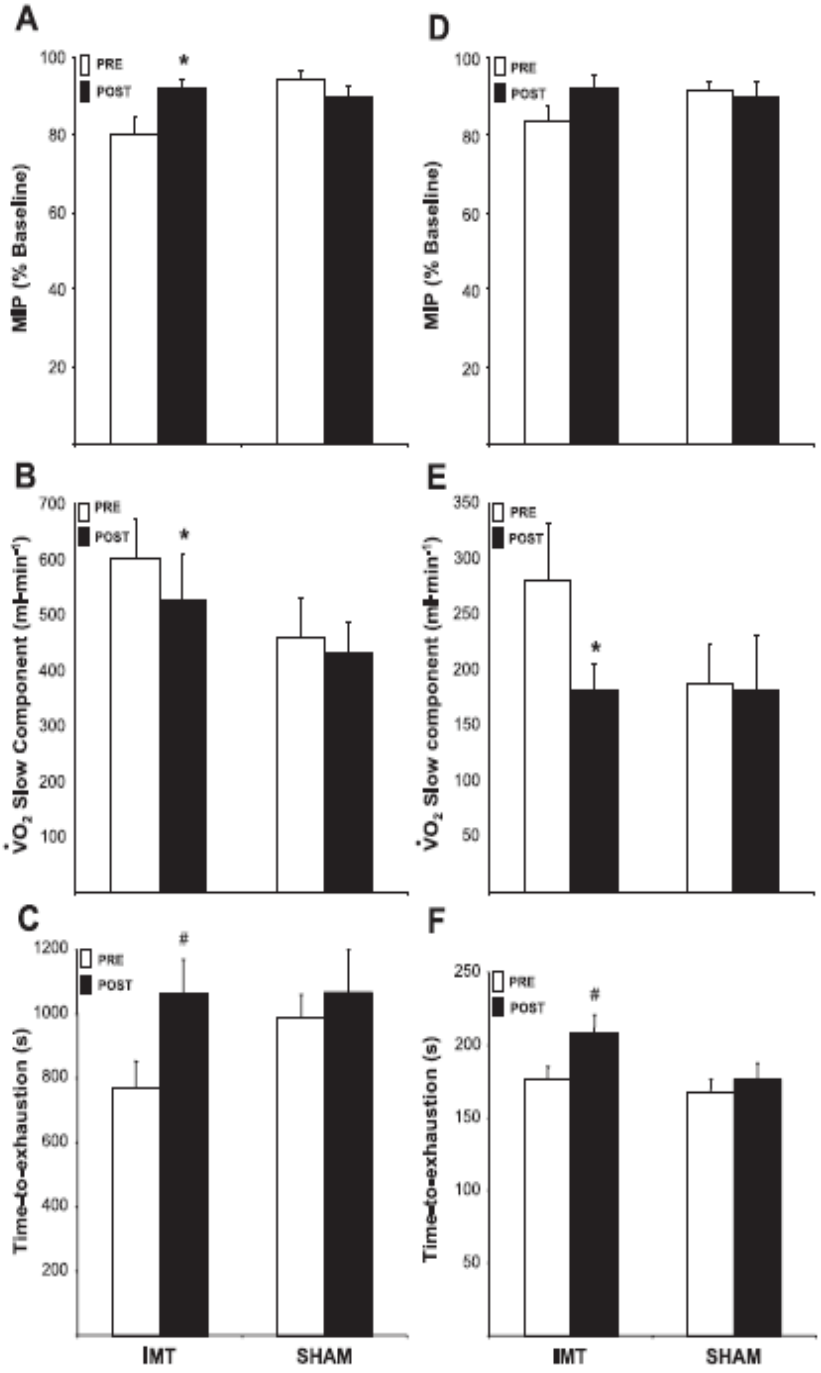

Fig. 4. Severe and maximal exercise inspiratory muscle fatigue, $\dot{V}O_2$ slow-component amplitude, and exercise tolerance in the IMT and Sham groups Pre and Post. *A–C*: severe-intensity exercise inspiratory muscle fatigue, $\dot{V}O_2$ slow-component amplitude, and exercise tolerance, respectively. *D–F*: maximal exercise inspiratory muscle fatigue, $\dot{V}O_2$ slow-component amplitude, and exercise tolerance, respectively. Open bars, Pre data. Solid bars, Post data. MIP, maximum inspiratory pressure. Results are group means ± SE. Note that post-IMT, inspiratory muscle fatigue and $\dot{V}O_2$ slow-component amplitude were reduced and exercise tolerance was significantly enhanced during both severe and maximal exercise. The parameters were not affected by Sham intervention. *Significantly different from Pre ($P < 0.05$); #significantly different from Pre ($P < 0.01$).

(4, 5), which, through reduced group III and IV afferent discharge, may reduce the sensations of respiratory and limb muscle discomfort and improve exercise tolerance (56). It should be noted, however, that despite the concomitant changes in $\dot{V}O_2$ dynamics and exercise tolerance after IMT, the changes in MIP were not significantly correlated with changes in $\dot{V}O_2$ kinetics or the time to task failure. It is possible that this lack of significant relationship was a function of the relatively low sample size ($n = 8$) and interindividual variability in the physiological responses to IMT.

Conclusions. Specific training of the inspiratory muscles increased baseline MIP, reduced inspiratory muscle fatigue and enhanced $\dot{V}O_2$ dynamics and exercise tolerance during severe- and maximal-intensity exercise. We propose that a reduction of inspiratory muscle fatigue after IMT spared the O_2 and blood-flow requirements of ventilation and offset the metaboreflex (61, 74), thereby increasing limb O_2 delivery (24). Increased muscle O_2 availability, in turn, resulted in a speeding of the overall $\dot{V}O_2$ dynamics (perhaps by reducing the rate of fatigue development and delaying the recruitment of low-

efficiency fibers) (9, 38) and enhancement of exercise tolerance (4, 14, 70).

Pressure-threshold IMT appears to present a practical and efficacious means for modulating the $\dot{V}O_2$ response to high-intensity exercise in healthy young people. These changes are likely to be, at least in part, responsible for the enhanced exercise tolerance after IMT that has been reported herein and in previous investigations. IMT therefore appears to have considerable potential as an adjunct to physical training for the enhancement of exercise performance. Further research is required to establish whether similar (or greater) effects on $\dot{V}O_2$ kinetics and exercise tolerance are possible in the elderly or in populations with ventilatory or cardiovascular impairments.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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Optimizing the "priming" effect: influence of prior exercise intensity and recovery duration on O_2 uptake kinetics and severe-intensity exercise tolerance

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Bailey SJ, Vanhatalo A, Wilkerson DP, DiMenna FJ, Jones AM. Optimizing the "priming" effect: influence of prior exercise intensity and recovery duration on O_2 uptake kinetics and severe-intensity exercise tolerance. *J Appl Physiol* 107: 1743–1756, 2009. First published October 1, 2009; doi:10.1152/japplphysiol.00810.2009.—It has been suggested that a prior bout of high-intensity exercise has the potential to enhance performance during subsequent high-intensity exercise by accelerating the O_2 uptake ($\dot{V}O_2$) on-response. However, the optimal combination of prior exercise intensity and subsequent recovery duration required to elicit this effect is presently unclear. Eight male participants, aged 18–24 yr, completed step cycle ergometer exercise tests to 80% of the difference between the preestablished gas exchange threshold and maximal $\dot{V}O_2$ (i.e., 80%Δ) after no prior exercise (control) and after six different combinations of prior exercise intensity and recovery duration: 40%Δ with 3 min (40-3-80), 9 min (40-9-80), and 20 min (40-20-80) of recovery and 70%Δ with 3 min (70-3-80), 9 min (70-9-80), and 20 min (70-20-80) of recovery. Overall $\dot{V}O_2$ kinetics were accelerated relative to control in all conditions except for 40-9-80 and 40-20-80 conditions as a consequence of a reduction in the $\dot{V}O_2$ slow component amplitude; the phase II time constant was not significantly altered with any prior exercise/recovery combination. Exercise tolerance at 80%Δ was improved by 15% and 30% above control in the 70-9-80 and 70-20-80 conditions, respectively, but was impaired by 16% in the 70-3-80 condition. Prior exercise at 40%Δ did not significantly influence exercise tolerance regardless of the recovery duration. These data demonstrate that prior high-intensity exercise (~70%Δ) can enhance the tolerance to subsequent high-intensity exercise provided that it is coupled with adequate recovery duration (≥9 min). This combination presumably optimizes the balance between preserving the effects of prior exercise on $\dot{V}O_2$ kinetics and providing sufficient time for muscle homeostasis (e.g., muscle phosphocreatine and H^+ concentrations) to be restored.

priming exercise; oxygen consumption kinetics; exercise performance; near-infrared spectroscopy; surface electromyography

AFTER THE ONSET of constant-work rate exercise, pulmonary O_2 uptake ($\dot{V}O_2$) rises with near-exponential kinetics to attain a steady state within 2–3 min in young healthy adults performing moderate-intensity [below the gas exchange threshold (GET)] exercise (60, 61). For exercise above the GET, the fundamental $\dot{V}O_2$ response is supplemented by a delayed-onset $\dot{V}O_2$ "slow component," which delays the attainment of a steady state during heavy-intensity exercise [performed below the critical power (CP)] or sets the $\dot{V}O_2$ on a trajectory toward its maximum during severe-intensity (>CP) exercise (50, 61, 64). The magnitude of the "O_2 deficit" incurred after the onset of exercise, which is compensated by increased substrate-level phosphorylation, is a function of both the rate at which $\dot{V}O_2$ rises after the onset of exercise ($\dot{V}O_2$ "kinetics") and the required "steady-state" amplitude. During high-intensity exercise, sparing the utilization of the finite anaerobic energy reserve and the accumulation of metabolites associated with the fatigue process by speeding overall $\dot{V}O_2$ kinetics and/or delaying the attainment of maximal $\dot{V}O_2$ ($\dot{V}O_{2max}$) by reducing the $\dot{V}O_2$ slow component would therefore be expected to result in enhanced exercise performance (3, 13, 59).

One acute intervention known to elicit an overall speeding of $\dot{V}O_2$ dynamics during supra-GET exercise is the performance of a prior bout of high-intensity "warm-up" or "priming" exercise. Gerbino et al. (26) demonstrated that prior heavy-intensity exercise (but not moderate-intensity exercise) resulted in faster overall $\dot{V}O_2$ kinetics during a second heavy-intensity exercise bout. Subsequent investigations established that this overall speeding of $\dot{V}O_2$ kinetics was chiefly consequent to a reduced $\dot{V}O_2$ slow component amplitude (8, 14, 40). It has been shown that although these effects on the $\dot{V}O_2$ response recede with time, they are preserved for at least 30–45 min after the completion of the prior exercise bout (9). The performance of higher-intensity exercise, such as constant-work rate severe-intensity exercise or single or repeated bouts of sprint exercise, can also speed the overall $\dot{V}O_2$ response during subsequent exercise (10, 11, 17, 22, 63). The mechanistic basis for the enhanced $\dot{V}O_2$ kinetics after prior high-intensity exercise remains a topic of intense debate (35, 49, 56). Such exercise induces a myriad of changes in skeletal muscle physiology, including increases in blood flow (4, 21, 41), oxygenation (19, 34, 43, 63), oxidative enzyme activity (15, 29, 53), O_2 extraction (19, 20, 25, 41, 43), and electromyographic (EMG) activity (7, 43), but which of these changes is essential for facilitating the speeding of $\dot{V}O_2$ kinetics during subsequent exercise is presently obscure.

While the aforementioned alterations in $\dot{V}O_2$ kinetics after prior exercise might be expected to enhance exercise tolerance, direct evidence for this is limited or equivocal (10, 17, 22, 36, 39, 63). This is due, in large part, to between-study differences in the intensities of the prior exercise and criterion exercise bouts and in the intervening recovery durations. The available evidence suggests that the tolerance to severe-intensity exercise is impaired after repeated all-out sprint exercise with 15 min of recovery (63) and prior severe-intensity exercise with 2 min of recovery (22). An unchanged severe-intensity exercise performance has been reported 10 min after a single 30-s sprint (10), 8 min after both low-intensity and high-intensity exercise (39), and 6 min after severe-intensity exercise (17). Conversely, improved tolerance to severe-intensity exercise has been reported 10 min after both moderate-intensity and heavy-intensity exercise (10) and 6 min (17) and 10 min (36) after heavy-intensity exercise. It should be noted here that the "heavy" exercise completed in these previous studies (10, 17, 36) was conducted at an intensity of "50%Δ" (i.e., GET plus

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50% of the difference between the GET and $\dot{V}O_{2max}$). This is problematic in that this intensity lies close to the CP (50, 64), and it is therefore possible that at least some subjects in these previous studies actually performed severe-intensity prior exercise.

The available data therefore indicate that prior exercise can be detrimental to subsequent exercise performance when it is excessively intense (63) or when insufficient recovery is provided (22, 57). However, given that effects on $\dot{V}O_2$ kinetics are greater after severe-intensity prior exercise than heavy-intensity prior exercise (35) and that these effects can be maintained for at least 30–45 min (9), it is possible that severe-intensity prior exercise combined with an extended recovery period [≥15 min (57)] might maximize the potential for exercise tolerance to be enhanced. Studies that provide information on the prior exercise/recovery combination that optimizes exercise tolerance are important because applied physiologists and coaches are becoming increasingly interested in manipulating precompetition warm-up regimes to enhance athletic performance (10, 30, 47).

The purpose of this investigation was to elucidate how prior exercise intensity and subsequent recovery duration interact to affect $\dot{V}O_2$ kinetics and the tolerance to severe-intensity exercise. We studied the physiological responses to severe-intensity exercise after no prior exercise (control) and after six different combinations of prior exercise (performed at either 40%Δ or 70%Δ, reflecting heavy and severe-intensity exercise, respectively) and subsequent recovery (of 3, 9, and 20 min in duration). We hypothesized that prior severe-intensity exercise followed by a 20-min recovery period would result in the greatest improvement in exercise tolerance, whereas prior severe-intensity exercise followed by a 3-min recovery period would impair tolerance to subsequent severe-intensity exercise. To provide insight into the physiological bases for the effects observed, we measured the dynamics of pulmonary gas exchange, heart rate (HR), muscle oxygenation [using near-infrared spectroscopy (NIRS)], blood [lactate], and EMG during all conditions.

METHODS

Subjects. Eight healthy male subjects (means ± SD; age: 21 ± 2 yr, height: 177 ± 4 cm, and body mass: 76 ± 6 kg) volunteered to participate in this study. Subjects participated in exercise at a recreational level, but were not highly trained, and were familiar with laboratory exercise testing procedures, having previously participated in studies using similar procedures in our laboratory. The study was approved by the University of Exeter Research Ethics Committee, and all subjects were required to give their written informed consent before the commencement of the study once the experimental procedures, associated risks, and potential benefits of participation had been explained. Subjects were instructed to arrive at the laboratory in a rested and fully hydrated state, at least 3 h postprandial, and to avoid strenuous exercise in the 24 h before each testing session. Each subject was also asked to refrain from caffeine and alcohol 6 and 24 h before each test, respectively. All tests were performed at the same time of day (±2 h).

Experimental design. Subjects were required to report to the laboratory on 15 occasions over a 6-wk period, and all tests were interspersed with at least 24 h of recovery. After an initial ramp incremental test, all subjects completed a number of "double-step" exercise tests during which pulmonary $\dot{V}O_2$ and HR kinetics, blood [lactate], parameters of muscle oxygenation (by NIRS), muscle activation [integrated EMG (iEMG)], and exercise tolerance were assessed. To determine the interactive influence of prior exercise intensity and recovery duration on subsequent exercise performance, we used a paradigm comprising two different exercise intensities and three different recovery durations.

Incremental test. On the first laboratory visit, subjects completed a ramp incremental exercise test for the determination of the $\dot{V}O_2$ peak and GET. All cycle tests were performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). Initially, subjects performed 3 min of baseline cycling at 0 W, after which the work rate was increased at a rate of 30 W/min until the limit of tolerance. Subjects cycled at a self-selected pedal rate (between 70 and 90 rpm), and this pedal rate, along with the saddle and handle bar height and configuration, was recorded and reproduced in subsequent tests. Breath-by-breath pulmonary gas exchange data were collected continuously during the incremental tests and averaged over consecutive 10-s periods. The $\dot{V}O_2$ peak was taken as the highest 30-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 $\dot{V}CO_2$ versus $\dot{V}O_2$, *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*) the increase in end-tidal PO_2 with no fall in end-tidal PCO_2. The work rates that would require 40%Δ [GET plus 40% of the difference between the work rate at the GET and $\dot{V}O_2$ peak (heavy exercise)], 70%Δ [GET plus 70% of the difference between the work rate at the GET and $\dot{V}O_2$ peak (severe exercise)], and 80%Δ [GET plus 80% of the difference between the work rate at the GET and $\dot{V}O_2$ peak (severe exercise)] were subsequently calculated.

Square-wave tests. A total of seven experimental conditions were investigated in this study, and these were administered in a randomized order. In the control condition, subjects completed 4 min of "unloaded" 20-W baseline cycling before an abrupt step increment to the target 80%Δ work rate was imposed. The remaining six conditions comprised double-step tests wherein the transitions to 80%Δ were preceded by bouts of prior exercise. The prior exercise conditions involved 4 min of baseline unloaded pedaling followed by step increments of 6-min duration to either 40%Δ (heavy exercise) or 70%Δ (severe exercise). A 3-min period of unloaded cycling took place before the 80%Δ criterion work rate, which was performed either immediately after the priming exercise or after 6 or 17 min of seated passive recovery. This scheme resulted in 3, 9, or 20 min of recovery, with the unloaded cycling considered part of the recovery duration. The six prior exercise permutations were, therefore, 40%Δ with 3 min (40-3-80), 9 min (40-9-80), and 20 min (40-20-80) of recovery and 70%Δ with 3 min (70-3-80), 9 min (70-9-80), and 20 min (70-20-80) of recovery. Each participant completed the seven protocols (control plus six prior exercise/recovery conditions) on two separate occasions. On one of these occasions, exercise at 80%Δ was continued until task failure as a measure of exercise tolerance, whereas on the other occasion the exercise was performed for 6 min only. The time to task failure was recorded when the pedal rate fell by >10 rpm below the required pedal rate. The $\dot{V}O_2$ responses from these like transitions were averaged before any analysis to enhance the signal-to-noise ratio and improve confidence in the parameters derived from the model fits (58).

Measurements. During all tests, pulmonary gas exchange and ventilation were measured continuously using a portable metabolic cart (MetaMax 3B, Cortex Biophysik, Leipzig, Germany), as previously described (3, 20). A DVT turbine digital transducer measured inspired and expired airflow, whereas an electrochemical cell O_2 analyzer and ND infrared CO_2 analyzer simultaneously measured expired gases. Subjects wore a nose clip and breathed through a low-dead space, low-resistance mouthpiece that was securely attached to the volume transducer. The inspired and expired gas volume and gas concentration signals were continuously sampled via a capillary line connected to the mouthpiece. The gas analyzers were calibrated

before each test with gases of known concentration, and the turbine volume transducer was calibrated using a 3-liter syringe (Hans Rudolph, Kansas City, MO). Pulmonary gas exchange and ventilation were calculated and displayed breath by breath. HR was measured during all tests using short-range radiotelemetry (Polar S610, Polar Electro Oy, Kempele, Finland).

During the 80%Δ criterion transitions, a blood sample was collected from a fingertip into a capillary tube over the 20 s before the step transition in work rate, at 6 min into the transition, and also at the limit of tolerance. These whole blood samples were subsequently analyzed to determine blood [lactate] (YSI 1500, Yellow Springs Instruments, Yellow Springs, OH) within 30 s of collection. Blood lactate accumulation (change in blood [lactate]) was calculated as the difference between the blood [lactate] at 6 min and blood [lactate] at baseline.

The oxygenation status of the vastus lateralis muscle of the right leg was monitored using a commercially available NIRS system (model NIRO 300, Hamamatsu Photonics, Hiugashi-ku, Japan). The system consisted of an emission probe that irradiates laser beams and a detection probe. Four different wavelength laser diodes provided the light source (776, 826, 845, and 905 nm), and the light returning from the tissue was detected by a photomultiplier tube in the spectrometer. The intensity of incident and transmitted light was recorded continuously at 2 Hz and used to estimate concentration changes from the resting baseline for oxygenated (HbO_2), deoxygenated (HHb), and total tissue hemoglobin/myoglobin (Hb_{tot}). Therefore, the NIRS data represent a relative change based on the optical density measured in the first datum collected. The [HHb] signal can be regarded as being essentially blood volume insensitive during exercise (18, 28) and was, therefore, assumed to provide an estimate of changes in the fractional O_2 extraction in the field of interrogation (19, 23, 28, 34). It should be noted here that the contribution of deoxygenated myoglobin to the NIRS signal is presently unclear, and, as such, the terms [Hb_{tot}], [HbO_2], and [HHb] used in this article should be considered to refer to the combined concentrations of total, oxygenated, and deoxygenated hemoglobin and myoglobin, respectively. The leg was initially cleaned and shaved around the belly of the muscle, and the optodes were placed in the holder, which was secured to the skin with adhesive at 20 cm above the lateral epicondyle. To secure the holder and wires in place, an elastic bandage was wrapped around the subject's leg. The wrap helped to minimize the possibility that extraneous light could influence the signal and also ensured that the optodes did not move during exercise. Pen marks were made around the holder to enable precise reproduction of the placement in subsequent tests. The probe gain was set with the subject at rest in a seated position with the leg extended at down stroke on the cycle ergometer before the first exercise bout, and NIRS data were collected continuously throughout the exercise protocols. The data were subsequently downloaded onto a personal computer, and the resulting text files were stored on disk for later analysis.

Neuromuscular activity of the vastus lateralis muscle of the left leg was measured using bipolar surface EMG. The leg was initially shaved and cleaned with alcohol around the belly of the muscle, and graphite snap electrodes (Unilect 40713, Unomedical, Stonehouse, UK) were adhered to the prepared area in a bipolar arrangement (interelectrode distance: 40 mm). A ground electrode was positioned on the rectus femoris equidistant from the active electrodes. The sites of electrode placement were chosen according to the recommendations provided in the EMG software (Mega Electronics). To secure electrodes and wires in place and to minimize movement during cycling, an elastic bandage was wrapped around the subject's leg. Pen marks were made around the electrodes to enable reproduction of the placement in subsequent tests. The EMG signal was recorded using a ME3000PB Muscle Tester (Mega Electronics). EMG measurements at a sampling frequency of 1,000 Hz were recorded throughout all exercise tests. The bipolar signal was amplified (amplifier input impedance >1 MΩ), and data were collected online in raw form and stored on a personal computer using MegaWin software (Mega Electronics). The raw EMG data were subsequently exported as an ASCII file and digitally filtered using a custom-designed filter developed through Labview 8.2 (National Instruments, Newbury, UK). Initially, the signals were filtered with a 20-Hz high-pass, second-order Butterworth filter to remove contamination from movement artifacts. The signal was then rectified and low pass filtered at a frequency of 500 Hz to produce a linear envelope. The average iEMG was calculated for 10-s intervals throughout the 80%Δ criterion exercise bout and the preceding baseline, with these values normalized to the average measured during 10–180 s of unloaded cycling before the initial prior exercise transition. Therefore, all iEMG data are presented as a percentage of the prior exercise unloaded cycling phase. Data from repeat trials were averaged, and $\Delta iEMG_{(2\ -\ 6\ min)}$ was defined as the difference between the average iEMG over the last 10 s of exercise and the average from 110–120 s.

Data analysis procedures. The breath-by-breath $\dot{V}O_2$ data from each test were initially examined to exclude errant breaths caused by coughing, swallowing, sighing, etc., and those values lying more than four standard deviations from the local mean were removed. The breath-by-breath data were subsequently linearly interpolated to pro-

Table 1. *$\dot{V}O_2$ kinetics during severe-intensity exercise in the control and variously primed conditions*

		Primed Conditions					
	Control	40-3-80	40-9-80	40-20-80	70-3-80	70-9-80	70-20-80
Baseline $\dot{V}O_2$, l/min	0.98±0.08	1.12±0.13[a,e,h]	1.03±0.16	1.03±0.12	1.31±0.14[b,c,d,e,g,h]	1.15±0.14[b,d,e,h]	1.01±0.11
End-exercise $\dot{V}O_2$ at 6 min, l/min	3.90±0.38	3.84±0.39	3.81±0.36	3.88±0.45	3.79±0.35	3.87±0.38	3.91±0.42
$\dot{V}O_2$ at exhaustion, l/min	3.92±0.41	3.87±0.37	3.85±0.36	3.85±0.45	3.80±0.37	3.90±0.40	3.95±0.46
Phase II τ, s	31±9	30±8	27±10	29±10	28±7	29±6	30±7
95% Confidence interval, s	5±3	5±2	4±3	3±1	6±3	4±2	4±1
Fundamental amplitude, l/min	2.27±0.33	2.30±0.32	2.26±0.20	2.35±0.39	2.21±0.24	2.36±0.30[f]	2.44±0.30[b]
Absolute fundamental amplitude, l/min	3.27±0.34	3.42±0.34[a]	3.29±0.19	3.38±0.40	3.51±0.31[a,d]	3.51±0.33[b,d,e]	3.45±0.34[b]
Fundamental gain, ml·min^{-1}·W^{-1}	8.6±0.7	8.7±0.8	8.6±0.7	8.9±0.9	8.4±0.4	9.0±0.7d	9.2±0.5[b]
Slow component amplitude, l/min	0.65±0.12	0.44±0.13[a,e]	0.53±0.26	0.54±0.14	0.30±0.14[b,c,e,h]	0.39±0.20[a]	0.48±0.17[b]
Slow component amplitude, %	22±6	16±4[a]	19±7	19±5	12±5[b,c,e]	14±6[a]	16±5[b]
Overall τ, s	88±22	72±12[a]	71±17	75±17	52±13[b,c,d,e,h]	60±10[b,c,e]	68±19[a]
End-exercise gain, ml·min^{-1}·W^{-1}	10.3±0.7	10.3±0.5	9.9±0.8	10.1±0.7	8.8±0.5	9.7±0.8	10.3±0.5

Values are means ± SD. The six prior exercise permutations were as follows: 40%Δ with 3 min (40-3-80), 9 min (40-9-80), and 20 min (40-20-80) of recovery and 70%Δ with 3 min (70-3-80), 9 min (70-9-80), and 20 min (70-20-80) of recovery. $\dot{V}O_2$, O_2 uptake; τ, time constant. [a]Significantly different from control ($P < 0.05$); [b]significantly different from control ($P < 0.01$); [c]significantly different from the 40-3-80 condition ($P < 0.05$); [d]significantly different from the 40-9-80 condition ($P < 0.05$); [e]significantly different from the 40-20-80 condition ($P < 0.05$); [f]significantly different from the 70-3-80 condition ($P < 0.05$); [g]significantly different from the 70-9-80 condition ($P < 0.05$); [h]significantly different from the 70-20-80 condition ($P < 0.05$).

vide second-by-second values, and, for each individual, identical repetitions were time aligned to the start of exercise and ensemble averaged. The first 20 s of data after the onset of exercise (i.e., the phase I response) were deleted (58), and a nonlinear least-square algorithm was used to fit the data thereafter. A single-exponential model was used to characterize the kinetics of the overall $\dot{V}O_2$ response to the criterion exercise bouts, and a biexponential model was used to characterize the $\dot{V}O_2$ response kinetics in its constituent fundamental and slow components, as described by the following equations:

$$\dot{V}O_2(t) = \dot{V}O_{2baseline} + A_p[1 - e^{-(t - TD_p)/\tau_p}] \quad (1)$$

$$\dot{V}O_2(t) = \dot{V}O_{2baseline} + A_p[1 - e^{-(t - TD_p)/\tau_p}] + A_s[1 - e^{-(t - TD_s)/\tau_s}] \quad (2)$$

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time t; $\dot{V}O_{2baseline}$ represents the mean $\dot{V}O_2$ in the baseline period; A_p, TD_p, and τ_p represent the amplitude, time delay, and time constant, respectively, describing the fundamental or phase II increase in $\dot{V}O_2$ above baseline; and A_s, TD_s, and τ_s represent the amplitude, time delay before the onset, and time constant describing the development of the $\dot{V}O_2$ slow component, respectively.

An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. $\dot{V}O_{2baseline}$ was defined as the mean $\dot{V}O_2$ measured over the final 90 s of baseline pedaling. The end-exercise $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 30 s of the 6-min exercise bouts, whereas the $\dot{V}O_2$ at exhaustion was defined as the mean $\dot{V}O_2$ measured over the 30 s before the subject's termination of exercise. The absolute fundamental component amplitude (absolute A_p) was defined as the sum of $\dot{V}O_{2baseline}$ and A_p. Because the asymptotic value (A_s) of the exponential term describing the $\dot{V}O_2$ slow com-

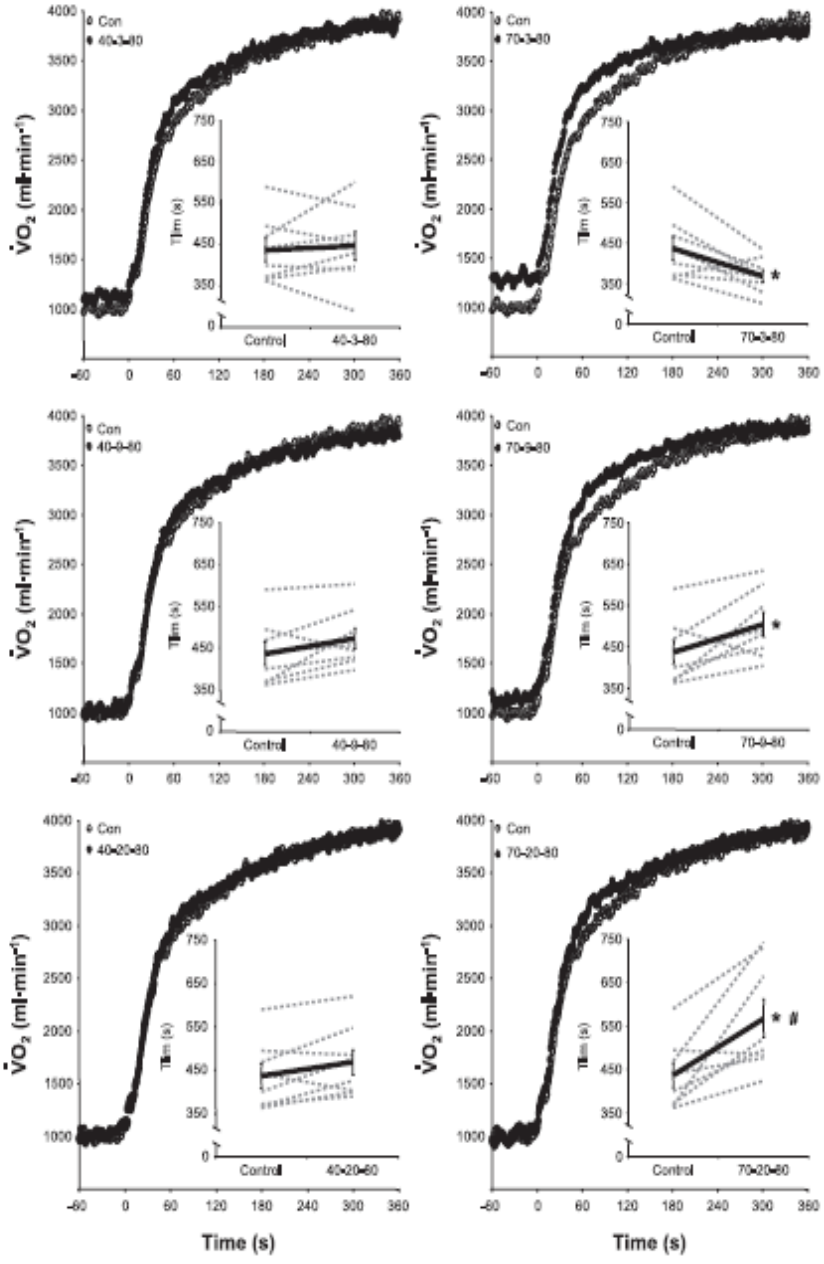

Fig. 1. Comparisons of the group mean pulmonary O_2 uptake ($\dot{V}O_2$) dynamics during a step increment from an unloaded baseline to a severe-intensity work rate during control (Con) and in the following priming conditions: 40-3-80 (*top left*), 40-9-80 (*middle left*), 40-20-80 (*bottom left*), 70-3-80 (*top right*), 70-9-80 (*middle right*), and 70-20-80 (*bottom right*). ○, responses in control; ●, responses to the various primed conditions. Error bars are omitted for clarity. Notice that the priming effect is greatest when the criterion bout is preceded by severe-intensity exercise and that it wanes with increasing recovery time after both heavy-intensity and severe-intensity prior exercise. The *insets* in the respective $\dot{V}O_2$ graphs show the individual (dashed lines) and group mean ± SE (solid lines) changes in the tolerance to severe-intensity exercise relative to control. *Significantly different from control ($P < 0.05$); #significantly different from the 70-9-80 condition ($P < 0.05$).

ponent may represent a higher value than is actually reached at the end of the exercise, the actual amplitude of the $\dot{V}O_2$ slow component at the end of exercise was defined as A_s'. The A_s' parameter was compared at the same isotime (360 s) pre- and postintervention. The amplitude of the slow component was also described relative to the entire $\dot{V}O_2$ response. In addition, the functional "gain" of the fundamental $\dot{V}O_2$ response was computed by dividing A_p by the change in work rate. The functional gain of the entire response (i.e., end-exercise gain) was calculated in a similar manner.

To provide information on muscle oxygenation, we also modeled the [HHb] response to exercise. [HHb] kinetics during the criterion exercise bouts in the fundamental phase were determined by fitting a biexponential model from the first data point, which was 1 SD above the baseline mean through the entire response. [HHb] TD and τ values were summed to provide information on overall [HHb] response dynamics in the fundamental phase of the response. The [HbO_2] and [Hb_{tot}] responses do not approximate an exponential (19) and were, therefore, not modeled. Rather, we assessed priming-induced changes in these parameters by determining the [HbO_2] and [Hb_{tot}] at baseline (90-s before the step transition) and at 60, 120, and 360 s (average response over the final 30 s) of exercise.

We also modeled the HR response to exercise in each condition. For this analysis, HR data were linearly interpolated to provide second-by-second values, and, for each individual, identical repetitions from like transitions were time aligned to the start of exercise and ensemble averaged. A nonlinear least squares monoexponential model without TD was used to fit the data to the criterion 80%Δ exercise, with the fitting window commencing at $t = 0$ s. The derived HR mean response time (MRT; equivalent to the overall τ for the response) provides an insight into the overall rate of adjustment of HR dynamics. Priming-induced changes in HR were also assessed through comparing the HR at baseline (90-s before the step transition) and at 60, 120, and 360 s (average response over the final 30 s) of exercise for each experimental condition.

Statistical analysis. One-way repeated-measures ANOVA was used to determine the effects on the relevant physiological variables elicited by the differing prior exercise permutations. Where the analysis revealed a significant difference, individual paired *t*-tests were used with a Fisher's least-significant difference correction to determine the origin of such effects. Pearson's product-moment correlation was used to examine the interrelationships between the parameters of $\dot{V}O_2$ kinetics, iEMG, muscle oxygenation, and exercise tolerance. All data are presented as means ± SD. Statistical significance was accepted when $P < 0.05$.

RESULTS

During the ramp incremental test, subjects attained a peak work rate of 354 ± 41 W and a $\dot{V}O_2$ peak of 3.80 ± 0.45 l/min, whereas the work rate and $\dot{V}O_2$ values at the GET were 107 ± 13 W and 1.54 ± 0.16 l/min, respectively. The 40%Δ and 70%Δ prior exercise work rates were calculated to be 186 ± 15 and 260 ± 27 W, respectively, and the work rate corresponding to 80%Δ (used in the criterion bout) was 284 ± 32 W.

$\dot{V}O_2$ kinetics. The parameters of $\dot{V}O_2$ dynamics during the control and primed criterion severe-intensity exercise bouts are reported in Table 1 and shown as group mean responses in Fig. 1. The baseline $\dot{V}O_2$ was significantly elevated above control in the 40-3-80, 70-3-80, and 70-9-80 prior exercise conditions, with the greatest elevation occurring in the 70-3-80 condition (Table 1 and Fig. 1). The end-exercise $\dot{V}O_2$ during the trial to exhaustion was similar among all experimental conditions, attaining a value of ~100–103% of the $\dot{V}O_2$ peak. Phase II τ was not significantly affected by any of the prior exercise conditions (Table 1). In the 70-20-80 condition only, the $\dot{V}O_2$ fundamental component amplitude was significantly elevated above control (control: 2.27 ± 0.33 l/min and 70-20-80: 2.44 ± 0.30 l/min, $P < 0.01$). The absolute fundamental component amplitude (baseline + fundamental component amplitude), however, was significantly elevated above control in the 40-3-80, 70-3-80, 70-9-80, and 70-20-80 conditions (Table 1 and Fig. 1). Compared with control, the amplitude of the $\dot{V}O_2$ slow component was significantly reduced in the 40-3-80, 70-3-80, 70-9-80, and 70-20-80 conditions (Table 1 and Fig. 1). The overall $\dot{V}O_2$ kinetics (as assessed with a

Table 2. *Near-infrared spectroscopy-derived [HHb], [HbO_2], and [Hb_{tot}] during severe-intensity exercise in the control and variously primed conditions*

		Primed Conditions					
	Control	40-3-80	40-9-80	40-20-80	70-3-80	70-9-80	70-20-80
[HHb]							
Baseline, AU	−95±58	−127±71	−97±59	−105±41	−116±87	−99±72	−92±60
End exercise, AU	252±125	297±151	258±116	263±132	266±160	249±105	268±133
Primary TD, s	5±1	2±2[b,d,e]	4±2	4±2	2±1[b,d,e,f]	3±1[b,d,e,f]	4±1
Primary τ, s	9±4	9±4	8±2	8±2	9±2	8±2	8±2
TD + τ, s	14±4	11±3[a]	12±2	13±2	11±2[b]	11±3[b,e]	12±2
Primary phase amplitude, AU	294±81	364±115[a]	307±92	316±110	360±82[b]	313±69	318±116[b]
Slow phase amplitude, AU	54±26	62±39	52±27	58±21	23±17[b]	39±27	47±19
[HbO_2]							
Baseline, AU	25±62	213±98[b,d,e,f,g]	127±71[b]	90±99	205±131[a]	96±63[a]	65±121
At 60 s, AU	−235±79	−175±69[a]	−152±72[b]	−189±55	−226±187	−197±68	−216±70
At 120 s, AU	−197±90	−157±66	−121±64[b,e,f,g]	−167±53	−208±196	−168±72	−189±71
End exercise, AU	−187±87	−159±71	−114±55[b,c]	−149±71	−215±190	−167±74	−178±87
[Hb_{tot}]							
Baseline, AU	−70±80	86±90[b,e]	30±112[b]	−15±105	89±90[b,g]	−4±86	−27±144
At 60 s, AU	−320±87	68±102[b,e]	61±95[b]	22±120	18±102	17±84	9±124
At 120 s, AU	20±70	108±111[a,e]	102±77[b]	55±119	42±107	54±81	48±115
End exercise, AU	65±77	138±113[a]	144±89[b]	114±109	51±101	82±82	91±119

Values are means ± SD. HHb, HbO_2, and Hb_{tot} are deoxygenated, oxygenated, and total hemoglobin/myoglobin. AU, arbitrary units; TD, time delay. [a]Significantly different from control ($P < 0.05$); [b]significantly different from control ($P < 0.01$); [c]significantly different from the 40-3-80 condition ($P < 0.05$); [d]significantly different from the 40-9-80 condition ($P < 0.05$); [e]significantly different from the 40-20-80 condition ($P < 0.05$); [f]significantly different from the 70-9-80 condition ($P < 0.05$); [g]significantly different from the 70-20-80 condition ($P < 0.05$).

monoexponential model) were significantly faster than control (88 ± 22 s) in the 40-3-80 (72 ± 12 s), 70-3-80 (52 ± 13 s), 70-9-80 (60 ± 10 s), and 70-20-80 (68 ± 19 s) conditions (Table 1 and Fig. 1).

NIRS and HR kinetics. The NIRS parameters during severe-intensity exercise in the control and primed conditions are shown in Table 2. [HHb] dynamics during exercise after each prior exercise condition are shown relative to control in Fig. 2. [HHb] during baseline and at the end of exercise were similar across the prior exercise conditions and did not differ from control (Table 2 and Fig. 2). While [HHb] τ was similar across the experimental conditions investigated in this study ($P > 0.05$), [HHb] TD was significantly reduced below control in the 40-3-80, 70-3-80, and 70-9-80 prior exercise conditions. As such, [HHb] MRT (TD + τ) was significantly faster in the 40-3-80 (11 ± 3 s), 70-3-80 (11 ± 2 s), and 70-9-80 (11 ± 3 s) prior exercise conditions relative to control (14 ± 4 s; Table 2). The [HHb] primary amplitude was significantly greater than control in the 40-3-80, 70-3-80, and 70-20-80 prior exercise conditions. However, the [HHb] slow component amplitude was only reduced significantly below the control value in the 70-3-80 condition (Table 2 and Fig. 2).

The priming-induced changes in [HbO_2] compared with control are shown in Fig. 3. [HbO_2] was significantly elevated during the baseline of the criterion bout in the 40-3-80, 40-9-80, 70-3-80, and 70-9-80 prior exercise conditions relative to control (Table 2 and Fig. 3). [Hb_{tot}] was significantly elevated during the baseline of the criterion bout in the 40-3-80, 40-9-

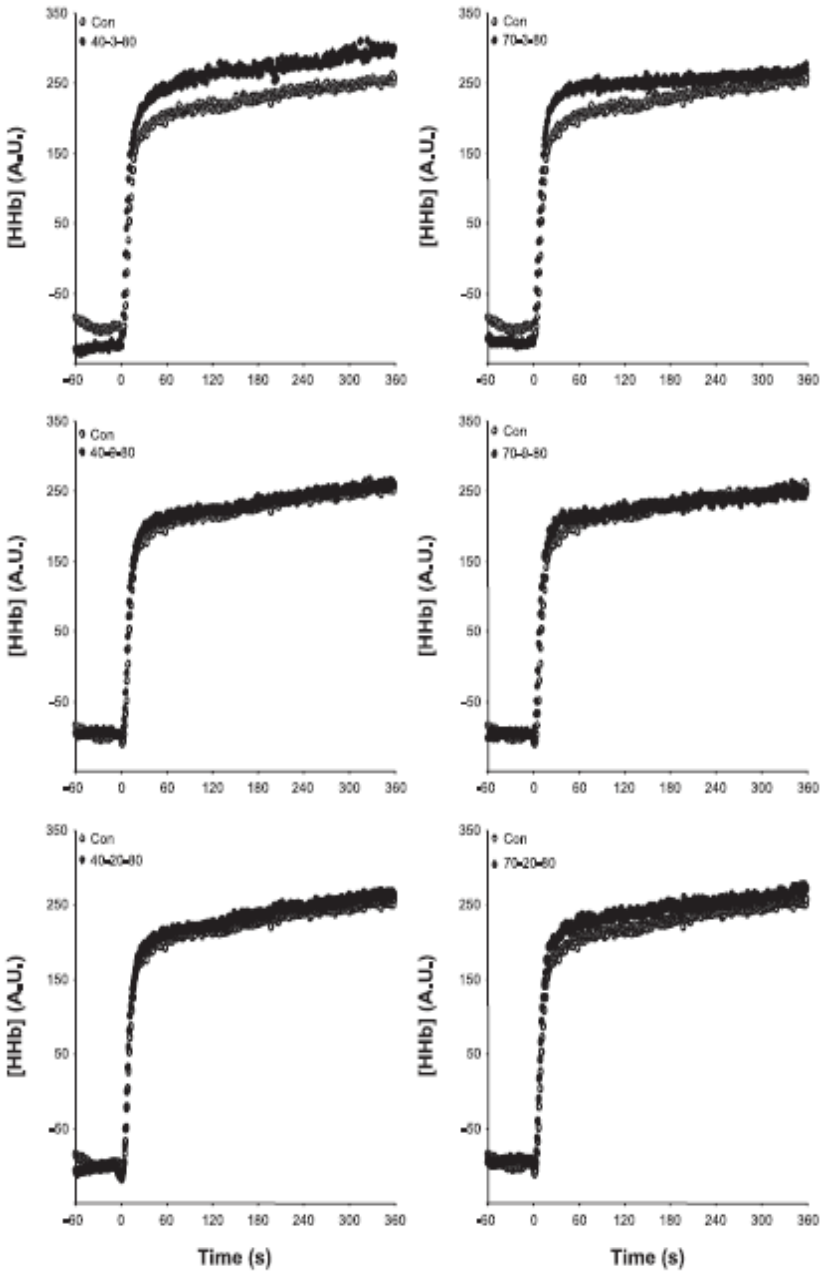

Fig. 2. Comparisons of group mean near-infrared spectroscopy (NIRS)-derived deoxygenated hemoglobin/myoglobin ([HHb]) dynamics during a step increment from an unloaded baseline to a severe-intensity work rate during control and in the following priming conditions: 40-3-80 (*top left*), 40-9-80 (*middle left*), 40-20-80 (*bottom left*), 70-3-80 (*top right*), 70-9-80 (*middle right*), and 70-20-80 (*bottom right*). ○, [HHb] responses in control; ●, [HHb] responses in the variously primed conditions. Error bars are omitted for clarity. Notice that the amplitude of the [HHb] response is increased after prior exercise, especially after 3 min of recovery. AU, arbitrary units.

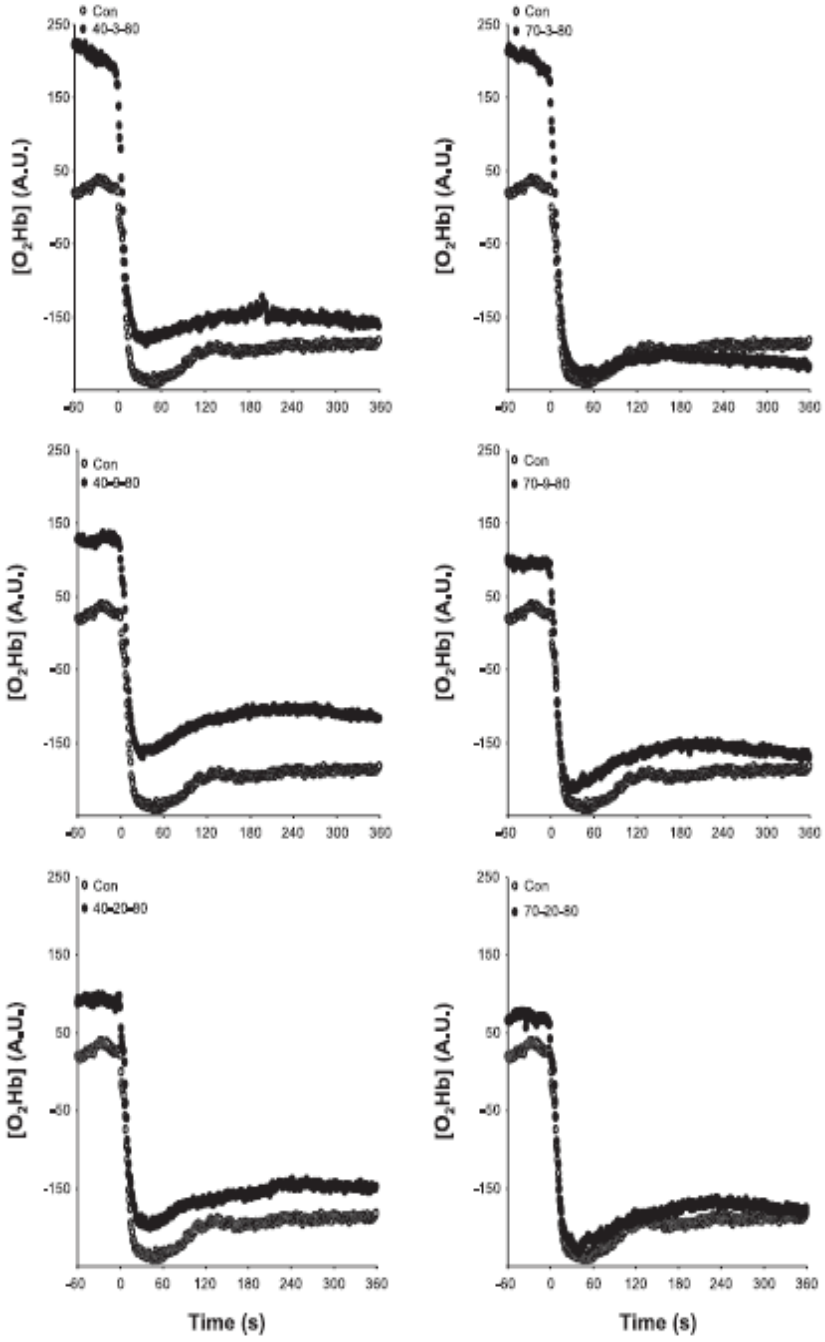

Fig. 3. Comparisons of group mean NIRS-derived oxygenated hemoglobin/myoglobin ($[HbO_2]$) dynamics during a step increment from an unloaded baseline to a severe-intensity work rate during control and in the following priming conditions: 40-3-80 (*top left*), 40-9-80 (*middle left*), 40-20-80 (*bottom left*), 70-3-80 (*top right*), 70-9-80 (*middle right*), and 70-20-80 (*bottom right*). ○, $[HbO_2]$ responses in control; ● $[HbO_2]$ responses in the variously primed conditions. Error bars are omitted for clarity. Notice that priming exercise results in increased $[HbO_2]$ at baseline and during subsequent exercise but that the effect wanes as recovery duration is increased.

80, and 70-3-80 prior exercise conditions relative to control (Table 2).

HR dynamics in the CON and primed conditions are reported in Table 3 and shown as group mean responses in Fig. 4. Compared with control, the HR at baseline and at 60 and 120 s of exercise was significantly elevated in all of the prior exercise conditions investigated. Overall kinetics of the HR response, on the other hand, were only significantly faster than control in the 40-9-80, 70-9-80, and 70-20-80 prior exercise conditions (Table 3 and Fig. 4).

Blood [lactate]. The baseline blood [lactate] was significantly elevated above control (1.1 ± 0.2 mM) during the 40-3-80, 40-9-80, 70-3-80, 70-9-80, and 70-20-80 prior exercise conditions (Table 3). The accumulation of blood lactate over the first 6 min of the criterion exercise bout was significantly reduced compared with control in the 70-3-80 and 70-9-80 conditions (Table 3).

iEMG response. iEMG responses during the criterion exercise bout in the control and experimental conditions are shown in Table 4 and Fig. 5. The average iEMG response over the first 60, 120, and 360 s of exercise as well as the absolute values at 120 and 360 s did not differ across the experimental conditions (Table 4). The change in iEMG from 2 to 6 min [$\Delta iEMG_{(2-6\ min)}$] was reduced relative to control (104 ± 115% increase) only in the

Table 3. *HR dynamics and blood [lactate] during severe-intensity exercise in the control and variously primed conditions*

	Control	Primed Conditions					
		40-3-80	40-9-80	40-20-80	70-3-80	70-9-80	70-20-80
HR, beats/min							
Baseline, beats/min	91±12	113±15[b,d,e]	104±13[b]	101±13[a]	128±13[b,c,d,e,g,h]	116±16[b,d,e,g]	108±11[b,e]
At 60 s, beats/min	140±5	156±11[b,e]	153±10[b]	149±11[a]	165±10[b,c,d,e,g,h]	160±13[b,e]	154±9[b,e]
At 120 s, beats/min	157±7	167±9[b]	165±9[b]	163±10[a]	176±9[b,c,d,e,g,h]	170±11[b,e,h]	166±8[b,e]
End exercise, beats/min	180±7	182±11	182±10	182±9	187±7[b,d,e,h]	186±9[e]	180±7
Mean response time, s	75±18	63±6	60±8[a]	62±12	64±6	59±10[a]	57±14[a]
Blood [lactate]							
Baseline, mM	1.1±0.2	3.1±0.9[b,d,e]	2.0±0.4[b]	1.3±0.3	6.7±0.9[b]	5.3±1.0[b]	3.0±0.8[b]
At 360 s, mM	7.3±1.5	9.2±1.2[a]	8.2±1.4	7.8±1.1	9.9±2.1[b,c,d,e,f,g,h]	9.3±1.8[b,c,d,e,h]	8.0±1.6[d,e]
Change in baseline − 360 s, mM	6.2±1.7	6.1±1.2	6.2±1.4	6.5±0.9	3.2±1.4[b,c,d,e,h]	4.0±1.3[a,c,d,e,h]	5.0±1.3[d,e]
Time to exhaustion, mM	10.1±1.6	9.4±1.3	10.1±1.8	9.9±1.8	9.8±1.5	9.9±1.9	10.1±1.6
Change in baseline − time to exhaustion, mM	8.9±1.7	6.3±1.0[b,d,e]	8.1±1.8	8.6±1.7	3.1±1.5[b,c,d,e,h]	4.5±1.5[b,c,d,e,h]	7.1±1.3[b,d,e]

Values are means ± SD. HR, heart rate. [a]Significantly different from control ($P < 0.05$); [b]significantly different from control ($P < 0.01$); [c]significantly different from the 40-3-80 condition ($P < 0.05$); [d]significantly different from the 40-9-80 condition ($P < 0.05$); [e]significantly different from the 40-20-80 condition ($P < 0.05$); [f]significantly different from the 70-3-80 condition ($P < 0.05$); [g]significantly different from the 70-9-80 condition ($P < 0.05$); [h]significantly different from the 70-20-80 condition ($P < 0.05$).

70-20-80 prior exercise condition (16 ± 45% increase, $P < 0.05$; Table 4 and Fig. 5). However, intracondition analyses revealed that the iEMG signal at 120 s was significantly lower than that observed at 360 s in the control and 40-9-80 conditions, whereas these values were similar at 120 and 360 s in the remaining prior exercise conditions (Table 4 and Fig. 5). In the 70-20-80 prior exercise condition, the reduced $\Delta iEMG_{(6-2\ min)}$ was significantly correlated with the reduction in the $\dot{V}O_2$ slow component amplitude ($r = 0.75$, $P < 0.05$).

Exercise tolerance. The changes in the tolerance of severe-intensity exercise consequent to the various prior exercise interventions were compared with control in the *insets* of the respective $\dot{V}O_2$ graphs in Fig. 1, and the group mean change is shown in Fig. 6. Prior exercise at 40%Δ did not significantly alter severe-intensity exercise tolerance, regardless of the recovery duration with which this intensity of prior exercise was coupled (Fig. 1). Significant improvements in exercise tolerance above control (437 ± 79 s) were observed in the 70-9-80 condition (504 ± 84 s, +15% improvement, $P < 0.05$) and in the 70-20-80 condition (567 ± 125 s, + 30% improvement, $P < 0.05$; Figs. 1 and 6). The time to exhaustion was significantly longer in the 70-20-80 condition compared with the 70-9-80 condition ($P < 0.05$). Exercise tolerance was significantly impaired in the 70-3-80 condition, being reduced 16% below control ($P < 0.05$; Fig. 6).

DISCUSSION

The principal original finding of this investigation was that severe-intensity prior exercise significantly improved subsequent severe-intensity exercise tolerance when it was coupled with a recovery period of 9 min (15% improved performance) or 20 min (30% improved performance; Fig. 6). In contrast, prior severe-intensity exercise followed by 3 min of recovery resulted in a significant 16% impairment in the tolerance of subsequent severe-intensity exercise. The performance of prior heavy-intensity exercise did not enhance performance during subsequent severe-intensity exercise irrespective of the intervening recovery duration. Prior severe-intensity exercise was more effective than prior heavy-intensity exercise in altering $\dot{V}O_2$ kinetics, but the magnitude of effect receded with time for both intensities of prior exercise. Overall $\dot{V}O_2$ kinetics were accelerated in the 40-3-80, 70-3-80, 70-9-80, and 70-20-80 conditions. That exercise tolerance was only enhanced in the latter two conditions indicates that a speeding of $\dot{V}O_2$ kinetics, per se, is not necessarily ergogenic. Rather, exercise tolerance was enhanced when the prior exercise bout was sufficiently intense to provoke an accelerated $\dot{V}O_2$ response and the subsequent recovery duration was sufficiently long for homeostasis (as reflected by the baseline $\dot{V}O_2$ and blood [lactate]) to return toward control values. Interestingly, in the 70-20-80 condition (where exercise tolerance was increased the most), $\dot{V}O_2$ kinetics were accelerated in association with a significantly blunted $\Delta iEMG_{(6-2\ min)}$ relative to control.

Physiological effects of prior exercise. Overall $\dot{V}O_2$ kinetics were significantly faster than control in all conditions other than the 40-9-80 and 40-20-80 conditions. Phase II $\dot{V}O_2$ kinetics were not altered by any combination of prior exercise intensity and recovery duration in the young healthy subjects who participated in our study, results that are consistent with a large number of previous studies (e.g., Refs. 5, 7–11, 14, 21, 33, 39, 40, 48, 53, 54, and 63). Rather, the faster overall $\dot{V}O_2$ adjustment was consequent to an increase in the absolute $\dot{V}O_2$ fundamental component amplitude and, in particular, to a reduction in the $\dot{V}O_2$ slow component amplitude. The latter was reduced below control in the 40-3-80 (by 32%), 70-3-80 (by 54%), 70-9-80 (by 40%), and 70-20-80 (by 26%) conditions, resulting, respectively, in an 11%, 41%, 32%, and 23% speeding of overall $\dot{V}O_2$ kinetics relative to control.

A novel finding in this study was that heavy-intensity prior exercise did not elicit the typical prior exercise effect on the $\dot{V}O_2$ response during subsequent exercise when the recovery duration separating the prior and criterion exercise bouts equaled or exceeded 9 min. This contrasts with previous reports (7, 8, 36). However, in the majority of the previous studies, "heavy-intensity" prior exercise was performed at 50%Δ (7, 8, 11, 14, 36), based on the initial study of Gerbino et al. (26). However, this is problematic as 50%Δ approximates the boundary between the heavy-intensity exercise domain, within which $\dot{V}O_2$ will eventually stabilize, and the severe-intensity exercise domain, within which $\dot{V}O_2$ will continue to rise with time until $\dot{V}O_{2max}$ is attained (50, 64). It is therefore possible that at least some of the subjects in these previous

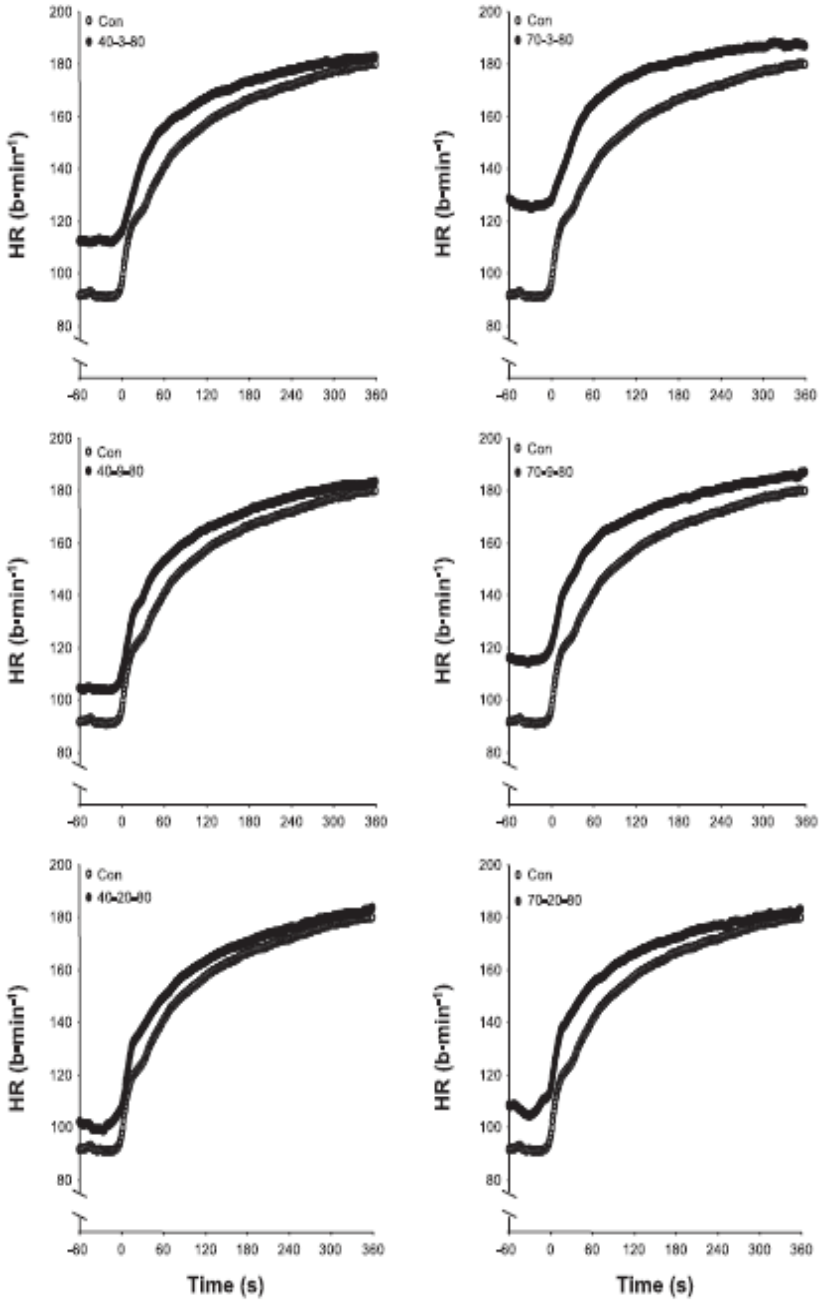

Fig. 4. Comparisons of group mean heart rate (HR) dynamics during a step increment from an unloaded baseline to a severe-intensity work rate during control and in the following priming conditions: 40-3-80 (*top left*), 40-9-80 (*middle left*), 40-20-80 (*bottom left*), 70-3-80 (*top right*), 70-9-80 (*middle right*), and 70-20-80 (*bottom right*). ○, HR responses in control; ●, HR responses in the variously primed conditions. Error bars are omitted for clarity. Notice that priming exercise results in increased HR at baseline and during subsequent exercise but that the effect wanes as recovery duration is increased.

studies performed prior exercise at an intensity that exceeded CP. We selected 40%Δ to represent heavy-intensity exercise in the present study, and, although we did not formally assess CP, the $\dot{V}O_2$ response profiles elicited by our prior exercise bouts at 40%Δ and 70%Δ were entirely characteristic of heavy-intensity and severe-intensity exercise (50, 64). Our data show that prior heavy-intensity exercise reduced the $\dot{V}O_2$ slow component during subsequent severe-intensity exercise after 3 min of recovery, but that the effect was lost when recovery was extended to 9 or 20 min. The results of the present study therefore indicate that prior severe-intensity exercise provides a more potent and longer-lasting stimulus than heavy-intensity exercise for reducing the $\dot{V}O_2$ slow component during subsequent exercise.

HR was significantly elevated above control in all the prior exercise conditions investigated in the study, both during the baseline period and over the first 120 s of exercise, as noted previously (e.g., Refs. 7, 25, 55, and 63). At the muscular level, however, $[Hb_{tot}]$ (an index of hyperemia) was significantly greater than control only in the baseline period of the 40-3-80, 40-9-80, and 70-3-80 prior exercise conditions. $[HbO_2]$ in the microcirculation was elevated during the baseline period in the 40-3-80, 40-9-80, 70-3-80, and 70-9-80 conditions relative to control. Despite this evidence for greater muscle O_2 availabil-

Table 4. *Surface iEMG during severe-intensity exercise in the control and variously primed conditions*

	Control	Primed Conditions					
		40-3-80	40-9-80	40-20-80	70-3-80	70-9-80	70-20-80
iEMG average							
From 0 to 60 s, %baseline	531±149	526±146	514±148	549±160	511±189	544±242	623±301
From 0 to 120 s, %baseline	536±146	541±163	522±155	558±175	523±191	558±251	629±306
From 0 to 360 s, %baseline	604±193	574±195	558±174	602±219	539±235	589±266	650±317
iEMG at 120 s, % baseline	559±157	560±184	540±177	572±193	549±209	574±265	640±322
iEMG at 360 s, %baseline	663±264†	607±225	600±210†	622±269	492±316	585±312	656±325
Change in iEMG from 6 to 2 min, %baseline	104±115	47±61	59±71	50±89	22±157	12±113	16±45*

Values are means ± SD. iEMG, integrated electromyography. *Significantly different from control ($P < 0.05$); †significantly different from the 120-s value in the respective experimental condition ($P < 0.05$).

ity, $\dot{V}O_2$ τ_p was not significantly different from control for any of the prior exercise conditions studied. In young healthy subjects performing high-intensity upright cycle exercise, $\dot{V}O_2$ τ_p appears to be intransigent to interventions that would be expected to enhance the muscle O_2 supply (Refs. 14, 33, 49, 62, and 65; cf. Ref. 29), indicating that the finite rate at which $\dot{V}O_2$ rises after the onset of exercise is determined principally by an intracellular limitation to the rate of O_2 utilization (27, 49, 52). It is of interest that overall $\dot{V}O_2$ kinetics were accelerated compared with control in the 70-9-80 and 70-20-80 conditions despite the baseline [Hb_{tot}] and [HbO_2] having returned to control values. These data suggest that an enhanced muscle vasodilatation at baseline after a prior exercise intervention is not necessary to invoke a speeding of overall $\dot{V}O_2$ kinetics. Similarly, prior contractile activity has been reported to result in the faster activation of muscle oxidative metabolism in the absence of enhanced muscle O_2 availability in animal models (6, 32). Also, although the MRT for HR kinetics was faster in the 70-9-80 and 70-20-80 conditions compared with control, this was also true for the 40-9-80 condition in which $\dot{V}O_2$ kinetics were not significantly altered, indicating a dissociation between changes in HR kinetics and $\dot{V}O_2$ kinetics.

The NIRS-derived [HHb] response reflects the balance between local O_2 delivery and utilization and has been used previously as an index of muscle fractional O_2 extraction (3, 19, 20, 23, 28, 29, 34). Relative to control, [HHb] TD and also [HHb] TD + τ was significantly reduced in the 40-3-80, 70-3-80, and 70-9-80 conditions. Moreover, the [HHb] primary amplitude was significantly increased above control in the 40-3-80, 70-3-80, and 70-20-80 conditions. The shorter TD + τ and/or greater [HHb] amplitude observed in these conditions indicates that, in the region of NIRS interrogation, muscle $\dot{V}O_2$ increased relatively more than blood flow in the transition to the higher metabolic rate in the primed condition. In the 70-20-80 condition, where [Hb_{tot}] and [HbO_2] were not different from control, the faster overall $\dot{V}O_2$ kinetics was apparently facilitated, in large part, by an increased muscle O_2 extraction. These data are consistent with previous reports showing that prior intense exercise increases muscle O_2 extraction during subsequent exercise (19, 20, 25, 41). The increased ability for muscle to extract O_2 after priming is presumably a consequence of an increased oxidative enzyme activity, which might be expected to reduce the intrinsic metabolic inertia to muscle O_2 utilization (27, 29, 49, 53). Certainly, the data indicate that the rate of change of $\dot{V}O_2$ exceeded the rate of change of blood flow to a greater extent in the presence, compared with the absence, of priming exercise. It should be noted that, like many of the other physiological responses measured in this study, the NIRS indexes of muscle O_2 extraction during exercise became less pronounced as the recovery interval increased (Fig. 2). This is consistent with the time course of recovery of muscle enzyme activity after exercise. For example, it has been reported that pyruvate dehydrogenase (PDH) activity, which has been suggested to represent a possible limitation to $\dot{V}O_2$ kinetics by restricting carbon substrate availability (15, 29, 56), recovers with a half-time of ~4 min after intense exercise (51). On this basis, PDH activity would have essentially recovered to baseline after 20 min of recovery, yet overall $\dot{V}O_2$ kinetics remained 23% faster than control in the 70-20-80 condition. This suggests a possible dissociation between PDH (and possibly other oxidative enzyme) activity and the observed changes in $\dot{V}O_2$ kinetics after prior exercise.

iEMG quantifies the gross electrical activity of the muscle and therefore provides information pertaining to motor unit recruitment and firing frequency during a given exercise task. It has been reported that, after a prior high-intensity exercise bout, iEMG is higher over the first 2 min of subsequent high-intensity exercise and then increases at a lower rate as exercise proceeds (7, 43). This has been interpreted to indicate that motor unit recruitment is increased after the onset of exercise in the primed condition such that the metabolic demand per fiber is reduced and the requirement for additional fiber recruitment as high-intensity exercise continues is reduced (7, 43). The development of the $\dot{V}O_2$ slow component has been ascribed to the progressive recruitment of motor units with time (7, 35, 42, 48, 58), and thus changes in motor unit recruitment profiles could explain the reciprocal changes noted in the $\dot{V}O_2$ fundamental and slow component amplitudes after prior exercise interventions (7, 12). While there is reasonable consensus that muscle fiber type and motor unit recruitment patterns play an important role in the development of the $\dot{V}O_2$ slow component, it should be noted that the contribution of progressive motor unit recruitment, per se, to the development of the $\dot{V}O_2$ slow component remains a matter of debate (see Refs. 20, 54, and 66 for a discussion).

In the present study, the iEMG values increased significantly between 2 and 6 min in the control and 40-9-80 conditions, with there being a strong trend for a similar pattern in the 40-20-80 condition (Fig. 5). However, the iEMG at 6 min was not significantly higher than at 2 min in the other prior exercise and recovery conditions, implying that the recruitment of additional motor units with time was attenuated. Interestingly, the $\dot{V}O_2$ slow component was reduced below control in these

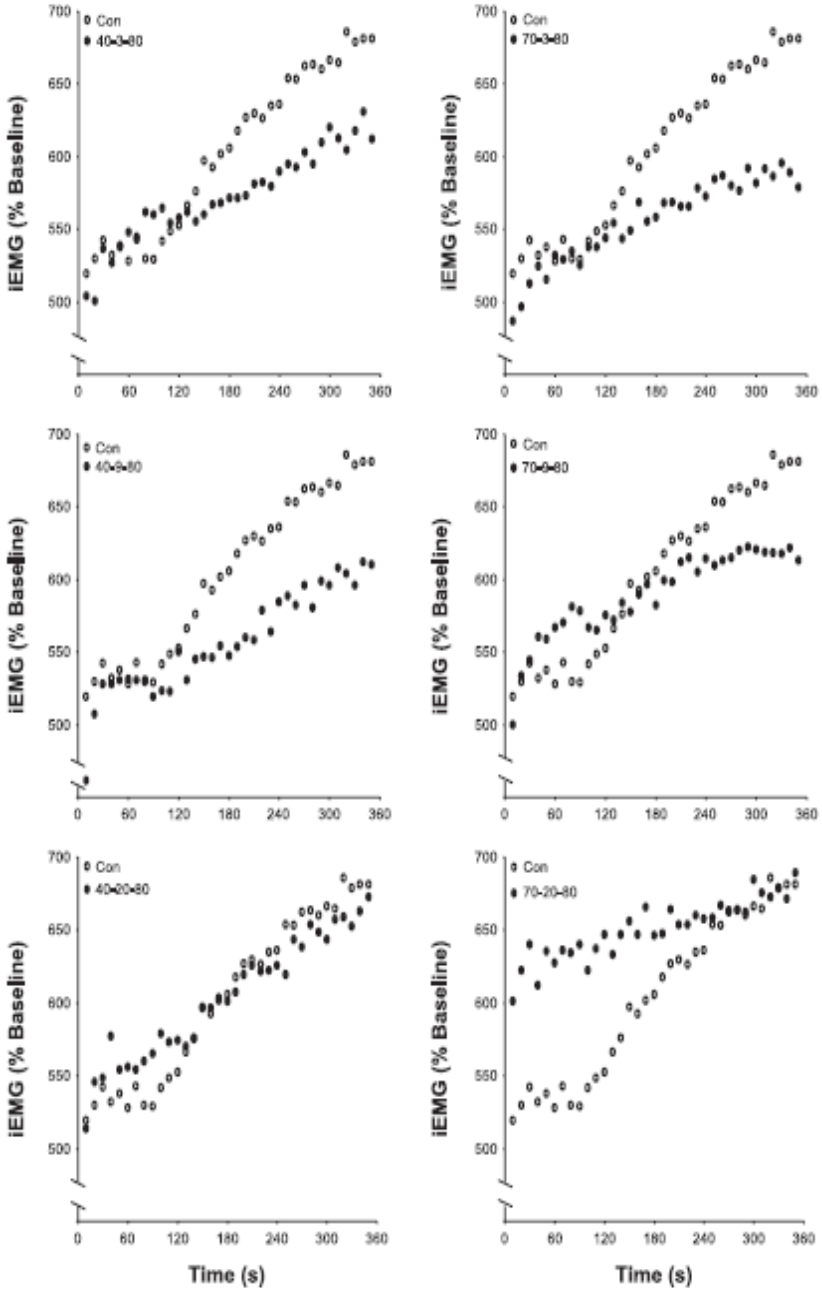

Fig. 5. Comparisons of the group mean surface integrated electromyography (iEMG) responses during a step increment from an unloaded baseline to a severe-intensity work rate during control and in the following priming conditions: 40-3-80 (*top left*), 40-9-80 (*middle left*), 40-20-80 (*bottom left*), 70-3-80 (*top right*), 70-9-80 (*middle right*), and 70-20-80 (*bottom right*). ○, iEMG responses in control; ●, iEMG responses in the variously primed conditions. Error bars are omitted for clarity. Notice that priming exercise tends to blunt the increase in iEMG with time during subsequent exercise.

same conditions, supporting the notion that the progressive recruitment of muscle fibers might contribute to the development of the $\dot{V}O_2$ slow component (42, 48, 58) and suggesting that alterations in muscle fiber recruitment might be, at least in part, responsible for the speeding of overall $\dot{V}O_2$ kinetics after prior exercise (7, 12, 35, 43). This was particularly the case for the 70-20-80 condition, in which there was a clear trend for iEMG to be higher over the first 2 min of exercise and in which $\Delta iEMG_{(6\ -\ 2\ min)}$ was significantly reduced. Therefore, in the 70-20-80 condition, the overall $\dot{V}O_2$ speeding appears to be related to an initial increase in muscle activity and fractional O_2 extraction and reduced recruitment of additional motor units with time. These data support the previous findings of Burnley et al. (7), who reported that muscle iEMG was 19% higher at the onset of the second of two bouts of severe-intensity exercise and noted that the iEMG responses (higher initial iEMG and a blunted increase in iEMG as exercise proceeded) were qualitatively similar to the changes in $\dot{V}O_2$ kinetics (increased $\dot{V}O_2$ fundamental component amplitude and reduced $\dot{V}O_2$ slow component) in the second bout. The mechanism responsible for these changes in iEMG is not clear. However, it has been shown that prior muscle activity reduces the "recruitment threshold" of motor units during subsequent contractions and that the restoration of the original thresholds may take considerably longer than the restoration of voluntary force-generating capacity (1, 16).

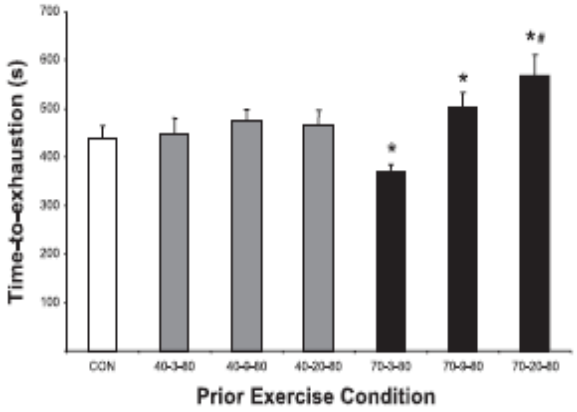

Fig. 6. Mean ± SE times to exhaustion during severe-intensity exercise in control and after the various prior exercise and recovery permutations. Relative to control, the time to exhaustion was increased by 2% in the 40-3-80 condition [nonsignificant difference (NSD)], by 8% in the 40-9-80 condition (NSD), by 7% in the 40-20-80 condition (NSD), by 15% in the 70-9-80 ($P < 0.05$), and by 30% in the 70-20-80 condition ($P < 0.05$). The time to exhaustion in the 70-20-80 condition was significantly greater than in the 70-9-80 condition ($P < 0.05$). Relative to control, the time-to-exhaustion was 16% lower in the 70-3-80 condition ($P < 0.05$).

Exercise tolerance. A significantly improved tolerance of severe-intensity exercise was observed after prior severe-intensity exercise coupled with 9 and 20 min of recovery but not after severe-intensity exercise and 3 min of recovery or after heavy-intensity prior exercise irrespective of the recovery duration. This potential for prior high-intensity exercise to enhance performance during subsequent exercise confirms some previous reports (10, 36). Importantly, the improved exercise tolerance in the 70-9-80 and 70-20-80 conditions manifested when the $\dot{V}O_2$ slow component amplitude was reduced and overall $\dot{V}O_2$ kinetics were speeded. While these data imply an interdependence between a speeding of overall $\dot{V}O_2$ kinetics and enhanced performance, the time to exhaustion in the 70-3-80 condition was significantly reduced despite the fact that overall $\dot{V}O_2$ dynamics were fastest in this condition. This suggests that, after prior exercise, the faster overall $\dot{V}O_2$ kinetics does not, in itself, determine the tolerable duration of exercise but instead might do so through interaction with other physiological parameters (13).

The performance of prior severe-intensity exercise does not appear to alter the CP or $\dot{V}O_{2max}$ but does have the potential to alter the finite quantity of work that can be performed above the CP (W') during subsequent high-intensity exercise (22, 36, 57). As such, it seems plausible that changes in W' and $\dot{V}O_2$ kinetics after prior exercise interact to determine the tolerance to subsequent high-intensity exercise. The magnitude of W' is determined by the phosphocreatine (PCr) and glycolytic (anaerobic) energy reserves along with a small contribution from stored O_2 (44, 45) and/or the accumulation of fatigue-related metabolites [e.g., H^+, P_i, $H_2PO_4^-$, and extracellular K^+ (2, 22, 34, 50, 57)]. During severe-intensity exercise, W' is gradually expended with time, and exercise cannot be continued at the same rate when W' has been entirely depleted (44, 50). It has been shown that severe-intensity exercise performance is impaired after a prior severe-intensity exercise bout and short recovery period as a consequence of a reduced W' at baseline (22, 57). Forbes et al. (24) reported that intramuscular [PCr] and pH were reduced below the control value 3 min after prior heavy-intensity plantar flexion exercise. When the recovery duration was extended to 6 and 15 min, however, [PCr] and pH were not different from control, with the pH recovering further between 6 and 15 min (24). In the present study, baseline $\dot{V}O_2$ was elevated above control in the 40-3-80, 70-3-80, and 70-9-80 conditions. An elevated baseline $\dot{V}O_2$ would be expected to reflect an incomplete [PCr] recovery after prior exercise given the close agreement between pulmonary $\dot{V}O_2$ and intramuscular [PCr] (52). It should be noted that a low intramuscular [PCr] might facilitate an acceleration of $\dot{V}O_2$ kinetics by reducing metabolic capacitance and hence enabling the stimuli to oxidative phosphorylation to rise more rapidly (37, 38); however, as noted above, a low [PCr] at the start of exercise might be expected to impair performance. It has been reported that the estimated W' is restored to baseline within ~15 min after prior sprint exercise (57) and that the effect of prior high-intensity exercise on $\dot{V}O_2$ dynamics is preserved for at least 30 min but declines in a time-dependent manner (9). Therefore, extending the recovery duration to restore W' (and hence the muscle energetic reserve and tissue homeostasis) after prior severe exercise, while preserving faster overall $\dot{V}O_2$ dynamics, appears to have facilitated the increased tolerance to severe-intensity exercise we observed.

The performance of prior high-intensity exercise results in an elevated baseline blood [lactate] and a reduced accumulation of blood lactate during the criterion bout (7, 14, 20, 36). We have previously reported that a baseline blood [lactate] of ~3 mM appeared to be associated with improvements in performance after prior exercise (10, 36, 47). Residual acidosis provides a stimulus for an increased O_2 availability through facilitating vasodilatation and a Bohr shift in the O_2 dissociation curve, potentially supporting faster overall $\dot{V}O_2$ kinetics as initially hypothesized by Gerbino et al. (26). Additionally, a small reduction in muscle pH might provide a stimulus for increased muscle excitability during the subsequent bout by offsetting the deleterious effects of muscle depolarization that accompanies repeated intense muscle contractions (2, 31, 46). Moderate-intensity prior exercise does not appreciably elevate blood [lactate] above resting values and does not enhance $\dot{V}O_2$ kinetics or performance during subsequent exercise (8, 14, 26, 39). In the present study, prior heavy-intensity exercise did not significantly enhance exercise tolerance irrespective of the recovery duration. For the 40-9-80 and 40-20-80 conditions, baseline blood [lactate] was low (~2.0 and 1.3 mM, respectively) and $\dot{V}O_2$ kinetics were not enhanced. Similarly, Burnley et al. (9) have shown that the effect of prior exercise on $\dot{V}O_2$ kinetics is lost when baseline blood [lactate] recovers to ≤2 mM. In the 40-3-80 condition, baseline blood [lactate] was elevated (~3.1 mM) and overall $\dot{V}O_2$ kinetics were speeded, but the 3-min recovery duration was unlikely to be sufficient to enable a full restoration of muscle [PCr] (24). Previous studies have shown that prior multiple sprint exercise or severe-intensity exercise with a short recovery, leading to a baseline blood [lactate] of >6–7 mM, enhances $\dot{V}O_2$ kinetics but is detrimental to subsequent exercise performance (22, 63). In these circumstances, it is likely that the high blood [lactate] reflects a situation in which W' has not been fully restored and/or the muscle fatigue-related metabolite concentration remains high. In the present study, the baseline blood [lactate] was ~7 mM in the 70-3-80 condition, where, relative to

control, performance was significantly impaired, ~5 mM in the 70-9-80 condition, where performance was enhanced by 15%, and ~3 mM in the 70-20-80 condition, where performance was enhanced by 30%. These results are therefore consistent with previous studies in suggesting that a baseline blood [lactate] in the range of ~3–5 mM is associated with enhanced performance during subsequent exercise (10, 36, 47), with the proviso that the recovery interval is sufficient to enable muscle homeostasis (including the concentration of fatigue-related metabolites) to be restored toward control values.

Conclusions. The appropriate combination of prior exercise intensity and recovery duration enables an acceleration of $\dot{V}O_2$ kinetics during subsequent severe-intensity exercise. This acceleration is principally the result of a reduction in the amplitude of the $\dot{V}O_2$ slow component. The present study shows that $\dot{V}O_2$ kinetics are not altered if the prior bout of exercise is not sufficiently intense or the recovery interval is too long (i.e., the 40-9-80 and 40-20-80 conditions), whereas the effect is greatest if a high-intensity prior exercise bout is coupled with a short recovery interval (the 70-3-80 condition). The mechanistic bases for the speeded overall $\dot{V}O_2$ kinetics after prior high-intensity exercise is complex and can potentially involve the interaction of a number of separate effects including increased muscle O_2 availability, greater muscle oxidative enzyme activity and carbon substrate supply, and altered motor unit recruitment profiles (19, 29, 35). All of these effects would be expected to recede as the recovery interval separating the priming and criterion bouts is extended. An important finding in the present study was that, after severe-intensity prior exercise, overall $\dot{V}O_2$ kinetics remained significantly speeded after 20 min of recovery, when the baseline [Hb_{tot}] and [HbO_2] had returned to control values. In this condition, the reduced $\dot{V}O_2$ slow component was associated with a reduced change in iEMG between 2 and 6 min of exercise, suggesting that changes in motor unit recruitment after prior exercise has a long latency and that such changes, along perhaps with increased activity of rate-limiting enzymes in the respiratory chain, might underpin the effects of prior exercise on $\dot{V}O_2$ kinetics and performance during subsequent exercise, at least in young physically active subjects.

The fact that exercise tolerance was significantly reduced in the 70-3-80 condition despite it producing the fastest overall $\dot{V}O_2$ response suggests that the presence of faster $\dot{V}O_2$ kinetics, per se, will not necessarily enhance performance during subsequent high-intensity exercise. Exercise tolerance was substantially improved in the 70-9-80 and 70-20-80 conditions and was greatest in the latter, in which the overall $\dot{V}O_2$ response was speeded relative to control and the baseline $\dot{V}O_2$ was restored. We suggest that the extent to which a prior exercise/recovery regimen will be ergogenic is determined by an interaction between the magnitude of the acceleration of overall $\dot{V}O_2$ kinetics and the magnitude of the reconstitution of W'. There is presently significant interest among applied physiologists in optimizing the precompetition warm-up to enhance athletic performance (30, 47). The present data suggest that a protocol involving a 6-min bout of severe-intensity exercise followed by a 20-min recovery period, enabling baseline $\dot{V}O_2$ to be restored and blood [lactate] to decline to ~3 mM, can significantly speed overall $\dot{V}O_2$ kinetics and improve the tolerance to subsequent severe-intensity exercise by as much as 30%, an effect that appears to be linked to changes in motor unit recruitment. This protocol appears to optimize the balance between maintaining faster overall $\dot{V}O_2$ kinetics and allowing complete or near-complete restoration of muscle energetic reserves and homeostasis, thereby facilitating performance gains.

DISCLOSURES

No conflicts of interest are declared by the author(s).

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Fast-Start Strategy Improves $\dot{V}O_2$ Kinetics and High-Intensity Exercise Performance

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ABSTRACT

BAILEY, S. J., A. VANHATALO, F. J. DIMENNA, D. P. WILKERSON, and A. M. JONES. Fast-Start Strategy Improves $\dot{V}O_2$ Kinetics and High-Intensity Exercise Performance. *Med. Sci. Sports Exerc.*, Vol. 43, No. 3, pp. 00–00, 2011. **Purpose**: The purpose of this study was to investigate the influence of pacing strategy on pulmonary $\dot{V}O_2$ kinetics and performance during high-intensity exercise. **Methods**: Seven males completed 3- and 6-min bouts of cycle exercise on three occasions with the bouts initiated using an even-start (ES; constant work rate), fast-start (FS), or slow-start (SS) pacing strategy. In all conditions, subjects completed an all-out sprint over the final 60 s of the test as a measure of performance. **Results**: For the 3-min exercise bouts, the mean response time (MRT) for the $\dot{V}O_2$ kinetics over the pacing phase was shortest in FS (35 ± 6 s), longest in SS (55 ± 14 s), and intermediate in ES (41 ± 10 s) ($P < 0.05$ for all comparisons). For the 6-min bouts, the $\dot{V}O_2$ MRT was longer in SS (56 ± 15 s) than that in FS and ES (38 ± 7 and 42 ± 6 s, respectively, $P < 0.05$). The $\dot{V}O_2$ at the end of exercise was not different from the $\dot{V}O_{2max}$ during the 6-min exercise bouts or three FS but was lower than $\dot{V}O_{2max}$ for three ES and three SS ($P < 0.05$). The end-sprint performance was significantly enhanced in three FS compared with three ES and three SS (mean power = 374 ± 68 vs 348 ± 61 and 345 ± 71 W, respectively; $P < 0.05$). However, end-sprint performance was unaffected by pacing strategy in the 6-min bouts. **Conclusions**: These data indicate that an FS pacing strategy significantly improves performance during 3-min bouts of high-intensity exercise by speeding $\dot{V}O_2$ kinetics and enabling the attainment of $\dot{V}O_{2max}$.
Key Words: $\dot{V}O_2$ DYNAMICS, CRITICAL POWER, ANAEROBIC CAPACITY, EXERCISE TOLERANCE

The rate of oxygen uptake ($\dot{V}O_2$) increases with exponential kinetics after the onset of exercise (40). The rate of muscle ATP turnover, on the other hand, increases instantaneously at exercise onset, with the energetic equivalent of the incurred "O_2 deficit" compensated by an increased rate of ATP resynthesis through phosphocreatine (PCr) degradation and anaerobic glycolysis. The tolerable duration of high-intensity exercise increases hyperbolically as power output declines, with the power asymptote of the power–duration curve termed the *critical power* (CP) (22,27,29). The curvature constant of this power–duration relationship, W', represents a finite amount of work that can be performed above CP and is related to the potential for ATP yield from substrate-level phosphorylation and/or the accumulation of fatigue-related metabolites (e.g., H^+, P_i, $H_2PO_4^-$, extracellular K^+) (16,24,27–29,36). For the same work rate, increasing the initial rate of oxidative energy production would be expected to reduce the depletion of the finite anaerobic energy reserves and the accumulation of fatiguing metabolites, thereby preserving W' and improving exercise performance (10,21). Accordingly, interventions that result in faster $\dot{V}O_2$ dynamics tend to result in improved performance during high-intensity exercise (5,6,25).

The pacing strategy adopted during an exercise bout, through determining the pattern of work rate distribution, has important implications for the activation and proportional contribution of oxidative metabolism to energy turnover. The total oxidative energy yield and the speed with which oxidative metabolism rises after the onset of exercise is increased when using all-out or fast-start (FS) pacing strategies compared with even-start (ES) or slow-start (SS) pacing strategies (2,3,8,19,25). The pacing strategy adopted during exercise therefore has important implications for exercise performance. During continuous athletic events of up to approximately 2–3 min in duration, the literature indicates that optimal performance is typically achieved with an all-out or positive pacing strategy (1,8,12,15,17,37). As the event duration is increased beyond 2–3 min, however, the optimal pacing strategy to enhance athletic performance becomes less clear with better performance having been reported after ES (15,32), all-out (2), FS (3), and FS followed by ES (12) pacing strategies. Thus, information on the extent to which event duration and pacing strategy interact to determine $\dot{V}O_2$ kinetics and exercise performance is presently limited.

In a recent study (25), we reported that the time to exhaustion during high-intensity exercise was significantly

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extended when subjects used an FS compared with an ES or SS pacing strategy. The $\dot{V}O_2$ kinetics were significantly faster in the FS condition compared with the ES and SS conditions. We interpreted these data to indicate that the FS pacing strategy enhanced high-intensity exercise performance by sparing the nonoxidative energy contribution to energy turnover across the rest-to-exercise transition, such that this energy equivalent was available for utilization later in the exercise bout (25). However, time-to-exhaustion trials do not adequately reflect the physiological demands or pacing strategy adopted in competition. For example, middle-to-long distance events are commonly terminated with and are often decided by performance during an "end sprint" (4,14,17,31). An investigation into the interaction of changes in $\dot{V}O_2$ kinetics and changes in exercise performance should therefore involve different initial pacing strategies (FS, ES, and SS) followed by a return to the same constant work rate and then an end-sprint phase in which subjects attempt to maximize the work done.

The purpose of this investigation was to assess how pacing strategy (FS, ES, and SS) and exercise duration (3 and 6 min) interact to determine end-sprint and hence overall high-intensity exercise performance. We hypothesized that an FS strategy would result in faster $\dot{V}O_2$ kinetics and an SS strategy would result in slower $\dot{V}O_2$ kinetics relative to ES for both exercise durations. To provide insight into the physiological bases of possible differences in $\dot{V}O_2$ kinetics between pacing conditions, we used near-infrared spectroscopy (NIRS) to assess differences in muscle oxygenation and estimated muscle fractional O_2 extraction between conditions (13,18). We also hypothesized that faster $\dot{V}O_2$ kinetics would be associated with improved end-sprint performance during both the 3- and the 6-min exercise trials.

METHODS

Subjects. Seven healthy males (mean ± SD: age = 21 ± 2 yr, stature = 1.80 ± 0.06 m, body mass = 80 ± 8 kg) volunteered to participate in this study. The subjects participated in exercise at a recreational level but were not highly trained and were familiar with laboratory exercise testing procedures, having previously participated in studies using similar procedures in our laboratory. The study was approved by the University of Exeter Research Ethics Committee. All subjects were required to give their written informed consent before the commencement of the study after the experimental procedures, associated risks, and potential benefits of participation had been explained. Subjects were instructed to arrive at the laboratory in a rested and fully hydrated state, at least 3 h postprandial, and to avoid strenuous exercise in the 24 h preceding each testing session. Each subject was also asked to refrain from caffeine and alcohol for 6 and 24 h before each test, respectively. All tests were performed at the same time of day (±2 h) at sea level in an air conditioned laboratory at 20°C.

Experimental overview. The subjects were required to report to the laboratory on eight occasions over a 3- to 4-wk period with the eight visits being separated by at least 24 h. After the completion of a ramp incremental test (visit 1) and a 3-min all-out test ([9,34]; visit 2), all subjects completed six paced exercise trials during which pulmonary $\dot{V}O_2$, heart rate (HR), blood (lactate), muscle oxygenation (by NIRS), and exercise performance (peak work rate and mean work rate achieved during the end sprint) were assessed. To assess the interactive influence of pacing strategy and exercise duration on exercise performance, we used a paradigm comprising three different pacing strategies (FS, ES, and SS) and two different exercise durations (3 and 6 min).

Incremental test. On the first laboratory visit, the subjects completed a ramp incremental exercise test for determination of the $\dot{V}O_{2max}$ and gas exchange threshold (GET). All cycle tests were performed on an electrically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). Initially, subjects performed 3 min of baseline cycling at "0 W," after which the work rate was increased by 30 $W \cdot min^{-1}$ until the limit of tolerance. The subjects cycled at a constant self-selected pedal rate (between 70 and 90 rpm), and the chosen pedal rate along with saddle and handle bar height and configuration was recorded and reproduced in subsequent tests. Breath-by-breath pulmonary gas exchange data were collected continuously during the incremental tests and averaged over consecutive 10-s periods. The $\dot{V}O_{2max}$ was taken as the highest 30-s mean 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 $\dot{V}CO_2$ versus 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 rate that would require 50%Δ (GET plus 50% of the difference between the work rate at the GET and $\dot{V}O_{2max}$) was subsequently calculated.

The 3-min all-out test. To estimate the parameters of the power–duration relationship (CP and W'), we used a 3-min all-out CP test (9,34). Before the test, subjects performed a 5-min warm-up at 90% GET, followed by 5 min of rest. The test then began with 3 min of unloaded baseline pedaling, followed by a 3-min all-out effort against a fixed resistance. Subjects were asked to accelerate to 110–120 rpm over the last 5 s of the baseline period. The resistance on the pedals during the 3-min all-out effort was set for each individual using the linear mode of the Lode ergometer so that the subject would attain the power output calculated to be 50%Δ on reaching their preferred cadence (linear factor = power/preferred cadence^2). Strong verbal encouragement was provided throughout the test, but subjects were not informed of the elapsed time to prevent pacing. To ensure an all-out effort, subjects were instructed to attain their peak power output as quickly as possible from the start of the test and to maintain the cadence as high as possible at all times throughout the

3 min. The CP was estimated as the mean power output over the final 30 s of the test and W' as the power-time integral above CP. The work rate that would be expected to lead to exhaustion in 3 min (3-tlim-WR) and 6 min (6-tlim-WR) was then calculated from the equation:

$$P = (W'/T_E) + \mathrm{CP} \quad [1]$$

where P is the target work rate, T_E is the time to exhaustion, CP is the critical power, and W' is the finite work capacity >CP in joules. For example, the work rate estimated to elicit a time to exhaustion of 180 s in a subject with W' of 20,000 J and CP of 250 W would be as follows: (20,000/180) + 250 = 361 W.

Pacing trials. The six experimental conditions were administered in a randomized order. Three of these exercise trials were of 3 min duration, and three were of 6 min duration. For each exercise duration, subjects completed the trial using ES, FS, and SS pacing strategies (Fig. 1). Each trial

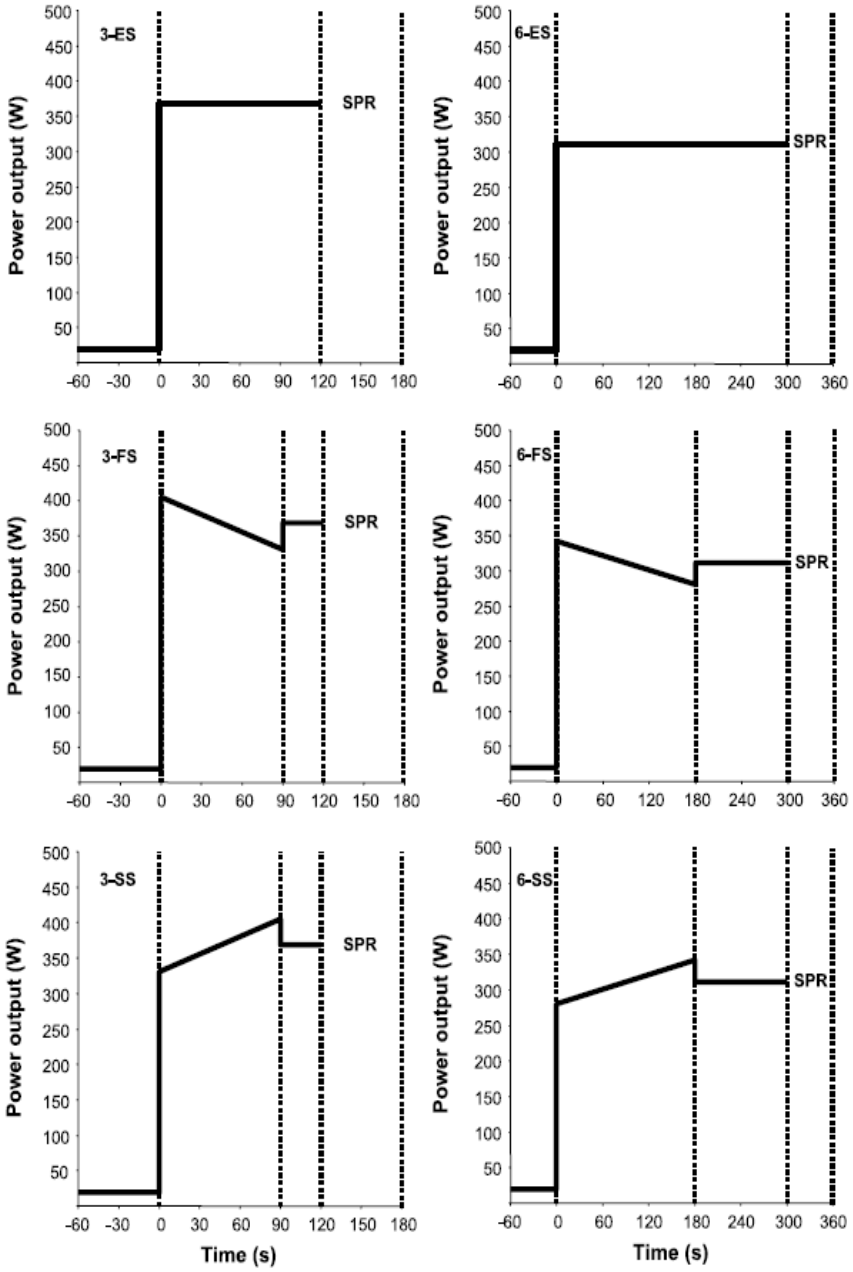

FIGURE 1—Schematic of the exercise protocol. Group mean work rates are shown for the 3-ES (*top left*), 3-FS (*middle left*), 3-SS (*bottom left*), 6-ES (*top right*), 6-FS (*middle right*), and 6-SS (*bottom right*) conditions. All protocols finished with a 1-min "end-sprint" phase (SPR) in which subjects were encouraged to complete as much work as possible. The dashed vertical lines demarcate the transition between the constituent phases specified by the particular protocol (for details, see Methods). Note that the mean work rate and hence the total work done were equal across the first 2 min of the 3-min trials and the first 5 min of the 6-min trials, irrespective of the pacing strategy used.

was preceded by 4 min of baseline cycling at 20 W before an abrupt step increment to the target work rate. The final minute of each trial required subjects to complete an all-out sprint against a fixed resistance, using the same linear factor as used in the 3-min all-out test, to determine the influence of the respective pacing strategy on exercise performance. In the 3-ES trial, subjects completed 2 min of constant work rate exercise at the 3-tlim-WR before the sprint, whereas in the 6-ES condition, the sprint was preceded by 5 min of constant work rate exercise at the 6-tlim-WR. In the FS and SS conditions, however, the work rate was initially not constant but rather decreased or increased with time, respectively, followed by a constant work rate "stabilization" phase before the all-out sprint (Fig. 1). In the 3-FS trial, the imposed work rate was initially 10% above the 3-tlim-WR, and this decreased linearly more than 90 s to 10% below the 3-tlim-WR; conversely, in the 3-SS trial, the work rate increased from 10% below to 10% above the 3-tlim-WR over the first 90 s of the test. After this "pacing" phase, a step increment (3-FS) or decrement (3-SS) was used to restore the work rate to the 3-tlim-WR, at which subjects completed 30 s of constant work rate cycling before initiation of the all-out sprint. A similar pattern of work rate imposition, relative to the test duration, was used in the 6-min trials (Fig. 1). Each subject completed an equal amount of work over the first 2 min of the 3-min trials and over the first 5 min of the 6-min trials, irrespective of the pacing strategy used. The pacing phase was used for one-half of the total exercise duration in the FS and SS conditions for both the 3- and the 6-min trials, and the final sprint was initiated from the same absolute work rate for the 3-min (~368 W) and the 6-min (~311 W) trials. Subjects were provided with a 5-s countdown before the sprint and were instructed to attain the peak power as quickly as possible and to continue exercising maximally for the duration of the sprint. No time feedback was given to the subjects at any point during the sprint.

Measurements. During all tests, pulmonary gas exchange and ventilation were measured breath by breath, with subjects wearing a nose clip and breathing through a low dead space, a low-resistance mouthpiece, and an impeller turbine assembly (Jaeger Triple V). The inspired and the expired gas volume and gas concentration signals were continuously sampled at 100 Hz, the latter using paramagnetic (O_2) and infrared (CO_2) analyzers (Jaeger Oxycon Pro, Hoechberg, Germany) via a capillary line connected to the mouthpiece. The gas analyzers were calibrated before each test with gases of known concentration, and the turbine volume transducer was calibrated with a 3-L syringe (Hans Rudolph, Kansas City, MO). The volume and the concentration signals were time aligned by accounting for the delay in the capillary gas transit and the analyzer rise time relative to the volume signal. Pulmonary gas exchange and ventilation were calculated and displayed breath by breath. HR was measured during all tests using short-range radiotelemetry (Polar S610; Polar Electro Oy, Kempele, Finland).

During the exercise trials, a blood sample was collected from a fingertip into a capillary tube over the 20 s preceding the step transition in work rate, the 20 s preceding the sprint, and also immediately after the sprint. These whole blood samples were subsequently analyzed to determine blood (lactate) (YSI 1500; Yellow Springs Instruments, Yellow Springs, OH) within 30 s of collection.

The oxygenation status of the musculus vastus lateralis of the right leg was monitored using a commercially available NIRS system (model NIRO 300; Hamamatsu Photonics KK, Hiugashi-ku, Japan). The system consisted of an emission probe that radiates laser beams and a detection probe. Four different wavelength laser diodes provided the light source (776, 826, 845, and 905 nm) and the light returning from the tissue was detected by a photomultiplier tube in the spectrometer. The intensity of incident and transmitted light was recorded continuously at 2 Hz and used to estimate concentration changes from the resting baseline for oxygenated, deoxygenated, and total tissue hemoglobin/myoglobin. Therefore, the NIRS data represent a relative change on the basis of the optical density measured in the first datum collected. The deoxygenated hemoglobin/myoglobin oncentration ([HHb]) signal was assumed to provide an estimate of changes in fractional O_2 extraction in the field of interrogation (5,13,18). The leg was initially cleaned and shaved around the belly of the muscle, and the optodes were placed in the holder that was secured to the skin with adhesive at 20 cm above the fibular head. To secure the holder and wires in place and to minimize the possibility that extraneous light could influence the signal, an elastic bandage was wrapped around the subject's leg. Indelible pen marks were made around the holder to enable precise reproduction of the placement in subsequent tests. The probe gain was set with the subject at rest in a seated position with the leg extended at down stroke on the cycle ergometer before the first exercise bout, and NIRS data were collected continuously throughout the exercise protocols. The data were subsequently downloaded onto a personal computer, and the resulting text files were stored on disk for later analysis.

Data analysis procedures. The breath-by-breath $\dot{V}O_2$ data from each test were initially examined to exclude errant breaths caused by coughing, swallowing, sighing, and so forth, and those values lying more than 4 SD from the local mean were removed. The breath-by-breath data were subsequently linearly interpolated to provide second-by-second values and time aligned to the start of exercise, and a nonlinear least square algorithm was used to fit the data thereafter. Given that the subjects only completed one trial in each condition, we did not consider it was justified to use a biexponential model to characterize the $\dot{V}O_2$ kinetics because statistical confidence in the derived parameters would be low. Therefore, a single-exponential model without time delay, with the fitting window commencing at t = 0 s (equivalent to the mean response time [MRT]), was used to characterize the kinetics of the overall $\dot{V}O_2$

response during the pacing trials as described in the following equation:

$$\dot{V}O_2(t) = \dot{V}O_{2\,baseline} + A(1-e^{-t/\tau}) \quad [2]$$

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time t, $\dot{V}O_{2baseline}$ represents the mean $\dot{V}O_2$ measured over the final 90 s of baseline pedaling, and A and τ represent the amplitude and time constant, respectively, describing the overall increase in $\dot{V}O_2$ above baseline. In addition, the same model was applied to the data between 20 and 120 s for all exercise bouts to provide an estimate of phase II. An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. We quantified the MRT and also the absolute $\dot{V}O_2$ at the end of the pacing (±5 s), stabilization (±5 s), and sprint (average over final 10 s) phases of the trials. The oxygen deficit was also calculated at these time points by multiplying the MRT and the $\Delta\ \dot{V}O_2$ at the specified time. The total O_2 consumed (in L) was also computed at these same time points, and the oxidative energy yield was estimated with the assumption that 1 L of O_2 consumed was equivalent to 20.9 kJ of energy expended (38).

To provide information on muscle oxygenation, we also modeled the [HHb] response to exercise. The [HHb] kinetics during the exercise were determined by fitting a monoexponential model from the first data point, which was 1 SD above the baseline mean through the entire response. The [HHb] TD and the τ values were summed to provide information on the overall [HHb] response dynamics. The [O_2Hb] and the [Hb_{tot}] responses do not approximate an exponential (18) and were not modeled. Rather, we assessed changes in these variables by determining the [O_2Hb] and the [Hb_{tot}] at baseline and after the pacing, stabilization, and sprint phases for both exercise durations. Pacing-induced changes in HR were assessed through comparing the HR at baseline and after the pacing, stabilization, and sprint phases for both exercise durations.

Performance during the end sprint was evaluated using the peak power output, time to peak power output, mean power output, and total sprint work done. The work done above the CP and the total work done in the 3- and 6-min tests were also calculated.

Statistical analysis. A one-way repeated-measures ANOVA was used to determine the effects on the relevant physiological and performance variables elicited by the pacing permutations for the 3- and 6-min trials. Where the analysis revealed a significant difference, individual paired t-tests were used with a Fisher's LSD to determine the origin of such effects. The influence of pacing strategy and event duration on the total work done above CP was explored by a two-way (pacing strategy × duration) repeated-measures ANOVA. Pearson's product-moment correlation was used to examine the interrelationships between changes in parameters of $\dot{V}O_2$ kinetics and exercise tolerance. All data are presented as mean ± SD. Statistical significance was accepted when $P < 0.05$.

RESULTS

During the ramp incremental test, subjects attained a peak work rate of 390 ± 66 W and a $\dot{V}O_{2max}$ of 4.17 ± 0.60 L·min^{-1}. The CP and the W' estimated from the 3-min all-out test were 253 ± 60 W and 20.7 ± 4.5 kJ, respectively, such that the 3-tlim-WR and the 6-tlim-WR were calculated to be 368 ± 67 and 311 ± 62 W, respectively (Fig. 1).

$\dot{V}O_2$ kinetics. The parameters of $\dot{V}O_2$ dynamics during the variously paced 3- and 6-min exercise trials are shown in Table 1 and illustrated as group mean responses in Figure 2. The overall $\dot{V}O_2$ kinetics during the 3-min trials when the fitting window was constrained to the end of the pacing phase (90 s) were fastest in the 3-FS condition, slowest in the 3-SS condition, and intermediate in the 3-ES condition, with the trials being significantly different from one another (Table 1, Fig. 2). When the fitting window was extended to the onset of the sprint (120 s), the MRT was reduced in the 3-FS versus the 3-SS condition (Table 1). The total O_2 consumed over the first 90 s of the 3-min trials was significantly greater in 3-FS and 3-ES compared with 3-SS, whereas the total O_2 consumed over the first 120 s of the 3-min trials was greater in 3-FS compared with 3-SS (Table 1).

The overall $\dot{V}O_2$ kinetics during the 6-min trials when the fitting window was constrained to the end of the pacing phase (180 s) were significantly faster in the 6-FS and 6-ES conditions compared with the 6-SS condition ($P < 0.05$), but there was no significant difference between 6-FS and 6-ES ($P = 0.09$) (Table 1, Fig. 2). However, $\dot{V}O_2$ kinetics were significantly different between all conditions, being fastest

TABLE 1. Pulmonary oxygen uptake kinetics during the ES, FS, and SS pacing strategies for the 3-and 6-min trials.

	ES	FS	SS
3-min Trial			
Baseline $\dot{V}O_2$ (L·min^{-1})	1.12 ± 0.15	1.11 ± 0.11	1.08 ± 0.17
$\dot{V}O_2$ at 90 s (L·min^{-1})	3.65 ± 0.54	3.69 ± 0.65	3.67 ± 0.57
MRT at 90 s (s)	41 ± 10	35 ± 6[a]	55 ± 14[a,b]
Oxygen deficit at 90 s (L)	1.7 ± 0.4	1.5 ± 0.3	2.3 ± 0.5[a,b]
Total $\dot{V}O_2$ more than 90 s (L)	4.4 ± 0.8	4.5 ± 0.8	4.2 ± 0.8[a,b]
Energy equivalent of 90 s $\dot{V}O_2$ (kJ)	91 ± 15	93 ± 168	87 ± 16[a,b]
$\dot{V}O_2$ at 120 s (L·min^{-1})	3.89 ± 0.51	3.93 ± 0.61	3.83 ± 0.60
MRT at 120 s (s)	42 ± 15	34 ± 5	49 ± 8[b]
"Phase II" τ (s)	23 ± 6	20 ± 5	32 ± 9[a,b]
Oxygen deficit at 120 s (L)	1.9 ± 0.7	1.6 ± 0.3	2.2 ± 0.4[b]
Total $\dot{V}O_2$ more than 120 s (L)	6.2 ± 1.0	6.4 ± 1.1	6.1 ± 1.1[b]
Energy equivalent of 120 s $\dot{V}O_2$ (kJ)	130 ± 21	133 ± 22	127 ± 22[b]
$\dot{V}O_2$ at 180 s (L·min^{-1})	3.95 ± 0.56[c]	3.98 ± 0.56	3.87 ± 0.57[c]
6-min Trial			
Baseline $\dot{V}O_2$ (L·min^{-1})	1.14 ± 0.13	1.07 ± 0.13	1.11 ± 0.15
"Phase II" τ (s)	27 ± 7	21 ± 4[a]	41 ± 19[a,b]
$\dot{V}O_2$ at 180 s (L·min^{-1})	3.71 ± 0.69	3.77 ± 0.69	3.83 ± 0.65
MRT at 180 s (s)	42 ± 6	38 ± 7	56 ± 15[a,b]
Oxygen deficit at 180 s (L)	1.8 ± 0.3	1.7 ± 0.5	2.5 ± 0.4[a,b]
Total $\dot{V}O_2$ more than 180 s (L)	9.4 ± 1.8	9.6 ± 1.7	9.3 ± 1.9[b]
Energy equivalent of 180 s $\dot{V}O_2$ (kJ)	197 ± 38	201 ± 36	194 ± 39[b]
$\dot{V}O_2$ at 300 s (L·min^{-1})	4.12 ± 0.64	4.13 ± 0.63	4.12 ± 0.66
MRT at 300 s (s)	52 ± 9	43 ± 7[a]	65 ± 13[a,b]
Oxygen deficit at 300 s (L)	2.5 ± 0.4	2.2 ± 0.5	3.2 ± 0.5[a,b]
Total $\dot{V}O_2$ more than 300 s (L)	17.3 ± 3.1	17.5 ± 3.1	17.3 ± 3.3
Energy equivalent of 300 s $\dot{V}O_2$ (kJ)	361 ± 66	366 ± 65	361 ± 68
$\dot{V}O_2$ at 360 s (L·min^{-1})	4.21 ± 0.54	4.08 ± 0.54	4.17 ± 0.66

[a] Significantly different from ES, $P < 0.05$.
[b] Significantly different from FS, $P < 0.05$.
[c] Significantly different from ramp test determined $\dot{V}O_{2max}$ $P < 0.05$.

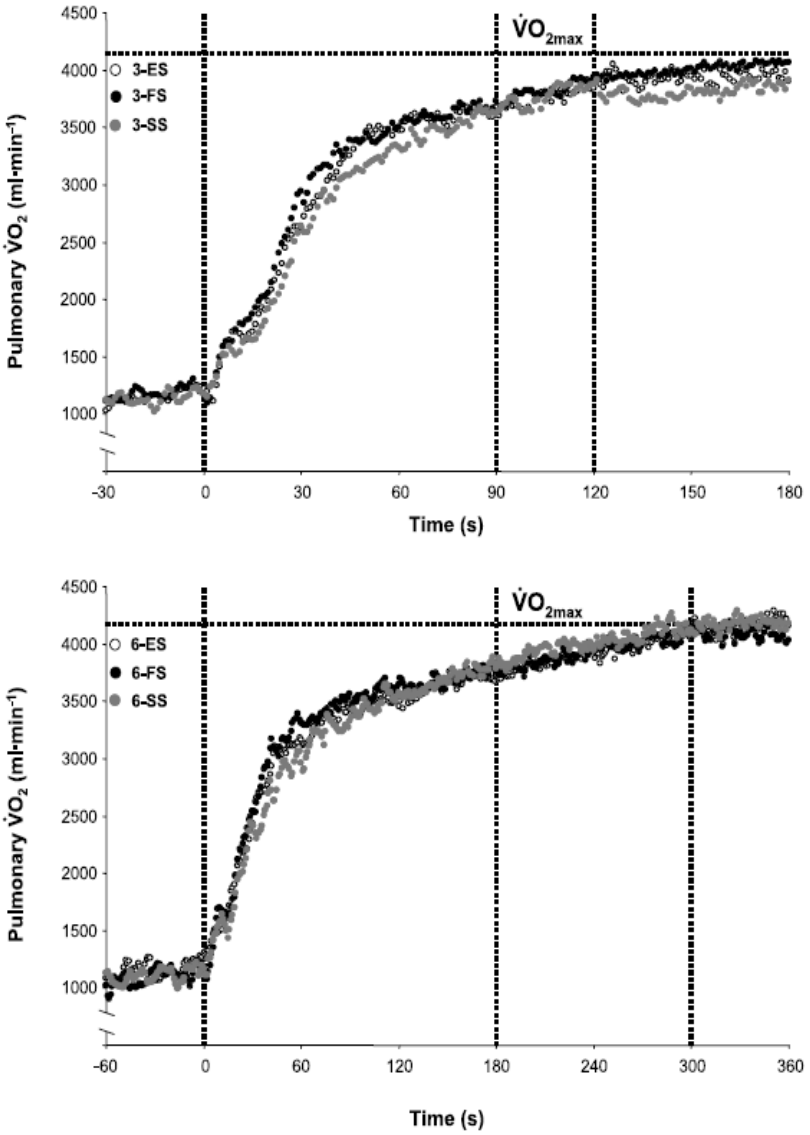

FIGURE 2—Group mean pulmonary $\dot{V}O_2$ dynamics during the ES (*open circles*), FS (*black circles*), and SS (*gray circles*) conditions are shown for the 3-min (*upper panel*) and 6-min (*lower panel*) exercise trials. Error bars are omitted for clarity. Note that the overall $\dot{V}O_2$ response dynamics are fastest in the FS condition and slowest in the SS condition during both the 3- and the 6-min trials.

in the 6-FS and slowest in the 6-SS, when the fitting window was extended to the start of the sprint (300 s; Table 1, Fig. 2). The total O_2 consumed over the first 180 s of the 6-min trials was significantly greater in the 6-FS than that in the 6-SS but not in the 6-ES (Table 1). However, the total O_2 consumed in the first 300 s of exercise was not influenced by the pacing strategy in the 6-min trials.

NIRS, HR, and blood (lactate) responses. The absolute [O_2Hb] and [Hb_{tot}] were not significantly different during the baseline or throughout exercise for any of the pacing strategies and exercise durations investigated. The [HHb] responses during the pacing trials are reported in Table 2 and illustrated in SDC 1 (Influence of pacing strategy and event duration on [HHb] response dynamics). During the 3-min pacing trials, the [HHb] τ + TD was significantly shorter in the 3-FS and 3-ES conditions compared with the 3-SS condition (Table 2). The [HHb] τ + TD was not significantly different during the 6-min trials irrespective of the pacing strategy or fitting procedures used. The absolute HR was not significantly different during the baseline or throughout exercise across all the pacing permutations investigated (Table 2). The blood (lactate) was not significantly different at any point during the 3-min pacing trials. However, the blood (lactate) before the sprint was significantly greater in the 6-SS compared with the 6-ES condition (Table 2).

Exercise performance. The exercise performance parameters during the various pacing conditions are reported

TABLE 2. NIRS-derived [HHb] kinetics, blood (lactate), and HR during the ES, FS, and SS pacing strategies for the 3- and 6-min trials.

	ES	FS	SS
3-min Trial			
[HHb]			
Baseline (A.U.)	−87 ± 70	−73 ± 64	−65 ± 70
90 s (A.U.)	267 ± 125	279 ± 137	267 ± 125
90 s τ + TD (s)	10.5 ± 1.3	9.7 ± 1.3	12.2 ± 2.4[a,b]
120 s (A.U.)	264 ± 132	275 ± 140	270 ± 130
120 s τ + TD (s)	10.4 ± 1.3	9.8 ± 1.3	12.3 ± 2.5[a,b]
Blood (lactate)			
Baseline (mM)	1.0 ± 0.3	1.0 ± 0.2	1.0 ± 0.3
120 s (mM)	3.6 ± 0.8	3.4 ± 0.7	3.9 ± 0.7
180 s (mM)	9.0 ± 1.7	8.6 ± 1.4	8.3 ± 2.4
HR			
Baseline (b·min^{-1})	91 ± 8	91 ± 12	94 ± 10
90 s (b·min^{-1})	161 ± 14	163 ± 13	163 ± 14
120 s (b·min^{-1})	168 ± 12	170 ± 13	170 ± 13
6-min Trial			
[HHb]			
Baseline (A.U.)	−62 ± 72	−72 ± 115	−59 ± 86
180 s (A.U.)	288 ± 148	304 ± 172	286 ± 176
180 s τ + TD (s)	13.0 ± 3.2	12.3 ± 2.7	13.8 ± 3.1
300 s (A.U.)	303 ± 141	321 ± 179	303 ± 170
300 s τ + TD (s)	14.8 ± 4.5	13.2 ± 3.6	15.3 ± 3.9
Blood [lactate]			
Baseline (mM)	0.9 ± 0.4	1.1 ± 0.4	1.1 ± 0.3
300 s (mM)	6.8 ± 1.3	8.0 ± 1.1	8.2 ± 1.0[a]
360 s (mM)	9.4 ± 1.4	9.6 ± 2.2	9.6 ± 1.9
HR			
Baseline (b·min^{-1})	91 ± 7	89 ± 10	87 ± 10
180 s (b·min^{-1})	164 ± 17	165 ± 13	168 ± 11
300 s (b·min^{-1})	175 ± 15	177 ± 12	179 ± 11
360 s (b·min^{-1})	182 ± 10	182 ± 11	183 ± 10

[a] Significantly different from ES, $P < 0.05$.
[b] Significantly different from FS, $P < 0.05$.

in Table 3, and the group mean power profile during the 60-s all-out sprint is shown in Figure 3. The peak power output attained in the sprint was significantly (~16%) greater in 3-FS compared with 3-ES and 3-SS, and the time to attain the peak work rate was significantly (~33%) shorter in 3-FS compared with 3-ES (Fig. 3). In addition, the mean power output during the sprint was significantly (~7%) greater in the 3-FS compared with the 3-ES and 3-SS conditions. Therefore, over the entire 3 min of exercise, the total work done was significantly greater in 3-FS compared with 3-ES and 3-SS (Table 3). In contrast, none of the parameters of exercise performance were enhanced during the 6-FS condition, with the values being similar to those observed in the 6-ES and 6-SS conditions (Table 3).

Comparison of the 3- and 6-min trials. The end-exercise $\dot{V}O_2$ in 3-FS, 6-ES, 6-FS, and 6-SS were not significantly different from one another or from the $\dot{V}O_{2max}$ attained in the ramp incremental test ($P > 0.05$; Table 1). However, the end-exercise O_2 in 3-ES and 3-SS was significantly lower than the $\dot{V}O_{2max}$ ($P < 0.05$). The total work done above the CP was not significantly different from the W' estimated in the 3-min all-out test for any of the six trials. However, the total work done above CP was significantly greater in the 6-min than the 3-min exercise trials ($P < 0.05$). Follow-up analyses revealed that the total work done above the CP in 3-FS (20.8 ± 5.4 kJ), 6-ES (22.1 ± 7.4 kJ), 6-FS (22.1 ± 7.9 kJ), and 6-SS (21.9 ± 6.9 kJ) was not significantly different from one another. However, the total work done above the CP was significantly lower in the 3-ES (19.2 ± 5.7 kJ) and 3-SS (19.0 ± 5.8 kJ) compared with the other conditions ($P < 0.05$).

DISCUSSION

The principal original finding of the present investigation was that an FS pacing strategy enhanced performance during the shorter (3 min) but not longer (6 min) exercise bouts, an effect that was linked to alterations in $\dot{V}O_2$ dynamics. The $\dot{V}O_2$ kinetics (and thus the magnitude of the O_2 deficit incurred over the initial transient phase) were influenced by the pacing strategy used during high-intensity exercise. Overall, an FS strategy resulted in faster $\dot{V}O_2$ kinetics than ES, with ES in turn resulting in faster $\dot{V}O_2$ kinetics than SS.

During the 3-min exercise bouts, an FS strategy significantly enhanced performance: compared with the ES condition, the peak power output attained in the end-sprint phase was 16% higher and the mean power output over the final 60 s was 7% higher. These results might be interpreted to indicate that the initial "sparing" of the W' due to the faster $\dot{V}O_2$ kinetics meant that a greater nonoxidative energy reserve was available later in exercise. However, although $\dot{V}O_2$ kinetics were slower in SS compared with ES in the 3-min exercise bouts, exercise performance was not impaired. Also, despite differences in $\dot{V}O_2$ kinetics between the three pacing conditions during the 6-min exercise bouts, there were no significant differences in exercise performance. The end-exercise $\dot{V}O_2$ was not significantly different from the $\dot{V}O_{2max}$ for 3-FS or for any of the 6-min exercise bouts but was significantly lower than the $\dot{V}O_{2max}$ in 3-ES and 3-SS. Similarly, the total work done above the CP was not different for 3-FS or for any of the 6-min exercise bouts but was significantly lower in 3-ES and 3-SS. These results therefore imply that FS resulted in an enhanced performance during short-term high-intensity exercise by enabling the attainment of $\dot{V}O_{2max}$ that, in turn, permitted more work to be done above the CP.

Influence of pacing strategy on $\dot{V}O_2$ kinetics. The overall $\dot{V}O_2$ kinetics were fastest in FS, slowest in SS, and

TABLE 3. Performance indices during the ES, FS, and SS pacing strategies for the 3- and 6-min trials.

	ES	FS	SS
3-min Trial			
Exercise performance			
Sprint peak work rate (W)	420 ± 67	487 ± 82[a,b]	415 ± 92
Sprint time to peak work rate (s)	12 ± 4	8 ± 1[a]	11 ± 3
Sprint mean work rate (W)	348 ± 61	374 ± 68[a,b]	345 ± 71
Total sprint work done (kJ)	20.7 ± 3.5	22.2 ± 3.9[a,b]	20.5 ± 4.1
Total work done (kJ)	64.8 ± 11.1	66.4 ± 11.6[a,b]	64.6 ± 11.7
6-min Trial			
Exercise performance			
Sprint peak work rate (W)	404 ± 85	413 ± 96	420 ± 85
Sprint time to peak work rate (s)	12 ± 4	17 ± 13	13 ± 6
Sprint mean work rate (W)	340 ± 77	339 ± 83	337 ± 76
Total sprint work done (kJ)	20.1 ± 4.6	20.1 ± 4.9	19.9 ± 4.5
Total work done (kJ)	113.3 ± 21.9	113.2 ± 21.6	113.1 ± 21.7

[a] Significantly different from ES, $P < 0.05$.
[b] Significantly different from SS, $P < 0.05$.

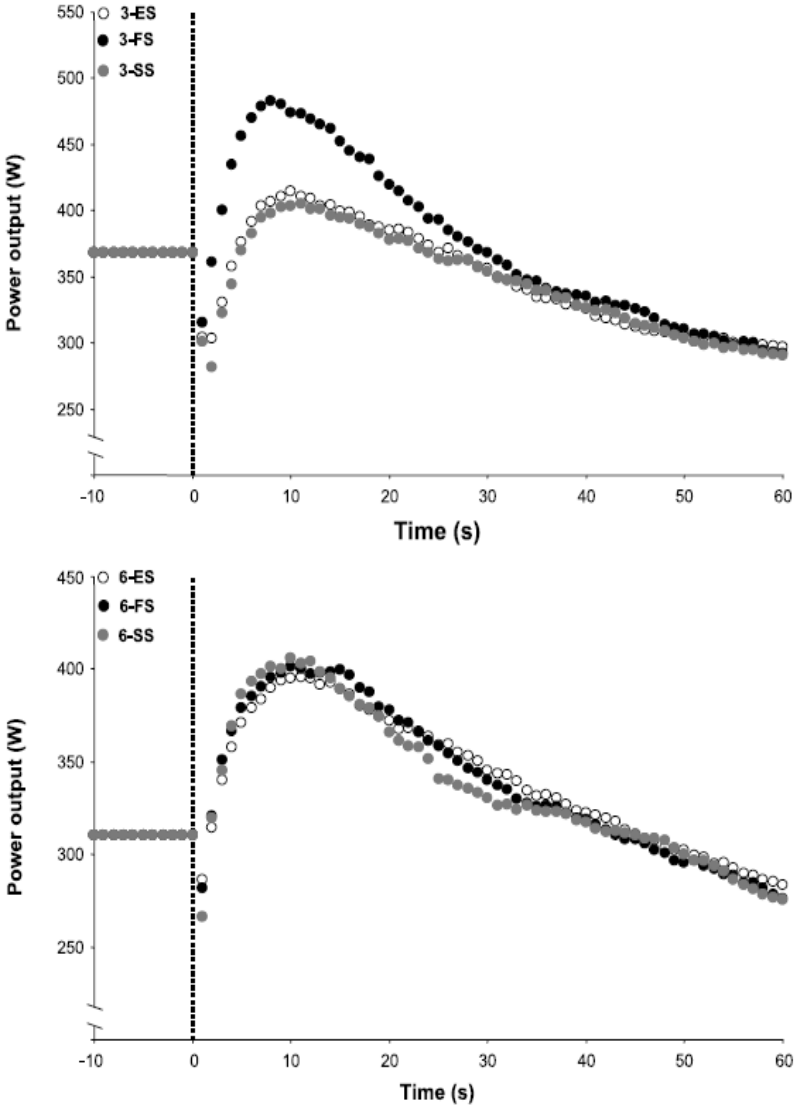

FIGURE 3—Group mean power output during the ES (*open circles*), FS (*black circles*), and SS (*grey circles*) for the 3-min (*upper panel*) and 6-min (*lower panel*) exercise trials. The dashed vertical lines demarcate the transition from a stable work rate to the onset of the all-out sprint at a fixed resistance. Note that the peak and mean power output is significantly greater in the 3-FS condition compared with the 3-ES and 3-SS conditions but that the effect is lost in the 6-min condition with all pacing strategies producing similar peak and mean power outputs.

intermediate for ES in both the 3- and the 6-min exercise trials. This is consistent with previous research, which has indicated that faster starting strategies can increase $\dot{V}O_2$ across the transition from rest to high-intensity exercise (2,3,8,19,25). However, these previous studies did not characterize the dynamics with which oxidative metabolism rose toward the projected steady state in the differently paced conditions.

Jones et al. (25) assessed the influence of pacing strategy on the dynamics of $\dot{V}O_2$ using exponential curve fitting and found that $\dot{V}O_2$ kinetics were fastest with an FS strategy, slowest with an SS strategy, and intermediate for an ES strategy. The results of the present study support these findings. As has been suggested previously (8,25), the greater initial rate of muscle ATP hydrolysis with an FS strategy would be expected to increase the "error signal" between the instantaneous supply and the required rates of oxidative phosphorylation (39). The muscle ATP turnover rate is proportional to the change in muscle PCr concentration per unit change in time ($\Delta[PCr]/\Delta t$), and therefore a greater rate of change of [PCr] at and after the onset of exercise should be associated with a more rapid increase in $\dot{V}O_2$ (30,39). Compared with ES, an FS pacing strategy would be expected to increased the $\Delta[PCr]/\Delta t$ and to increase the concentrations of ADP, P_i, and Ca^{2+}, thus augmenting several of the stimuli believed to be responsible for an acceleration of oxidative phosphorylation (7,11,30). Conversely, compared with ES, an SS pacing strategy would be expected to reduce the initial error signal and result in a blunting of the $\dot{V}O_2$ response dynamics.

To elucidate the mechanistic bases for the faster $\dot{V}O_2$ kinetics with faster starting strategies, we measured [Hb_{tot}] and [HbO_2] to provide information on total blood volume and oxygenation within the NIRS area of interrogation and [HHb] kinetics to estimate muscle O_2 extraction dynamics (13,18). The faster $\dot{V}O_2$ kinetics in the FS and ES compared with the SS condition during the 3-min trials were accompanied by a shorter [HHb] τ + TD, but there were no differences in [Hb_{tot}], [HbO_2], or HR between the conditions. These results suggest that the improved $\dot{V}O_2$ dynamics that are evident with faster starting strategies may be linked to increased muscle O_2 extraction. However, although the $\dot{V}O_2$ kinetics were altered in a similar fashion under the three pacing strategies when the exercise duration was extended to 6 min, the mechanisms that governed these adjustments were less clear. That is, the [Hb_{tot}], the [HbO_2], and the [HHb] were all similar across the various pacing conditions. For the 6-min exercise bouts, the unchanged [HHb] kinetics imply that the faster $\dot{V}O_2$ kinetics were linked to improvements in both muscle O_2 supply and utilization.

During high-intensity exercise of short duration (≤3 min), exhaustion often ensues before the $\dot{V}O_{2max}$ is attained (20,41). Consistent with this, in the present study, the end-exercise $\dot{V}O_2$ for 3-ES and 3-SS was significantly lower than the $\dot{V}O_{2max}$ measured in the ramp incremental test. However, in 3-FS and in all three of the 6-min pacing conditions, the end-exercise $\dot{V}O_2$ was not significantly different from the $\dot{V}O_{2max}$. Therefore, one consequence of the faster $\dot{V}O_2$ kinetics that attend the adoption of an FS pacing strategy is that it permits the attainment of $\dot{V}O_{2max}$ during "extreme" exercise bouts when this is ordinarily not possible (20,41).

Exercise performance. To investigate the influence of pacing strategy and event duration on exercise performance, we had subjects perform 3- and 6-min exercise trials initiated with FS, ES, and SS pacing strategies, with all trials culminating in an all-out sprint over the final minute of the bout. After the pacing phase, the work rate returned to a constant work rate (ES work rate), which was maintained until the onset of the sprint so that all sprints were initiated from the same absolute work rates and the total work done before the sprint was matched between conditions. It was therefore expected that the same fixed amount of work could be achieved during the end-sprint phase for all conditions, unless one or more of the initial pacing conditions predisposed to improved exercise performance.

Of all the pacing permutations investigated, exercise performance was only enhanced in the 3-FS condition. Specifically, the peak and the mean power outputs during the end sprint were higher, the time to reach the peak power output was reduced, and the total work done during the end sprint and over the entire bout was increased. Intriguingly, however, performance was not impaired in the SS condition compared with the ES condition. These data are consistent with a previous report (25) and suggest the existence of an "asymmetry" in the physiological consequences of FS and SS pacing strategies during short-term high-intensity exercise; that is, relative to ES, an FS strategy is clearly ergogenic, whereas an SS strategy is not necessarily ergolytic. Exercise performance was not different between the pacing strategies during the 6-min trials, indicating that the potential for an FS pacing strategy to enhance exercise performance recedes as the event duration is extended (14).

In the present study, $\dot{V}O_2$ kinetics were faster and exercise performance was superior in 3-FS compared with 3-ES and 3-SS. These data might suggest that exercise performance was enhanced in the 3-FS condition consequent to the faster $\dot{V}O_2$ kinetics sparing the W' over the transient region; this additional nonoxidative energy would then be available for utilization at the commencement of the end-sprint phase, enabling a greater total work output. Indeed, the additional oxidative energy yield associated with the greater O_2 consumed in the first 90 s of exercise in 3-FS compared with 3-ES (equivalent to 2.1 kJ on average; Table 1) was not significantly different from the greater work done over the end-sprint phase (1.6 kJ on average; Table 3). This supports the suggestion that $\dot{V}O_2$ kinetics and the W' are inherently linked in the determination of exercise tolerance (10,21). In contrast, the greater oxidative energy yield in the first 90 s of exercise in 3-ES compared with 3-SS (3.8 kJ on average; Table 1) was significantly greater than the increased work done over the end-sprint phase in 3-ES compared with 3-SS (0.2 kJ on average; Table 3). With the assumptions that the total ATP requirement and muscle efficiency were identical for the first 120 s of exercise in all three conditions (cf. [26]), these data indicate complex interrelationships between oxidative and nonoxidative metabolic contributions to energy turnover during high-intensity exercise, which are sensitive to the pattern of work rate imposition (for discussion, see Jones et al. [25]).

Exercise performance was not different across the 6-min trials, irrespective of the imposed pacing strategy. The FS strategy, through increasing the initial ATP turnover rate, would be expected to increase both oxidative and nonoxidative energy turnover. The key difference between the influence of an FS strategy on performance during shorter-term (~3 min) compared with longer-term (~6 min) high-intensity exercise, at least in the present study, might be the duration that the higher-than-average work rate is sustained. For example, the work rate during FS was higher than that during ES and SS for 90 s in the 6-min trials compared with 45 s in the 3-min trials. It is possible that the greater oxidative energy yield in the 6-FS condition was offset by an increased nonoxidative energy turnover such that W' was not significantly spared before the commencement of the end sprint. In particular, it is likely that anaerobic glycolysis was activated to a greater extent during the FS phase of the 6-min compared with the 3-min exercise bouts. In this regard, an FS that is of short duration relative to the event duration might be considered optimal in both shorter and longer duration high-intensity exercise bouts because it would increase $\Delta[PCr]/\Delta t$ and drive a rapid increase in mitochondrial respiration without risking a precipitous drop

in pH early in the event. Overall, the data suggest that pacing strategy, at least in the forms administered herein, has little effect on exercise performance during maximal exercise of around 6 min duration.

An alternative, but attractive, interpretation of the performance data in the present study requires a reconsideration of the physiological meaning of the parameters derived from the power–duration relationship (i.e., CP and W'). It is noteworthy that, despite differences in the MRT, the $\dot{V}O_2$ attained at the end of each of the 6-min pacing trials was not different from the preestablished $\dot{V}O_{2max}$, and there was no difference in the amount of work done above CP or in overall performance between the conditions. In contrast, for the ES and SS conditions in the 3-min trials, the end-exercise $\dot{V}O_2$ was significantly lower than the $\dot{V}O_{2max}$, and both the work done above the CP and the overall performance were significantly reduced compared with the FS condition. If the attainment of $\dot{V}O_{2max}$ is a major determinant of W' (10,33,35), then it is possible that the pacing strategy chosen will not significantly impact on high-intensity exercise performance, provided that the $\dot{V}O_{2max}$ is attained, as was the case for the 6-min exercise trials. However, for shorter duration high-intensity exercise bouts in which the $\dot{V}O_{2max}$ cannot be attained when an ES strategy is used, the W' will not be fully manifested such that performance might be "suboptimal." In such a situation, an intervention such as the adoption of an FS strategy, which speeds $\dot{V}O_2$ kinetics and enables the attainment of $\dot{V}O_{2max}$, would enable a more complete utilization of the W' and consequently better overall performance. This interpretation is supported by other lines of evidence. For example, Jones et al. (23) compared the $\dot{V}O_2$ response and the time to exhaustion during extreme exercise (120% $\dot{V}O_{2max}$) with and without a preceding "priming" bout of heavy exercise. Without priming, the end-exercise $\dot{V}O_2$ reached ~89% $\dot{V}O_{2max}$ (significantly lower than $\dot{V}O_{2max}$), and the time to exhaustion was 139 ± 18 s; after priming, $\dot{V}O_2$ kinetics were faster, the end-exercise $\dot{V}O_2$ was increased to 98% $\dot{V}O_{2max}$ (not significantly different from the $\dot{V}O_{2max}$), and the time to exhaustion was extended to 180 ± 29 s.

CONCLUSIONS

In both the 3- and 6-min trials, $\dot{V}O_2$ kinetics were fastest in FS, slowest in SS, and intermediate in ES. These findings indicate that the rate at which $\dot{V}O_2$ increases after the onset of exercise is sensitive to the pattern of work rate imposition, even when the mean work rate is identical. A higher initial work rate and thus muscle ATP turnover rate would result in a greater initial $\Delta[PCr]/\Delta t$ and a more rapid accumulation of metabolites that stimulate oxidative phosphorylation. In 3-FS, the energy equivalent of the additional O_2 consumed across the transient was subsequently expended in the end-sprint phase, such that performance was significantly enhanced. Conversely, the relatively slow $\dot{V}O_2$ kinetics across the transient phase in 3-SS did not impair end-sprint or overall exercise performance relative to 3-ES. Alongside an earlier report (25), this indicates the existence of an asymmetry in the bioenergetic response to the pattern of work rate allocation during short-term high-intensity exercise. Although differences in $\dot{V}O_2$ kinetics between the three pacing strategies persisted during the 6-min exercise bouts, there were no differences in performance indices. During short-term "extreme" exercise, an FS pacing strategy might enhance performance by enabling the attainment of $\dot{V}O_{2max}$ when this is not ordinarily possible and by permitting a more complete utilization of the W'. However, during longer-term "severe" exercise in which the $\dot{V}O_{2max}$ is normally attained and the W' is fully expended, differences in pacing strategy have a relatively small effect on performance outcomes.

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Acute L-arginine supplementation reduces the O_2 cost of moderate-intensity exercise and enhances high-intensity exercise tolerance

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Bailey SJ, Winyard PG, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, Jones AM. Acute L-arginine supplementation reduces the O_2 cost of moderate-intensity exercise and enhances high-intensity exercise tolerance. *J Appl Physiol* 109: 1394–1403, 2010. First published August 19, 2010; doi:10.1152/japplphysiol.00503.2010.—It has recently been reported that dietary nitrate (NO_3^-) supplementation, which increases plasma nitrite (NO_2^-) concentration, a biomarker of nitric oxide (NO) availability, improves exercise efficiency and exercise tolerance in healthy humans. We hypothesized that dietary supplementation with L-arginine, the substrate for NO synthase (NOS), would elicit similar responses. In a double-blind, crossover study, nine healthy men (aged 19–38 yr) consumed 500 ml of a beverage containing 6 g of L-arginine (Arg) or a placebo beverage (PL) and completed a series of "step" moderate- and severe-intensity exercise bouts 1 h after ingestion of the beverage. Plasma NO_2^- concentration was significantly greater in the Arg than the PL group (331 ± 198 vs. 159 ± 102 nM, $P < 0.05$) and systolic blood pressure was significantly reduced (123 ± 3 vs. 131 ± 5 mmHg, $P < 0.01$). The steady-state O_2 uptake ($\dot{V}O_2$) during moderate-intensity exercise was reduced by 7% in the Arg group (1.48 ± 0.12 vs. 1.59 ± 0.14 l/min, $P < 0.05$). During severe-intensity exercise, the $\dot{V}O_2$ slow component amplitude was reduced (0.58 ± 0.23 and 0.76 ± 0.29 l/min in Arg and PL, respectively, $P < 0.05$) and the time to exhaustion was extended (707 ± 232 and 562 ± 145 s in Arg and PL, respectively, $P < 0.05$) following consumption of Arg. In conclusion, similar to the effects of increased dietary NO_3^- intake, elevating NO bioavailability through dietary L-Arg supplementation reduced the O_2 cost of moderate-intensity exercise and blunted the $\dot{V}O_2$ slow component and extended the time to exhaustion during severe-intensity exercise.

exercise economy; muscle efficiency; oxygen uptake; nitric oxide; exercise performance; oxygen uptake kinetics

AT THE ONSET OF MODERATE-INTENSITY exercise [i.e., exercise performed at work rates below the gas exchange threshold (GET)], pulmonary O_2 uptake ($\dot{V}O_2$) rises in an exponential fashion to attain a "steady state" within ~2–3 min in healthy humans (74). In this exercise intensity domain, the $\dot{V}O_2$ steady state reflects the rate of ATP turnover within the recruited myocytes and is linearly related to the external work rate, with the functional "gain" (i.e., increase in $\dot{V}O_2$ per unit increment in external work rate) approximating 10 $ml \cdot min^{-1} \cdot W^{-1}$ during cycle ergometry (32). The steady-state $\dot{V}O_2$ for a given moderate-intensity work rate is impervious to a variety of acute exercise, nutritional, and pharmacological interventions and is only minimally impacted by age and training (3, 16, 42, 75). During supra-GET exercise, muscle contractile efficiency progressively declines and a $\dot{V}O_2$ "slow component," which delays the attainment of steady state during heavy-intensity exercise (performed below the critical power) or sets the $\dot{V}O_2$ on a trajectory toward its maximum [peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$)] during severe-intensity exercise (above critical power), is manifest (60, 74). Interventions that reduce the $\dot{V}O_2$ slow component amplitude have been reported to improve severe-intensity exercise tolerance (1–4, 15, 31).

The signaling molecule nitric oxide (NO) is produced by the NO synthase (NOS) family of enzymes, which catalyze the oxidation of L-arginine, yielding NO and L-citrulline (13, 54–56). A complementary, NOS-independent pathway for NO production, involving the reduction of inorganic nitrite (NO_2^-) to NO, particularly in acidic/hypoxic conditions, has also been described (21, 26). It is well known that changes in NO production can affect vasodilatation and blood pressure (1, 4, 21, 25, 47, 48, 67), but there is increasing evidence that interventions that influence NO bioavailability can also alter the O_2 cost of exercise in humans (1, 4, 34, 47, 48) and other mammals (37, 53, 65). For example, it has recently been reported that 3–6 days of pharmacological (sodium nitrate) (47, 48) or dietary (beetroot juice) (1, 4) nitrate (NO_3^-) administration can reduce the steady-state $\dot{V}O_2$ during submaximal cycle exercise. Moreover, during severe-intensity exercise, the amplitude of the $\dot{V}O_2$ slow component is reduced, and exercise tolerance is enhanced following dietary NO_3^- supplementation (1, 4). Conversely, the amplitude of the $\dot{V}O_2$ slow component is increased following infusion of the NOS inhibitor nitro-L-arginine methyl ester (L-NAME) (33). L-NAME administration has also been shown to increase tissue $\dot{V}O_2$ in dogs (37, 65).

Given the findings described above, it is possible that dietary supplementation with the NOS substrate L-arginine might reduce the O_2 cost of moderate-intensity exercise and the amplitude of the $\dot{V}O_2$ slow component and enhance exercise tolerance during severe-intensity exercise. Despite the presence of an abundant intracellular L-arginine concentration, which can be as high as 0.1–1.0 mM, significantly exceeding the K_m of endothelial NOS (eNOS) for L-arginine of 2.9 μmol/l (76), exogenous L-arginine causes NO-mediated biological effects, a phenomenon that has been termed the arginine paradox (12, 45, 76). For example, exogenous L-arginine administration has been reported to increase urinary NO_3^- concentration ($[NO_3^-]$) (53), plasma NO_2^- concentration ($[NO_2^-]$) + $[NO_3^-]$ (NOx) (77), and L-citrulline concentration ([L-citrulline]) (64) and to reduce resting systolic blood pressure (67). Conversely, an arginine-free diet has been shown to reduce plasma arginine flux and NO synthesis (18). Despite this evidence that L-arginine can alter NO production, recent reports suggest that dietary L-arginine supplementation does not influence the

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steady-state O_2 cost of moderate-intensity cycle exercise (41) or submaximal treadmill running (8). However, markers of NO bioavailability were not demonstrably increased in these two earlier studies (8, 41), possibly because the supplementation regimens involved the ingestion of a relatively small amount of L-arginine on a number of occasions each day. In this respect, it is possible that the ingestion of an acute "bolus" of L-arginine might result in a rapid rise in markers of NO bioavailability, with corresponding physiological effects. It has been reported that the maximal concentration of plasma L-arginine is reached 90 min following the ingestion of 6 g of L-arginine (9). Also, whereas L-arginine supplementation has been reported to improve exercise tolerance in patient populations (7, 22, 58), the effects in healthy humans are less clear. Specifically, improvements in repeated sprint performance (14) or muscle power and fatigue resistance (17, 69) have been observed in some studies following L-arginine supplementation. However, other studies have reported no effect of L-arginine supplementation on intermittent anaerobic exercise (52) or marathon running performance (19). The L-arginine supplementation regimen differed considerably between these studies, and markers of NO bioavailability were either not measured (14, 17, 19, 69) or were found to be not different following L-arginine supplementation (52). Therefore, the possible influence of dietary L-arginine supplementation on the physiological responses to exercise and on exercise tolerance is controversial.

The purpose of the present study was to investigate the effects of acute L-arginine ingestion on indexes of NO synthesis and the physiological responses to low- and high-intensity exercise. The pharmacokinetic relationship between acute L-arginine ingestion and plasma L-arginine concentration (9) was used to inform the study design. We hypothesized that acute L-arginine supplementation would elevate plasma [NO_2^-] and reduce systolic blood pressure, consistent with an enhanced NO bioavailability (38, 51). On the basis of the results of previous studies that used dietary NO_3^- to enhance NO bioavailability (1, 4), we also hypothesized that L-arginine supplementation would reduce the O_2 cost of moderate-intensity exercise and improve severe-intensity exercise tolerance by reducing the $\dot{V}O_2$ slow component amplitude.

METHODS

Subjects

Nine healthy, recreationally active men (mean ± SD: 26 ± 6 yr old, 1.81 ± 0.04 m height, 84 ± 5 kg body mass) volunteered to participate in the study after responding to poster advertisements placed around the University of Exeter campus. None of the subjects were tobacco smokers or users of dietary supplements. All subjects were fully familiar with laboratory exercise-testing procedures, having previously participated in studies employing cycle ergometry in our laboratory. The procedures were approved by the Institutional Research Ethics Committee. All subjects gave their written informed consent after the experimental procedures, associated risks, and potential benefits of participation had been explained. Subjects were instructed to arrive at the laboratory ≥3 h postprandial and to refrain from caffeine and alcohol intake 6 and 24 h before each test, respectively. All tests were performed at the same time of day (±2 h) to minimize the effects of diurnal biological variation on physiological responses and exercise performance.

Procedures

The subjects were required to report to the laboratory on seven occasions, over a 4- to 5-wk period. During the first visit to the laboratory, the subjects performed a ramp incremental exercise test for determination of $\dot{V}O_{2peak}$ and GET. All cycle tests were performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). Initially, each subject completed 3 min of "unloaded" baseline cycling; then the work rate was increased by 30 W/min until the subject was unable to continue. The subjects cycled at a self-selected pedal rate (70–90 rpm), and this pedal rate, along with the saddle and handlebar height and configuration, was recorded and reproduced in subsequent tests. The breath-by-breath pulmonary gas exchange data were collected continuously during the incremental tests and averaged over consecutive 10-s periods. $\dot{V}O_{2peak}$ was taken as the highest 30-s mean value attained prior to the subject's volitional exhaustion. GET was determined as described previously (1–3). The work rates that would require 80% of the GET (moderate-intensity exercise) and 70%Δ (70% of the difference between the power output at the GET and $\dot{V}O_{2peak}$ plus the power output at GET; i.e., severe-intensity exercise) were subsequently calculated, with account taken of the mean response time (MRT) for $\dot{V}O_2$ during ramp exercise.

After completion of the ramp test, subjects were randomly assigned, using a double-blind cross-over design, to receive 3 consecutive days of dietary supplementation with a commercially available L-arginine product (Arg; ARK 1, Arkworld International; administered in 0.5 liter of water) or placebo (PL; 0.5 liter of blackcurrant-flavor cordial). Participants received a 20-g dose of the ARK 1 supplement, which contained 6 g of L-arginine, along with trace amounts of vitamins (E, C, B_6, and B_{12}), other amino acids (L-glutamine, L-leucine, L-valine, L-carnitine, L-citrulline, L-cysteine, and L-isoleucine), and fructose (11 g) at a dose that would not be expected to elicit performance gains (30). A 10-day washout period separated the supplementation periods. The order between the Arg and PL supplementation periods was randomized. The subjects were not aware of the experimental hypotheses to be tested but were informed that the purpose of the study was to compare the physiological responses to exercise following the consumption of two commercially available beverages. Throughout the study period, subjects were instructed to maintain their normal daily activities and diet. The subjects kept a food diary and consumed an identical diet during the two periods of exercise testing.

On the 3 days of supplementation, the subjects completed "step" exercise tests from a 20-W baseline to moderate- and severe-intensity work rates for the determination of pulmonary $\dot{V}O_2$ dynamics. After arrival at the laboratory, the subjects rested for 20 min and then were instructed to consume the Arg or PL beverage within a 5-min period; exercise tests were initiated 1 h after ingestion. On *day 1* of supplementation the subjects completed two 6-min bouts of moderate-intensity cycling, on *day 2* they completed one 6-min bout of moderate-intensity cycling followed by one 6-min bout of severe-intensity cycling, and on *day 3* they completed one 6-min bout of moderate-intensity cycling followed by one bout of severe-intensity cycling that was continued until task failure as a measure of exercise tolerance. The two bouts of exercise on each day were separated by 25 min of rest. The time to task failure was recorded when the pedal rate fell by >10 rpm below the required pedal rate. Before each exercise bout, blood pressure was measured and venous blood samples were collected for subsequent determination of plasma [NO_2^-] (see *Measurements*). The supplementation protocol was based on the pharmacokinetics of L-arginine: it has been reported that the maximal concentration of plasma L-arginine is reached 90 min following the ingestion of 6 g of L-arginine (9).

Measurements

During all tests, pulmonary gas exchange and ventilation were measured continuously using a portable metabolic cart (MetaMax 3B,

Cortex Biophysik, Leipzig, Germany), as described previously (1–3). A digital volume transducer turbine measured inspired and expired airflow, while an electrochemical cell O_2 analyzer and a nondispersive infrared CO_2 analyzer measured expired gases. The inspired and expired gas volume and gas concentration signals were continuously sampled via a capillary line connected to the mouthpiece and displayed breath-by-breath. Heart rate (HR) was measured during all tests via short-range radiotelemetry (Polar S610, Polar Electro, Kempele, Finland). During one of the transitions to moderate- and severe-intensity exercise, for both supplementation periods, a blood sample was collected from a fingertip into a capillary tube over the 20 s preceding the step transition in work rate and within the last 20 s of exercise. A capillary blood sample was also collected at the limit of tolerance for the severe-intensity exercise bout performed on *day 3* of each supplementation period. These whole blood samples were subsequently analyzed to determine blood lactate concentration ([lactate]; YSI 1500, Yellow Springs Instruments, Yellow Springs, OH) within 30 s of collection. Blood lactate accumulation (Δblood [lactate]) was calculated as the difference between blood [lactate] at end exercise and blood [lactate] at baseline.

The blood pressure in the brachial artery was measured with subjects in a rested, seated position prior to each exercise bout via an automated sphygmomanometer (Dinamap Pro, GE Medical Systems, Tampa, FL). Three measurements were taken, and the mean of the second and third blood pressure measurements was recorded. Venous blood samples were also drawn into lithium-heparin tubes prior to each exercise bout and centrifuged at 4,000 rpm and 4°C for 10 min, within 3 min of collection. Plasma was subsequently extracted and immediately placed in a freezer at −80°C for later analysis of [NO_2^-] via chemiluminescence, as described previously (1).

Data Analysis Procedures

The breath-by-breath $\dot{V}O_2$ data from each test were initially examined to exclude errant breaths caused by coughing, swallowing, sighing, etc., and those values lying >4 SDs from the local mean were removed. The breath-by-breath data were subsequently linearly interpolated to provide second-by-second values, and, for each individual, identical repetitions were time-aligned to the start of exercise and ensemble-averaged. In this way, the $\dot{V}O_2$ responses to the four moderate-intensity and the two severe-intensity exercise bouts were averaged prior to analysis to reduce breath-to-breath noise and enhance confidence in the parameters derived from the modeling process (46). The first ~20–25 s of data after the onset of exercise (i.e., the phase I response) were deleted, and a nonlinear least-squares algorithm was used to fit the data thereafter. A single-exponential model was used to characterize the $\dot{V}O_2$ responses to moderate-intensity exercise, and a biexponential model was used for severe-intensity exercise, as described in *Eqs. 1* and *2* for moderate- and severe-intensity exercise, respectively

$$\dot{V}O_2(t) = \dot{V}O_{2baseline} + A_p\left[1 - e^{-(t-TD_p)/\tau_p}\right] \quad (1)$$

$$\dot{V}O_2(t) = \dot{V}O_{2baseline} + A_p\left[1 - e^{-(t-TD_p)/\tau_p}\right] + A_s\left[1 - e^{-(t-TD_s)/\tau_s}\right] \quad (2)$$

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time t; $\dot{V}O_{2baseline}$ represents the mean $\dot{V}O_2$ in the baseline period; A_p, TD_p, and τ_p represent the amplitude, time delay (TD), and time constant (τ), respectively, describing the phase II (primary) increase in $\dot{V}O_2$ above baseline; and A_s, TD_s, and τ_s represent the amplitude, TD before the onset, and τ describing the development, of the $\dot{V}O_2$ slow component, respectively.

An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. $\dot{V}O_{2baseline}$ was defined as the mean $\dot{V}O_2$ measured over the final 90 s of baseline pedaling. The "end-exercise" $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 30 s of the 6-min exercise bouts (i.e., between 330 and 360 s); the $\dot{V}O_2$ at exhaustion for the severe-intensity exercise bouts was calculated as the mean $\dot{V}O_2$ over the final 30 s before the subjects reached the limit of tolerance. Because the asymptotic value (A_s) of the exponential term describing the $\dot{V}O_2$ slow component may represent a higher value than is actually reached at the end of the exercise, the actual amplitude of the $\dot{V}O_2$ slow component at the end of exercise was defined as A'_s. The A'_s parameter was compared at isotime (360 s) under both supplementation periods. The amplitude of the $\dot{V}O_2$ slow component was also described relative to the entire $\dot{V}O_2$ response.

Because of possible concerns over the inclusion of nonessential parameters in *Eq. 2*, we also fitted the severe-intensity exercise data using the methods of Rossiter et al. (63). Briefly, with use of a purpose-designed program (LabView, version 6.1, National Instruments, Newbury, UK), *Eq. 1* was initially fit up to the first 60 s of exercise and then increased iteratively by 1 s to 360 s of exercise. The best-fit curve for the phase II portion of the response was established using *1*) a plot of the $\dot{V}O_2$ time constant (τ) against time to identify the point at which the influence of the $\dot{V}O_2$ slow component lengthened the estimated τ following an initial plateau and *2*) deviation from an optimal fitting of the model as judged by a systematic departure of the model's residuals. The magnitude of the $\dot{V}O_2$ slow component was then calculated as the difference between the phase II asymptote and the mean $\dot{V}O_2$ value between 330 and 360 s of exercise.

For moderate-intensity exercise, the functional gain (i.e., the reciprocal of "delta" efficiency) of the $\dot{V}O_2$ response was computed by dividing A_p by the Δwork rate. In addition, to determine the overall kinetics of the $\dot{V}O_2$ response to moderate- and severe-intensity exercise, data were fit with a monoexponential model from 0 s to the end of exercise, without TD. For moderate-intensity exercise, the MRT so derived was used in the computation of the O_2 deficit ($A_p \cdot MRT/60$).

We also modeled the HR response to exercise in each condition. For this analysis, HR data were linearly interpolated to provide second-by-second values, and, for each individual, identical repetitions from like-transitions were time-aligned to the start of exercise and ensemble-averaged. Nonlinear least-squares mono- and biexponential models without TD were used to fit the data to moderate- and severe-intensity exercise, respectively, with the fitting window commencing at 0 s.

Statistics

Differences in the cardiorespiratory variables between conditions were analyzed with two-tailed, paired-samples *t*-tests. Alterations in blood pressure and plasma [NO_2^-] were determined via a two-tailed, two-way (supplement × time), repeated-measures ANOVA. Significant effects were further explored using Fisher's least significant difference. Relationships between variables were assessed using Pearson's product moment correlation coefficient. Data are presented as means ± SD. Statistical significance was accepted when $P < 0.05$.

RESULTS

During the ramp incremental test, subjects attained a peak work rate of 367 ± 32 W and $\dot{V}O_{2peak}$ of 4.03 ± 0.37 l/min. The moderate- and severe-intensity work rates used in the main part of the study were 82 ± 14 and 274 ± 21 W, respectively.

Plasma [NO_2^-] and Blood Pressure

The group mean plasma [NO_2^-] values obtained before each of the two exercise bouts completed on each of *days 1*, *2*, and *3* of the Arg and PL supplementation periods are illustrated in Fig. 1. There was a significant main effect for supplement ($P < 0.05$), while the main effect for time and the interaction effect were not significant ($P > 0.05$). Plasma [NO_2^-] was significantly higher during Arg supplementation than in the PL

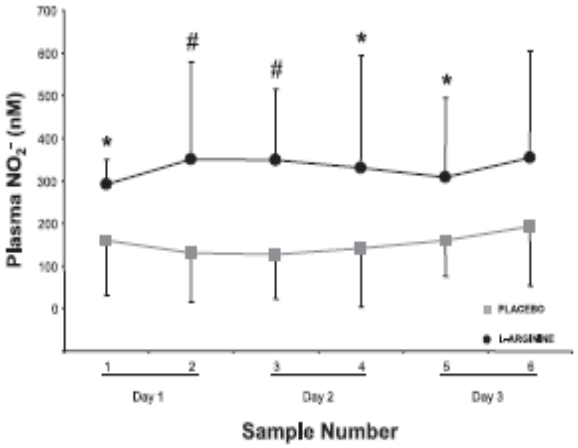

Fig. 1. Plasma nitrite (NO_2^-) concentration ([NO_2^-]) following acute L-arginine or placebo supplementation over 3 consecutive days. The 2 sample points within each day represent measurements obtained before the 1st and 2nd exercise bouts. Values are group means ± SD. Note significantly greater plasma [NO_2^-] following L-arginine supplementation. Significantly different from placebo: *$P < 0.05$; #$P < 0.01$.

condition for the first five sample points (Fig. 1). On average, across the six sample points, plasma [NO_2^-] was 108% greater during Arg supplementation than in the PL condition ($P < 0.01$). The Arg-induced elevations in plasma [NO_2^-] were not different between *days 1, 2,* and *3*.

The mean systolic and diastolic blood pressure values measured before each of the six exercise bouts during Arg supplementation and the PL condition are shown in Fig. 2. Similar to the plasma [NO_2^-] response to Arg supplementation, we observed a significant main effect for supplement ($P < 0.05$), while the main effect for time and the interaction effect were not significant ($P > 0.05$). The ingestion of Arg significantly reduced systolic blood pressure at three of the six sample points relative to PL (Fig. 2). Overall, systolic blood pressure was reduced by 8 mmHg when averaged over the six sample points ($P < 0.01$ compared with PL). The Arg-induced reductions in systolic blood pressure were not significantly different between *days 1, 2,* and *3*. The ~3-mmHg reduction in diastolic blood pressure following Arg ingestion was not significantly different from PL (Fig. 2).

$\dot{V}O_2$ Dynamics and Exercise Tolerance

Moderate-intensity exercise. The pulmonary $\dot{V}O_2$ response during moderate-intensity exercise is shown in Fig. 3, and the parameters derived from the model fit are presented in Table 1. Arg supplementation resulted in a 10% reduction in the amplitude of the pulmonary $\dot{V}O_2$ response, relative to PL, following a step increment to the same absolute moderate-intensity cycling work rate (0.67 ± 0.13 and 0.60 ± 0.13 l/min for PL and Arg, respectively, $P < 0.05$; Fig. 3), with no difference in $\dot{V}O_2$ during the baseline period of very low-intensity (20-W) cycling. Accordingly, the functional gain (i.e., the ratio of the increase in O_2 consumed per minute to the increase in external work rate) was reduced from 10.8 ml·min^{-1}·W^{-1} in the PL condition to 9.7 ml·min^{-1}·W^{-1} following Arg supplementation. The absolute $\dot{V}O_2$ over the final 30 s of moderate-intensity exercise was also significantly lower following Arg ingestion (1.59 ± 0.13 and 1.48 ± 0.12 l/min for PL and Arg, respectively, $P < 0.05$; Fig. 3), as was the O_2 deficit (0.45 ± 0.15 and 0.39 ± 0.12 liter for PL and Arg, respectively, $P < 0.05$). The phase II τ was not significantly altered by Arg supplementation (27 ± 5 and 26 ± 8 s for PL and Arg, respectively, $P > 0.05$). The 95% confidence interval for the estimation of the phase II τ was 3 ± 1 s for both conditions. The baseline and end-exercise values of HR, CO_2 output, respiratory exchange ratio, minute ventilation, and blood [lactate] were not significantly different between the conditions (Tables 1 and 2).

Severe-intensity exercise. The pulmonary $\dot{V}O_2$ response during severe-intensity exercise is shown in Fig. 4, and the parameters derived from the biexponential fit (*Eq. 2*) are presented in Table 1. In contrast to the effects observed for moderate-intensity exercise, the primary $\dot{V}O_2$ amplitude during severe-intensity exercise was significantly elevated following Arg supplementation (2.27 ± 0.14 and 2.45 ± 0.12 l/min for PL and Arg, respectively, $P < 0.01$; Table 1, Fig. 4). The phase II τ was not significantly different following Arg supplementation relative to the PL condition (34 ± 10 and 39 ± 12 s for PL and Arg, respectively, $P > 0.05$; Table 1). The 95% confidence intervals for the estimation of the phase II τ were 5 ± 2 and 4 ± 2 s in the PL condition and following Arg

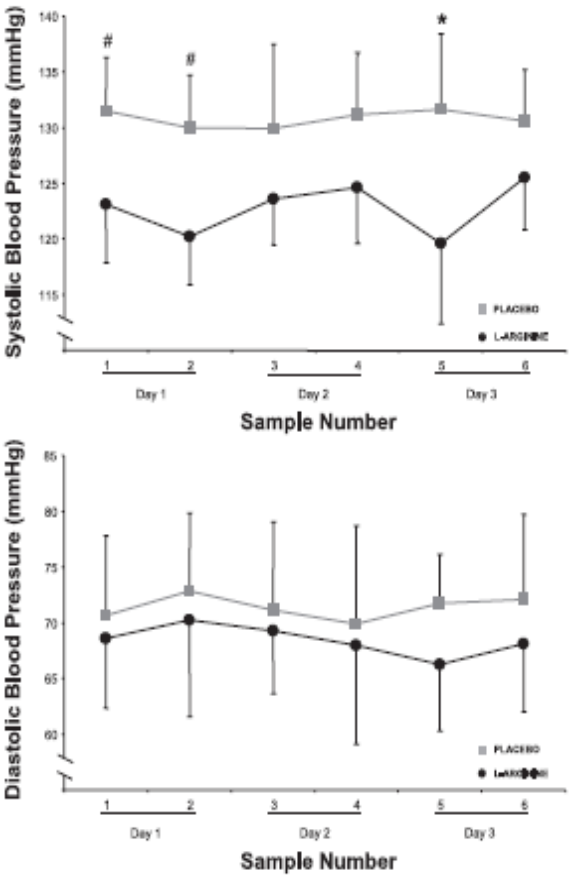

Fig. 2. Systolic (*top*) and diastolic (*bottom*) blood pressure following acute L-arginine or placebo supplementation over 3 consecutive days. The 2 sample points within each day represent measurements obtained before the 1st and 2nd exercise bouts. Values are group means ± SD. Systolic blood pressure was significantly reduced following L-arginine supplementation. Significantly different from placebo: *$P < 0.05$; #$P < 0.01$.

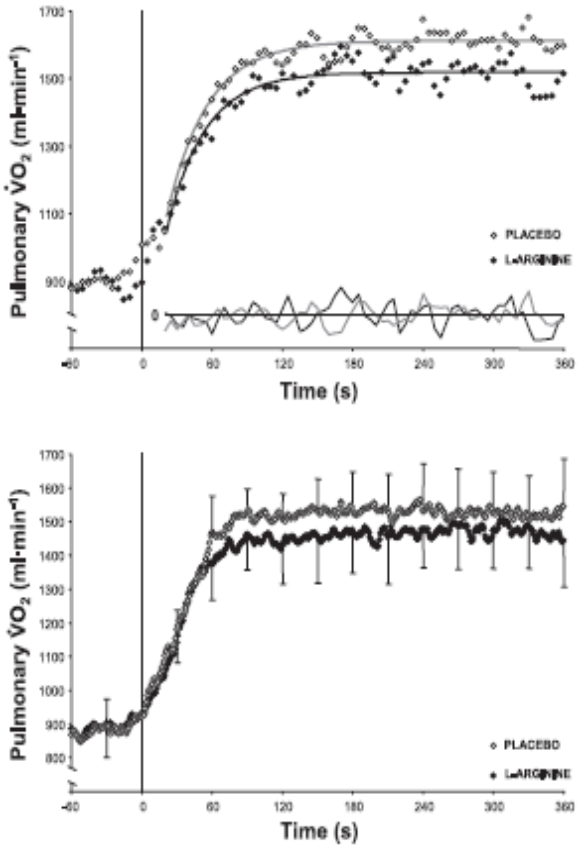

Fig. 3. Pulmonary O_2 uptake ($\dot{V}O_2$) following L-arginine and placebo supplementation after a step increment to a moderate-intensity work rate. Dotted vertical line represents abrupt imposition of the moderate-intensity work rate from a baseline of cycling at 20 W. *Top*: $\dot{V}O_2$ response of a representative individual (data are shown at 5-s intervals). Curve fits are shown as solid lines, and residuals are shown at the foot of the panel. *Bottom*: group mean $\dot{V}O_2$ response, with SD bars shown every 30 s for clarity. Steady-state $\dot{V}O_2$ during moderate-intensity exercise was reduced following L-arginine supplementation.

supplementation, respectively. The amplitude of the $\dot{V}O_2$ slow component was significantly smaller following Arg supplementation (0.76 ± 0.29 and 0.58 ± 0.23 l/min for PL and Arg, respectively, $P < 0.05$; Fig. 4) and, therefore, represented a smaller proportion of the overall $\dot{V}O_2$ response (20 ± 6 and 15 ± 6% for PL and Arg, respectively, $P < 0.05$). The essential results were the same when the alternative modeling procedure of Rossiter et al. (63) was employed. Specifically, the phase II τ was not significantly different (30 ± 11 and 33 ± 7 s for PL and Arg, respectively, $P > 0.05$), the primary $\dot{V}O_2$ amplitude was increased (2.12 ± 0.19 and 2.24 ± 0.27 l/min for PL and Arg, respectively, $P < 0.05$), and the $\dot{V}O_2$ slow component was reduced (0.87 ± 0.23 and 0.71 ± 0.25 l/min for PL and Arg, respectively, $P < 0.01$) following Arg supplementation. The $\dot{V}O_2$ at exhaustion in the constant work rate tests was not significantly different between supplements or from the $\dot{V}O_{2peak}$, as determined in the initial ramp incremental test. The baseline and end-exercise values of CO_2 output, respiratory exchange ratio, and minute ventilation were not significantly different between the conditions (Table 1). Blood [lactate] at 6 min of exercise and at exhaustion, as well as HR dynamics, were not significantly different between the conditions (Table 2).

Exercise tolerance was enhanced following Arg supplementation, as demonstrated by the 20% increase in the time to task failure (562 ± 145 and 707 ± 232 s for PL and Arg, respectively, $P < 0.05$). After Arg supplementation, exercise tolerance was significantly related to the absolute plasma [NO_2^-] prior to the exhaustive severe-intensity exercise bout ($r = 0.82$, $P < 0.05$). However, there were no significant correlations between the changes in plasma [NO_2^-] following Arg and changes in $\dot{V}O_2$ kinetics or exercise tolerance.

DISCUSSION

The principal novel finding of this investigation was that acute L-arginine supplementation, which significantly increased plasma [NO_2^-] and reduced systolic blood pressure, resulted in a reduced O_2 cost of moderate-intensity cycle exercise, along with a reduced $\dot{V}O_2$ slow component amplitude and improved time to task failure during severe-intensity exercise. These findings confirm our experimental hypotheses and are consistent with the improvement in the physiological and performance parameters we observed previously following

Table 1. *Gas exchange and ventilation responses during moderate- and severe-intensity exercise following supplementation with L-arginine and placebo*

	Placebo	L-Arginine
	Moderate-intensity exercise	
O_2 uptake, l/min		
Baseline	0.92 ± 0.10	0.89 ± 0.07
End-exercise	1.59 ± 0.14	1.48 ± 0.12*
Phase II time constant, s	27 ± 5	26 ± 8
Mean response time, s	40 ± 6	39 ± 4
Primary amplitude, l/min	0.67 ± 0.13	0.60 ± 0.13*
O_2 deficit, liter	0.45 ± 0.15	0.39 ± 0.12*
CO_2 output, l/min		
Baseline	0.86 ± 0.08	0.83 ± 0.12
End-exercise	1.32 ± 0.18	1.31 ± 0.14
Minute ventilation, l/min		
Baseline	26 ± 2	25 ± 4
End-exercise	36 ± 4	36 ± 5
Respiratory exchange ratio		
Baseline	0.93 ± 0.10	0.93 ± 0.11
End-exercise	0.83 ± 0.06	0.88 ± 0.03
	Severe-intensity exercise	
O_2 uptake, l/min		
Baseline	0.97 ± 0.08	0.99 ± 0.06
End-exercise	4.00 ± 0.26	4.02 ± 0.29
Phase II time constant, s	34 ± 10	39 ± 12
Primary amplitude, l/min	2.27 ± 0.14	2.45 ± 0.12†
Slow component amplitude		
l/min	0.76 ± 0.29	0.58 ± 0.23*
%	20 ± 6	15 ± 6*
CO_2 output, l/min		
Baseline	0.88 ± 0.10	0.90 ± 0.10
End-exercise	4.07 ± 0.35	4.05 ± 0.32
Minute ventilation, l/min		
Baseline	25 ± 5	26 ± 4
End-exercise	142 ± 17	147 ± 22
Respiratory exchange ratio		
Baseline	0.91 ± 0.13	0.91 ± 0.09
End-exercise	1.02 ± 0.04	1.01 ± 0.04

Values are means ± SD. Significantly different from placebo: *$P < 0.05$; †$P < 0.01$.

Table 2. *Heart rate and blood [lactate] responses to moderate- and severe-intensity exercise following supplementation with L-arginine and placebo*

	Placebo	L-Arginine
	Moderate-intensity exercise	
Heart rate, beats/min		
Baseline	80 ± 9	81 ± 8
End-exercise	96 ± 10	98 ± 13
Time constant, s	29 ± 14	31 ± 16
Amplitude, beats/min	17 ± 6	16 ± 17
Blood [lactate], mM		
Baseline	0.8 ± 0.3	0.9 ± 0.1
End-exercise	1.0 ± 0.4	1.1 ± 0.3
Δ	0.2 ± 0.2	0.2 ± 0.4
	Severe-intensity exercise	
Heart rate, beats/min		
Baseline	86 ± 7	88 ± 8
End-exercise	172 ± 7	170 ± 8
Time constant, s	15 ± 7	17 ± 6
Blood [lactate], mM		
Baseline	1.0 ± 0.2	1.0 ± 0.1
End-exercise	6.8 ± 1.2	6.5 ± 1.3
Δ	5.8 ± 1.3	5.5 ± 1.4
Exhaustion	10.3 ± 1.7	9.2 ± 1.6

Values are means ± SD. [lactate], Lactate concentration.

dietary NO_3^- supplementation (1). The similar physiological and performance effects following L-arginine and NO_3^- supplementation suggest that these effects are attributable, in large part, to the increased NO availability afforded by these dietary interventions.

Effects of L-Arginine Supplementation on Indexes of NO Production

NO is synthesized through the activity of the NOS family of enzymes, which includes three major isoforms: eNOS, neuronal NOS (nNOS), and inducible NOS (68). These enzymes produce NO and L-citrulline through catalysis of the five-electron oxidation of L-arginine in a reaction requiring O_2 (13). eNOS and nNOS are constitutively expressed in skeletal muscle (39). Skeletal muscles produce NO at low levels at rest and at higher levels during contraction, resulting in a significant increase in NO synthesis during exercise (5, 38). The in vivo quantification of NO is technically challenging; therefore, changes in the plasma concentration of the oxidation products of NO, namely, NO_2^- and NO_3^-, are often measured to provide an indication of NO production (38, 50, 51). NO_2^- is formed by the enzymatic oxidation of NO by ceruloplasmin (66) and through the binding of NO to the Cu^{2+} active site of cytochrome *c* oxidase (20), while NO_3^- is derived through the reaction of NO with oxyhemoglobin (23). Of these NO markers, NO_2^- is considered to provide the best indication of eNOS activity in humans (51, 61). In the present study, plasma [NO_2^-] was essentially doubled following acute L-arginine supplementation, and this is indicative of an increased NOS activity and NO production. L-Arginine administration has also been reported to increase urinary [NO_3^-] (53), plasma [NO_2^-] and [NO_3^-] (77), and plasma [L-citrulline] (64), consistent with an enhanced NO production.

While NO is involved in a multitude of physiological processes in metazoan species (68), a reduction in blood pressure is one of the most recognized manifestations of an increased NO bioavailability (1, 4, 47, 48, 67). Indeed, NO is known to be an important endothelium-relaxing factor, through its activation of guanylate cyclase, which subsequently metabolizes GTP to cGMP, culminating in smooth muscle relaxation (28). In keeping with the significantly elevated plasma [NO_2^-], we observed a ~8-mmHg reduction in systolic blood pressure following acute L-arginine administration, consistent with the observations of Siani et al. (67). Taken together, these findings confirm that L-arginine administration resulted in an enhanced NO production in healthy humans, as reported elsewhere in healthy mammals (53, 77). As well as serving as a substrate for NOS, L-arginine has a number of important antioxidant properties that can also serve to increase NO bioavailability through NOS-independent mechanisms. Although the physiological relevance is unclear, these mechanisms include the reaction between arginine and H_2O_2 to form NO (57) and the L-arginine-induced inhibition of superoxide (O_2^-) production and increased O_2^- scavenging (72). O_2^- reacts rapidly with NO to form the highly reactive peroxynitrite anion. A reduction in the concentration of O_2^- could potentially lower the amount of

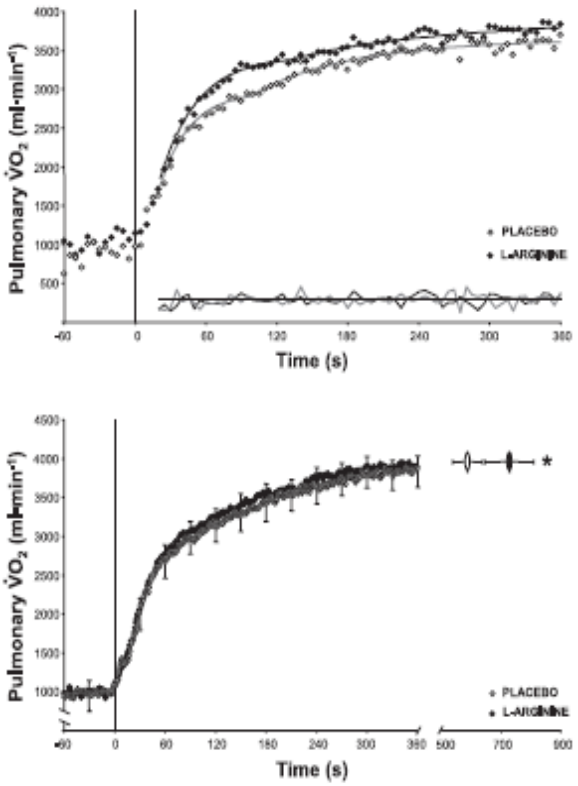

Fig. 4. Pulmonary $\dot{V}O_2$ following L-arginine and placebo supplementation after a step increment to a severe-intensity work rate. Dotted vertical line represents abrupt imposition of the severe-intensity work rate from a baseline of cycling at 20 W. *Top*: $\dot{V}O_2$ response of a representative individual (data are shown at 5-s intervals). Curve fits are shown as solid lines, and residuals are shown at the foot of the panel. *Bottom*: group mean $\dot{V}O_2$ response to 6 min of severe-intensity exercise, with SD bars shown every 30 s for clarity; group mean ± SD $\dot{V}O_2$ at task failure is also shown. Primary $\dot{V}O_2$ amplitude is elevated and $\dot{V}O_2$ slow component is reduced following L-arginine supplementation, effects that are akin to those of priming exercise (2, 15). *Time to exhaustion is significantly different from placebo ($P < 0.05$).

scavenging of NO by O_2^-, thereby increasing NO bioavailability.

Effects of L-Arginine on the Physiological Responses to Moderate-Intensity Exercise

After acute L-arginine supplementation, the amplitude of the $\dot{V}O_2$ response from baseline cycling to the steady state during moderate-intensity exercise was significantly reduced (by 10%), the absolute steady-state $\dot{V}O_2$ was significantly reduced (by 7%), and Δefficiency was increased from ~27% to ~30%. A reduction in the O_2 cost of low-intensity exercise has also been reported following pharmacological and dietary NO_3^- administration in association with increases in markers of NO synthesis (1, 4, 47, 48). Moreover, administration of the NOS inhibitor L-NAME has been associated with an increased O_2 cost of muscular contraction in dogs (65). While two recent studies have reported that the O_2 cost of low-intensity cycling (41) and running (8) was unaffected by L-arginine administration, it is noteworthy that markers of NO synthesis were not significantly altered in either study. In the study of Bescós et al. (8), plasma [NO_3^-] was not significantly different between three diets in which dietary L-arginine was manipulated with a combination of foodstuffs and supplementation (5.5, 9.0, and 20.5 g/day for 3 days). In the study of Koppo et al. (41), 14 days of supplementation with L-arginine hydrochloride capsules (7.2 g/day in 3 equal doses) significantly increased serum arginine concentration but did not alter resting blood pressure or urinary [NO_3^-]. In contrast, in the present study, plasma [NO_2^-] was significantly increased and systolic blood pressure was significantly reduced (consistent with increased NO bioavailability and production) ~60–90 min following the consumption of a beverage containing 6 g of L-arginine.

It is possible that the differences in the effects of L-arginine on $\dot{V}O_2$ dynamics and exercise tolerance between the present study and previous studies (8, 41) are related to the supplementation regimen (i.e., acute bolus vs. more regular, but smaller, doses of L-arginine given over 3–14 days) and the timing of the exercise test in relation to the administration of the supplement. Specifically, with chronic administration of L-arginine, it is unlikely that a sufficiently large dose of L-arginine is ingested at any time to result in a substantial increase in plasma L-arginine (9) or NO bioavailability (e.g., as estimated by plasma [NO_2^-]), such that no differences in resting blood pressure or the physiological responses to exercise would be expected. It is also possible that continued L-arginine supplementation in previous studies (8, 41) resulted in increased production of asymmetric dimethylarginine, a naturally occurring endogenous L-arginine metabolite that can inhibit all NOS isoforms (71). Consequently, the net effect of prolonged L-arginine supplementation may be no alteration in NO synthesis, as the potential for increased NOS activity with L-arginine supplementation may be offset by NOS inhibition by asymmetric dimethylarginine. The results of the present study, by contrast, indicate that ingestion of an acute bolus of L-arginine within 60–90 min of the start of exercise increases NO bioavailability (as indicated by increased plasma [NO_2^-] and reduced systolic blood pressure) and positively influences the physiological responses to exercise and exercise tolerance.

Although a 10% reduction in the primary $\dot{V}O_2$ amplitude during moderate-intensity exercise following acute L-arginine supplementation is impressive, we previously reported an even greater reduction (19%) following dietary NO_3^- supplementation (1). The NOS enzymes that catalyze L-arginine conversion to NO require O_2 as an essential cosubstrate. The eNOS and nNOS isoforms have been reported to have a K_m for O_2 of 2.3 and 202 Torr, respectively (70). In human skeletal muscle, P_{O_2} can fall to ~2–5 Torr during exercise (62). Therefore, while the activity of eNOS will be largely preserved during exercise, the activity of nNOS might be reduced, potentially limiting the total NO yield through this pathway. The NO_3^--NO_2^--NO pathway has fewer rate-limiting steps, such that it is possible that this pathway might be preferable for NO production during exercise (26). This might explain the greater effects of NO_3^- than L-arginine supplementation on exercise efficiency. However, the effects of dietary NO_3^- supplementation on exercise efficiency in our earlier studies (1, 4) were likely amplified, because habitual dietary NO_3^- intake was restricted throughout the study period.

Given that dietary NO_3^- and L-arginine supplementation reduce the O_2 cost of low-intensity exercise and that both of these dietary regimens increase indexes of NO production, it seems reasonable to conclude that it is the increased NO generation afforded by these dietary interventions that elicits the improvement in exercise efficiency. To have lowered the O_2 cost of low-intensity exercise, L-arginine administration would have been required to reduce the ATP cost of force production (i.e., to improve muscle contractile efficiency), the O_2 cost of ATP resynthesis (i.e., increased ratio of mitochondrial phosphorylated ADP to $\dot{V}O_2$), or both. To investigate the mechanistic bases of the reduced O_2 cost of moderate-intensity exercise following dietary NO_3^- supplementation, we recently determined skeletal muscle energetics (using ^{31}P-MRS) and pulmonary gas exchange dynamics during knee-extension exercise (4). This study confirmed that dietary NO_3^- supplementation reduced steady-state $\dot{V}O_2$ and demonstrated that this effect was proportionately similar to the blunted changes in intramuscular phosphocreatine, P_i, and ADP concentrations measured across the transition from rest to moderate-intensity exercise (4). Moreover, the estimated total ATP turnover rate was significantly reduced following dietary NO_3^- supplementation (4). These data suggest that the improved exercise efficiency afforded by a NO_3^--rich diet might be consequent to a reduced ATP cost of force production. Given that both dietary NO_3^- and L-arginine yield NO, it is possible that the reduced O_2 cost of moderate-intensity exercise following L-arginine supplementation has a similar mechanistic basis, although this remains to be confirmed.

In addition to its effects on the steady-state O_2 cost of moderate-intensity exercise, NO can also influence the kinetics with which $\dot{V}O_2$ rises following the onset of exercise. Indeed, faster $\dot{V}O_2$ kinetics have been reported following L-NAME administration (33, 34, 36), an effect that was ascribed to the alleviation of the competitive inhibition of cytochrome *c* oxidase by NO (20). Consistent with this finding, dietary NO_3^- supplementation resulted in significantly slower phase II $\dot{V}O_2$ kinetics in our previous study (1). Surprisingly, Koppo et al. (41) recently reported a slight (<2 s), but statistically significant, acceleration of phase II $\dot{V}O_2$ kinetics following L-arginine supplementation. However, this was countered by an increased TD_p of similar magnitude, such that the overall kinetics (i.e., MRT) were similar (~32 s) and the magnitude of the O_2 deficit

was unaltered (41). In the present study, we also found no significant difference in the MRT; however, the significant reduction in the primary $\dot{V}O_2$ amplitude resulted in a significantly reduced O_2 deficit following L-arginine supplementation. The explanation for the effects on $\dot{V}O_2$ kinetics in the present study compared with other studies that have attempted to alter NO bioavailability (1, 33, 34, 36, 41) is unclear but may be related to the potency of the intervention.

Effects of L-Arginine on the Physiological Responses to Severe-Intensity Exercise

In contrast to the reduction in the primary $\dot{V}O_2$ amplitude observed during moderate-intensity exercise, L-arginine supplementation resulted in an increased primary $\dot{V}O_2$ amplitude and a reduced $\dot{V}O_2$ slow component amplitude during severe-intensity exercise. These findings are consistent with, and of similar magnitude to, our recent findings with dietary NO_3^- supplementation (1). In our recent study (1), we also observed a reduced steady-state $\dot{V}O_2$ during moderate-intensity cycle exercise but an elevated primary $\dot{V}O_2$ amplitude and reduced $\dot{V}O_2$ slow component during severe-intensity cycle exercise. In contrast, during knee-extension exercise in which the work rates were not classified relative to the GET, $\dot{V}O_2$ was lower during "low-intensity" and "high-intensity" exercise following dietary NO_3^- supplementation compared with placebo (4). The influence of NO bioavailability on the O_2 cost of exercise, therefore, appears to be intensity domain and, perhaps, also exercise modality, specific. It is possible that these differential effects are related to differences in the limitations to $\dot{V}O_2$ kinetics for moderate-intensity compared with severe-intensity exercise (32). It is well documented that an increased NO production elevates blood flow (68) and that interventions that increase blood flow are associated with an increased $\dot{V}O_2$ primary component and a reduced $\dot{V}O_2$ slow component amplitude (1, 2, 16, 40). We previously observed an increased $\dot{V}O_2$ slow component amplitude following L-NAME infusion (34) and a reduced $\dot{V}O_2$ slow component amplitude following dietary NO_3^- supplementation (1, 4). These data indicate that manipulation of NO synthesis influences the magnitude of the $\dot{V}O_2$ slow component. Given the regulatory influence of NO on blood flow (68), it is conceivable that these effects on the $\dot{V}O_2$ slow component are related to changes in muscle perfusion. This, in turn, would be expected to influence the rate of muscle fatigue development and the pattern of motor unit recruitment; the latter has been suggested to be mechanistically linked to the $\dot{V}O_2$ slow component phenomenon (32).

Dietary supplementation with L-arginine has previously been reported to reduce blood lactate and ammonium accumulation (64) and to increase maximum $\dot{V}O_2$ in healthy mammals (53). These factors would be expected to predispose to improved exercise tolerance (15, 31). However, the influence of L-arginine supplementation on exercise performance in healthy humans is equivocal (14, 17, 19, 52, 69). In the present study, blood [lactate] and the $\dot{V}O_2$ measured at 360 s of severe-intensity exercise or at exhaustion (which was not significantly different from the maximum $\dot{V}O_2$ attained in the ramp incremental test) were not significantly altered by L-arginine ingestion. However, the time to task failure was extended by 20% following L-arginine administration; this improvement in exercise tolerance was accompanied by an increase in plasma $[NO_2^-]$ prior to exercise and improved $\dot{V}O_2$ dynamics. There is a growing appreciation that plasma $[NO_2^-]$, as an indicator of NOS activity (38, 50, 51, 61) and through its role as a reservoir for NO production (26), provides an important indication of the capacity to tolerate high-intensity exercise (1, 4, 47, 48, 61). Our recent studies indicate that interventions that increase plasma $[NO_2^-]$ can improve $\dot{V}O_2$ dynamics (1, 4; present study). Indeed, the reduced $\dot{V}O_2$ slow component amplitude observed in the present study following L-arginine supplementation and, in previous studies following NO_3^- supplementation (1, 4), would be expected to spare the utilization of the anaerobic reserves (4, 43, 63) and the accumulation of metabolites related to the fatigue process (4), leading to improved exercise tolerance (1–4, 15, 31).

Although the enhanced exercise tolerance following L-arginine supplementation is striking, relatively small changes in oxidative function can result in substantial changes in exercise tolerance due to the hyperbolic nature of the power-duration relationship for severe-intensity exercise (73). Nevertheless, the effect is equivalent to a 1–2% reduction in the time taken to complete a set distance (where the duration in the control condition is ~10 min) and is therefore likely to be meaningful for athletic performance (29). Ageing and several pathologies result in impaired endothelial function, which limits the capacity for NO generation and may explain, at least in part, the reduced exercise capacity in these populations (24, 27, 49). There is evidence that L-arginine administration increases maximal walking distance in patients with peripheral arterial disease (10, 11) and that the increase in exercise capacity following exercise training in congestive heart failure patients is associated with an increased L-arginine transport (59). The possibility that L-arginine administration might enhance exercise efficiency and performance in senescent and patient populations requires further study.

A limitation of the present study was that the commercial L-arginine supplement contained small amounts of other compounds that might also be considered "active" or might have acted synergistically with L-arginine. While we cannot exclude this possibility, we consider it to be unlikely, given the similarity of our results to other studies in which NO availability was enhanced to a similar degree with use of pharmacological (47, 48) or dietary (1, 4) NO_3^- interventions.

Conclusions

Acute dietary supplementation with 6 g of L-arginine, which increased indexes of NO synthesis, reduced the steady-state $\dot{V}O_2$ during moderate-intensity exercise and also reduced the $\dot{V}O_2$ slow component and increased the time to task failure during severe-intensity exercise in healthy adults. These findings are similar to recent observations in which NO availability was increased via dietary NO_3^- supplementation (1, 4, 47, 48). Therefore, a diet rich in the amino acid L-arginine and/or NO_3^-, which increases plasma $[NO_2^-]$, a key marker of NO bioavailability, appears to reduce systolic blood pressure and to improve exercise efficiency and exercise tolerance in healthy humans. While the precise mechanisms responsible for the latter effects remain to be elucidated, they likely involve an increased muscle O_2 supply and direct effects of NO on muscle contractile efficiency and/or mitochondrial function.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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Dietary nitrate supplementation reduces the O_2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans

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Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, Tarr J, Benjamin N, Jones AM. Dietary nitrate supplementation reduces the O_2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J Appl Physiol* 107: 1144–1155, 2009. First published August 6, 2009; doi:10.1152/japplphysiol.00722.2009.—Pharmacological sodium nitrate supplementation has been reported to reduce the O_2 cost of submaximal exercise in humans. In this study, we hypothesized that dietary supplementation with inorganic nitrate in the form of beetroot juice (BR) would reduce the O_2 cost of submaximal exercise and enhance the tolerance to high-intensity exercise. In a double-blind, placebo (PL)-controlled, crossover study, eight men (aged 19–38 yr) consumed 500 ml/day of either BR (containing 11.2 ± 0.6 mM of nitrate) or blackcurrant cordial (as a PL, with negligible nitrate content) for 6 consecutive days and completed a series of "step" moderate-intensity and severe-intensity exercise tests on the last 3 days. On *days 4–6*, plasma nitrite concentration was significantly greater following dietary nitrate supplementation compared with PL (BR: 273 ± 44 vs. PL: 140 ± 50 nM; $P < 0.05$), and systolic blood pressure was significantly reduced (BR: 124 ± 2 vs. PL: 132 ± 5 mmHg; $P < 0.01$). During moderate exercise, nitrate supplementation reduced muscle fractional O_2 extraction (as estimated using near-infrared spectroscopy). The gain of the increase in pulmonary O_2 uptake following the onset of moderate exercise was reduced by 19% in the BR condition (BR: 8.6 ± 0.7 vs. PL: 10.8 ± 1.6 ml·min^{-1}·W^{-1}; $P < 0.05$). During severe exercise, the O_2 uptake slow component was reduced (BR: 0.57 ± 0.20 vs. PL: 0.74 ± 0.24 l/min; $P < 0.05$), and the time-to-exhaustion was extended (BR: 675 ± 203 vs. PL: 583 ± 145 s; $P < 0.05$). The reduced O_2 cost of exercise following increased dietary nitrate intake has important implications for our understanding of the factors that regulate mitochondrial respiration and muscle contractile energetics in humans.

exercise economy; muscle efficiency; O_2 uptake; exercise performance; fatigue

A FUNDAMENTAL TENET OF HUMAN exercise physiology is a predictable oxygen (O_2) cost for a given submaximal work rate. Upon the initiation of moderate-intensity exercise [i.e., exercise performed at work rates below the gas exchange threshold (GET)], pulmonary O_2 uptake ($\dot{V}O_2$), which closely reflects O_2 consumption in the skeletal muscles (2, 29, 38), rises in an exponential fashion to attain a "steady state" within ~2–3 min in healthy humans (64). The steady-state increase in $\dot{V}O_2$ is linearly related to the increase in external work rate; is essentially independent of factors such as age, health status or aerobic fitness; and approximates 10 ml O_2·min^{-1}·W^{-1} of external power output during cycle ergometry (i.e., 10 ml·min^{-1}·W^{-1}; Ref. 36). During supra-GET exercise, $\dot{V}O_2$ dynamics become more complex, owing, in part, to the development of a delayed-onset $\dot{V}O_2$ "slow component", which elevates the O_2 cost of exercise above 10 ml·min^{-1}·W^{-1} (36, 64).

Whereas it is known that interventions such as training and the inspiration of hyperoxic gas can reduce the O_2 cost of heavy (above the GET but below critical power; Ref. 52) and severe (above critical power) exercise by reducing the amplitude of the $\dot{V}O_2$ slow component, the steady-state $\dot{V}O_2$ during moderate exercise is unaffected by these and other interventions in healthy humans (1, 15, 36, 51, 65). Surprisingly, however, it was recently reported that 6 days of dietary supplementation with pharmacological sodium nitrate reduced the O_2 cost of submaximal cycling at work rates expected to require 45–80% maximum $\dot{V}O_2$ ($\dot{V}O_{2max}$) (45). That this effect occurred without any increase in estimated nonoxidative energy production (as reflected by an unchanged blood [lactate]) (where brackets denote concentration) suggested that sodium nitrate ingestion improved the efficiency of muscle oxidative metabolism. It is known that tolerance to high-intensity exercise is, in certain respects, a function of $\dot{V}O_{2max}$ and submaximal exercise economy (20). Therefore, assuming that $\dot{V}O_{2max}$ is not altered, it is feasible that dietary nitrate supplementation might enhance exercise tolerance. However, this possibility has not been investigated.

The nitrate anion (NO_3^-) is relatively inert, and thus any biological effects are likely conferred via its conversion to the bioactive nitrite anion (NO_2^-). Inorganic nitrate is rapidly absorbed from the gut and is concentrated in saliva at least 10-fold. In the mouth, facultative anaerobic bacteria on the surface of the tongue reduce NO_3^- to NO_2^- (23). Nitrite can be converted to nitric oxide (NO) in the stomach (6, 47), but it is also clear that some is absorbed to increase circulating plasma [nitrite] (21, 46). We and several other groups have shown that NO_2^- can be converted to NO under appropriate physiological conditions (6). The requisite one-electron reduction has variously been reported to be catalyzed via xanthine oxidoreductase, hemoglobin, myoglobin, endothelial NO synthase, and the mitochondrial electron transfer complexes (61).

There are at least two mechanisms by which NO derived from NO_2^- (rather than from the much better known synthesis of NO from L-arginine by the NO synthase family of enzymes) might influence O_2 utilization by contracting skeletal muscle. First, as all of the known mechanisms for NO_2^- reduction are facilitated by hypoxia, it may be that more NO (which is a potent vasodilator) is generated in parts of muscle that are receiving less or using more O_2, and, therefore, this mechanism would help to match local blood flow to O_2 requirement, providing a more homogenous distribution of O_2 within skel-

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etal muscle. However, while this might be beneficial in terms of muscle function, it would not explain a reduced O_2 cost during exercise. A second possible mechanism involves the roles of NO_2^- and NO as regulators of cellular O_2 utilization. For example, NO is known to be an important inhibitor of cytochrome oxidase activity (10). More recently, it has been suggested that NO might enhance the efficiency of oxidative phosphorylation by reducing "slippage" of the mitochondrial proton pumps (17). There is also evidence that NO_2^- can serve as an alternative electron acceptor, theoretically replacing the role of O_2 in respiration (3).

The diet constitutes the main source of NO_3^- in humans, with vegetables accounting for 60–80% of daily NO_3^- intake in a Western diet (67). Given the reported ability of pharmacological sodium nitrate to reduce the O_2 cost of exercise (45), we sought to determine whether similar effects are observed when the NO_3^- dose is administered in the form of nitrate-rich beetroot juice (BR). This is important because sodium nitrate is a pharmaceutical product, whereas BR is a natural food product that can be readily ingested as part of the normal diet. We, therefore, investigated the influence of BR ingestion on plasma [nitrite], blood pressure (BP), muscle oxygenation [assessed with near-infrared spectroscopy (NIRS)] and the $\dot{V}O_2$ response to step transitions to moderate- and severe-intensity exercise. We hypothesized that dietary BR supplementation would reduce the O_2 cost of moderate-intensity exercise and increase exercise tolerance (assessed as the time-to-task failure) during severe-intensity exercise.

METHODS

Subjects

Eight healthy men (mean ± SD, age 26 ± 7 yr, height 180 ± 3 cm, body mass 82 ± 6 kg; $\dot{V}O_{2max}$ 49 ± 5 $ml \cdot kg^{-1} \cdot min^{-1}$) who were recreationally active in sporting activities volunteered to participate in this study. None of the subjects was a tobacco smoker or user of dietary supplements. All subjects were fully familiar with laboratory exercise testing procedures, having previously participated in studies employing cycle ergometry in our laboratory. The procedures employed in this study were approved by the Institutional Research Ethics Committee. All subjects gave their written, informed consent before the commencement of the study, after the experimental procedures, associated risks, and potential benefits of participation had been explained. Subjects were instructed to arrive at the laboratory in a rested and fully hydrated state, at least 3 h postprandial, and to avoid strenuous exercise in the 24 h preceding each testing session. Each subject was also asked to refrain from caffeine and alcohol intake 6 and 24 h before each test, respectively. All tests were performed at the same time of day (±2 h).

Procedures

The subjects were required to report to the laboratory on seven occasions, over a 4- to 5-wk period. During the first visit to the laboratory, subjects performed a ramp incremental exercise test for determination of the peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$) and GET. All cycle tests were performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). Initially, subjects completed 3 min of "unloaded" baseline cycling, after which the work rate was increased at a rate of 30 W/min until the subject was unable to continue. The participants cycled at a self-selected pedal rate (70–90 rpm), and this pedal rate, along with saddle and handle bar height and configuration, was recorded and reproduced in subsequent tests. The breath-by-breath pulmonary gas-exchange data were collected continuously during the incremental tests and averaged over consecutive 10-s periods. The $\dot{V}O_{2peak}$ was taken as the highest 30-s average value attained before the subject's volitional exhaustion. The GET was determined as described previously (1, 5). The work rates that would require 80% of the GET (moderate exercise) and 70% Δ (70% of the difference between the power output at the GET and $\dot{V}O_{2peak}$, severe exercise) were subsequently calculated, with account taken of the mean response time for $\dot{V}O_2$ during ramp exercise (i.e., two-thirds of the ramp rate was deducted from the power output at GET and peak; Ref. 63).

Following completion of the ramp test, subjects were randomly assigned in a crossover design, to receive 6 days of dietary supplementation with either nitrate (NO_3^-; 5.5 mmol/day; administered as 0.5 liter organic BR/day; Beet It, James White Drinks, Ipswich, UK) or "placebo" (PL; low-calorie black currant juice cordial with negligible nitrate content). The subjects were instructed to sip the beverages at regular intervals throughout the day. A 10-day washout separated the supplementation periods. The order between the nitrate and PL supplementation periods was balanced. The subjects were provided with a list of foods rich in nitrates and asked to abstain from consuming these foods for the duration of the study. The subjects were not aware of the experimental hypotheses to be tested but were informed that the purpose of the study was to compare the physiological responses to exercise following the consumption of two commercially available beverages. The personnel administering the exercise tests were not aware of the type of beverage being consumed by the subjects.

On *days 4*, *5*, and *6* of the supplementation periods, the subjects completed "step" exercise tests from a 20-W baseline to moderate- and severe-intensity work rates for the determination of pulmonary $\dot{V}O_2$ dynamics. On the 4th day of supplementation, subjects completed two bouts of moderate cycling, while on *days 5* and *6* the subjects completed one bout of moderate cycling and one bout of severe cycling. The two bouts of exercise on each day were separated by 25 min of passive recovery. All exercise bouts were of 6-min duration, with the exception of the severe exercise bout on the final day, which was continued until task failure as a measure of exercise tolerance. The time to task failure was recorded when the pedal rate fell by >10 rpm below the self-selected pedal rate. In these bouts, the subjects were verbally encouraged to continue for as long as possible. The $\dot{V}O_2$ responses to the four moderate and two severe exercise bouts were averaged before analysis to reduce breath-to-breath noise and enhance confidence in the parameters derived from the modeling process (44). Before each exercise bout, BP was measured, and venous blood samples were collected for subsequent determination of plasma [nitrite] (see Measurements below).

Measurements

During all tests, pulmonary gas exchange and ventilation were measured continuously using a portable metabolic cart (MetaMax 3B, Cortex Biophysik, Leipzig, Germany), as described previously (1). A turbine digital transducer measured inspired and expired airflow, while an electrochemical cell O_2 analyzer and an infrared CO_2 analyzer simultaneously measured expired gases. Subjects wore a nose clip and breathed through a low-dead-space, low-resistance mouthpiece that was securely attached to the volume transducer. The inspired and expired gas volume and gas concentration signals were continuously sampled via a capillary line connected to the mouthpiece and displayed breath by breath. Heart rate (HR) was measured during all tests using short-range radiotelemetry (Polar S610, Polar Electro Oy, Kempele, Finland). During one of the transitions to moderate and severe exercise, for both supplementation periods, a blood sample was collected from a fingertip into a capillary tube over the 20 s preceding the step transition in work rate and within the last 20 s of exercise. A capillary blood sample was also collected at the limit of tolerance for the severe bout performed on *day 6* of each supplementation period.

These whole blood samples were subsequently analyzed to determine blood [lactate] (YSI 1500, Yellow Springs Instruments, Yellow Springs, OH) within 30 s of collection. Blood lactate accumulation (Δblood [lactate]) was calculated as the difference between blood [lactate] at end-exercise and blood [lactate] at baseline.

The oxygenation status of the *m. vastus lateralis* of the right leg was monitored using a commercially available NIRS system (model NIRO 300, Hamamatsu Photonics KK, Hiugashi-ku, Japan). The system consisted of an emission probe that irradiates laser beams and a detection probe. Four different wavelength laser diodes provided the light source (776, 826, 845, and 905 nm), and the light returning from the tissue was detected by a photomultiplier tube in the spectrometer. The intensity of incident and transmitted light was recorded continuously at 2 Hz and used to estimate concentration changes from the resting baseline for oxygenated, deoxygenated, and total tissue hemoglobin/myoglobin. Therefore, the NIRS data represent a relative change based on the optical density measured in the first datum collected. The deoxyhemoglobin concentration ([HHb]) signal can be regarded as being essentially blood-volume insensitive during exercise and so was assumed to reflect the balance between local O_2 supply and utilization and to provide an estimate of changes in O_2 extraction in the field of interrogation (1, 22, 24, 30). The leg was initially cleaned and shaved around the belly of the muscle, and the probes were placed in the holder, which was secured to the skin with adhesive at 20 cm above the fibular head. To secure the holder and wires in place, an elastic bandage was wrapped around the subject's leg. The wrap helped to minimize the possibility that extraneous light could influence the signal and also ensured that the optodes did not move during exercise. Pen marks were made around the probes to enable precise reproduction of the placement in subsequent tests. The probe gain was set with the subject at rest in a seated position with the leg extended at down stroke on the cycle ergometer before the first exercise bout, and NIRS data were collected continuously throughout the exercise protocols. The data were subsequently downloaded onto a personal computer, and the resulting text files were stored on disk for later analysis.

BP of the brachial artery was measured with subjects in a rested, seated position before each exercise bout using an automated sphygmomanometer. Three measurements were taken at each sample point with the mean of the second and third BP measurements being recorded. Venous blood samples were also drawn into lithium-heparin tubes before each exercise bout and centrifuged at 4,000 rpm and 4°C for 10 min, within 3 min of collection. Plasma was subsequently extracted and immediately frozen at −80°C for later analysis of NO_2^- via chemiluminescence (4).

All glass wear, utensils, and surfaces were rinsed with deionized water to remove residual NO_2^- before analysis. After thawing at room temperature, plasma samples were initially deproteinized before analysis using the procedures of Higuchi and Motomizu (33). Initially, 100 μl of sample were placed in a microcentrifuge tube, along with 200 μl of deionized H_2O and 300 μl of 0.3 N NaOH, and left to stand at room temperature for 5 min. Then 300 μl of 5% by weight aqueous $ZnSO_4$ was added to the mixture, after which the sample was vortexed and left to stand at room temperature for a further 10 min. Thereafter, samples were centrifuged at 4,000 rpm for 15 min, and the supernatant was removed for subsequent analysis. The [NO_2^-] of the deproteinized plasma samples was determined by its reduction to NO in the presence of 5-ml glacial acetic acid and 1% NaI under nitrogen at room temperature in a gas-sealed purging vessel. Samples were introduced to the vessel via injection into the septum at the top of the vessel. The NO content was quantified by a chemiluminescence NO analyzer (Sievers, 280i NO analyzer). The reaction of NO with ozone in the chemiluminescent reaction chamber yielded electronically excited NO_2 (nitrogen dioxide), which emits light at the infrared region of the electromagnetic spectrum. Ozone was generated from an O_2 supply via an electrostatic ozone generator and high-voltage transformer. To minimize the interference of the chemiluminescent reactions of sulfur-containing compounds, an optical filter transmitted only red wavelengths (>600 nm), since the light emitted by sulfur-containing compounds is of shorter wavelengths. The intensity of the filtered infrared light was quantified by a red-sensitive photomultiplier tube and amplified producing an analog millivolt output signal. The [NO_2^-] was derived from the integral of the NO-generated millivolt signal over time compared with those obtained for $NaNO_2^-$ standards.

Data Analysis Procedures

The breath-by-breath $\dot{V}O_2$ data from each test were initially examined to exclude errant breaths caused by coughing, swallowing, sighing, etc., and those values lying more than 4 SDs from the local mean were removed. The breath-by-breath data were subsequently linearly interpolated to provide second-by-second values, and, for each individual, identical repetitions were time aligned to the start of exercise and ensemble averaged. The two severe exercise bouts were of different duration (6 min for the first bout and >6 min in the second bout, which was performed to task failure), and so, at this intensity, only the first 6 min of data were averaged together and modeled. The first 20 s of data after the onset of exercise (i.e., the phase I response) were deleted, and a nonlinear least squares algorithm was used to fit the data thereafter. A single-exponential model was used to characterize the $\dot{V}O_2$ responses to moderate exercise, and a biexponential model was used for severe exercise, as described in the following equations:

$$\dot{V}O_2(t) = \dot{V}O_{2\,baseline} + A_p\left[1 - e^{-(t-TD_p/\tau_p)}\right] \quad \text{(moderate)} \qquad (1)$$

$$\dot{V}O_2(t) = \dot{V}O_{2\,baseline} + A_p\left[1 - e^{-(t-TD_p/\tau_p)}\right] + A_s\left[1 - e^{-(t-TD_s/\tau_s)}\right] \quad \text{(severe)} \qquad (2)$$

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time t; $\dot{V}O_{2baseline}$ represents the mean $\dot{V}O_2$ in the baseline period; A_p, TD_p, and τ_p represent the amplitude, time delay, and time constant, respectively, describing the phase II increase in $\dot{V}O_2$ above baseline; and A_s, TD_s, and τ_s represent the amplitude of, time delay before the onset of, and time constant describing the development of the $\dot{V}O_2$ slow component, respectively.

An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. $\dot{V}O_{2baseline}$ was defined as the mean $\dot{V}O_2$ measured over the final 90 s of baseline pedaling. The end-exercise $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 30 s of exercise. Because the asymptotic value (A_s) of the exponential term describing the $\dot{V}O_2$ slow component may represent a higher value than is reached at the end of the exercise, the actual amplitude of the $\dot{V}O_2$ slow component at the end of exercise was defined as A_s'. The A_s' parameter was compared at the same iso-time (360 s) under both supplementation periods. The amplitude of the slow component was also described relative to the entire $\dot{V}O_2$ response. In addition, the functional "gain" of the fundamental $\dot{V}O_2$ response was computed by dividing A_p by the Δwork rate. The functional gain of the entire response (i.e., end-exercise gain) was calculated in a similar manner. To determine the overall kinetics of the $\dot{V}O_2$ response to both moderate- and severe-intensity exercise, data were fit with a monoexponential model from 0 s to end exercise, without TD.

To provide information on muscle oxygenation, we also modeled the [HHb] response to exercise. Mono- and biexponential models, similar to those described above, were applied to the ensemble-averaged data, with the exception that the fitting window commenced at the time at which the [HHb] signal increased 1 SD above the baseline mean (22). The [HHb] kinetics for moderate exercise were determined by constraining the fitting window to the point at which monoexponentiality became distorted, consequent to a gradual fall in [HHb] (1), as determined by visual inspection of the residual plots. The [HHb] kinetics for severe exercise were determined by fitting a biexponential model from the first data point, which was 1 SD above

the baseline mean through the entire response. The [HHb] TD and time constant values were summed to provide information on the overall [HHb] response dynamics in the fundamental phase of the response. The oxyhemoglobin concentration ($[HbO_2]$) and total hemoglobin concentration ($[Hb_{tot}]$) responses do not approximate an exponential (22) and were, therefore, not modeled. Rather, we assessed changes in these parameters by determining the HbO_2 and $[Hb_{tot}]$ at baseline (90-s preceding step transition), 120 s, and end exercise (average response over the final 30 s of exercise).

We also modeled the HR response to exercise in each condition. For this analysis, HR data were linearly interpolated to provide second-by-second values, and, for each individual, identical repetitions from like transitions were time aligned to the start of exercise and ensemble averaged. Nonlinear least squares monoexponential and biexponential models without TD were used to fit the data to moderate- and severe-intensity exercise, respectively, with the fitting window commencing at $t = 0$ s. The HR τ_P so derived provides information on the overall HR response dynamics.

Statistics

Differences in the cardiorespiratory and NIRS-derived variables between conditions were analyzed with two-tailed, paired-samples *t*-tests. Alterations in BP and plasma $[NO_2^-]$ were determined via a two-tailed, two-way (supplement × time) repeated-measures ANOVA. Significant effects were further explored using simple contrasts, with the α-level adjusted via a Bonferroni correction. Correlations were assessed via Pearson's product-moment correlation coefficient. Data are presented as means ± SD, unless otherwise stated. Statistical significance was accepted when $P < 0.05$.

RESULTS

The BR supplementation regimen implemented in this study was well tolerated with no deleterious side effects. Subjects did, however, report beeturia (red urine) and red stools, consistent with a previous study (62).

Plasma $[NO_2^-]$ and BP

The group mean plasma $[NO_2^-]$ values obtained at the two sample points on each of *days 4*, *5*, and *6* of the BR and PL supplementation periods are illustrated in Fig. 1. Participants showed elevations in plasma $[NO_2^-]$ during the BR supplementation compared with PL at all sample points (Fig. 1). On average, across the six sample points, BR ingestion increased plasma $[NO_2^-]$ by 96%. The BR-induced elevations in plasma $[NO_2^-]$ were not different across *days 4–6*.

The group mean systolic BP values measured at the six BR and PL sample points are shown in Fig. 2. The ingestion of BR significantly reduced systolic BP at five of the six sample points, relative to PL. Overall, systolic BP was reduced by 6 mmHg across the six samples points (Fig. 2); however, similar to plasma $[NO_2^-]$, the BR-induced reductions in systolic BP were not significantly different among *days 4–6*. The systolic BP was significantly related to the plasma $[NO_2^-]$ on *day 5* ($r = -0.71$, $P < 0.05$), but no relationships were detected between systolic BP and plasma $[NO_2^-]$ on *days 4* or *6*. Diastolic BP (~72 ± 8 mmHg) and mean arterial pressure (~91 ± 5 mmHg) were not significantly affected by BR ingestion.

NIRS Measurements

Moderate exercise. The $[HbO_2]$, [HHb], and $[Hb_{tot}]$ values measured during moderate exercise are shown in Table 1, and the group mean responses are shown in Fig. 3. A 13% reduction in the [HHb] amplitude was observed following BR ingestion, indicating that fractional O_2 extraction was reduced (PL: 88 ± 38 vs. BR: 78 ± 34 AU; $P < 0.05$; Fig. 3). The $[HbO_2]$ within the microvasculature was increased at baseline and at 2 min into exercise, but was not significantly different at the end of exercise (Table 1). BR ingestion elevated $[Hb_{tot}]$ (an index of vascular red blood cell content) at baseline; however, this effect was not maintained during exercise (Table 1).

Severe exercise. The $[HbO_2]$, [HHb], and $[Hb_{tot}]$ values measured during severe exercise are shown in Table 1, and the group mean response is shown in Fig. 4. In contrast to the BR-induced changes in indexes of muscle oxygenation during moderate exercise, the [HHb], $[HbO_2]$, and $[Hb_{tot}]$ parameters were unaffected by BR ingestion during severe exercise.

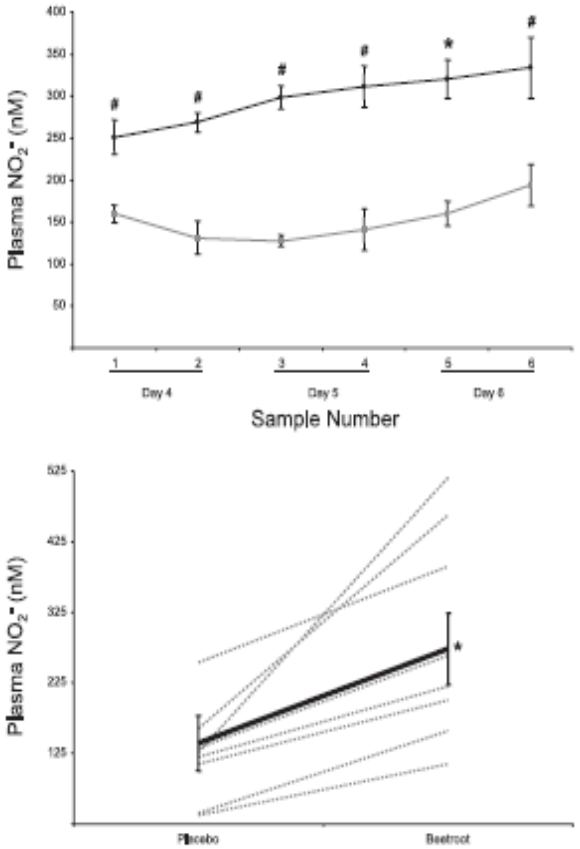

Fig. 1. Plasma nitrite concentration ($[NO_2^-]$) following 4–6 days of dietary nitrate or placebo supplementation. *Top*: group mean (±SE) values of plasma NO_2^- on *days 4*, *5*, and *6* of supplementation, with either nitrate (solid circles) or placebo (shaded squares). The blood samples for $[NO_2^-]$ determination were taken before each of the six exercise bouts that were completed in each condition (*bouts 1*, *2*, *3*, and *5* were moderate, and *bouts 4* and *6* were severe; see text for further details). Note the significantly greater plasma $[NO_2^-]$ following dietary nitrate supplementation. Significant difference from placebo at corresponding time point at the #5% and *1% levels of significance are given. *Bottom*: individual (dotted shaded lines) and mean ± SE (solid line) values for plasma $[NO_2^-]$ measured over *days 4*, *5*, and *6*. *$P < 0.01$.

$\dot{V}O_2$ Dynamics and Exercise Tolerance

Moderate exercise. The pulmonary $\dot{V}O_2$ response during moderate exercise is illustrated in Fig. 5, and the parameters derived from the model fit are presented in Table 2. Dietary BR

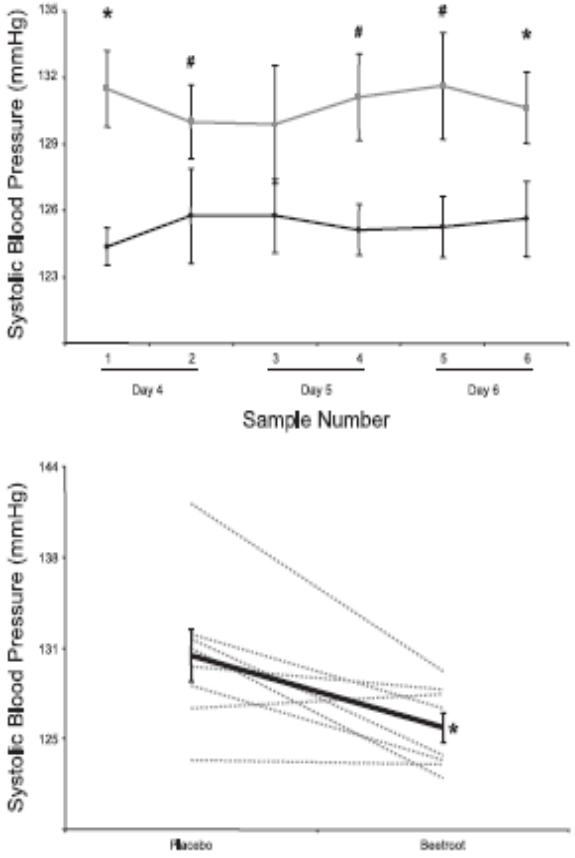

Fig. 2. Systolic blood pressure (SBP) following 4–6 days of dietary nitrate or placebo supplementation. *Top*: group mean (±SE) values of SBP on *days 4*, *5*, and *6* of supplementation with either nitrate (solid circles) or placebo (shaded squares). Note the significantly lower SBP following dietary nitrate supplementation. Significant difference from placebo at corresponding time point at the #5% and *1% levels of significance are given. *Bottom*: individual (dotted shaded lines) and mean ± SE (solid line) values for SBP measured over *days 4*, 5, and 6. *$P < 0.01$.

supplementation resulted in a 19% reduction in the amplitude of the pulmonary $\dot{V}O_2$ response, relative to PL, following a step increment to the same absolute moderate-intensity cycling work rate (PL: 640 ± 146 vs. BR: 521 ± 153 ml/min; $P < 0.01$; Fig. 5), with there being no difference in $\dot{V}O_2$ during the baseline period of very-low-intensity (20 W) cycling. Accordingly, the functional gain (i.e., the ratio of the increase in O_2 consumed per minute to the increase of external power output) was reduced from 10.8 $ml \cdot min^{-1} \cdot W^{-1}$ following PL supplementation to 8.6 $ml \cdot min^{-1} \cdot W^{-1}$ following BR supplementation. The absolute $\dot{V}O_2$ value over the final 30 s of moderate exercise was also significantly lower following BR ingestion (PL: 1,517 ± 123 vs. BR: 1,448 ± 129 ml/min; $P < 0.01$; Fig. 5). The phase II time constant was not significantly altered by BR supplementation (PL: 26 ± 7 vs. BR: 29 ± 6 s; $P > 0.05$). The 95% confidence interval for the estimation of the phase II time constant was 3 ± 1 s for both conditions. The baseline and end-exercise values of CO_2 production, minute ventilation ($\dot{V}E$), respiratory exchange ratio (RER), HR, and blood [lactate] were not significantly between the conditions (Tables 2 and 3).

Severe exercise. The pulmonary $\dot{V}O_2$ response during severe exercise is shown in Fig. 6, and the parameters derived from the bi-exponential fit are presented in Table 2. In contrast to the effects observed for moderate exercise, the primary $\dot{V}O_2$ amplitude during severe exercise was significantly elevated following BR supplementation (PL: 2,158 ± 168 vs. BR: 2,345 ± 179 ml/min; $P < 0.05$). Additionally, the phase II time constant was significantly greater following BR supplementation relative to PL (PL: 33 ± 11 vs. BR: 40 ± 13 s; $P < 0.05$; Table 2). The 95% confidence intervals for the estimation of the phase II time constant were 5 ± 2 and 6 ± 3 s for the PL and BR conditions, respectively. The amplitude of the $\dot{V}O_2$ slow component was significantly smaller following BR supplementation (PL: 739 ± 242 vs. BR: 568 ± 195 ml/min; $P < 0.05$), and, therefore, represented a smaller proportion of the overall $\dot{V}O_2$ response (PL: 25 ± 6 vs. BR: 19 ± 6%; $P < 0.05$). The $\dot{V}O_2$ attained at task failure was not different, either between the conditions or from the $\dot{V}O_{2max}$ recorded during the initial ramp incremental test. Exercise tolerance was enhanced following BR supplementation, as demonstrated by the increased time to task failure (PL: 583 ± 145 vs. BR: 675 ± 203 s; $P < 0.05$; Table 2). However, the

Table 1. *Near-infrared spectroscopy-derived HHb, HbO_2, and Hb_{tot} dynamics during moderate- and severe-intensity exercise following supplementation with nitrate and placebo*

	Placebo	Nitrate
Moderate-intensity exercise		
[HHb]		
Baseline, AU	−132±84	−131±96
120 s, AU	−41±56	−54±74
End, AU	−51±55	−55±83
Mean response time, s	32±8	29±9
Amplitude, AU	88±38	78±34*
[HbO_2]		
Baseline, AU	−29±74	21±51*
120 s, AU	−80±72	−15±30*
End, AU	−5±67	25±39
[Hb_{tot}]		
Baseline, AU	−160±129	−110±89*
120 s, AU	−47±89	−29±70
End, AU	−57±92	−30±81
Severe-intensity exercise		
[HHb]		
Baseline, AU	−142±95	−104±89
120 s, AU	176±107	202±125
End, AU	215±110	246±126
Primary time constant, s	9±2	11±2
Primary time delay, s	8±1	8±2
Primary amplitude, AU	300±70	287±103
Slow-phase amplitude, AU	63±27	67±16
[HbO_2]		
Baseline, AU	96±119	57±91
120 s, AU	−147±66	−176±68
End, AU	−133±59	−166±65
[Hb_{tot}]		
Baseline, AU	−46±116	−47±69
120 s, AU	29±121	26±105
End, AU	82±110	80±104

Values are means ± SD. [HHb], deoxygenated hemoglobin concentration; [HbO_2], oxygenated hemoglobin concentration; [Hb_{tot}], total hemoglobin concentration; AU, arbitrary units. *Significantly different from placebo, $P < 0.05$.

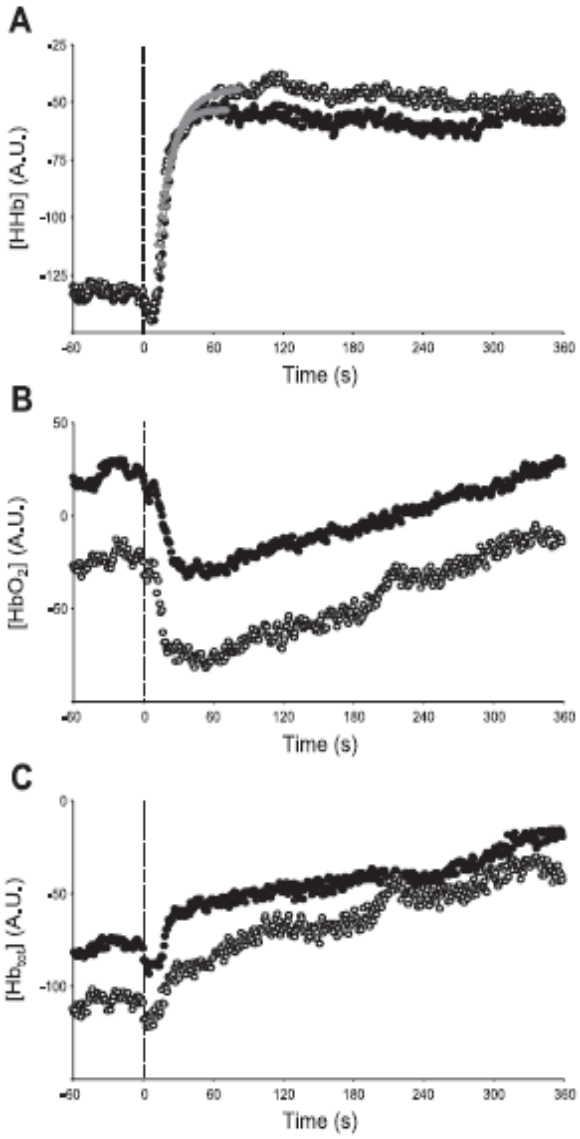

Fig. 3. Group mean changes in the parameters of muscle oxygenation following nitrate and placebo supplementation before and during a step increment to a moderate-intensity cycle work rate. Responses following nitrate supplementation are shown as solid circles, while the placebo responses are shown as open circles. The dotted vertical line represents the abrupt imposition of a moderate work rate from a baseline of "unloaded" cycling. *A*: deoxyhemoglobin concentration ([HHb]). *B*: oxyhemoglobin concentration ([HbO_2]). *C*: total hemoglobin concentration ([Hb_{tot}]). For each individual, the responses to four like-transitions were averaged together before analysis. Note the reduction in the amplitude of the [HHb] response and the greater [HbO_2] and [Hb_{tot}] before and during moderate exercise following dietary nitrate supplementation. Error bars are not shown for clarity, but see Table 1 for further details. AU, arbitrary units.

increased time to task failure was not correlated with the reduction of the $\dot{V}O_2$ slow component ($r = -0.14$; $P = 0.70$). The baseline and end-exercise values of CO_2 production, $\dot{V}E$, RER, and HR were not significantly different between the conditions (Tables 2 and 3). Blood [lactate] at 6 min of exercise and at task failure was also not significantly different between the conditions (Table 3).

DISCUSSION

The principal original finding of this investigation is that 3 days of dietary supplementation with nitrate-rich BR (which doubled the plasma [nitrite]) significantly reduced the O_2 cost of cycling at a fixed submaximal work rate and increased the time to task failure during severe exercise. These findings were consistent with our experimental hypotheses. The O_2 cost of cycling at a fixed moderate work rate is known to be highly consistent in human populations, irrespective of factors such as age and training status (36, 49). That an acute nutritional

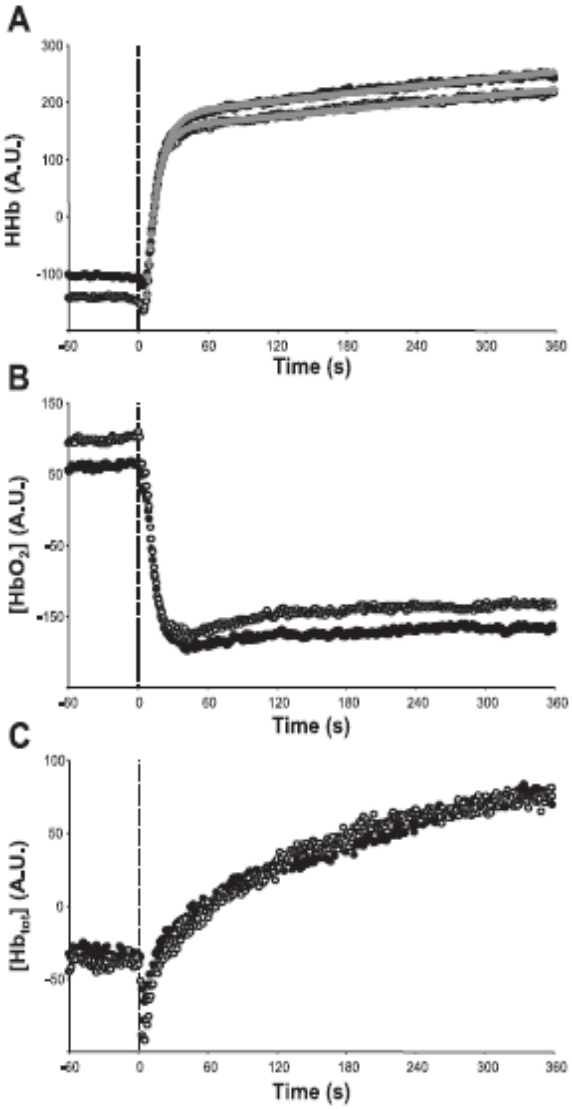

Fig. 4. Group mean changes in the parameters of muscle oxygenation following nitrate and placebo supplementation before and during a step increment to a severe-intensity cycle work rate. Responses following nitrate supplementation are shown as solid circles, while the placebo responses are shown as open circles. The dotted vertical line represents the abrupt imposition of a severe work rate from a baseline of "unloaded" cycling. *A*: [HHb]. *B*: [HbO_2]. *C*: [Hb_{tot}]. For each individual, the responses to two like-transitions were averaged together before analysis. The near-infrared spectroscopy-derived parameters were not appreciably different before or during severe exercise following dietary nitrate supplementation. Error bars are not shown for clarity, but see Table 1 for further details.

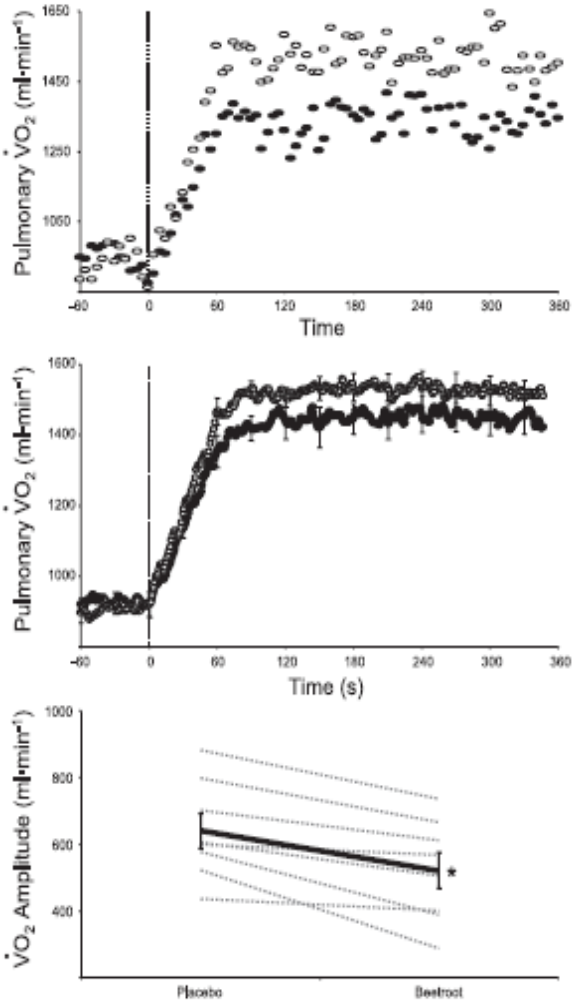

Fig. 5. Pulmonary oxygen uptake ($\dot{V}_{O_2}$) response following nitrate and placebo supplementation during a step increment to a moderate-intensity work rate. Responses following nitrate supplementation are shown as solid circles, while the placebo responses are shown as open circles. The dotted vertical line represents the abrupt imposition of the moderate work rate from a baseline of "unloaded" cycling. *Top*: $\dot{V}_{O_2}$ response of a representative individual (data are shown at 5-s intervals). *Middle*: group mean $\dot{V}_{O_2}$ response with error bars shown every 30 s for clarity. The oxygen cost of moderate exercise was significantly reduced following beetroot supplementation. *Bottom*: individual changes in the amplitude of the $\dot{V}_{O_2}$ response to moderate exercise following nitrate supplementation (dotted shaded lines), along with the group mean change (solid line). For each individual, the responses to four like-transitions were averaged together before analysis. Note that the effect was observed in all participants. *$P < 0.01$.

intervention (i.e., dietary supplementation with a natural food product that is rich in nitrate) can reduce the O_2 cost of a given increment in work rate by ~20% is, therefore, remarkable.

Short-term (i.e., 4–6 days) dietary supplementation with BR increased plasma [NO_2^-] by ~96% in this investigation. Consistent with these findings, dietary NO_3^- supplementation has previously been shown to increase plasma [NO_2^-] when administered as either sodium nitrate (45) or BR (62). Importantly, interrupting the entero-salivary circulation by spitting out saliva thwarted the rise in plasma [NO_2^-] (62), while administration of antibacterial mouthwash before sodium nitrate ingestion also prevented the rise in plasma [NO_2^-] by decreasing the NO_3^--reducing bacteria counts in the oral cavity (28). Collectively, these data highlight the dependence of the NO_3^--to-NO_2^- conversion pathway on the commensal bacterial nitrate reductases present in the human oral cavity. The bacterially derived NO_2^- can increase circulating plasma NO_2^- and undergo reduction to yield NO in hypoxia or acidosis (21, 46).

Effects of dietary nitrate on BP. In the present study, dietary supplementation with BR reduced systolic BP by an average of 6 mmHg, but without altering diastolic BP or mean arterial pressure. In contrast, both systolic and diastolic BP were reduced following 3 days of dietary NO_3^- supplementation in the study of Larsen et al. (45). Recent data indicate that, following BR ingestion, peak reductions in systolic and diastolic BP are observed 2.5 and 3 h postingestion, respectively (62). Furthermore, the BR-induced reduction in systolic BP persisted for 24 h postingestion, while diastolic BP had returned toward baseline (62). Collectively, these data suggest that systolic BP is more amenable to nitrate-induced change than is diastolic BP. The reduced BP observed with a diet rich in nitrates

Table 2. *Mean ± SD ventilatory and gas exchange dynamics during moderate- and severe-intensity exercise following supplementation with nitrate and placebo*

	Placebo	Nitrate
Moderate-intensity exercise		
$\dot{V}_{O_2}$		
Baseline, l/min	0.91±0.09	0.93±0.05
End-exercise, l/min	1.52±0.12	1.45±0.13*
Phase II time constant, s	26±7	29±6
Mean response time, s	39±8	45±4
Primary amplitude, l/min	0.64±0.15	0.52±0.15*
Primary gain, ml·min^{-1}·W^{-1}	10.8±1.6	8.6±0.7†
$\dot{V}_{CO_2}$		
Baseline, l/min	0.85±0.08	0.84±0.05
End-exercise, l/min	1.31±0.15	1.32±0.15
$\dot{V}_E$		
Baseline, l/min	25±2	24±1
End-exercise, l/min	36±4	34±3
Respiratory exchange ratio		
Baseline	0.93±0.07	0.90±0.07
End-exercise	0.90±0.05	0.91±0.02
Severe-intensity exercise		
$\dot{V}_{O_2}$		
Baseline, l/min	0.99±0.10	0.96±0.07
End-exercise, l/min	3.87±0.29	3.82±0.28
Phase II time constant, s	33±11	40±13†
Primary amplitude, l/min	2.19±0.17	2.35±0.18†
Primary gain, ml·min^{-1}·W^{-1}	9.0±0.7	9.4±0.6
Slow-phase amplitude, l/min	0.74±0.24	0.57±0.20†
Slow-component amplitude, %	25±6	19±6†
Overall gain, ml·min^{-1}·W^{-1}	11.6±0.9	10.8±0.8*
Overall mean response time, s	75±16	71±16
$\dot{V}_{CO_2}$		
Baseline, l/min	0.85±0.23	0.86±0.10
End-exercise, l/min	3.99±0.32	4.03±0.43
$\dot{V}_E$		
Baseline, l/min	24±7	25±3
End-exercise, l/min	140±14	139±21
Respiratory exchange ratio		
Baseline	0.86±0.17	0.89±0.09
End-exercise	1.04±0.05	1.05±0.05

Values are means ± SD. $\dot{V}_{O_2}$, oxygen uptake; $\dot{V}_{CO_2}$, expired carbon dioxide; $\dot{V}_E$, minute ventilation. Significantly different from placebo: *$P < 0.01$, †$P < 0.05$.

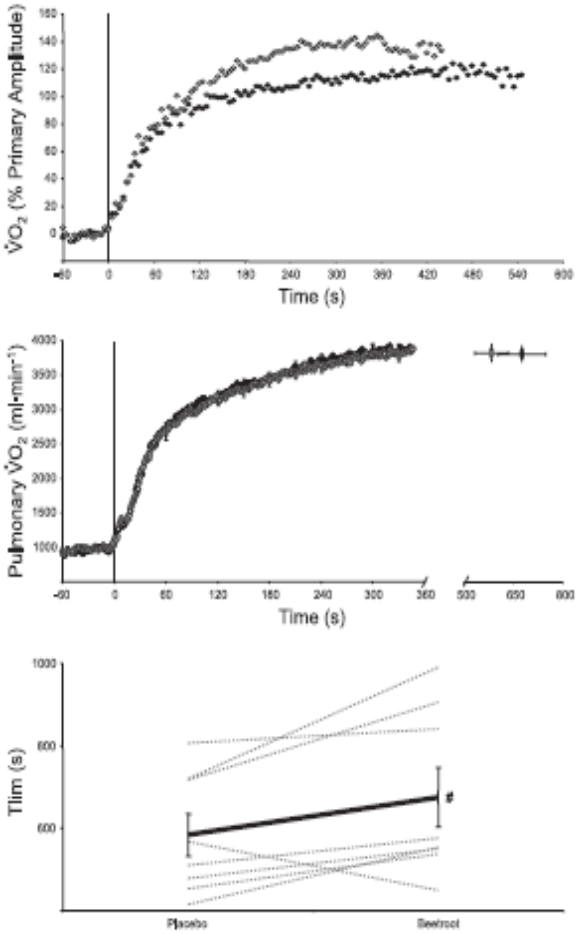

Fig. 6. Pulmonary $\dot{V}O_2$ response following nitrate and placebo supplementation during a step increment to a severe-intensity work rate. Responses following nitrate supplementation are shown as solid circles, while the placebo responses are shown as open circles. The dotted vertical line represents the abrupt imposition of the severe work rate from a baseline of "unloaded" cycling. *Top*: $\dot{V}O_2$ response of a representative individual (data are shown at 5-s intervals). The data are plotted as a fraction of the $\dot{V}O_2$ fundamental component amplitude to more clearly illustrate the slower phase II $\dot{V}O_2$ kinetics and reduced $\dot{V}O_2$ slow component following nitrate supplementation. *Middle*: group mean $\dot{V}O_2$ response with error bars shown every 30 s for clarity. The group mean ± SE $\dot{V}O_2$ at task failure is also shown. *Bottom*: individual changes in the tolerance of severe exercise following nitrate supplementation (dotted shaded lines) along with the group mean change (solid line). #$P < 0.05$.

suggests that this "natural" approach has the potential to maintain or enhance aspects of human cardiovascular health.

The NO_3^--induced reduction in systolic BP is likely mediated via its conversion to NO_2^- and thence NO. NO is known to be an important endothelial relaxing factor, through its role as a secondary messenger in cyclic guanosine monophosphate synthesis, culminating in smooth muscle relaxation (31). Conventionally, NO production was considered to be derived, universally, via the NO synthase family of enzymes in humans. These enzymes produce NO through catalyzing the five-electron oxidation of L-arginine in a reaction requiring O_2 and NADPH (9). More recent research has identified an O_2-independent pathway for the generation of NO, via the reduction of NO_2^- to NO in an acidic milieu (6). Importantly, this pathway preserves blood and tissue NO production during hypoxia when the enzymatic activity of the NO synthases are rate limited by the lack of O_2 availability. As such, a considerable body of evidence now supports a biological role for NO_2^- in hypoxic vasodilation (26), which serves to protect tissues from ischemia and reperfusion injury (27).

Effects of dietary nitrate on the physiological responses to moderate exercise. Increased dietary NO_3^- consumption altered indexes of muscle oxygenation as investigated using NIRS. During moderate exercise, [Hb_{tot}] was elevated at baseline, and [HbO_2] was elevated both at baseline and over the first 120 s of exercise following BR ingestion. The increased blood volume in the region of interrogation at baseline following BR ingestion is presumably a consequence of enhanced muscle vasodilatation, resulting from increased NO production from NO_2^-. The NIRS-derived [HHb] response reflects the balance between local O_2 delivery and utilization and has been used previously as an index of muscle fractional O_2 extraction (1, 22, 24, 30). In the present study, the amplitude of the [HHb] response was reduced by 13% following BR ingestion. Conversely, inhibition of NO synthesis via N^G-nitro-L-arginine methyl ester (L-NAME) administration has been shown to increase muscle O_2 extraction in the exercising horse (40). By the Fick equation, for the same $\dot{V}O_2$, an increased muscle O_2 delivery would be expected to enable a reduced muscle fractional O_2 extraction. However, in the present study, in which $\dot{V}O_2$ was reduced by dietary BR supplementation, an alternative interpretation is that less O_2 extraction was required consequent to a reduced aerobic energy turnover or muscle energy utilization.

Perhaps the most striking finding of the present investigation was the significant reduction in the O_2 cost of submaximal exercise following increased dietary NO_3^- intake. While the $\dot{V}O_2$ response during the unloaded baseline cycling period was unaffected, a 19% reduction in the amplitude of the pulmonary

Table 3. *Heart rate and blood lactate responses to moderate- and severe-intensity exercise following supplementation with nitrate and placebo*

	Placebo	Nitrate
Moderate-intensity exercise		
Heart rate		
Baseline, beats/min	81±9	81±8
End, beats/min	98±12	98±13
Time constant, s	28±12	31±16
Blood [lactate]		
Baseline, mM	1.0±0.5	0.9±0.3
End, mM	1.2±0.7	1.1±0.2
Δ, mM	0.2±0.2	0.2±0.2
Severe-intensity exercise		
Heart rate		
Baseline, beats/min	85±8	88±8
End, beats/min	170±8	170±8
Time constant, s	16±10	17±5
Blood [lactate]		
Baseline, mM	1.0±0.2	1.1±0.5
End, mM	6.9±1.6	6.9±1.2
Δ, mM	5.9±1.6	5.7±1.0
Exhaustion, mM	10.0±1.9	10.0±1.7

Values are means ± SD. [Lactate], lactate concentration; Δ, change.

$\dot{V}O_2$ response, relative to PL, was evident following NO_3^- supplementation during a step increment to the same absolute moderate-intensity cycling work rate. Accordingly, the functional gain (i.e., the ratio of the increase in O_2 consumed per minute to the increase of external power output and the reciprocal of delta efficiency; Ref. 64) was reduced from 10.8 $ml \cdot min^{-1} \cdot W^{-1}$ following PL supplementation to 8.6 $ml \cdot min^{-1} \cdot W^{-1}$ following NO_3^- supplementation. Moreover, the gross O_2 cost of exercise (comprising resting metabolic rate, the O_2 cost of moving the limbs during baseline pedaling, and the O_2 cost of muscle contraction to meet the imposed work rate) was reduced by ~5%. The magnitude of effect was similar to that reported by Larsen et al. (45) using sodium nitrate supplementation. While we therefore suggest that the reduced O_2 cost of submaximal exercise in our study was consequent to the increased dietary nitrate content, we cannot presently exclude the possibility that other substances contained in BR contributed to the results we obtained. Importantly, the reduction in $\dot{V}O_2$, and thus of ATP resynthesis through oxidative phosphorylation, was not compensated by elevations in glycolytic ATP provision, as inferred, albeit crudely, from the similar blood [lactate] values between the BR and PL conditions. These findings extend those of Larsen et al. (45) by demonstrating that the O_2 cost of submaximal exercise is reduced following NO_3^- ingestion in the form of a natural food product. That HR and $\dot{V}E$ were not significantly different between treatments suggests that the reduction in $\dot{V}O_2$ originated from the skeletal muscles and not from alterations in the energetic cost of cardiorespiratory support processes. Moreover, the similar RER between the conditions indicates that substrate utilization (which can influence the O_2 cost of exercise) was not altered by the intervention.

The mechanistic bases for the reduced O_2 cost of submaximal exercise following increased NO_3^- intake, either by pharmacological (45) or natural dietary means (present study), are unclear. The inhibition of NO synthesis has previously been shown to increase steady-state $\dot{V}O_2$ in dogs (56), but not humans (38) or horses (41). It is widely accepted, however, that NO is involved in the regulation of mitochondrial O_2 consumption. In particular, it has been established that NO has a strong affinity for cytochrome-*c* oxidase (CytOX; Ref. 10), but there is also evidence that NO has the potential to modulate other aspects of mitochondrial and muscle contractile function (11, 57, 58).

A reduction in the O_2 cost of mitochondrial ATP resynthesis would require either more protons pumped per O_2 molecule reduced, or the use of an alternative terminal electron acceptor in place of O_2. It has been proposed that mitochondrial efficiency is intimately linked to the process of uncoupled respiration in which mitochondrial proton leak results in energy dissipation as heat instead of conversion to ATP (8). In this regard, the improved O_2 efficiency noted in the present study following BR ingestion might be related to a reduction of mitochondrial proton leak or proton pump slippage. There is evidence to suggest that NO increases the efficiency of oxidative phosphorylation in isolated mitochondria by reducing slipping of the proton pumps (17). Another possibility is that NO_2^- could be acting in place of O_2 as the final electron acceptor in the respiratory chain, thereby reducing the requirement for O_2 consumption (3). An intra-mitochondrial NO_2^- regeneration pathway would be critical in this scenario, given the limited NO_2^- concentration within the mitochondria. Under conditions of low electron flux, NO can inhibit CytOX by binding to the Cu^{2+} active site yielding nitrosonium (NO^+), which is subsequently hydrated to NO_2^- (18). One possible scenario is that the NO_2^- so produced could be reduced to NO by accepting an electron from CytOX, and this NO could subsequently bind to the Cu^{2+} active site, completing the cyclical process to regenerate NO_2^-. This possibility is intriguing, as the hydration of NO^+ to NO_2^- yields an electron that can be redistributed within CytOX (19, 60). Subsequently, this electron may be accepted by NO_2^-, potentially utilizing the electron derived from its synthesis, and could contribute to proton pumping and ATP synthesis, in an efficiently coupled process.

The reduction in O_2 consumption with increased dietary NO_3^- intake could also be attributed, in part, to a reduced ATP cost of force production, requiring less flux through oxidative phosphorylation. One of the most energetically costly processes during skeletal muscle contraction is sarcoplasmic reticulum Ca^{2+} pumping, which may account for up to 50% of the total ATP turnover (7). The presence of reactive oxygen species increases the opening probability of the sarcoplasmic reticulum Ca^{2+} release channels (48), and the active reuptake of the elevated cytosolic Ca^{2+} would present a considerable energetic challenge (7). NO donors that invoke small elevations in NO might protect the channel against oxidation-induced Ca^{2+} release, without significantly altering channel function (53). Therefore, the BR-induced elevations in NO may have prevented an excess of Ca^{2+} release and subsequently reduced the considerable energetic cost of its resequestration. These suggestions are naturally speculative at the present time and await further investigation.

Effects of dietary nitrate on the physiological responses to severe exercise. The physiological responses during severe exercise were different from those observed during moderate exercise following BR supplementation, suggesting that the influence of NO_2^- and/or NO on muscle function is specific to the exercise intensity domain being investigated. First, there were no significant differences in NIRS-derived indexes of muscle oxygenation between the NO_3^- and PL conditions during severe exercise, although this might be a function of the fact that severe exercise always followed moderate exercise in our experiments. Moreover, in contrast to the reduced steady-state $\dot{V}O_2$ observed during moderate exercise following increased NO_3^- consumption, the amplitude of the primary or fundamental component $\dot{V}O_2$ response during severe exercise was increased (by ~7%), and the amplitude of the subsequent $\dot{V}O_2$ slow component was reduced (by ~23%), with the $\dot{V}O_2$ at the point of task failure being not significantly different between the conditions. These changes in $\dot{V}O_2$ kinetics during high-intensity exercise following dietary NO_3^- supplementation resemble those that are observed following an initial "priming" bout of high-intensity exercise (15, 35, 45), effects that have been attributed, either separately or in combination, to increased muscle O_2 delivery, increased oxidative metabolic enzyme activity and carbon substrate availability, and altered motor unit recruitment patterns (15, 35, 45). We have previously reported that NO synthase inhibition with L-NAME significantly increased the amplitude of the $\dot{V}O_2$ slow component and speculated that this might be related to changes in muscle O_2 delivery or its distribution and/or to (related)

changes in motor unit recruitment patterns (39). It is presently unclear which, if any, of the above-named mechanisms contributed to the altered $\dot{V}O_2$ kinetics during severe exercise following dietary NO_3^- supplementation. Myocytes in close proximity to a capillary are advantaged with respect to muscle O_2 availability, whereas myocytes situated further away are increasingly less well supplied with O_2, creating an O_2 pressure gradient within the contracting muscles. The hypoxic and acidic milieu within and surrounding the distal myocytes might stimulate NO_2^- reduction to NO facilitating vasodilatation and thus delivery of O_2. The NO so produced is capable of diffusion and may inhibit mitochondrial O_2 consumption in the myocytes proximal to the capillary bed, promoting deeper diffusion of the available O_2 (32, 59) and, therefore, enabling a more appropriate matching of local O_2 delivery to O_2 requirement. Another possibility is that the increased NO availability following increased dietary NO_3^- intake promotes mitochondrial biogenesis (16, 50). Greater mitochondrial volume would enable the same rate of mitochondrial respiration to be accomplished with a reduced perturbation of adenine nucleotides. However, irrespective of the mechanism(s) involved, the return of $\dot{V}O_2$ kinetics toward first-order linear system dynamics during severe exercise will likely limit the rate at which metabolites that have been associated with the fatigue process (e.g., H^+, P_i, ADP) accumulate in skeletal muscle (14), effects that would be expected, in turn, to portend enhanced exercise tolerance.

The τ_p was significantly longer (i.e., phase II pulmonary $\dot{V}O_2$ kinetics was slower) following NO_3^- supplementation relative to PL for severe exercise (group mean τ_p 40 vs. 33 s for BR and PL). This is consistent with our laboratory's previous reports that the τ_p was significantly faster when NOS activity was inhibited by L-NAME (38, 39, 66). The τ_p was also slightly longer during moderate exercise (group mean τ_p 29 vs. 26 s for BR and PL), but this difference was not statistically significant. While it is acknowledged that changes in muscle blood flow, which might occur with greater or lesser NO availability, have the potential to dissociate the normally close relationship between muscle and pulmonary $\dot{V}O_2$ kinetics (2), our data suggest that NO might have an important regulatory influence on the inertia of $\dot{V}O_2$ dynamics following the onset of exercise (36, 51). It is of interest that NO_3^- supplementation resulted in changes in the amplitudes of the $\dot{V}O_2$ response to exercise (i.e., lower steady-state $\dot{V}O_2$ during moderate exercise and higher $\dot{V}O_2$ fundamental component and reduced $\dot{V}O_2$ slow component during severe exercise), while also slowing the phase II $\dot{V}O_2$ kinetics. While the mechanistic bases of this effect is not clear, it is possible that increased NO availability results, simultaneously, in a slowing of the rate at which $\dot{V}O_2$ rises following the onset of exercise (via competitive inhibition of CytOX; Refs. 10, 38, 39, 41, 56) and changes in the amplitudes of the fundamental and slow components of $\dot{V}O_2$ (through effects on the efficiency of muscle oxidative metabolism and/or contractile function; Refs. 3, 11, 17, 57).

The kinetics of $\dot{V}O_2$ are considered to be an important determinant of exercise tolerance (14, 36). However, in this study, we observed a 16% improvement in the time to task failure during severe exercise in the NO_3^- condition, despite the slower phase II $\dot{V}O_2$ kinetics. Another parameter of $\dot{V}O_2$ dynamics considered to influence exercise tolerance is the slow component rise in $\dot{V}O_2$ observed during supra-GET exercise, since this parameter is associated with greater utilization of the finite phosphocreatine (55) and glycogen (42) reserves. Indeed, a reduction in the $\dot{V}O_2$ slow-component amplitude has been associated with improved exercise tolerance (1, 14). However, in the present study, the improvement in severe exercise tolerance with NO_3^- supplementation was not significantly correlated with the reduction of the $\dot{V}O_2$ slow-component amplitude. The $\dot{V}O_2$ at task failure (which was equivalent to the preestablished $\dot{V}O_{2max}$) was not different between conditions, suggesting that the $\dot{V}O_{2max}$ was reached more slowly and/or could be sustained for longer following NO_3^- supplementation. It is noteworthy that the $\dot{V}O_{2max}$ during severe exercise was not impaired with NO_3^- supplementation, although the steady-state $\dot{V}O_2$ was reduced during submaximal exercise. These results differ from those obtained with NO synthase inhibition. With the latter, $\dot{V}O_{2max}$ and exercise tolerance are impaired during both ramp incremental exercise (37) and supramaximal step exercise (66). Although the mechanism for the enhanced performance observed in the present study is uncertain, an interesting possibility is that an elevation of tissue s-nitrosothiols with increased dietary nitrate intake (13) prevents those nitrosylated thiols undergoing irreversible oxidative modification as a consequence of the production of reactive oxygen species (12, 54), an effect that is known to compromise skeletal muscle force production (25). We wish to stress here that, while the 16% improvement in the time to task failure during severe constant work rate exercise is impressive, the magnitude of effect would be expected to be much smaller during time trial exercise tasks in which a given distance is completed in the shortest possible time (34). Nevertheless, it is possible that the effect might still be meaningful in terms of performance enhancement.

Applications and conclusions. A short period of dietary NO_3^- supplementation through a natural food product resulted in increased plasma [nitrite] and reduced systolic BP in the normotensive young adult men who participated in our study. During exercise at a fixed moderate work rate, increased NO_3^- intake resulted in improvements in NIRS-derived indexes of muscle oxygenation and a significant reduction in pulmonary $\dot{V}O_2$. It should be stressed that the remarkable reduction in the O_2 cost of submaximal cycle exercise following dietary supplementation with inorganic nitrate in the form of a natural food product cannot be achieved by any other known means, including long-term endurance exercise training (1, 15, 49, 65). Although not directly tested in the present study, the results suggest that increased dietary NO_3^- intake has the potential to enhance exercise tolerance during longer term endurance exercise. Moreover, in certain human populations (including the senescent and those with cardiovascular, respiratory, or metabolic diseases), the activities of daily living are physically difficult because they have an energy requirement that represents a high fraction of the $\dot{V}O_{2max}$. A reduction in the $\dot{V}O_2$ associated with such activities following dietary nitrate supplementation, therefore, has the potential to improve exercise tolerance and the quality of life in these groups. During exercise at a fixed severe work rate, BR ingestion reduced the amplitude of the $\dot{V}O_2$ slow component and increased the time to task failure by ~16%, suggesting that dietary nitrate supplementation might enhance high-intensity exercise performance. Further research is required to investigate the mechanistic bases for the reduced O_2 cost of submaximal exercise

observed with increased dietary nitrate intake in this study and previously (Ref. 45; i.e., reduced ATP cost of force production and/or increased mitochondrial P/O ratio; i.e., ratio of phosphate radicals esterified to atoms of oxygen consumed). Finally, the possible ergogenicity of dietary nitrate supplementation during different types of exercise in humans is likely to be a fertile area for further research.

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Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans

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Bailey SJ, Fulford J, Vanhatalo A, Winyard PG, Blackwell JR, DiMenna FJ, Wilkerson DP, Benjamin N, Jones AM. Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *J Appl Physiol* 109: 135–148, 2010. First published May 13, 2010; doi:10.1152/japplphysiol.00046.2010.— The purpose of this study was to elucidate the mechanistic bases for the reported reduction in the O_2 cost of exercise following short-term dietary nitrate (NO_3^-) supplementation. In a randomized, double-blind, crossover study, seven men (aged 19–38 yr) consumed 500 ml/day of either nitrate-rich beetroot juice (BR, 5.1 mmol of NO_3^-/day) or placebo (PL, with negligible nitrate content) for 6 consecutive days, and completed a series of low-intensity and high-intensity "step" exercise tests on the last 3 days for the determination of the muscle metabolic (using ^{31}P-MRS) and pulmonary oxygen uptake ($\dot{V}O_2$) responses to exercise. On *days 4–6*, BR resulted in a significant increase in plasma [nitrite] (mean ± SE, PL 231 ± 76 vs. BR 547 ± 55 nM; $P < 0.05$). During low-intensity exercise, BR attenuated the reduction in muscle phosphocreatine concentration ([PCr]; PL 8.1 ± 1.2 vs. BR 5.2 ± 0.8 mM; $P < 0.05$) and the increase in $\dot{V}O_2$ (PL 484 ± 41 vs. BR 362 ± 30 ml/min; $P < 0.05$). During high-intensity exercise, BR reduced the amplitudes of the [PCr] (PL 3.9 ± 1.1 vs. BR 1.6 ± 0.7 mM; $P < 0.05$) and $\dot{V}O_2$ (PL 209 ± 30 vs. BR 100 ± 26 ml/min; $P < 0.05$) slow components and improved time to exhaustion (PL 586 ± 80 vs. BR 734 ± 109 s; $P < 0.01$). The total ATP turnover rate was estimated to be less for both low-intensity (PL 296 ± 58 vs. BR 192 ± 38 μM/s; $P < 0.05$) and high-intensity (PL 607 ± 65 vs. BR 436 ± 43 μM/s; $P < 0.05$) exercise. Thus the reduced O_2 cost of exercise following dietary NO_3^- supplementation appears to be due to a reduced ATP cost of muscle force production. The reduced muscle metabolic perturbation with NO_3^- supplementation allowed high-intensity exercise to be tolerated for a greater period of time.

bioenergetics; muscle metabolism; ^{31}P-MRS; fatigue; efficiency; respiratory control

AT THE ONSET of moderate-intensity exercise (that is, exercise performed at work rates below the gas exchange threshold, GET), pulmonary O_2 uptake ($\dot{V}O_2$) rises in an exponential fashion to attain a steady state within approximately 2–3 min in healthy humans (76, 77). In the steady state, the rate of ATP catabolism is in equilibrium with the rate of ATP resynthesis through oxidative phosphorylation. For moderate-intensity cycle exercise, the steady-state $\dot{V}O_2$ is linearly related to the external work rate with the functional "gain" (increase in $\dot{V}O_2$ per unit increment in external work rate) approximating 10 ml·min^{-1}·W^{-1} (38, 76). During supra-GET exercise, however, $\dot{V}O_2$ dynamics become more complex due to the development of a delayed-onset $\dot{V}O_2$ "slow component" which elevates the O_2 cost of exercise above that predicted from linear extrapolation of the moderate-intensity $\dot{V}O_2$-work rate relationship (38, 76, 77).

The steady-state $\dot{V}O_2$ for a given moderate-intensity work rate during cycle ergometry has been considered to be intransigent to a variety of acute exercise and pharmacological interventions and to be essentially unaltered with age and training (2, 10, 16, 40, 78). However, recent research has shown that 3–6 days of either pharmacological (sodium nitrate; 52) or dietary (beetroot juice; 3) nitrate (NO_3^-) administration can reduce the steady-state $\dot{V}O_2$ during submaximal cycle exercise in young healthy participants. During severe-intensity exercise, dietary NO_3^- supplementation reduced the $\dot{V}O_2$ slow component, delaying the attainment of the maximal $\dot{V}O_2$ ($\dot{V}O_{2\,max}$) and increasing the tolerable duration of exercise (3).

The mechanism(s) by which NO_3^- administration reduces the O_2 cost of submaximal exercise and enhances exercise tolerance is/are presently unclear. Given that NO_3^- is relatively inert, the effect is unlikely to be mediated by an elevated [NO_3^-], per se, but rather, through the action of its bioactive nitrogen derivative, nitrite (NO_2^-), or the subsequent bioconversion of NO_2^- to nitric oxide (NO). Ingested inorganic NO_3^- is rapidly absorbed from the gut and concentrated in the saliva; facultative anaerobic bacteria on the surface of the tongue then reduce NO_3^- to NO_2^- (21). Swallowed NO_2^- can be converted to NO in the stomach (9, 55) but some is absorbed to increase the circulating plasma [NO_2^-] (19, 54). We (9) and others (56) have shown that NO_2^- can be converted to NO, with the requisite one-electron reduction being catalyzed via xanthine oxidoreductase, hemoglobin, myoglobin, endothelial nitric oxide synthase, and the mitochondrial electron transfer complexes (see 15 for review).

To reduce the O_2 cost of moderate-intensity exercise, NO_2^-/NO would be required to *1*) increase the mitochondrial P/O ratio (i.e., reduce the O_2 cost of mitochondrial ATP resynthesis); *2*) improve the coupling between ATP hydrolysis and muscle force production (i.e., reduce the ATP cost of force production); or *3*) inhibit mitochondrial ATP production, in which case a compensatory increase in energy provision through substrate-level phosphorylation would be required. An elevation in NO_2^-/NO may increase the P/O ratio through a reduction in "slippage" at the mitochondrial proton pumps (18) or through the potential role for NO_2^- as an alternative electron acceptor (7). The ATP cost of contraction in skeletal myocytes is essentially the sum of ATP consumption via the interaction between actin and myosin (actomyosin-ATPase) and calcium (Ca^{2+}) pumping in the sarcoplasmic reticulum (Ca^{2+}-ATPase), with membrane depolarization (Na^+-K^+-ATPase) making a further small contribution to the total ATP turn-

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over (6). NO has been demonstrated to slow cross-bridge cycling kinetics (25, 34), reduce ryanodine activity, and therefore Ca^{2+} release (33) and inhibit Ca^{2+}-ATPase activity (71), and NO may therefore have a regulatory influence on the ATP cost of force production. Previous studies have suggested that the reduced O_2 cost of exercise with dietary NO_3^- supplementation was not compensated by an elevation in anaerobic metabolism, as inferred from an unchanged blood [lactate] (3, 52). However, it is acknowledged that blood [lactate] represents a very indirect and incomplete assessment of muscle anaerobic energy turnover. Therefore, it is presently unclear which of these potential mechanisms underpins the reduced O_2 cost of exercise and improved exercise tolerance following dietary NO_3^- supplementation.

Phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS) facilitates the assessment of human muscle metabolism noninvasively, in vivo, and with a high temporal and spatial resolution (39, 44, 61, 79). Utilization of ^{31}P-MRS, to provide information on tissue changes in [phosphocreatine] ([PCr]), [ADP], and pH, in conjunction with pulmonary $\dot{V}O_2$ dynamics, which closely reflects skeletal muscle O_2 consumption (31, 47), enables estimation of changes in total ATP turnover rate and the proportional contribution of PCr hydrolysis, glycolysis, and oxidative phosphorylation during exercise following dietary NO_3^- supplementation (42, 43, 51, 53). A reduction in the steady-state $\dot{V}O_2$ amplitude following NO_3^- administration, as observed previously (3, 52), with no change in energy derived through the PCr and glycolytic pathways, would be indicative of an increased mitochondrial P/O ratio. Alternatively, a reduction in the steady-state $\dot{V}O_2$ amplitude with accompanying reductions in energy derived through one or both of the PCr or glycolytic pathways might suggest a reduced ATP cost of force production following NO_3^- administration, i.e., a reduction in ATP turnover for the same external work rate rather than an improvement in the efficiency of mitochondrial oxidative ATP synthesis, per se. However, a greater fall in pH or greater [PCr] degradation following NO_3^- administration would indicate that the reduction in aerobic ATP yield was compensated through an increased anaerobic ATP yield.

The purpose of the present study was to elucidate the mechanism by which NO_3^- administration resulted in a reduced O_2 cost of submaximal exercise and an improved tolerance of high-intensity exercise (3). We hypothesized that NO_3^-, administered as nitrate-rich beetroot juice (BR), would reduce the O_2 cost of both low-intensity and high-intensity exercise and that this would be accompanied by reductions in the extent of intramuscular PCr degradation of a similar magnitude (reflecting a reduced high-energy phosphate cost of force production), with no change in pH. We used quantitative ^{31}P-MRS (42) to investigate changes in the rates of ATP resynthesis deriving from PCr hydrolysis, glycolysis, and oxidative phosphorylation, and the total muscle ATP turnover rate (51, 53), with and without dietary NO_3^- supplementation. Given that the termination of high-intensity, constant-work-rate exercise is associated with the attainment of a consistent muscle metabolic milieu (i.e., low [PCr] and pH and high P_i concentration, [P_i]; 70), we also hypothesized that exercise tolerance would be enhanced following dietary NO_3^- supplementation due to a sparing of the finite [PCr] reserves and reduced muscle metabolic perturbation.

METHODS

Subjects

Seven healthy, recreationally active males (mean ± SD, age 28 ± 7 yr, height 1.80 ± 0.02 m, body mass 81 ± 7 kg) volunteered to participate in this study. None of the subjects were tobacco smokers or users of dietary supplements, and all were familiar with the experimental procedures used in this study. The procedures employed in this study were approved by the Institutional Research Ethics Committee. All subjects gave their written informed consent before the commencement of the study, after the experimental procedures, associated risks, and potential benefits of participation had been explained. Subjects were instructed to arrive at the laboratory in a rested and fully hydrated state, at least 3 h postprandial, and to avoid strenuous exercise in the 24 h preceding each testing session. Each subject was also asked to refrain from caffeine and alcohol 6 and 24 h before each test, respectively, and to abstain from the consumption of foods rich in nitrates for the duration of the study. All tests were performed at the same time of day (±2 h).

Procedures

The subjects were required to report to the laboratory on seven occasions over a 4-wk period. During the first visit to the laboratory, subjects provided a venous blood sample for determination of plasma [nitrite], and resting blood pressure (BP) was measured. The subjects then completed three maximum voluntary isometric contractions (MVC) of the quadriceps; each MVC was 3 s in duration and was separated by 1 min of passive recovery. Five minutes after completion of the final MVC, subjects commenced an incremental test using a two-legged knee-extension ergometer to establish the peak work rate (WR_{peak}). These and subsequent exercise tests were conducted in the prone position, with subjects secured to the ergometer bed via Velcro straps at the thigh, buttocks, lower back, and middle back to minimize extraneous movement. The custom-designed ergometer consisted of a nylon frame that fitted onto the bed in alignment with the subject's feet and a base unit that was positioned behind the bed. Cuffs with Velcro straps were secured to the subject's feet, and ropes were attached to the cuffs. These ropes passed around pulleys housed within the frame to points of attachment on chains that meshed with a cassette of sprockets on the base unit. The sprocket arrangement was such that when a bucket containing nonmagnetic weights was attached to each chain it provided a concentric-only resistive load for each leg. This allowed for the performance of rhythmic two-legged knee-extension exercise in a contralateral alternating manner over a distance of ~0.22 m. Subjects lifted the weight in accordance with a visual cue at a frequency of 40/min. The load for the first step increment was 4 kg per leg and the load was increased by 1 kg for each subsequent increment until the limit of tolerance.

Throughout the MVCs and incremental test, muscle activity (integrated electromyography, iEMG) of the right vastus lateralis muscle was recorded. The leg was initially shaved and cleaned with alcohol around the belly of the muscle, and graphite snap electrodes (Unilect 40713, Unomedical, Stonehouse, UK) were then adhered to the prepared area in a bipolar arrangement (interelectrode distance: 40 mm). A ground electrode was positioned on the rectus femoris muscle equidistant from the active electrodes. To secure electrodes and wires in place and to minimize movement, an elastic bandage was wrapped around the subject's leg. Indelible pen marks were made around the electrodes to enable reproduction of the placement in subsequent tests. The EMG signal was recorded using a ME3000PB Muscle Tester (Mega Electronics). EMG measurements at a sampling frequency of 1,000 Hz were recorded throughout all exercise tests. The bipolar signal was amplified (amplifier input impedance > 1 MΩ), and data were collected in raw form and stored on a personal computer using MegaWin software (Mega Electronics). The work rates that would require 15% and 30% of the MVC iEMG signal were calculated and

applied as the low- and high-intensity work rates, respectively, in subsequent tests.

Following completion of the step incremental test, subjects were randomly assigned, in a double-blind, crossover fashion, to receive 6 days of dietary supplementation with nitrate [NO_3^-; 5.1 mmol/day; administered as 0.5 liter of organic beetroot juice (BR) per day; Beet It, James White Drinks, Ipswich, UK] and placebo (PL; low-calorie black-currant juice cordial with negligible NO_3^- content) with a 10-day washout separating the supplementation periods. The concentration of NO_3^- in the beetroot juice was determined by its reduction to NO in the presence of VCl_3 at 90°C using chemiluminescence (8). The subjects were not aware of the experimental hypotheses to be tested but were informed that the purpose of the study was to compare the physiological responses to exercise following the consumption of two commercially available beverages. This study was completed before the publication of our initial study (3) such that the subjects were not aware that beetroot juice might be ergogenic.

On *days 4* and *5* of the supplementation periods, the subjects completed "step" exercise tests from a resting baseline to low-intensity and high-intensity work rates for the determination of breath-by-breath pulmonary $\dot{V}O_2$ dynamics and muscle activity. Pulmonary gas exchange and ventilation ($\dot{V}E$) were measured breath by breath with subjects wearing a nose clip and breathing through a low-dead-space, low-resistance mouthpiece and impeller turbine assembly (Jaeger Triple V). The inspired and expired gas volume and gas concentration signals were continuously sampled at 100 Hz, the latter using paramagnetic (O_2) and infrared (CO_2) analyzers (Jaeger Oxycon Pro, Hoechberg, Germany) via a capillary line connected to the mouthpiece. The gas analyzers were calibrated before each test with gases of known concentration, and the turbine volume transducer was calibrated with a 3-liter syringe (Hans Rudolph, Kansas City, MO). The volume and concentration signals were time aligned by accounting for the delay in the capillary gas transit and the analyzer rise time relative to the volume signal. Pulmonary gas exchange and $\dot{V}E$ were calculated and displayed breath by breath. Heart rate (HR) was measured during all tests using short-range radiotelemetry (Polar S610, Polar Electro Oy, Kempele, Finland). Subjects completed two 4-min bouts of low-intensity exercise and one bout of high-intensity exercise with 6 min passive recovery separating each exercise bout. On *day 4*, the high-intensity work rate was continued until task failure as a measure of exercise tolerance whereas on *day 5*, the high-intensity work rate was discontinued after 6 min. The time to task failure was recorded when the subjects were unable to sustain the required contraction frequency. The coefficient of variation for time to task failure in this mode of exercise in our laboratory is 5–7%. The $\dot{V}O_2$ responses to the four moderate and two severe exercise bouts were averaged before analysis to reduce breath-to-breath noise and enhance confidence in the parameters derived from the modeling process (50). EMG data were also recorded during testing on *days 4* and *5* using the setup as previously described.

On *day 6* of the nitrate and placebo supplementation periods, subjects repeated the testing protocol completed on *day 4*, but on this occasion, the step exercise tests were performed with the ergometer placed in the bore of a 1.5-T superconducting magnet (Intera, Philips, The Netherlands) at the Peninsula Magnetic Resonance Research Centre (Exeter, UK) while simultaneously undertaking ^{31}P-MRS for the determination of in vivo skeletal muscle energetics. The time to task failure was therefore calculated as the mean of the times recorded on *days 4* and *6*, for both experimental conditions. Before the exercise protocol commenced, absolute baseline concentrations of metabolites were established via a technique similar to that described by Kemp et al. (42) using a 6-cm ^{31}P transmit/receive surface coil. First, spatially localized spectroscopy was undertaken to determine the relative signal intensities obtained from a phosphoric acid source within the scanner bed and P_i from the subject's right quadriceps muscle, which was centered over the coil. A subsequent scan was obtained comparing the signals obtained from the phosphoric acid standard and an external P_i solution, where the localized voxel sampled within the external solution was of the same dimensions and distance from the coil as the muscle, allowing the calculation of muscle P_i concentration following corrections for relative coil loading. Absolute values of PCr and ATP concentrations were subsequently calculated via the ratio of P_i:PCr and P_i:ATP.

For the exercise protocol, the knee-extension rate was set in unison with the magnetic pulse sequence to ensure the quadriceps muscles were positioned in the same phase of contraction during each MR pulse acquisition. The subjects were visually cued via a display consisting of two vertical bars, one that moved at a constant frequency of 0.67 Hz and one that monitored foot movement via a sensor in the ergometer pulley system. Initially, fast-field echo images were acquired to determine whether the muscle was positioned correctly relative to the coil. This was aided by placing cod liver oil capsules, which yield high-intensity signal points within the image, adjacent to the coil, allowing its orientation relative to the muscle volume under examination to be assessed. A number of preacquisition steps were carried out to optimize the signal from the muscle under investigation. Matching and tuning of the coil was performed and an automatic shimming protocol was then undertaken within a volume that defined the quadriceps muscle. Before and during exercise, data were acquired every 1.5 s, with a spectral width of 1,500 Hz and 1 K data points. Phase cycling with four phase cycles was employed, leading to a spectrum being acquired every 6 s. The subsequent spectra were quantified via peak fitting, assuming prior knowledge, using the jMRUI (version 3) software package employing the AMARES fitting algorithm (*69*). Spectra were fitted assuming the presence of the following peaks: P_i, phosphodiester, PCr, α-ATP (2 peaks, amplitude ratio 1:1), γ-ATP (2 peaks, amplitude ratio 1:1), and β-ATP (3 peaks, amplitude ratio 1:2:1). In all cases, relative amplitudes recorded during exercise were corrected for partial saturation by obtaining a baseline spectrum before exercise with long repetition time (TR = 20 s) in which the relative unsaturated peak amplitudes could be determined. Intracellular pH was calculated using the chemical shift of the P_i spectral peak relative to the PCr peak (67). Resting and end-exercise values of [PCr], [P_i], and pH were calculated over the last 30 s of the rest or exercise period. ADP was calculated via knowledge of P_i, PCr, and pH values, as described by Kemp et al. (43) taking into account the dependency of rate constants on pH.

The total ATP turnover rate (ATP_{total}) was calculated as the sum of the total ATP turnover deriving from PCr hydrolysis, glycolysis, and oxidative phosphorylation (ATP_{PCr}, ATP_{Gly}, and ATP_{Ox}, respectively) using the methods of Lanza et al. (51) and Layec et al. (53). ATP_{PCr} was obtained from the rate of change in [PCr] from the modeling of [PCr] values acquired at each time point during the exercise protocol. ATP_{Gly} was determined by proton flux, assuming that the production of 1 mol of H^+ yields 1.5 mol of ATP. Proton flux calculations were based on determining the protons consumed by the creatine kinase (CK) reaction and buffering capacity and those produced via oxidative phosphorylation and cellular efflux. ATP_{Ox} was determined based on the hyperbolic relationship between ATP production rate and free cytosolic ADP concentration, requiring the calculation of the first-order PCr recovery rate constant determined from fitting PCr to a single-exponential function.

Before exercise on *days 4*, *5*, and *6*, resting BP was measured and a venous blood sample was collected for subsequent determination of plasma [nitrite]. BP of the brachial artery was measured with subjects in a rested, seated position before each exercise bout using an automated sphygmomanometer (Dinamap Pro, GE Medical Systems, Tampa, FL). Following 10 min of rest, four measurements were taken with the mean of the measurements being recorded. Venous blood samples were also drawn into lithium-heparin tubes before each exercise bout and centrifuged at 4,000 rpm and 4°C for 10 min, within 3 min of collection. Plasma was subsequently extracted and immediately frozen at −80°C, for later analysis of [nitrite] (NO_2^-) via chemiluminescence (8) as described previously (3).

Data Analysis Procedures

Oxygen uptake. The breath-by-breath $\dot{V}O_2$ data from each test on *days 4* and *5* were initially examined to exclude errant breaths caused by coughing, swallowing, sighing, etc., and those values lying more than four SDs from the local mean were removed. The breath-by-breath data were subsequently linearly interpolated to provide second-by-second values, and, for each individual, identical repetitions were time-aligned to the start of exercise and ensemble-averaged. The first 20 s of data after the onset of exercise (i.e., the phase I response) was deleted, and a nonlinear least-squares algorithm was used to fit the data thereafter. A single-exponential model was used to characterize the $\dot{V}O_2$ responses to moderate exercise and a biexponential model was used for severe exercise, as described in the following equations:

$$\dot{V}O_2(t) = \dot{V}O_{2\,\text{baseline}} + A_p(1 - e^{-(t-TD_p)/\tau_p}) \quad \text{(moderate)} \quad (1)$$

$$\dot{V}O_2(t) = \dot{V}O_{2\,\text{baseline}} + A_p(1 - e^{-(t-TD_p)/\tau_p}) + A_s(1 - e^{-(t-TD_s)/\tau_s}) \quad \text{(severe)} \quad (2)$$

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time t; $\dot{V}O_{2\text{baseline}}$ represents the mean $\dot{V}O_2$ in the baseline period; A_p, TD_p, and τ_p represent the amplitude, time delay, and time constant, respectively, describing the phase II increase in $\dot{V}O_2$ above baseline; and A_s, TD_s, and τ_s represent the amplitude of, time delay before the onset of, and time constant describing the development of the $\dot{V}O_2$ slow component, respectively.

An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. $\dot{V}O_{2\text{baseline}}$ was defined as the mean $\dot{V}O_2$ measured over the final 90 s of the resting baseline period. The end-exercise $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 30 s of exercise. Because the asymptotic value (A_s) of the exponential term describing the $\dot{V}O_2$ slow component may represent a higher value than is actually reached at the end of the exercise, the actual amplitude of the $\dot{V}O_2$ slow component at the end of exercise was defined as A_s'. The A_s' parameter was compared at the same iso-time (360 s) for both supplementation periods. To determine the overall kinetics of the $\dot{V}O_2$ response to both moderate- and severe-intensity exercise, the data were also fit with a monoexponential model from 0 s to end exercise without time delay.

Energy expenditure (EE) at rest and in the "steady state" was calculated from $\dot{V}O_2$ taking into account the energetic value of oxygen based on the respiratory exchange ratio (RER). Work efficiency (WE) was subsequently calculated by dividing the exercise work rate by the difference between the exercise EE and the baseline EE.

Phosphorous metabolites. To enhance the signal-to-noise properties and therefore the underlying features of the [PCr], [P_i], [ADP], and pH response profiles before kinetic parameter estimation, each subject's low-intensity exercise transitions were time-aligned to the onset of exercise ($t = 0$ s), averaged, and interpolated yielding a single second-by-second response. The [PCr] responses were subsequently modeled using nonlinear least-squares regression techniques. We used a procedure similar to that of Rossiter et al. (60) as described in the following equation:

$$\Delta PCr(t) = [PCr]ss(1 - e^{-bt}) \quad (3)$$

where (t) represents the absolute metabolite concentration at a given time, ss is the projected asymptotic value, and τ is the time constant of the response. The low-intensity data manifested a monoexponential time course and were thus fit from $t = 0$ through the entire 240 s of the response. Data analysis became more complex during the high-intensity exercise bouts owing to the existence of a delayed-onset, secondary component response. Therefore, the fitting window was constrained to an initial start point of 60 s and increased iteratively thereafter until there was a clear departure of the measured data from the model fit, as judged from visual inspection of a plot of the residuals. In this way, the best-fit exponential for the fundamental component of the response was established. The magnitude of the [PCr] slow component was then calculated as the difference between the asymptotic amplitude of the fundamental response and the mean value measured over the last 30 s of exercise for that condition. Metabolite concentrations (for PCr, ADP, P_i, and also pH) at end exercise (low intensity) and task failure (high intensity) were taken as the mean values measured over the final 30 s of exercise.

EMG. Raw EMG data were exported as an ASCII file and digitally filtered using a custom-designed filter developed through Labview 8.2 (National Instruments, Newbury, UK). Initially, the signals were filtered with a 20-Hz high-pass, second-order Butterworth filter to remove contamination from movement artifacts. The signal was then rectified and low-pass filtered at a frequency of 500 Hz to produce a linear envelope. The average iEMG was calculated at 1-s intervals during the MVCs while the average iEMG was calculated at 15-s intervals throughout the low- and high-intensity exercise bouts, with these values normalized to the highest 1-s average value attained in the three MVCs. Therefore, all iEMG data are presented as a percentage of the MVC iEMG response attained before supplementation. Data from repeat trials were averaged.

Statistics

Differences in BP and plasma [NO_2^-] were assessed using one-way repeated-measures ANOVA. Significant effects were further explored using simple contrasts with the α-level adjusted via a Fisher's LSD. Differences in the cardiorespiratory, muscle activity, and muscle metabolic responses between conditions were analyzed with two-tailed, paired-samples *t*-tests. Correlations were assessed via Pearson's product-moment correlation coefficient. Data are presented as means ± SE. Statistical significance was accepted when $P < 0.05$, while a tendency was noted when $P < 0.10$.

RESULTS

The NO_3^- supplementation regimen employed in this study was well tolerated with no deleterious side effects. Subjects did, however, report beeturia (red urine) and red stools, consistent with previous studies (3, 74).

Plasma [NO_2^-] and BP

The group mean plasma [NO_2^-] values obtained before exercise in control (CON) and on each of *days 4*, *5*, and *6* of the NO_3^- and PL supplementation periods are shown in Table 1. Plasma [NO_2^-] was elevated during the NO_3^- supplementation compared with CON and to PL at all sample points (Table 1). The mean plasma [NO_2^-] obtained over the three samples was 137% greater following NO_3^- compared with PL supplementation (Table 1). However, the elevations in plasma [NO_2^-] with NO_3^- supplementation were not different across *days 4–6*.

The mean blood pressure parameters measured at CON and at the three NO_3^- and PL sample points are shown in Table 1. NO_3^- supplementation significantly reduced the systolic BP on *day 6* and the mean arterial pressure on *day 4* relative to PL (Table 1). Over the three sample points, NO_3^- supplementation resulted in significant reductions in systolic BP (−5 mmHg), diastolic BP (−2 mmHg), and mean arterial pressure (−2 mmHg) relative to PL, with systolic BP and mean arterial pressure also being reduced below CON values (Table 1). Similar to plasma [NO_2^-], the reductions in systolic and dia-

Table 1. *Blood pressure and plasma [nitrite] before exercise in the control condition and following dietary supplementation with nitrate (beetroot juice) or placebo*

		Placebo				Nitrate			
	Control	*Day 4*	*Day 5*	*Day 6*	Mean	*Day 4*	*Day 5*	*Day 6*	Mean
Plasma nitrite, nM	219 ± 65	257 ± 76	231 ± 80	206 ± 93	231 ± 76	591 ± 41*†	406 ± 57*†	643 ± 110*†	547 ± 55*†
Systolic blood pressure, mmHg	125 ± 2	123 ± 2	124 ± 2	124 ± 2	124 ± 2	119 ± 2	120 ± 2	118 ± 2*†	119 ± 2*†
Diastolic blood pressure, mmHg	73 ± 4	70 ± 3	68 ± 2	67 ± 1	68 ± 2	66 ± 2	66 ± 2	66 ± 2	66 ± 2†
Mean arterial pressure, mmHg	91 ± 2	90 ± 2	89 ± 1	89 ± 1	89 ± 1	87 ± 1†	87 ± 1	87 ± 1	87 ± 1*†

Values are means ± SE. *Significantly different from control ($P < 0.05$). †Significantly different from placebo ($P < 0.05$).

stolic BP with NO_3^- supplementation were not significantly different across *days 4–6*.

Pulmonary $\dot{V}O_2$ Dynamics

Low-intensity exercise. There was no significant difference between the NO_3^- and PL conditions for muscle activity as assessed by iEMG (PL 17 ± 3 vs. BR 16 ± 2% MVC). End-exercise HR was not significantly different between conditions (PL 85 ± 4 vs. BR 86 ± 4 beats/min).

The group mean pulmonary $\dot{V}O_2$ responses during low-intensity exercise following both NO_3^- and PL supplementation are shown in Fig. 1*A*, and the response parameters are reported in Table 2. The increase in pulmonary $\dot{V}O_2$ from rest to low-intensity exercise was reduced by 25% following dietary NO_3^- supplementation (PL 484 ± 41 vs. BR 362 ± 30 ml/min; $P < 0.05$) and the end-exercise $\dot{V}O_2$ was also reduced (PL 870 ± 42 vs. BR 778 ± 38 ml/min; $P < 0.05$). However, neither the resting baseline $\dot{V}O_2$ nor the τ, which characterizes

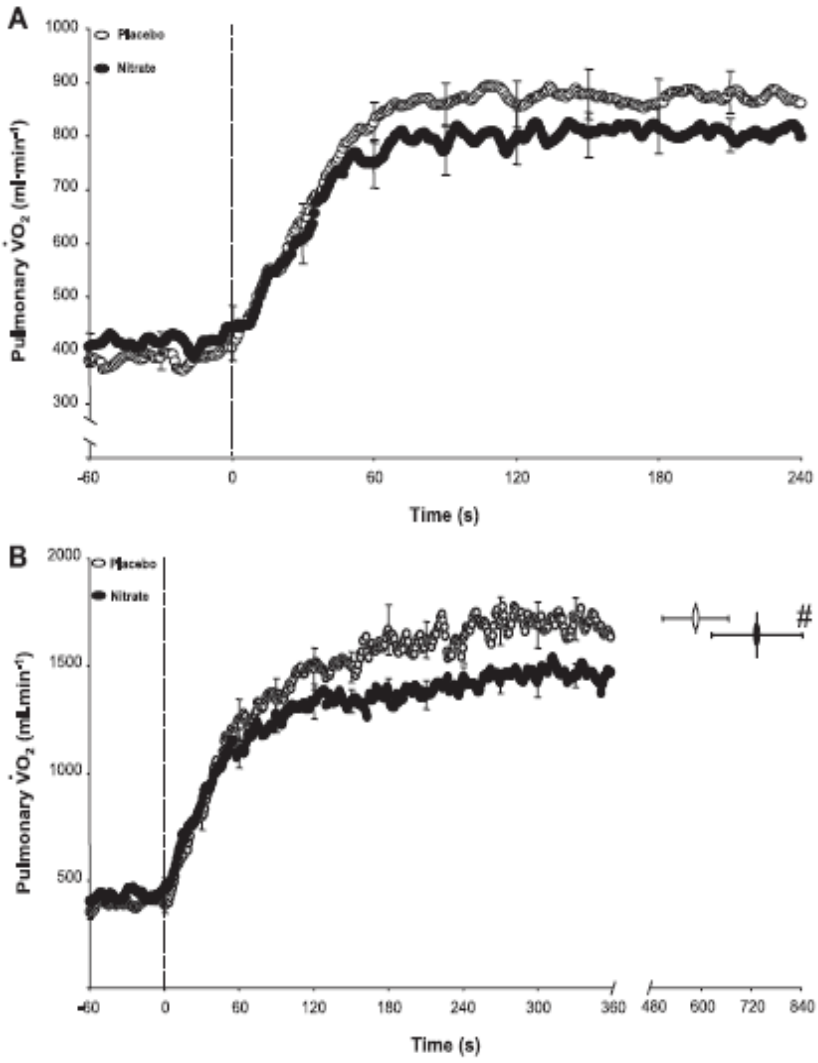

Fig. 1. Group mean ± SE pulmonary oxygen uptake ($\dot{V}O_2$) response during low-intensity (*A*) and high-intensity (*B*) exercise following dietary nitrate and placebo supplementation. The dashed vertical line represents the abrupt imposition of the work rate from a resting baseline. Note that the oxygen cost of both low-intensity and high-intensity exercise was substantially spared following nitrate supplementation (see Table 2) and that the time to task failure was extended in the latter. #Time to task failure significantly different from placebo ($P < 0.01$).

Table 2. *Pulmonary $\dot{V}o_2$ dynamics during low-intensity and high-intensity exercise following dietary supplementation with nitrate (beetroot juice) or placebo*

	Placebo	Nitrate
Low-intensity exercise		
$\dot{V}o_2$		
Baseline, ml/min	389 ± 16	429 ± 16
Primary amplitude, ml/min	484 ± 41	362 ± 30*
End exercise, ml/min	870 ± 42	778 ± 38*
Phase II time constant, s	22 ± 2	21 ± 5
Mean response time, s	33 ± 3	32 ± 2
High-intensity exercise		
$\dot{V}o_2$		
Baseline, ml/min	391 ± 8	426 ± 15
Primary amplitude, ml/min	1,116 ± 83	947 ± 53
At 360 s of exercise, ml/min	1,692 ± 70	1,460 ± 54*
At task failure, ml/min	1,726 ± 65	1,647 ± 100
Phase II time constant, s	37 ± 6	40 ± 8
Slow component amplitude, ml/min	209 ± 30	100 ± 26*
Mean response time, s	69 ± 8	58 ± 13

Values are means ± SE; $n = 6$ for values at task failure. $\dot{V}o_2$, oxygen uptake. *Significantly different from placebo ($P < 0.05$).

the rate of $\dot{V}o_2$ adjustment toward the required steady state, was significantly altered by dietary NO_3^- supplementation (Table 2). The end-exercise values of CO_2 production ($\dot{V}co_2$) (PL 726 ± 21 vs. BR 682 ± 25 ml/min), $\dot{V}E$ (PL 23 ± 2 vs. BR 22 ± 2 l/min), and RER (PL 0.84 ± 0.03 vs. BR 0.87 ± 0.02) were not significantly different between the conditions. WE was significantly greater following NO_3^- supplementation (PL 10.1 ± 0.6 vs. BR 13.0 ± 1.0%; $P < 0.05$).

High-intensity exercise. There was no significant difference between the NO_3^- and PL conditions for muscle activity as assessed by iEMG (PL 36 ± 3 vs. BR 33 ± 3% MVC). End-exercise HR was not significantly different between conditions (PL 117 ± 3 vs. BR 113 ± 3 beats/min). One subject exercised for 21–22 minutes during high-intensity exercise following NO_3^- supplementation, exceeding the 20 min available for data storage during this bout in the magnet. Therefore, the "end-exercise" data for this subject were excluded from analysis.

The group mean pulmonary $\dot{V}o_2$ responses during high-intensity exercise following both NO_3^- and PL supplementation are shown in Fig. 1*B*, and the parameters derived from the biexponential model fits are presented in Table 2. The primary component $\dot{V}o_2$ amplitude tended to be lower following dietary NO_3^- supplementation (PL 1,116 ± 83 vs. BR 947 ± 53 ml/min; $P = 0.08$; Fig. 1). The baseline $\dot{V}o_2$ and $\dot{V}o_2$ τ were not significantly different between the conditions (Table 2). The amplitude of the $\dot{V}o_2$ slow component was significantly reduced (by 50%) following NO_3^- supplementation (PL 209 ± 30 vs. BR 100 ± 26 ml/min; $P < 0.05$; Fig. 1), resulting in a significantly reduced $\dot{V}o_2$ at 360 s (PL 1,692 ± 70 vs. BR: 1,460 ± 54 ml/min; $P < 0.05$; Fig. 1), but there was no significant difference in $\dot{V}o_2$ at the limit of tolerance (Table 2). The end-exercise values of $\dot{V}co_2$ (PL 1,529 ± 28 vs. BR 1,452 ± 51 ml/min) and $\dot{V}E$ (PL 48 ± 3. vs. BR 44 ± 3 l/min) were not significantly different between the conditions. WE was significantly greater following NO_3^- supplementation (PL 6.3 ± 0.3 vs. BR 7.7 ± 0.3%; $P < 0.05$).

Muscle Metabolic Measurements In Vivo

Low-intensity exercise. The muscle metabolic effects of dietary NO_3^- supplementation during low-intensity exercise are reported in Table 3 and illustrated in Fig. 2. NO_3^- supplementation resulted in a 36% reduction in the amplitude of PCr degradation during low-intensity exercise (PL 8.1 ± 1.2 vs. BR 5.2 ± 0.8 mM; $P < 0.05$). This effect was similar in magnitude to the reduction in the $\dot{V}o_2$ amplitude with NO_3^- supplementation (Fig. 3). Consistent with the low-intensity $\dot{V}o_2$ response, the baseline [PCr] and [PCr] τ were not different following NO_3^- and PL supplementation (Table 3). A 21% reduction in the accumulation of [P_i] was observed with NO_3^- supplementation (PL 4.4 ± 0.8 vs. BR 3.5 ± 0.8 mM; $P < 0.05$; Fig. 2). The estimated [ADP] amplitude was significantly reduced following NO_3^- supplementation (PL 17.0 ± 2.8 vs. BR 10.3 ± 1.7 μM; $P < 0.05$; Fig. 2). Muscle pH at baseline and throughout exercise was not different between NO_3^- and PL supplementation (Table 3).

The estimated mean ATP_{total}, ATP_{Ox}, ATP_{PCr} and ATP_{Gly} for low-intensity exercise are shown in Fig. 4. The estimated total ATP turnover rate (ATP_{total}) at resting baseline was not significantly different between conditions (PL 75 ± 9 vs. BR 57 ± 4 μM/s). However, the ATP_{total} averaged over the entire 4-min exercise bout was significantly reduced by NO_3^- supplementation (PL 296 ± 58 vs. BR 192 ± 38 μM/s; $P < 0.05$). The oxidative ATP turnover rate (ATP_{Ox}) averaged over 4 min was also reduced with NO_3^- supplementation (PL 199 ± 37 vs. BR 120 ± 15 μM/s; $P < 0.05$). The amplitude of ATP_{Ox} from rest to steady-state exercise was lower following NO_3^- supplementation (PL 127 ± 35 vs. BR 65 ± 14 μM/s; $P < 0.05$), an effect which was consistent with the reduction in the amplitude of the pulmonary $\dot{V}o_2$ response (Fig. 1*A*). The mean ATP turnover rate from PCr hydrolysis (ATP_{PCr}) over the entire low-intensity bout was lower following NO_3^- supplementation

Table 3. *Muscle metabolic response during low-intensity exercise following dietary supplementation with nitrate (beetroot juice) and placebo*

	Placebo	Nitrate
[PCr]		
Baseline, mM	35.8 ± 1.5	34.9 ± 1.7
120 s, mM	27.9 ± 1.9	29.9 ± 2.4
240 s, mM	27.7 ± 2.0	29.7 ± 2.5
Time constant, s	24 ± 3	30 ± 4
Amplitude, mM	8.1 ± 1.2	5.2 ± 0.8*
[P_i]		
Baseline, mM	4.0 ± 0.4	4.0 ± 0.2
120 s, mM	8.2 ± 0.7	7.5 ± 0.7
240 s, mM	8.2 ± 0.7	7.3 ± 0.7
Amplitude, mM	4.4 ± 0.8	3.5 ± 0.8*
[ADP]		
Baseline, μM	6.1 ± 0.7	6.1 ± 0.7
120 s, μM	23.4 ± 2.5	16.3 ± 1.4*
240 s, μM	22.4 ± 2.2	15.8 ± 1.3*
Amplitude, μM	17.0 ± 2.8	10.3 ± 1.7*
pH		
Baseline	7.05 ± 0.01	7.03 ± 0.01
120 s	7.06 ± 0.02	7.05 ± 0.01
240 s	7.04 ± 0.02	7.03 ± 0.01
ΔBaseline − 240 s	−0.01 ± 0.02	0.00 ± 0.01

Values are mean ± SE. PCr, phosphocreatine. *Significantly different from placebo ($P < 0.05$).

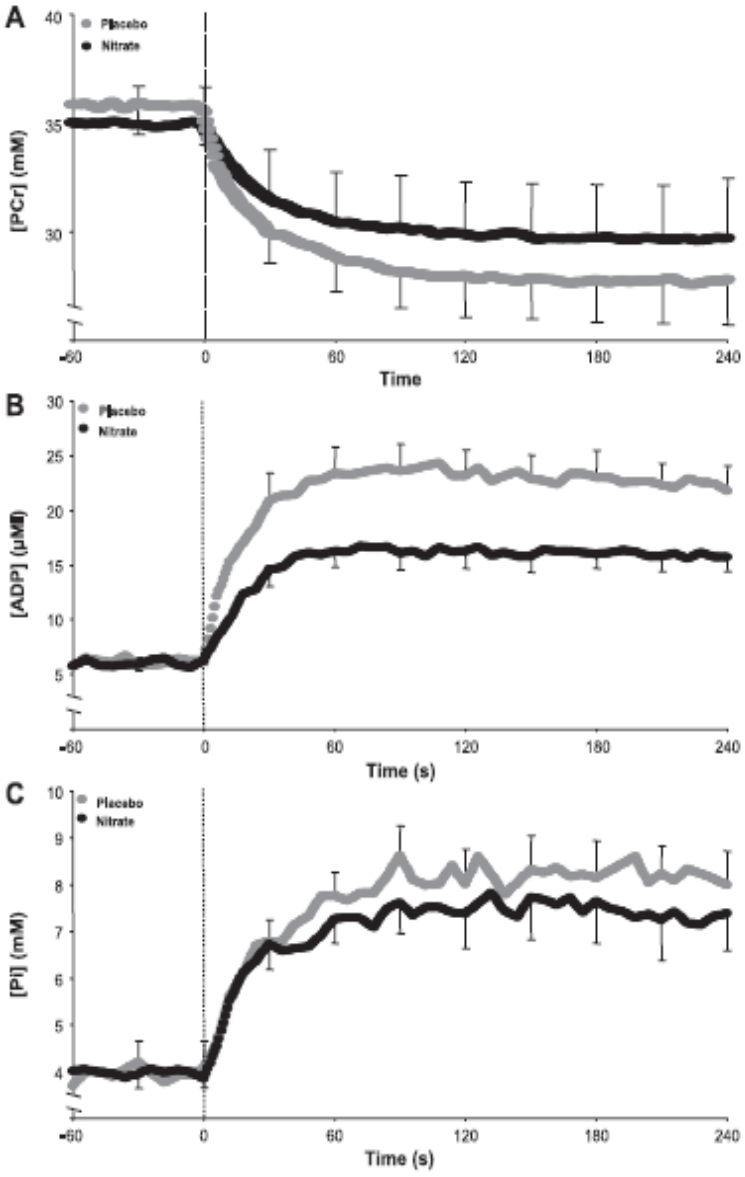

Fig. 2. Group mean ± SE muscle metabolic responses to low-intensity exercise following dietary nitrate and placebo supplementation. The change in muscle phosphocreatine concentration ([PCr]) (*A*), [ADP] (*B*), and [P_i] (*C*) from rest to the steady state was significantly reduced following nitrate supplementation (see Table 3). The dashed vertical line represents the abrupt imposition of the work rate from a resting baseline.

(PL 34 ± 5 vs. BR 22 ± 3 μM/s; $P < 0.05$), but the mean glycolytic rate (ATP_{Gly}) was not different between conditions (Fig. 4). The relative contribution of ATP_{Ox} (PL 70 ± 3 vs. BR 69 ± 6%), ATP_{PCr} (PL 13 ± 2 vs. BR 13 ± 2%), and ATP_{Gly} (PL 17 ± 4 vs. BR 18 ± 6%) to ATP_{total} over the 4-min bout of low-intensity exercise was not significantly different between conditions.

High-intensity exercise. The muscle metabolic effects of dietary NO_3^- supplementation during high-intensity exercise are reported in Table 4 and illustrated in Fig. 5. The primary [PCr] amplitude, [PCr] baseline, and τ values were not different between the NO_3^- and PL conditions. However, the [PCr] slow component amplitude was significantly reduced (by 59%) following NO_3^- supplementation (PL 3.9 ± 1.1 vs. BR 1.6 ± 0.7 mM; $P < 0.05$; Fig. 5*A*), consistent with the reduced $\dot{V}O_2$ slow component (Fig. 3). The relative magnitude of the changes in [PCr] with NO_3^- supplementation was not different from the relative magnitude of the changes in $\dot{V}O_2$ either in the fundamental or slow phases of the response. There were no significant differences in any of the dynamic parameters of [P_i] and [ADP] between conditions (Table 4). Muscle pH was not significantly different between the NO_3^- and PL conditions either at baseline or during exercise (Table 4).

The estimated mean ATP_{total}, ATP_{Ox}, ATP_{PCr}, and ATP_{Gly} for high-intensity exercise are shown in Fig. 6. The estimated ATP_{total} averaged over the entire exercise bout was significantly reduced by NO_3^- supplementation (PL 607 ± 65 vs. BR 436 ± 43 μM/s; $P < 0.05$). The mean ATP_{Ox} (PL 468 ± 49 vs. BR 329 ± 18 μM/s; $P < 0.05$) and ATP_{PCr} (PL 44 ± 8 vs. BR 25 ± 7 μM/s; $P < 0.05$) were also lower following NO_3^- supplementation. The mean ATP_{Gly} was not significantly affected (PL 95 ± 21 vs. BR 82 ± 35 μM/s). The amplitude of change in ATP_{total} from resting baseline to end exercise (averaged over the final 30 s) was lower following NO_3^- supple-

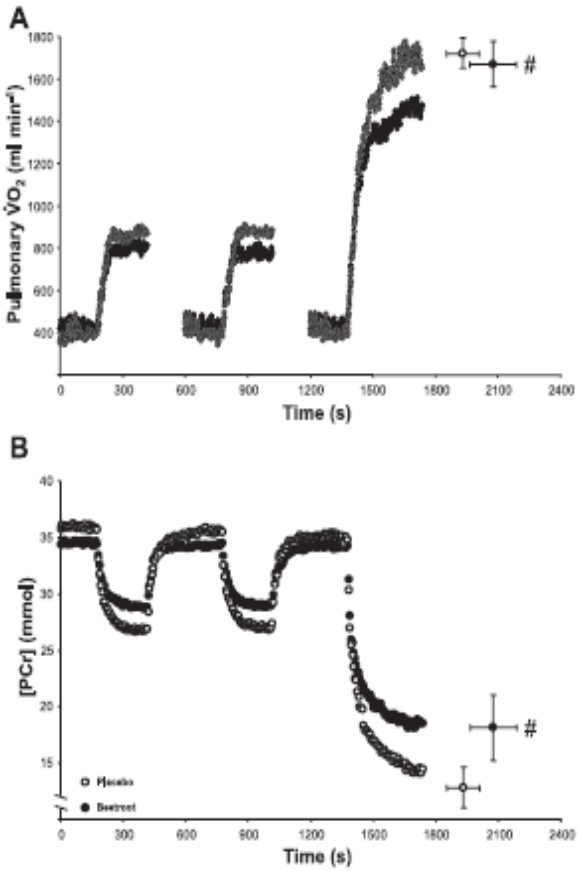

Fig. 3. Pulmonary $\dot{V}o_2$ (*A*) and intramuscular [PCr] (*B*) responses to the experimental protocol which involved 2 bouts of low-intensity exercise and 1 bout of high-intensity exercise. Note the proportionately similar sparing in $\dot{V}o_2$ and [PCr] across the 3 exercise bouts. Models of respiratory control predict that a reduced decrement in [PCr] (presumably as a consequence of a reduced muscle ATP turnover requirement for the same work rate) would result in a reduced stimulation of oxidative phosphorylation. See text for further details. #Time to task failure significantly different from placebo ($P < 0.01$).

mentation (PL 491 ± 71 vs. BR 334 ± 39 μM/s; $P < 0.05$), an effect that was also evident in the ATP_{Ox} response (PL 414 ± 47 vs. BR 275 ± 20 μM/s; $P < 0.05$). The relative contribution of ATP_{Ox} (PL 77 ± 3 vs. BR 78 ± 5%) and ATP_{Gly} (PL 15 ± 2 vs. BR 16 ± 5%) to ATP_{total} over the 6-min bout of high-intensity exercise was not significantly different between conditions but the relative contribution from ATP_{PCr} was smaller following NO_3^- supplementation (PL 8 ± 2 vs. BR 6 ± 2%; $P < 0.05$).

Exercise Tolerance

Exercise tolerance was enhanced following NO_3^- supplementation as demonstrated by the 25% increased time to task failure (PL 586 ± 80 vs. BR 734 ± 109 s; $P < 0.01$). All seven subjects had a longer time to task failure in the BR condition. Following NO_3^- supplementation, the time to task failure was correlated with the average plasma [NO_2^-] over *days 4–6*, although this relationship did not attain statistical significance ($r = 0.73$, $P = 0.06$). The time to task failure with NO_3^- supplementation tended to be related to the [PCr]

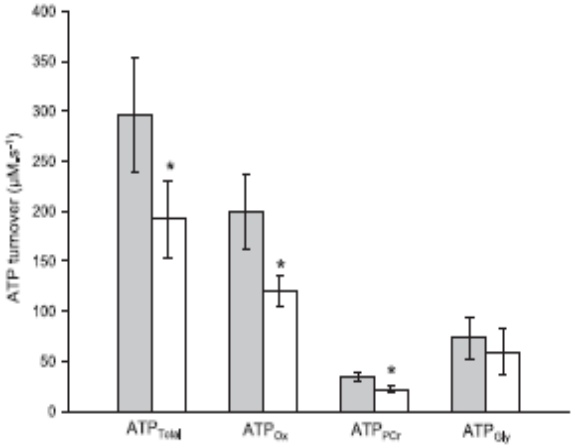

Fig. 4. Group mean ± SE ATP resynthesis rate averaged over the entire exercise bout during low-intensity knee-extension exercise following dietary nitrate (white bars) and placebo (gray bars). Total ATP turnover (ATP_{Total}), ATP derived from oxidative phosphorylation (ATP_{Ox}), ATP derived from PCr splitting (ATP_{PCr}), and ATP derived from glycolysis (ATP_{Gly}) are shown. The reductions in ATP_{Total}, ATP_{Ox}, and ATP_{PCr} were statistically significant. See text for further details. *Significantly different from placebo ($P < 0.05$).

remaining at 120 s ($r = 0.72$, $P = 0.07$) and 360 s ($r = 0.71$, $P = 0.07$) of exercise. The intramuscular [PCr] and pulmonary $\dot{V}o_2$ at task failure were not significantly different between the placebo and NO_3^--supplemented conditions ($n = 6$).

Table 4. *Muscle metabolic response during high-intensity exercise following dietary supplementation with nitrate (beetroot juice) and placebo*

	Placebo	Nitrate
[PCr]		
Baseline, mM	35.2 ± 1.5	35.0 ± 1.8
120 s, mM	18.0 ± 2.2	21.6 ± 2.4
360 s, mM	15.3 ± 2.2	20.0 ± 3.0
At task failure, mM	12.8 ± 1.8	18.1 ± 2.9
Time constant, s	22 ± 2	26 ± 5
Primary amplitude, mM	15.8 ± 2.0	13.3 ± 0.1
SC amplitude, mM	3.9 ± 1.1	1.6 ± 0.7*
[P_i]		
Baseline, mM	3.7 ± 0.5	3.9 ± 0.3
120 s, mM	14.9 ± 1.5	12.1 ± 1.7
360 s, mM	16.3 ± 2.2	13.7 ± 2.7
At task failure, mM	17.1 ± 2.4	13.7 ± 2.7
Primary amplitude, mM	11.1 ± 1.5	8.5 ± 2.0
SC amplitude, mM	1.7 ± 0.7	1.7 ± 1.0
[ADP]		
Baseline, μM	7.1 ± 0.6	6.2 ± 0.5
120 s, μM	74.7 ± 18.4	42.9 ± 5.9
360 s, μM	97.7 ± 31.8	42.6 ± 28.5
At task failure, μM	100.2 ± 31.7	46.2 ± 6.3
Primary amplitude, μM	96.0 ± 41.6	36.7 ± 6.6
SC amplitude, μM	2.8 ± 2.8	1.2 ± 1.2
pH		
Baseline	7.04 ± 0.01	7.01 ± 0.01
120 s	7.04 ± 0.03	7.02 ± 0.03
360 s	6.95 ± 0.05	6.92 ± 0.04
At task failure	6.84 ± 0.08	6.85 ± 0.08
ΔBaseline − 360 s	−0.09 ± 0.05	−0.09 ± 0.04
Δ 360 s − Tlim	−0.11 ± 0.07	−0.07 ± 0.05

Values are mean ± SE; $n = 6$ for values at task failure. SC, slow component. Tlim = time to task failure. *Significantly different from placebo ($P < 0.05$).

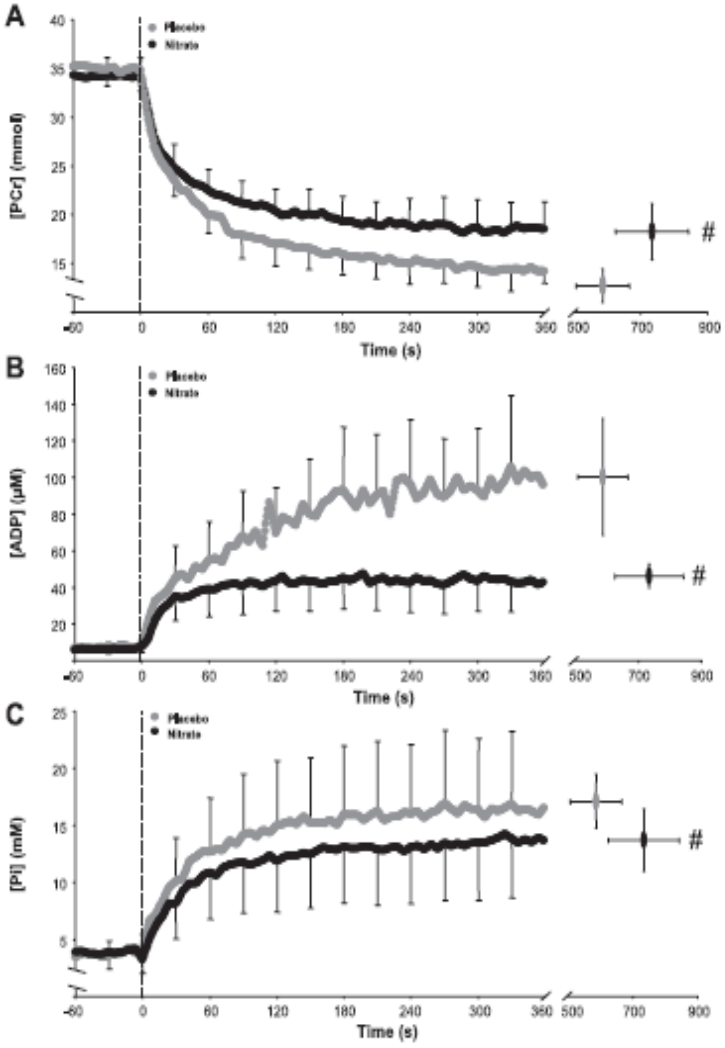

Fig. 5. Group mean ± SE muscle metabolic responses to high-intensity exercise following dietary nitrate and placebo supplementation. The change in muscle [PCr] (*A*), [ADP] (*B*), and [P_i] (*C*) are illustrated. The dashed vertical line represents the abrupt imposition of the work rate from a resting baseline. #Time to task failure significantly different from placebo ($P < 0.01$).

DISCUSSION

The major novel finding of this study was that dietary supplementation with nitrate-rich BR (which more than doubled plasma [NO_2^-]) reduced both the O_2 cost and the degree of PCr degradation during both low- and high-intensity exercise, without affecting muscle pH. Moreover, NO_3^- supplementation significantly reduced the estimated ATP_{total} during both low- and high-intensity exercise. These findings are consistent with our experimental hypotheses and suggest that NO_3^- supplementation predominantly reduces the O_2 cost of exercise (improves exercise efficiency) through reducing the total ATP cost of muscle force production rather than by increasing the mitochondrial P/O ratio. Moreover, dietary NO_3^- supplementation appears to improve the tolerance of high-intensity exercise by reducing muscle metabolic perturbation as reflected, for example, in the extent to which the finite muscle PCr reserve is depleted over time. These findings are important as they provide the first step in elucidating the intramuscular mechanism(s) by which dietary NO_3^- supplementation improves exercise efficiency and exercise tolerance in young healthy humans.

Effects of Dietary Nitrate on Plasma NO_2^- and BP

Dietary NO_3^- supplementation elevated plasma [NO_2^-] on *days 4*, *5*, and *6* of the supplementation regime, with a mean 137% increase in the mean plasma [NO_2^-] over the 3 days. This finding corroborates previous observations that NO_3^- administration substantially elevates plasma [NO_2^-] in humans (3, 52, 74). The differences in absolute plasma [NO_2^-] that have been reported in this and in previous studies likely reflect the relative complexity and technical challenges associated with its measurement. It is clear that NO_2^- can be converted to NO under appropriate physiological conditions (9, 56) and therefore the elevated plasma [NO_2^-] would be expected to increase NO bioavailability during exercise. An elevation in extracellular NO activates guanylate cyclase, which synthesizes cyclic guanosine monophosphate (cGMP) from guanosine triphosphate, leading to smooth muscle relaxation (32). As such, reductions in both systolic and diastolic BP have been noted following NO_3^- administration (52, 74). In our previous study, we observed a reduction in systolic but not diastolic BP following BR ingestion (3). In the present study, we observed

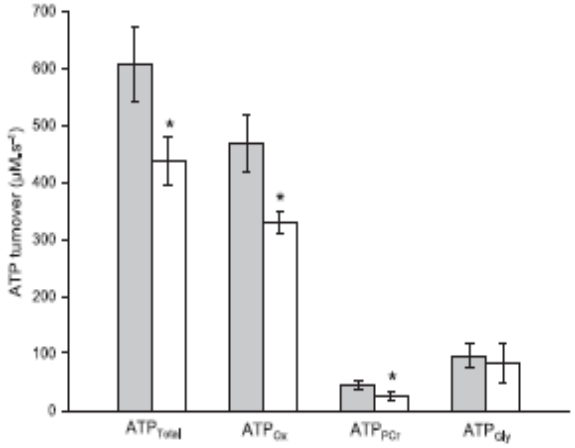

Fig. 6. Group mean ± SE ATP resynthesis rate averaged over the entire exercise bout during high-intensity knee-extension exercise following dietary nitrate (white bars) and placebo (gray bars) supplementation. Total ATP turnover (ATP_{Total}), ATP derived from oxidative phosphorylation (ATP_{Ox}), ATP derived from PCr splitting (ATP_{PCr}), and ATP derived from glycolysis (ATP_{Gly}) are shown. The reductions in ATP_{Total}, ATP_{Ox}, and ATP_{PCr} were statistically significant. See text for further details. *Significantly different from placebo ($P < 0.05$).

reductions in the mean systolic BP (−5 mmHg) and diastolic BP (−2 mmHg) as well as mean arterial pressure (−2 mmHg) over *days 4–6*, supporting the notion that both systolic and diastolic BP can be reduced following NO_3^- administration (52, 74). These data, along with evidence for a beneficial role of NO_2^- in hypoxic vasodilation (27) and in the protection of tissues from ischemia-reperfusion injury (28, 73), suggest that a diet rich in vegetables containing a high nitrate content might confer benefits to cardiovascular health (26, 36).

Effects of Dietary Nitrate on Pulmonary Gas Exchange

During low-intensity exercise, the pulmonary $\dot{V}O_2$ amplitude was reduced by 25% during prone knee extension exercise following NO_3^- supplementation, consistent with our previous study during upright cycling (3). In our previous study, this reduction in the pulmonary $\dot{V}O_2$ amplitude was accompanied by an increased blood volume in the thigh (consistent with vasodilatation and increased muscle O_2 availability) and a reduction in muscle fractional O_2 extraction, as estimated using near-infrared spectroscopy (3). A reduction in the O_2 cost of low-intensity submaximal exercise has also been observed following pharmacological NO_3^- administration (52). Conversely, inhibition of nitric oxide synthase (NOS) has been reported to increase $\dot{V}O_2$ in dogs at rest (62). Neither of the two previous human studies assessed the influence of dietary NO_3^- on resting $\dot{V}O_2$, and thus it was unclear whether a possible reduction in resting $\dot{V}O_2$ contributed to the reduced $\dot{V}O_2$ during submaximal exercise. An important finding in the present study was that $\dot{V}O_2$ and the calculated ATP_{Ox} at rest were unaffected by NO_3^- ingestion; thus the reduced O_2 cost appears to be manifest only during skeletal muscle contraction. If NO_3^- supplementation had improved mitochondrial respiratory efficiency, a reduction in resting $\dot{V}O_2$ might have been expected given that there is considerable mitochondrial activity both in skeletal and nonskeletal muscle tissues during resting conditions.

Consistent with our previous results (3), neither $\dot{V}E$ nor HR was significantly altered by dietary NO_3^- supplementation. This suggests that the reduction in $\dot{V}O_2$ is not a consequence of a reduction of the energy cost of cardiorespiratory processes but is specific to the contracting skeletal muscles. Another intriguing observation from the present study was that the 5–6% reduction in "steady-state" $\dot{V}CO_2$ following nitrate supplementation was not statistically significant and was less than the reduction in steady-state $\dot{V}O_2$, leading to a small but nonsignificant increase in RER. A slight shift in substrate utilization toward a relatively greater use of carbohydrate, perhaps as a consequence of a NO-mediated increase in myocyte glucose uptake (64), might provide a partial explanation for this effect. Additional muscle metabolic studies are required to resolve this issue.

During high-intensity knee-extension exercise, there was a nonsignificant tendency for the primary $\dot{V}O_2$ amplitude to be reduced following NO_3^- supplementation (15%); in contrast, we previously observed an increased primary $\dot{V}O_2$ amplitude during severe-intensity cycling following BR ingestion (3). The reason for this disparity is unclear. However, it may be related to the intensity at which the subjects were exercising. Larsen et al. (52) previously reported that NO_3^- administration was only effective in reducing $\dot{V}O_2$ at intensities up to 80% of the $\dot{V}O_{2\,max}$. Therefore, it is possible that NO_3^- supplementation is effective in reducing the primary $\dot{V}O_2$ amplitude only during exercise that is below the so-called critical power (38). In contrast to our previous study, in which we observed a slower phase II $\dot{V}O_2$ time constant (τ) following NO_3^- supplementation (3), the $\dot{V}O_2$ τ was unchanged in the present study. Differences in exercise modality and body position may explain the differences in the effects on $\dot{V}O_2$ kinetics between the studies.

Following dietary NO_3^- supplementation, the amplitude of the $\dot{V}O_2$ slow component was significantly reduced (by 52%) but the $\dot{V}O_2$ attained at the termination of exercise was not different, results that are consistent with our previous study (3). Interestingly, inhibition of NOS has been shown to have the opposite effect, i.e., to increase the amplitude of the $\dot{V}O_2$ slow component during high-intensity exercise in humans (41). In animal models, NOS inhibition has been reported to reduce mechanical efficiency and impair exercise performance in rats (49) but to reduce the fatigue index during high-intensity contractions in an isolated canine muscle model (30). Although the results are inconsistent and controversial (23), it is clear that NO plays a potentially important role in regulating muscle force production and the dynamic $\dot{V}O_2$ response to muscular exercise (23, 59).

Effects of Dietary Nitrate on Muscle Energetics

During low-intensity exercise, we observed a reduction in the steady-state amplitude of muscle [PCr] degradation and [P_i] and [ADP] accumulation following NO_3^- supplementation, while muscle pH was not significantly altered. It was also calculated that muscle ATP_{total} was significantly reduced with NO_3^- supplementation as a consequence of a reduced ATP_{Ox} and ATP_{PCr}. It should be noted that these estimates of muscle ATP turnover rates involve numerous assumptions and may be subject to considerable error. However, the ^{31}P-MRS data, in conjunction with a reduction in the $\dot{V}O_2$ steady state, suggest that the liberation of energy from the CK reaction and oxida-

tive metabolism was reduced, with the rate of anaerobic glycolysis, which is not expected to make a significant energetic contribution during low-intensity exercise, being essentially unchanged. Collectively, these data suggest that the reduced O_2 cost of exercise following NO_3^- supplementation is consequent to an improved coupling between ATP hydrolysis and skeletal muscle force production rather than an increased mitochondrial P/O ratio. This is highlighted in Fig. 7, which indicates that the slope of the relationship between "steady-state" $\dot{V}O_2$ and muscle [PCr] is similar in the placebo and NO_3^--supplemented conditions, i.e., the changes in $\dot{V}O_2$ and [PCr] are broadly proportional following NO_3^- supplementation. If NO_3^- supplementation had reduced the O_2 cost of exercise exclusively through a specific reduction in the mitochondrial O_2 cost of ATP synthesis, muscle [PCr] degradation and ADP accumulation would not have been expected to change. It appears, instead, that NO_3^- supplementation reduced ATP hydrolysis for the same work rate, which, in turn, reduced PCr degradation and ADP and P_i accumulation. Based on established models of respiratory control, these changes would reduce the stimulus/stimuli for oxidative phosphorylation (12, 13, 17, 57), consistent with the reduced steady-state $\dot{V}O_2$ we have observed (Fig. 3).

During high-intensity exercise, the effect of NO_3^- supplementation on the [PCr] response was analogous to the changes observed in $\dot{V}O_2$. Specifically, there was a clear trend for a reduction in the primary [PCr] amplitude (16%) with no change in the kinetic parameters, and an appreciable reduction in the [PCr] slow component amplitude (59%). Similar to low-intensity exercise, NO_3^- supplementation did not affect pH dynamics during high-intensity exercise. The calculated muscle ATP_{total} was significantly reduced with NO_3^- supplementation as a consequence of a reduced ATP_{Ox} and ATP_{PCr}. Despite there being a clear trend for NO_3^- supplementation to reduce [ADP] and [P_i] accumulation during high-intensity exercise, these changes did not attain statistical significance.

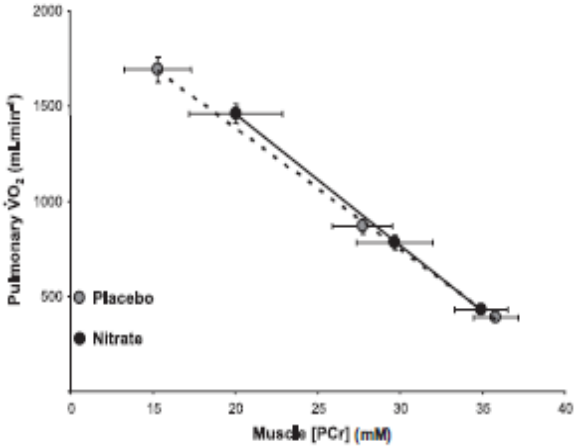

Fig. 7. Group mean ± SE muscle [PCr] plotted against pulmonary $\dot{V}O_2$ at rest, after 4 min of low-intensity exercise and after 6 min of high-intensity exercise following dietary nitrate and placebo supplementation. Note that the lines of best fit for these responses are similar, suggesting reciprocal changes in [PCr] and $\dot{V}O_2$ following dietary nitrate supplementation. This suggests that the improved exercise efficiency following nitrate supplementation is a consequence of a reduced ATP cost of muscle contraction rather than to changes in the mitochondrial P/O ratio.

Overall, the changes in the pulmonary $\dot{V}O_2$ and muscle metabolic responses with NO_3^- supplementation were similar during low-intensity and high-intensity exercise, which suggests that a reduction in the total ATP cost of force production was primarily responsible for the observed effects at both intensities (Fig. 7). During intense constant-work-rate exercise, the rate of ATP turnover within the recruited myocytes is not constant but increases as exercise proceeds such that there is a progressive loss of efficiency (5, 61). NO_3^- supplementation appears to have ameliorated this effect, as evidenced by the commensurate reductions in the $\dot{V}O_2$ and [PCr] slow component amplitudes.

Effects of Dietary Nitrate on Exercise Tolerance

Dietary NO_3^- supplementation resulted in a 25% improvement in the time to task failure during high-intensity exercise. This confirms our previous report in which NO_3^- supplementation resulted in a 17% increase in the time to task failure during high-intensity cycle exercise (3). In the present study, the time to task failure tended to be correlated with the mean plasma [NO_2^-] measured over *days 4–6* of NO_3^- supplementation ($r = 0.73$; $P = 0.06$). The reduced [PCr] slow component amplitude resulted in a sparing of [PCr] over the first 360 s of exercise, and this also tended to be related to the time to task failure ($r = 0.71$; $P = 0.07$). Therefore, dietary NO_3^- supplementation, by reducing the ATP cost of force production, facilitated a sparing of the finite PCr stores and a reduction in the O_2 cost of exercise (particularly in the slow phase), culminating in an improved tolerance of intense exercise. It has been shown that at the termination of high-intensity constant-work-rate exercise, intramuscular [PCr] and pH reach consistently low values, and [P_i] reaches a consistently high value, that may limit continued muscle function (70). Interventions that reduce the $\dot{V}O_2$ or [PCr] slow component amplitude have previously been associated with improved exercise performance (2, 4, 78; see 37 for review). While the causes of skeletal muscle fatigue during intense exercise are debated (75), the reduced muscle metabolic perturbation (i.e., reduced fall in [PCr] and reduced accumulation of [ADP] and [P_i]) following NO_3^- supplementation appears to have enabled high-intensity exercise to be sustained for longer before these metabolites reached "critical" values.

Possible Mechanisms for the Reduced ATP Cost of Force Production with Dietary Nitrate

The results of this study suggest that the improvements in exercise efficiency and exercise tolerance associated with NO_3^- supplementation are a result of a reduced rate of muscle ATP turnover for a given work rate. The pertinent question is therefore: by which mechanism(s) does NO_3^- supplementation reduce the ATP cost of muscle force production? The ATP turnover rate in the contracting skeletal myocytes is predominantly determined by two ATPase pathways: actomyosin-ATPase, which facilitates the interaction between actin and myosin, and Ca^{2+}-ATPase, which is responsible for sarcoplasmic reticulum (SR) Ca^{2+} pumping; membrane depolarization (Na^+-K^+-ATPase) is responsible for a further small (~7%) fraction of the total ATP turnover (6). While the relative energetic requirements of actomyosin-ATPase and Ca^{2+}-ATPase during contraction is equivocal (6, 72, 80), it is clear

that the O_2 cost of contraction is reduced by interventions that inhibit both the actomyosin-ATPase (46, 72) and the Ca^{2+}-ATPase (65, 66). There is evidence that NO reduces Ca^{2+} cycling (33, 71) and slows cross-bridge cycling kinetics (25, 34), indicating that NO may modulate the ATP cost of force production. Our observation of a reduced O_2 cost of exercise following dietary NO_3^- supplementation might therefore be related to a reduction in the ATP cost of cross-bridge cycling and/or Ca^{2+} handling. However, whereas NO reduces muscle force development in in vitro preparations (25, 34), in the present study it enabled the same submaximal power output to be sustained for longer.

We are not able to exclude the possibility that NO_3^- supplementation also increased the mitochondrial P/O ratio during exercise and/or that some other factor associated with BR ingestion caused a reduced PCr breakdown. For example, greater homogeneity of perfusion relative to metabolic rate, perhaps as a consequence of an extension of the tissue O_2 gradient due to NO-mediated inhibition of mitochondria closest to the capillary (14, 68), might reduce muscle metabolic perturbation. NO-mediated mitochondrial biogenesis (58) might also reduce muscle metabolic perturbation for the same work rate (35, 39). However, these changes would not be expected to reduce the steady-state $\dot{V}O_2$ for a given submaximal work rate. CK, the enzyme which catalyzes [PCr] splitting, is a dimer containing several sulfhydryl groups (24). Administration of NO donors results in reversible inhibition of CK via *S*-nitrosylation (1), which might also explain our observed reductions in [PCr] degradation. However, the proportionally similar reductions in $\dot{V}O_2$ and [PCr] suggests that the likely effect of NO_3^- supplementation is a reduced ATP cost of force production during skeletal muscle contraction.

The results of this study have a number of potentially important implications. For example, it is possible that the protective effect of NO_2^- on infarct size that has been reported in experimental models of myocardial ischemia (20, 63, 73) might be due to an NO-mediated reduction in the energy (and O_2) cost of contraction rather than, or perhaps in addition to, enhanced perfusion of ischemic areas. Moreover, it has recently been reported that Tibetans residing at 4,200-m altitude have >10-fold-higher circulating concentrations of bioactive NO products including NO_2^- compared with low-altitude dwellers (24). While enhanced NO bioavailability might facilitate blood flow and tissue O_2 delivery in hypobaric hypoxia through effects on peripheral vasodilation (24), it is also possible that the physiological adaptation to high altitude involves an NO-mediated reduction in the energy (and O_2) cost of physical activity. This would be consistent with reports that altitude training can improve submaximal cycling efficiency (29).

We have attributed the various physiological changes observed in the present study to increased dietary nitrate consumption consequent to beetroot juice consumption. This interpretation is based on the similarity of our results (increased plasma [nitrite] and improved exercise efficiency) to those observed when the diet is supplemented with sodium nitrate (52). However, we wish to stress that beetroot juice is also rich in antioxidants and phenols (48), and it is possible that these compounds contributed either independently, or synergistically with nitrate, to the results obtained. Additional studies are required to identify the extent to which other metabolically active compounds in beetroot juice influence BP and the physiological responses to exercise.

Conclusions

Dietary nitrate supplementation effectively doubled plasma [nitrite] and reduced both systolic and diastolic BP, effects that are consistent with an elevated NO bioavailability. The $\dot{V}O_2$ required for the same work rate was reduced during both low-intensity and high-intensity knee-extension exercise following NO_3^- supplementation. On average, during low-intensity exercise, the amplitude of the increase in $\dot{V}O_2$ above the resting baseline was reduced by 25% and the absolute $\dot{V}O_2$ (including the resting baseline) was reduced by 11%. Similarly, following 6 min of high-intensity exercise, the $\dot{V}O_2$ amplitude was reduced by 21% and the absolute $\dot{V}O_2$ was reduced by 14%, although the $\dot{V}O_2$ at the termination of exercise was not different between conditions. At both exercise intensities, the reduction in $\dot{V}O_2$ was accompanied by a reduction in muscle [PCr] of similar magnitude, while pH was unchanged. These in vivo data suggest that the reduction in $\dot{V}O_2$ following NO_3^- supplementation is principally a result of a reduced rate of ATP turnover in the contracting myocytes, i.e., a blunting of the changes in high-energy phosphates would reduce the stimuli to oxidative phosphorylation. The reduction in muscle metabolic perturbation with NO_3^- supplementation, including a sparing of the rate of depletion of the finite PCr reserve, was associated with an improved tolerance of high-intensity exercise.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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Influence of N-acetylcysteine administration on pulmonary O_2 uptake kinetics and exercise tolerance in humans

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ABSTRACT

We investigated the influence of the antioxidant N-acetylcysteine (NAC) on plasma nitrite concentration ($[NO_2^-]$), pulmonary oxygen uptake ($\dot{V}_{O_2}$) kinetics and exercise tolerance. Eight males completed 'step' moderate- and severe-intensity cycle exercise tests following infusion of either NAC (125 mg kg^{-1} h^{-1} for 15 min followed by 25 mg kg^{-1} h^{-1} until the termination of exercise) or Placebo (PLA; saline). Following the initial loading phase, NAC infusion elevated plasma free sulfhydryl groups compared to placebo (PLA: 4 ± 2 vs. NAC: 13 ± 3 μM g^{-1}; $P < 0.05$) and this elevation was preserved throughout the protocol. The administration of NAC did not significantly influence plasma $[NO_2^-]$ or $\dot{V}_{O_2}$ kinetics during either moderate- or severe-intensity exercise. Although NAC did not significantly alter severe-intensity exercise tolerance at the group mean level (PLA: 776 ± 181 vs. NAC: 878 ± 284 s; $P > 0.05$), there was appreciable inter-subject variability in the response: four subjects had small reductions in exercise tolerance with NAC compared to PLA (−4%, −8%, −11%, and −14%) while the other four showed substantial improvements (+24%, +24%, +40%, and +69%). The results suggest that exercise-induced redox perturbations may contribute to fatigue development in recreationally-active adults.

1. Introduction

The initiation of exercise provokes an immediate increase in the ATP turnover rate, but an exponential increase in oxygen consumption within the contracting myocytes. This initial discrepancy between the rates of muscle ATP utilization and ATP supply through oxidative phosphorylation mandates a compensatory energy liberation from both phosphocreatine (PCr) degradation and anaerobic glycolysis (Krogh and Lindhard, 1920; Poole et al., 2008). While pulmonary oxygen uptake ($\dot{V}_{O_2}$), which provides a close approximation of muscle $\dot{V}_{O_2}$ (Grassi et al., 1996; Krustrup et al., 2009), attains a 'steady-state' within 2–3 min following the onset of moderate-intensity exercise performed below the gas exchange threshold (GET; Whipp and Wasserman, 1972; Whipp et al., 1982), a supplementary $\dot{V}_{O_2}$ 'slow component' emerges during supra-GET exercise. This $\dot{V}_{O_2}$ slow component delays the attainment of the steady-state during heavy-intensity exercise (below the critical power; CP), or prevents the attainment of a steady state during severe-intensity exercise (above CP) (Poole et al., 1988). The development of the $\dot{V}_{O_2}$ slow component is associated with greater muscle PCr (Rossiter et al., 2002) and glycogen (Krustrup et al., 2004) utilization. Therefore, interventions that modulate the dynamic $\dot{V}_{O_2}$ response during exercise, through determining the rate at which the energetic reserves are depleted and fatiguing metabolites accumulated, have important implications for the tolerable duration of exercise (Burnley and Jones, 2007; Bailey et al., 2009a,b,c; Jones and Burnley, 2009).

The causes of fatigue during exercise are known to be manifold and complex (Westerblad and Allen, 2003) but the possibility that the production of reactive oxygen species (ROS) during exercise contributes to fatigue development has begun to receive significant attention (Shindoh et al., 1990; Reid et al., 1992a; Moopanar and Allen, 2005; Ferreira and Reid, 2008; Powers and Jackson, 2008; Reid, 2008; Reardon and Allen, 2009). While the administration of nutritional antioxidants fails to reduce muscle fatigue development (Ferreira and Reid, 2008), administration of the pharmacological antioxidant, N-acetylcysteine [NAC], has been shown to delay fatigue in small muscle mass (Reid et al., 1994; Travaline et al., 1997; Koechlin et al., 2004; Matuszczak et al., 2005) and whole body (Medved et al., 2004b; McKenna et al., 2006) exercise in humans. The antioxidant role of NAC is twofold: firstly, NAC has direct antioxidant properties and can scavenge a number of ROS (Aruoma et al., 1989; Benrahmoune et al., 2000); and, secondly,

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NAC serves as a donor of reduced cysteine (CYS; Cotgreave, 1997) which is subsequently metabolized to reduced glutathione (GSH) by the action of γ-glutamylcysteine synthase (Sen, 1997). Both CYS and GSH have direct antioxidant properties with GSH also serving as the substrate for the enzymatic antioxidant, glutathione peroxidase. How exactly these biological actions of NAC are related to its ability to retard the rate of fatigue development is presently unclear. In a series of experiments (Medved et al., 2003, 2004a,b; McKenna et al., 2006), McKenna and colleagues applied a two-phase intravenous NAC administration procedure in humans that was well-tolerated and demonstrated to increase [NAC], [CYS] and [GSH], to decrease oxidized glutathione concentration ([GSSG]) in blood, and to increase [NAC] and [CYS] in skeletal muscle. While this improved capacity to tolerate exercise-induced redox perturbations has been accompanied by an improved exercise tolerance (Medved et al., 2004b; McKenna et al., 2006), it is presently unclear if this effect is related to changes in $\dot{V}_{O_2}$ dynamics.

Manipulating the bioavailability of the multi-functional physiological signaling molecule, nitric oxide (NO), has important effects on the $\dot{V}_{O_2}$ kinetics during both the fundamental (Jones et al., 2003, 2004; Wilkerson et al., 2004; Bailey et al., 2009a, 2010) and slow component (Jones et al., 2004; Bailey et al., 2009a, 2010) phases of the response in humans. Moreover, interventions that increase NO bioavailability have also been demonstrated to improve high-intensity exercise tolerance in conjunction with a reduced $\dot{V}_{O_2}$ slow component (Bailey et al., 2009a, 2010). Muscle contraction results in an increased production of ROS (Reid et al., 1992a,b, 1993; McArdle et al., 2005), as well as NO (Balon and Nadler, 1994; Kobzik et al., 1994), and it has been reported that ROS are capable of scavenging NO (Huang et al., 2007) and thwarting the enzymatic activity of the nitric oxide synthase (NOS) enzymes (Huang et al., 2001). Accordingly, interventions that scavenge exercise-induced ROS may increase NO synthesis and bioavailability during exercise, resulting in improved $\dot{V}_{O_2}$ dynamics and exercise tolerance. NAC and its derivatives CYS and GSH, not only have non-specific antioxidant properties but are also capable of reacting with reactive nitrogen species (RNS), derived from NO, to generate a reservoir of relatively stable NO that is carried in the form of S-nitrosothiols (Taylor and Winyard, 2007). However, the extent to which these S-nitrosothiols might serve as NO donors during exercise is obscure. The influence of NAC on NO synthesis and bioavailability during exercise is highly complex and in need of clarification.

The purpose of this investigation was therefore to determine the influence of NAC administration on NO synthesis and bioavailability (as reflected by plasma [nitrite]), pulmonary $\dot{V}_{O_2}$ kinetics and exercise tolerance in humans. We reasoned that the potential for NAC to enhance NO synthesis would result in greater NO bioavailability. We therefore hypothesized that the $\dot{V}_{O_2}$ slow component would be reduced and severe-intensity exercise tolerance improved following the administration of NAC.

2. Methods

2.1. Subjects

Eight healthy males (mean ± SD, age 27 ± 8 yr, height 180 ± 2 cm, body mass 80 ± 7 kg; $\dot{V}_{O_2}$ max; 51 ± 9 mL kg^{-1} min^{-1}) volunteered to participate in this study. None of the subjects were tobacco smokers or users of dietary supplements. The subjects were physically trained (engaging in 4–6 h of recreational exercise per week) but were not competitive athletes. All subjects were fully familiar with laboratory exercise testing procedures, having previously participated in studies employing cycle ergometry in our laboratory. The procedures employed in this study were approved by the University of Exeter Research Ethics Committee. All subjects gave their written informed consent prior to the commencement of the study, after the experimental procedures, associated risks, and potential benefits of participation had been explained. Subjects were instructed to arrive at the laboratory in a rested and fully hydrated state, at least 3 h postprandial, and to avoid strenuous exercise in the 24 h preceding each testing session. Each subject was also asked to refrain from caffeine and alcohol 6 h and 24 h, respectively before each test. All exercise tests were performed at the same time of day (±2 h).

2.2. Procedures

The subjects were required to report to the laboratory on three occasions, over a 5 week period. During the first visit to the laboratory, subjects performed a ramp incremental exercise test for determination of the $\dot{V}_{O_2}$ max and GET. All cycle tests were performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). Initially, subjects completed 3 min of 'unloaded' baseline cycling, after which the work rate was increased at a rate of 30 W min^{-1} until the subject was unable to continue. The participants cycled at a self-selected pedal rate (70–90 rpm), and this pedal rate along with saddle and handle bar height and configuration were recorded and reproduced in subsequent tests. The breath-by-breath pulmonary gas exchange data were collected continuously during the incremental tests and averaged over consecutive 10 s periods. The $\dot{V}_{O_2}$ max was taken as the highest 30 s mean value attained prior to the participant's volitional exhaustion. The GET was determined as described previously (Bailey et al., 2009a,b,c). The work rates that would require 80% of the GET (moderate-intensity exercise) and 65% Δ (65% of the difference between the power output at the GET and $\dot{V}_{O_2}$ max plus the GET, severe-intensity exercise) were subsequently calculated with account taken of the mean response time for $\dot{V}_{O_2}$ during ramp exercise (i.e., two thirds of the ramp rate was deducted from the power output at GET and peak).

Following completion of the ramp test, subjects were randomly assigned, using a double-blind, cross-over design, to receive, on two separate occasions, an intravenous infusion of N-acetylcysteine (Parvolex, solution for infusion; UCB Pharma, Slough, UK) and an intravenous infusion of a placebo (PLA; 0.9% saline) with a 14 day washout period separating the two experiments. Participants were instructed to complete a food diary for the seven days preceding their first experimental trial and asked to consume the same foods in the seven days preceding the second experimental trial. The order between the NAC and PLA supplementation periods was balanced. We utilized the NAC infusion procedures employed by McKenna and colleagues (Medved et al., 2003, 2004a,b; McKenna et al., 2006) as this protocol has been well tolerated and has been demonstrated to elevate blood, plasma and muscle NAC (Medved et al., 2004b).

Following arrival at the laboratory, two intravenous cannulae (BD Venflon, Becton Dickinson, Helsingborg, Sweden) were inserted under local anaesthesia (Marcain 0.5%, Astrazeneca, Cheshire), one in each forearm. One (18 g) was used for intravenous infusion of the NAC or PLA and the other (20 g) was used for blood sampling. The cannula used for blood sampling was kept patent with an infusion of 0.9% saline at 10 mL h^{-1} using a syringe driver (Graseby 3200 Syringe Pump, Graseby Medical, Watford). Subjects then rested for 30 min in the semi recumbent position. The infusions were given by a medical practitioner who was not blinded. NAC was infused for 15 min at a rate of 125 mg kg^{-1} h^{-1} (diluted in 0.9% saline at a concentration of 80 mg mL^{-1}) followed by a constant infusion rate of 25 mg kg^{-1} h^{-1} until the termination of the final exercise bout using an infusion pump (Baxter Colleague, Baxter Healthcare Ltd., Northampton, UK). The PLA consisted of a corresponding volume of 0.9% saline given over the same period.

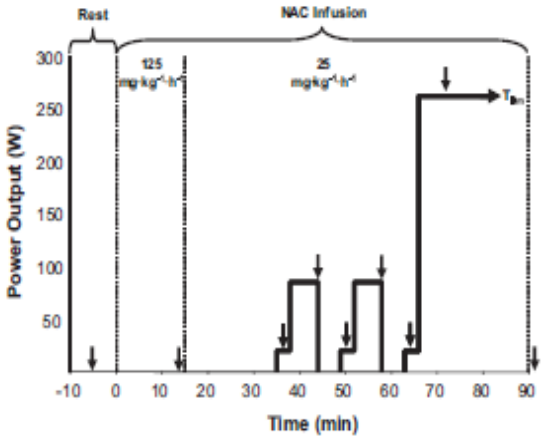

Fig. 1. A schematic timeline of the infusion regime and exercise protocol employed in this study. The dashed vertical lines indicate a change in the rate of N-acetylcysteine infusion, and the vertical arrows indicate the blood sampling points. The arrow on the horizontal line of the severe-intensity exercise bout indicates that this exercise bout was continued to Tlim, the limit of tolerance.

Twenty min into the constant infusion phase, participants initiated a series of 'step' cycle exercise tests that comprised two bouts of moderate-intensity exercise and one bout of severe-intensity exercise as illustrated in Fig. 1. Each step test was preceded by 3 min of baseline pedalling at 20 W and each bout was interspersed with a 5 min passive recovery period. The severe-intensity work rate was continued until task failure as a measure of exercise tolerance with the time to task failure noted when the pedal rate fell by >10 rpm below the required pedal rate. The $\dot{V}_{O_2}$ responses to the two moderate-intensity exercise bouts were averaged prior to analysis to reduce breath-to-breath noise and enhance confidence in the parameters derived from the modelling process (Lamarra et al., 1987). Blood was sampled at rest and following the initial infusion phase, following the baseline period for each of the three exercise bouts, at end-exercise for the two moderate-intensity bouts, at 360 s of exercise and at exhaustion for the severe-intensity bouts.

2.3. Measurements

During all tests, pulmonary gas exchange and ventilation were measured breath-by-breath with subjects wearing a nose clip and breathing through a low-dead-space, low-resistance mouthpiece and impeller turbine assembly (Jaeger Triple V). The inspired and expired gas volume and gas concentration signals were continuously sampled at 100 Hz, the latter using paramagnetic (O_2) and infrared (CO_2) analyzers (Jaeger Oxycon Pro, Hoechberg, Germany) via a capillary line connected to the mouthpiece. The gas analyzers were calibrated before each test with gases of known concentration and the turbine volume transducer was calibrated with a 3-liter syringe (Hans Rudolph, Kansas City, MO). The volume and concentration signals were time-aligned by accounting for the delay in the capillary gas transit and the analyzer rise time relative to the volume signal. Pulmonary gas exchange and ventilation were calculated and displayed breath-by-breath. Heart rate (HR) was measured during all tests using short-range radiotelemetry (Polar S610, Polar Electro Oy, Kempele, Finland).

Blood was drawn into 2 mL lithium–heparin tubes and an aliquot was used to determine lactate concentration ([lactate]) (YSI 1500, Yellow Springs Instruments, Yellow Springs, OH) within 30 s of collection. The blood samples were then centrifuged at 4000 rpm and 4 °C for 10 min. A 250 μL plasma aliquot was immediately analyzed for determination of the plasma potassium concentration ($[K^+]$) using an automated ion-selective electrode analyzer (9180 Electrolyte Analyzer, Roche Diagnostics, Mannheim, Germany). The remaining plasma was divided into 250 μL aliquots and immediately frozen at −80 °C, for later analysis.

Total plasma solubilized [protein] was determined using a microplate protein assay kit (Bio-Rad, München, Germany) using the method of Bradford (Bradford, 1976). Briefly, 160 μL of each sample was added to separate micotiter plate wells followed by 40 μL of dye reagent concentrate. The sample and reagent were subsequently mixed for 10 min using a microplate mixer and incubated at room temperature for a further 10 min prior to analysis. Thereafter, sample absorbance at 595 nm was measured using a microplate reader (Dynex MRX microplate reader, Dynex Technologies, Chantilly, VA, USA).

Plasma free sulfhydryl groups were determined using the method of Ellman (1959) as later modified by Hu et al. (1993). Briefly, 1 mL of buffer containing 0.1 mol L^{-1} Tris, 10 mmol L^{-1} EDTA, pH 8.2 and 50 μL plasma were added to cuvettes, followed by 50 μL of 10 mmol L^{-1} DTNB in methanol. Sample absorbance was measured at 412 nm using a spectrophotometer (Jenway 6310 visible spectrophotometer, Jenway, Essex, UK) following a 15 min incubation period at room temperature. Blanks were run for each sample, with no DTNB in the methanol, and for the reagents and these absorbance values were subtracted from the absorbance of each sample. The concentration of free sulfhydryl groups was subsequently determined using the TNB molar extinction coefficient of 14,100 M^{-1} cm^{-1}, yielding results in μM (Riddles et al., 1979). Total plasma free sulfhydryl groups are presented herein as micromolar per gram protein per litre (μM g^{-1}). Plasma nitrite concentrations ($[NO_2^-]$) were determined via chemiluminescence as described previously (Bailey et al., 2009a).

2.4. Data analysis procedures

The breath-by-breath $\dot{V}_{O_2}$ data from each test were initially examined to exclude errant breaths caused by coughing, swallowing, sighing, etc., and those values lying more than four standard deviations from the local mean were removed. The breath-by-breath data were subsequently linearly interpolated to provide second-by-second values and, for each individual, identical repetitions were time-aligned to the start of exercise and ensemble-averaged. The first 20 s of data after the onset of exercise (i.e., the phase I response) were deleted and a nonlinear least-square algorithm was used to fit the data thereafter. A single-exponential model was used to characterize the $\dot{V}_{O_2}$ responses to moderate-intensity exercise and a bi-exponential model was used for severe-intensity exercise, as described in the following equations:

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2\text{baseline}} + A_p(1 - e^{-(t-TD_p/\tau_p)}) \quad \text{(moderate)} \tag{1}$$

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2\text{baseline}} + A_p(1 - e^{-(t-TD_p/\tau_p)}) + A_s(1 - e^{-(t-TD_s/\tau_s)}) \quad \text{(severe)} \tag{2}$$

where $\dot{V}_{O_2}(t)$ represents the absolute $\dot{V}_{O_2}$ at a given time t; $\dot{V}_{O_2\text{baseline}}$ represents the mean $\dot{V}_{O_2}$ in the baseline period; A_p, TD_p, and τ_p represent the amplitude, time delay, and time constant, respectively, describing the phase II increase in $\dot{V}_{O_2}$ above baseline; and A_s, TD_s, and τ_s represent the amplitude of, time delay before the onset of, and time constant describing the development of, the $\dot{V}_{O_2}$ slow component, respectively.

An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. $\dot{V}_{O_2\text{baseline}}$ was defined as the mean $\dot{V}_{O_2}$ measured over the final 90 s of baseline pedalling. The end-exercise $\dot{V}_{O_2}$ was defined as the mean $\dot{V}_{O_2}$ measured over the final 30 s of exercise. Because

the asymptotic value (A_s) of the exponential term describing the $\dot{V}_{O_2}$ slow component may represent a higher value than is actually reached at the end of the exercise, the amplitude of the $\dot{V}_{O_2}$ slow component at the end of exercise was defined as A_s'. The amplitude of the $\dot{V}_{O_2}$ slow component was also described relative to the entire $\dot{V}_{O_2}$ response. To determine the overall kinetics of the $\dot{V}_{O_2}$ response to both moderate- and severe-intensity exercise, data were fit with a mono-exponential model from 0 s to end-exercise, without time delay.

We also modelled the HR response to exercise in each condition. For this analysis, HR data were linearly interpolated to provide second-by-second values, and, for each individual, identical repetitions from like-transitions were time-aligned to the start of exercise and ensemble-averaged. Nonlinear least squares mono-exponential and bi-exponential models without TD were used to fit the data to moderate- and severe-intensity exercise, respectively. The HR τ_p so derived was used to provide information on the overall HR response dynamics.

2.5. Statistics

Differences in the cardio-respiratory variables and exercise tolerance between conditions were analyzed with two-tailed, paired-samples *t*-tests. Alterations in blood parameters was determined via two-tailed, two-way (treatment × time) repeated-measures ANOVA. Significant effects were further explored using simple contrasts. Relationships between variables were assessed using Pearson's product moment correlation coefficient. Data are presented as mean ± SD, unless otherwise stated. Statistical significance was accepted when $P < 0.05$.

3. Results

The NAC administration regime employed in this investigation was well tolerated with no adverse reactions. During the ramp incremental test, subjects attained a peak work rate of 376 ± 61 W and a $\dot{V}_{O_2}$ max of 4.07 ± 0.72 L min^{-1}, while the work rate and $\dot{V}_{O_2}$ values at the GET were 127 ± 20 W and 1.77 ± 0.20 L min^{-1}, respectively.

3.1. Total plasma sulfhydryl groups and plasma [NO_2^-]

The group mean total plasma sulfhydryl groups across the nine sample points for the NAC and PLA trials are illustrated in Fig. 2. There were significant main effects for time and treatment, as well as a significant interaction effect all ($P < 0.01$). At pre-infusion, the total plasma sulfhydryl groups did not differ during the PLA and NAC trials (PLA: 4 ± 2 vs. NAC: 4 ± 1 μM g^{-1}; $P > 0.05$; Fig. 2). The total plasma sulfhydryl groups did not differ from the pre-infusion value following or during PLA administration ($P > 0.05$). However, the total plasma sulfhydryl groups increased by 225% following the 125 mg kg^{-1} h^{-1} loading phase of NAC (PLA: 4 ± 2 vs. NAC: 13 ± 3 μM g^{-1}; $P < 0.01$; Fig. 2) and remained significantly elevated above the pre-infusion value and the corresponding PLA values for the remainder of the protocol ($P < 0.01$). The group mean plasma [NO_2^-] data following NAC and PLA administration are shown in Table 1. There was no time, treatment or interaction effect on plasma [NO_2^-] ($P > 0.05$).

3.2. Blood [lactate] and plasma [potassium]

We observed a significant main effect of time on both blood [lactate] and plasma [potassium] ($P < 0.01$); however, there was no effect of treatment and no interaction (both $P > 0.05$; Table 2). Follow-up analyses revealed that neither blood [lactate] nor plasma [potassium] was significantly different between the NAC and PLA conditions at the time points investigated.

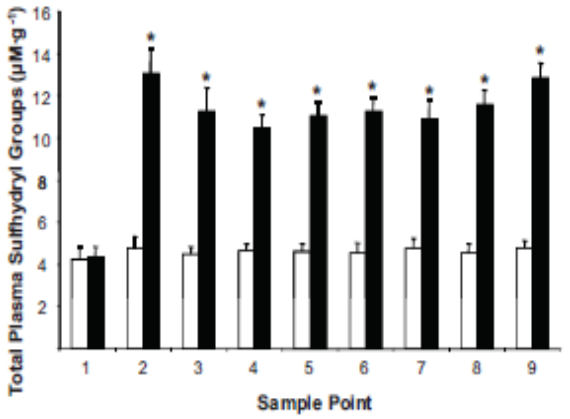

Fig. 2. Group mean ± SEM plasma free sulfhydryl groups following NAC and PLA administration over the nine sample points: 1 = resting, pre-infusion; 2 = post NAC loading phase; 3 = baseline (immediately before the start of the first bout of moderate-intensity exercise); 4 = end of first moderate-intensity exercise bout; 5 = baseline (immediately before the start of the second bout of moderate-intensity exercise); 6 = end of second moderate-intensity exercise bout; 7 = baseline (immediately before the start of the severe-intensity exercise bout); 8 = 6 min into the severe-intensity exercise bout; 9 = the point of exhaustion during severe-intensity exercise. Note the significant increase in plasma free sulfhydryl groups following the NAC loading phase and that this elevation is preserved during the NAC constant infusion phase.* denotes significantly different from pre-infusion and the corresponding placebo value ($P < 0.05$).

3.3. $\dot{V}_{O_2}$ dynamics and exercise tolerance

The group mean pulmonary $\dot{V}_{O_2}$ response during moderate- and severe-intensity exercise is illustrated in Fig. 3 and the parameters derived from the model fits are shown in Table 3. During severe-intensity exercise, neither the phase II τ (PLA: 25 ± 6 vs. NAC: 23 ± 7 s; $P > 0.05$; Table 3) nor the fundamental $\dot{V}_{O_2}$ amplitude (PLA: 0.65 ± 0.18 vs. NAC: 0.61 ± 0.16 L min^{-1}; $P > 0.05$; Table 3) were influenced by the administration of NAC. The 95% confidence interval surrounding the estimate of the phase II τ for moderate-intensity exercise was 4 ± 1 s in both the PLA and NAC conditions. The phase II τ was not different between conditions (PLA: 30 ± 9 vs. NAC: 23 ± 14 s; $P > 0.05$; paired-samples 95% confidence interval −309, 104). The fundamental $\dot{V}_{O_2}$ amplitude (PLA: 2.24 ± 0.61 vs. NAC: 2.20 ± 0.59 L min^{-1}; $P > 0.05$) and $\dot{V}_{O_2}$ slow component amplitude (PLA: 0.49 ± 0.21 vs. NAC: 0.45 ± 0.16 L min^{-1}; $P > 0.05$) were not significantly altered by NAC administration (Table 3). The 95% confidence interval surrounding the estimate of the phase II τ

Table 1
Mean ± SD plasma [nitrite] prior to and post moderate- and severe-intensity exercise following N-acetylcysteine (NAC) and placebo (PLA) administration.

	PLA	NAC
Rest (nM)	195 ± 119	208 ± 104
Post 125 mg kg^{-1} h^{-1} (nM)	229 ± 120	240 ± 174
Moderate-intensity exercise bout 1		
Baseline (nM)	257 ± 139	283 ± 114
End (nM)	272 ± 178	242 ± 146
Moderate-intensity exercise bout 2		
Baseline (nM)	197 ± 160	238 ± 75
End (nM)	225 ± 105	268 ± 114
Severe-intensity exercise bout		
Baseline (nM)	186 ± 172	167 ± 84
360 s (nM)	212 ± 125	234 ± 110*
Exhaustion (nM)	251 ± 158*	231 ± 102

* Significantly greater than severe-intensity baseline $P < 0.05$.

Table 2
Mean ± SD blood [lactate] and plasma [potassium] during moderate- and severe-intensity exercise following administration of N-acetylcysteine (NAC) and placebo (PLA).

	PLA	NAC
Blood [lactate] (mM)		
Rest	0.9 ± 0.2	0.9 ± 0.3
Post 125 mg kg^{-1} h^{-1}	0.8 ± 0.2	0.8 ± 0.2
Moderate-intensity exercise bout 1		
Baseline	0.8 ± 0.1	0.9 ± 0.2
End	1.3 ± 0.3	1.1 ± 0.3
Moderate-intensity exercise bout 2		
Baseline	1.1 ± 0.2	1.0 ± 0.2
End	1.1 ± 0.2	1.1 ± 0.1
Severe-intensity exercise bout		
Baseline	1.0 ± 0.2	1.0 ± 0.2
360 s	4.2 ± 1.6	4.1 ± 1.6
Exhaustion	8.6 ± 2.2	8.6 ± 2.1
Plasma [potassium] (mM)		
Rest	3.9 ± 0.4	3.8 ± 0.4
Post 125 mg kg^{-1} h^{-1}	3.8 ± 0.3	3.8 ± 0.3
Moderate-intensity exercise bout 1		
Baseline	4.1 ± 0.4	4.0 ± 0.4
End	4.1 ± 0.3	4.0 ± 0.3
Moderate-intensity exercise bout 2		
Baseline	4.2 ± 0.3	4.1 ± 0.3
End	4.3 ± 0.3	4.2 ± 0.3
Severe-intensity exercise bout		
Baseline	4.2 ± 0.3	4.1 ± 0.3
360 s	4.9 ± 0.5	4.7 ± 0.4
Exhaustion	5.2 ± 0.5	5.2 ± 0.7

Table 3
Mean ± SD oxygen uptake and heart rate dynamics during moderate- and severe-intensity exercise following administration of N-acetylcysteine (NAC) and placebo (PLA).

	PLA	NAC
Moderate-intensity exercise		
Oxygen uptake ($\dot{V}_{O_2}$)		
Baseline (L min^{-1})	0.98 ± 0.10	1.02 ± 0.08
End-exercise (L min^{-1})	1.64 ± 0.18	1.65 ± 0.19
Phase II time constant (s)	25 ± 6	23 ± 7
Mean response time (s)	39 ± 8	35 ± 7
Fundamental amplitude (L min^{-1})	0.65 ± 0.18	0.61 ± 0.16
Heart rate		
Baseline (b min^{-1})	93 ± 15	95 ± 12
End-exercise (b min^{-1})	112 ± 14	115 ± 12
Mean response time (s)	26 ± 11	30 ± 12
Severe-intensity exercise		
Oxygen uptake ($\dot{V}_{O_2}$)		
Baseline (L min^{-1})	1.02 ± 0.11	1.16 ± 0.21
360 s (L min^{-1})	3.72 ± 0.68	3.79 ± 0.68
Exhaustion (L min^{-1})	3.97 ± 0.68	3.93 ± 0.70
Phase II time constant (s)	30 ± 9	23 ± 14
Fundamental amplitude (L min^{-1})	2.24 ± 0.61	2.20 ± 0.59
Slow component amplitude (L min^{-1})	0.49 ± 0.21	0.45 ± 0.16
Slow component amplitude (%)	18 ± 6	17 ± 6
Overall mean response time (s)	56 ± 12	49 ± 17
Heart rate		
Baseline (b min^{-1})	101 ± 17	105 ± 13
End-exercise (b min^{-1})	173 ± 12	172 ± 11
Mean response time (s)	58 ± 20	61 ± 22

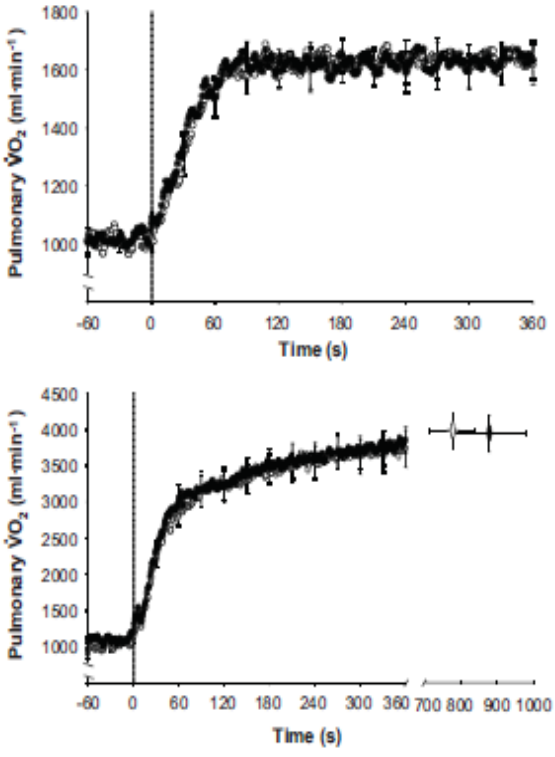

Fig. 3. Group mean ± SEM pulmonary oxygen uptake ($\dot{V}_{O_2}$) response during moderate-intensity (upper panel) and severe-intensity (lower panel) exercise following NAC and PLA administration. The dashed vertical line represents the abrupt imposition of the work rate from a resting baseline. Open symbols represent responses following PLA and closed symbols represent responses following NAC. Note that pulmonary $\dot{V}_{O_2}$ kinetics were not affected by NAC administration for either moderate-intensity or severe-intensity exercise.

for severe-intensity exercise was 5 ± 3 s in the PLA condition and 4 ± 2 s in the NAC condition. The baseline and end-exercise values for minute ventilation ($\dot{V}_E$), heart rate, carbon dioxide production ($\dot{V}_{CO_2}$) and respiratory exchange ratio (RER) were not significantly altered during either moderate- or severe-intensity exercise following NAC administration compared to PLA.

The tolerable duration of severe-intensity exercise was not significantly different between conditions (PLA: 776 ± 181 vs. NAC: 878 ± 284 s; $P > 0.05$; Fig. 4; paired-samples 95% confidence interval −5.8, 18.9). However, there was substantial inter-subject variability in the influence of NAC on exercise tolerance: four subjects had small reductions in exercise tolerance with NAC compared to PLA (−4%, −8%, −11%, and −14%) while the other four showed substantial improvements (+24%, +24%, +40%, and +69%). The difference (Δ) in total plasma sulfhydryl groups between the NAC and PLA conditions following the loading phase was positively correlated

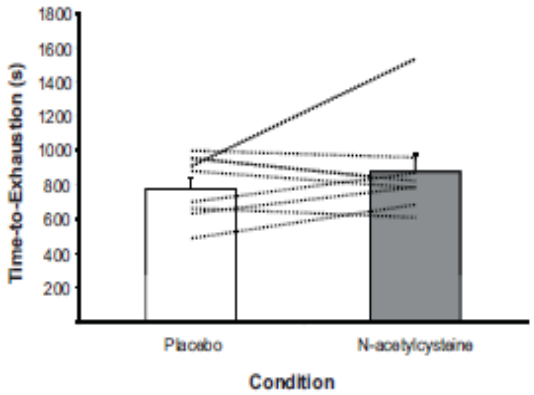

Fig. 4. Group mean ± SEM time-to-exhaustion during severe-intensity exercise following NAC and PLA administration. The mean ± SEM responses following NAC administration are shown as a grey filled bar and the PLA responses are shown as open bars. Individual changes in the tolerance of severe-intensity exercise between the two experimental conditions are shown as dashed lines. Note the inter-subject variability in the response to NAC administration.

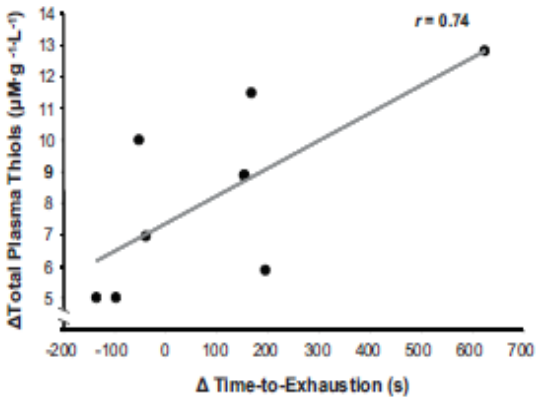

Fig. 5. The relationship between the difference (Δ) in total plasma sulfhydryl groups following the loading phase and the Δ in exercise tolerance between the NAC and PLA conditions. Note that the Δ total plasma sulfhydryl groups were positively correlated with the Δ exercise tolerance ($r = 0.74$, $P < 0.05$).

with the Δ exercise tolerance ($r = 0.74$, $P < 0.05$; Fig. 5), although this relationship was not significant when the subject with the greatest positive response was excluded. In contrast, the Δ exercise tolerance was not correlated with the changes in $\dot{V}O_2$ kinetics or plasma $[NO_2^-]$.

4. Discussion

This is the first investigation to assess the influence of NAC administration on indices of NO synthesis, pulmonary $\dot{V}O_2$ kinetics and exercise tolerance in humans. The principal original findings were that intravenous infusion of the potent antioxidant, NAC, which significantly increased total plasma sulfhydryl groups, had no significant influence on plasma $[NO_2^-]$, $\dot{V}O_2$ kinetics or exercise tolerance in healthy adult humans. The unchanged plasma $[NO_2^-]$ suggests that NAC administration did not appreciably alter NO synthesis and bioavailability; in turn, no effects on $\dot{V}O_2$ kinetics might be expected. However, it is important to note that while the overall 13% improvement in exercise tolerance following NAC administration was not statistically significant, this result conceals the appreciable changes in exercise tolerance that were evident at an individual subject level. The significant positive correlation between the elevation in plasma sulfhydryl groups and the change in exercise tolerance suggests that the magnitude of the redox perturbations incurred during intense exercise may be linked to the ability to tolerate severe-intensity exercise.

4.1. Total plasma sulfhydryl groups and plasma $[NO_2^-]$

In addition to its direct antioxidant properties (Aruoma et al., 1989; Benrahmoune et al., 2000), NAC undergoes rapid deacetylation to yield CYS (Deneke, 2000), which is the substrate for the GSH producing enzyme, γ-glutamylcysteine synthase (Sen, 1997). Administration of NAC would therefore be expected to increase the thiols, CYS and GSH, which comprise sulfhydryl groups and thereby confer direct antioxidant properties. Indeed, it has been demonstrated that intravenous NAC administration increased plasma [NAC] (Medved et al., 2003, 2004a,b; Merry et al., 2010), [GSH] (Medved et al., 2003) and [CYS] (Medved et al., 2003, 2004b; Merry et al., 2010). Consistent with this, the total plasma sulfhydryl groups increased by 225% following the loading phase of NAC infusion in this study and remained significantly elevated above rest and the corresponding PLA values throughout the protocol during the constant NAC infusion phase. Importantly, utilizing the same NAC administration procedures applied herein has been demonstrated to increase muscle [NAC] (Medved et al., 2004b; Merry et al., 2010), [CYS] (Medved et al., 2004b; Merry et al., 2010) and to reduce protein S-glutathionylation (Merry et al., 2010). During exercise, ROS are produced at an increased rate (Reid et al., 1992a,b, 1993; McArdle et al., 2005) resulting in an increased thiol (sulfhydryl) oxidation, which can influence the structure and function of numerous proteins (Ferreira and Reid, 2008). Therefore, the elevation in exogenous free sulfhydryl groups achieved via NAC herein may have preserved the function of several regulatory proteins through buffering the oxidation of the critical thiol constituents of these proteins.

One enzyme whose catalytic activity is impaired upon oxidative thiol modification is endothelial NOS (eNOS; Huang et al., 2001). An increased presence of the ROS, hydrogen peroxide (H_2O_2), also impairs NO production through the oxidative inactivation of essential eNOS co-factors (Jaimes et al., 2001), as well as the synthesized NO (Thomas et al., 2006), whereas neuronal NOS (nNOS) activity is increased following NAC administration (Pechanova et al., 2009). Given that NAC can scavenge superoxide (O_2^-) (Benrahmoune et al., 2000), NO bioavailability may also be increased through reducing the O_2^- scavenging of NO (Powers and Jackson, 2008). Collectively, these data suggest that the reduction in exercise-induced oxidative stress following NAC administration (Medved et al., 2003; Merry et al., 2010) may increase NO synthesis and bioavailability. However, administration of NAC increases muscle and plasma concentrations of the thiols, NAC, CYS and GSH (Medved et al., 2003, 2004a,b; Merry et al., 2010), whose antioxidant properties are non-specific and which can therefore react with NO, as well as ROS, to yield the S-nitrosothiols SNAC, CySNO and GSNO, respectively. An obvious limitation of the present study is therefore that S-nitrosothiols were not measured. However, the determination of individual –SNO– containing amino acids and peptides in complex materials such as plasma is difficult and few viable methods are currently available (Torta et al., 2010). Moreover, many small S-nitrosothiols are likely to be unstable in stored plasma samples. The extent to which these S-nitrosothiols liberate NO is highly complex and poorly understood (Jenkins et al., 1993; Hogg et al., 1996; Singh et al., 1996; Wong et al., 1998; Steffen et al., 2001; Zeng et al., 2001) and it is unclear if the formation of S-nitrosothiols serves to scavenge NO or acts as a storage reservoir of NO for later release.

Of the biomarkers that are typically measured to determine NO synthesis, plasma $[NO_2^-]$ has been suggested to provide the best indication of eNOS activity in humans (Lauer et al., 2001, 2002; Rassaf et al., 2007) and other mammals (Kleinbongard et al., 2003). The quantification of plasma $[NO_2^-]$ can therefore provide information on NO synthesis and bioavailability in humans. In the present study, plasma $[NO_2^-]$ increased over the course of the severe-intensity exercise bout, consistent with an enhanced NO production (Balon and Nadler, 1994; Kobzik et al., 1994; Rassaf et al., 2007). However, plasma $[NO_2^-]$ was not significantly different between the NAC and PLA conditions at any time in the experimental protocol. Therefore, these data suggest either that NAC did not increase NO synthesis or that any potential for NAC to increase NO synthesis and bioavailability was offset by an increased formation of S-nitrosothiols, which were unable to release sufficient NO, such that net NO bioavailability was unaffected.

4.2. Pulmonary $\dot{V}O_2$ kinetics

The assessment of pulmonary $\dot{V}O_2$ kinetics enables non-invasive characterization of muscle $\dot{V}O_2$ kinetics (Grassi et al., 1996; Krustrup et al., 2009). NAC has the potential to modulate several of the factors that determine $\dot{V}O_2$ (Poole et al., 2008). Firstly, NAC elicits a NO or S-nitrosothiol-mediated vasodilatory response that provokes an increase in muscle blood flow (Andrews et al., 2001).

During supra-GET exercise, both the fundamental and slow component $\dot{V}_{O_2}$ amplitudes appear to be sensitive to interventions which would be expected to increase muscle blood flow (Bailey et al., 2009a,b; Koga et al., 1999), indicating that these parameters may be altered by NAC administration. Additionally, ROS have been shown to impair mitochondrial metabolism and disrupt mitochondrial proteins (Reid, 2008), which may compromise flux through oxidative phosphorylation and thus impact on $\dot{V}_{O_2}$ kinetics. The accumulation of ROS can also disrupt sarcoplasmic reticulum (SR) calcium (Ca^{2+}) release channels, SR Ca^{2+}-ATPase, and actin, myosin and myofilament interaction (Reid et al., 1993); ROS accumulation therefore has the potential to influence Ca^{2+}-ATPase and actomyosin-ATPase, the predominant determinants of myocyte ATP consumption (Barclay et al., 2007).

Despite this potential for NAC to modulate several of the processes that might influence $\dot{V}_{O_2}$, the present results indicate that $\dot{V}_{O_2}$ kinetics was not significantly altered during either moderate- or severe-intensity exercise following the administration of NAC. However, it is possible that the young healthy adults investigated in this study had sufficient residual enzymatic and non-enzymatic antioxidant defence mechanisms such that elevating plasma, and presumably muscle, thiols (Medved et al., 2004b; Merry et al., 2010) did not appreciably increase ROS scavenging. However, ROS synthesis is potentiated in the elderly and in patient groups (Maccarrone and Ullrich, 2004) and it therefore remains possible that administration of NAC, or other antioxidants, may influence oxidative metabolism in these populations.

4.3. Exercise tolerance

ROS exert a complex influence on skeletal muscle force production. In the unfatigued skeletal muscle, the presence of ROS is essential for normal force production (Reid, 2001). Indeed, administration of antioxidants, resulting in the scavenging of ROS, can compromise force production (Reid et al., 1993), while mild, but not high, elevations in ROS can potentiate force production (Reid et al., 1993). Therefore, according to the theoretical model of Reid et al. (1993), there appears to be an optimal cellular redox state, deviation from which results in an impaired skeletal muscle force production and, ultimately, fatigue. During repeated contractions there is an increased rate of ROS production (Reid et al., 1992a,b, 1993; McArdle et al., 2005), which would result in a more oxidized redox state and thus sub-optimal force production. Accordingly, the administration of NAC, through prolonging the duration for which the optimal cellular redox state can be maintained, has been suggested to delay fatigue during small muscle mass (Reid et al., 1994; Travaline et al., 1997; Koechlin et al., 2004; Matuszczak et al., 2005) and whole body (Medved et al., 2004b; McKenna et al., 2006) exercise in humans. However, the recent data of Herspring et al. (2008) demonstrate that antioxidant administration impaired force production in aged rat spinotrapezius muscle, despite the expectation that these aged muscles would generate large quantities of ROS during contraction. These data therefore indicate that, even in an oxidized cellular redox state, antioxidant supplementation which shifts the cellular redox state beyond the optimal point and into a sub-optimal reduced state can impair muscle force production.

The fatigue associated with exercise-induced ROS is multifaceted and can potentially involve numerous regulatory proteins (Ferreira and Reid, 2008; Reid, 2008). One protein whose activity is preserved following NAC administration is the Na^+/K^+-ATPase pump (Medved et al., 2004a; McKenna et al., 2006). Indeed, McKenna and colleagues have demonstrated an increased Na^+/K^+-ATPase pump activity (Medved et al., 2004a) resulting in a reduced plasma K^+ accumulation (Medved et al., 2004a; McKenna et al., 2006) in exercising humans following NAC administration. This would be expected to reduce the extent of skeletal muscle fatigue (Juel, 1988). However, plasma $[K^+]$ was not affected by NAC administration in this study, despite our employment of the same NAC infusion procedures applied by McKenna and colleagues (Medved et al., 2004a; McKenna et al., 2006).

The tolerable duration of severe-intensity exercise was not significantly altered by NAC administration in the present study, but was enhanced in the earlier investigations of McKenna and associates (Medved et al., 2004b; McKenna et al., 2006). This discrepancy may be ascribed, in part, to the differences in the exercise protocols employed and the training status of the subjects investigated. Specifically, in the investigations of McKenna and colleagues (Medved et al., 2004b; McKenna et al., 2006), participants first exercised for 45 min at ~70% $\dot{V}_{O_2}$ max before cycling to exhaustion at ~90% $\dot{V}_{O_2}$ max, resulting in a total exercise duration of around 50–51 min. In contrast, in the present study, participants exercised at a constant-work-rate equivalent to 65% Δ (~80% $\dot{V}_{O_2}$ max) until exhaustion, resulting in much shorter total exercise duration of 14–15 min. Moreover, McKenna and colleagues found NAC to be ergogenic during the aforementioned exercise test when utilizing trained (Medved et al., 2004b; McKenna et al., 2006) but not untrained (Medved et al., 2004a) participants. It has recently been reported that, despite possessing greater levels of enzymatic and non-enzymatic antioxidants, athletes exhibited greater oxidative stress when compared to less-trained individuals (Teixeira et al., 2009). Therefore, athletes might be expected to benefit more from antioxidant interventions than would lesser trained individuals, consistent with the findings of the present study.

Although the 13% improvement in severe-intensity exercise tolerance following NAC administration was not statistically significant, there was substantial inter-individual variability in the response. Four of the eight subjects in the present study demonstrated substantially improved exercise tolerance following NAC (from +24% to +69%), with the other four having similar or slightly worsened performance (from −4% to −14%). The mechanistic bases for these differential effects require further investigation. However, it is possible that differences in response to NAC may be a consequence of the subjects occupying different positions on Reid's (1993) cellular redox state model. Alternatively, it is possible that the NAC dose used in the present study was insufficient to elicit an ergogenic effect in the 'non-responsive' subjects. This suggestion is strengthened by our observation of a significant positive correlation between the Δ total plasma sulfhydryl groups and the Δ exercise tolerance between the NAC and placebo interventions. This correlation suggests that the individuals who experienced the greatest antioxidant augmentation with NAC were better able to tolerate severe-intensity exercise. This finding therefore supports the notion that the degree of redox perturbation may be an important determinant of exercise tolerance in humans. However, the present data suggest that possible ergogenic effects of antioxidant supplementation may not be mediated by changes in NO synthesis or bioavailability, at least in healthy adults. Determination of the parameters of the hyperbolic power–duration relationship (i.e. its asymptote, CP, and its curvature constant, W′; Poole et al., 1988) might provide mechanistic insight into possible positive effects of antioxidant supplementation on fatigue resistance.

5. Summary

The administration of NAC more than doubled plasma free sulfhydryl (thiol) groups in this investigation, consistent with the observations of an increase in plasma thiols (NAC, CYS and GSH) reported by others using the same NAC administration procedure (Medved et al., 2003, 2004a,b; Merry et al., 2010). While plasma $[NO_2^-]$ increased over the course of the severe-intensity exercise bout, in line with an enhanced NO synthesis during exercise (Balon and Nadler, 1994; Kobzik et al., 1994; Rassaf et al., 2007),

NAC infusion did not alter the plasma [NO_2^-] compared to PLA at any time during the experimental protocol. Similarly, NAC administration did not measurably impact on pulmonary $\dot{V}_{O_2}$ kinetics during either moderate- or severe-intensity exercise. Overall, exercise tolerance was not significantly altered by NAC administration; however, the effects were highly variable between subjects, suggesting that exercise-induced redox perturbations may contribute to fatigue development.

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Chapter 12 General Discussion

Since the first assessment of breath-by-breath pulmonary $\dot{V}O_2$ in exercising humans (Wasserman *et al*. 1967), tremendous strides have been made to advance the discipline of $\dot{V}O_2$ kinetics. Indeed, the data analysis procedures, including sophisticated mathematical modelling techniques, have been rigorously developed for accurate quantification of $\dot{V}O_2$ kinetics during various intensities of exercise. Extensive efforts have also been directed towards elucidating the determinants of the various response phases of $\dot{V}O_2$ kinetics and while there is not unanimous agreement on these factors, a number of candidates have been identified (Tschakovsky and Hughson, 1999; Jones and Poole, 2005; Poole *et al*., 2008).

Through determining the rate and source of substrate utilisation and metabolite accumulation, the dynamics of $\dot{V}O_2$ has clear potential to influence the tolerable duration of exercise. Surprisingly, however, empirical research investigations on this topic are relatively sparse. While understanding the determinants of $\dot{V}O_2$ kinetics has important implications for our understanding of oxidative metabolism, understanding the extent to which $\dot{V}O_2$ kinetics influence human exercise tolerance will have direct functional implications. Moreover, if $\dot{V}O_2$ kinetics is identified as an important determinant of exercise tolerance then interventions that improve $\dot{V}O_2$ kinetics could be implemented to enhance the functional capacity in the elderly, diseased and sedentary individuals in order to improve their quality of life, and to improve exercise performance in elite and recreational athletes. Accordingly, there is considerable merit in determining the extent to which $\dot{V}O_2$ kinetics can influence human exercise tolerance.

To demonstrate that $\dot{V}O_2$ kinetics is an important determinant of exercise tolerance requires evidence that improving $\dot{V}O_2$ kinetics improves exercise tolerance and that impairing $\dot{V}O_2$ kinetics compromises exercise tolerance. There is evidence that $\dot{V}O_2$ kinetics is faster in elite athletes compared to club level athletes (Ingham *et al*., 2007), in moderately trained athletes compared to untrained subjects (Koppo *et al*., 2004) and that $\dot{V}O_2$ kinetics is slowed with ageing (Barstow and Scheuerman, 2005) and in various disease states (Poole *et al*., 2005). These findings indicate that improved physical fitness is accompanied by faster $\dot{V}O_2$ kinetics, while ageing and pathology is accompanied by a slowing in $\dot{V}O_2$ kinetics; these changes in $\dot{V}O_2$ kinetics are accompanied by reciprocal changes in exercise tolerance. Taken together, these findings support the notion that $\dot{V}O_2$ kinetics is an important determinant of exercise tolerance. However, there are a variety of physiological modulations that accompany an enhanced fitness level, and ageing and pathogenesis, that may alter exercise tolerance independent of changes in $\dot{V}O_2$ kinetics. It is, therefore, unclear if these changes are causal or coincidental.

It has been reported that neither the phase II τ nor the MRT during moderate exercise is related to 5 km running speed in runners of a similar performance level (n = 10) but that a moderate relationship exists between $\dot{V}O_2$ kinetics and exercise performance in a larger (n = 36) heterogeneous fitness group (Kilding *et al*., 2006). A limitation of this investigation is that $\dot{V}O_2$ kinetics was assessed in the moderate domain with exercise performance assessed in the severe domain; thus, it is unclear how the $\dot{V}O_2$ kinetics in the actual performance trial influenced exercise performance. Moreover, this investigation did not determine the influence of the $\dot{V}O_2$ slow component on exercise performance. As such, it was not possible to exclude $\dot{V}O_2$ kinetics as a determinant of exercise tolerance. To address these issues, the investigations that comprise this thesis imposed a variety of interventions to manipulate

aspects of $\dot{V}O_2$ kinetics and attempted to elucidate how these changes influenced exercise tolerance. Importantly, $\dot{V}O_2$ kinetics and exercise tolerance/performance were assessed in the same exercise test for a valid investigation of the issue.

Research Questions Addressed

To investigate $\dot{V}O_2$ kinetics as a determinant of exercise performance, this thesis set about answering the following questions in the eight experimental chapters:

1) Does RST elicit greater adaptations in $\dot{V}O_2$ kinetics and exercise tolerance than does work-matched conventional endurance training?
2) Does IMT improve exercise tolerance through modulating aspects of $\dot{V}O_2$ kinetics?
3) What is the optimal prior exercise/recovery regime to elicit maximal gains in exercise tolerance and what is the $\dot{V}O_2$ profile that accompanies this enhanced performance?
4) How does the pacing strategy adopted and the event duration interact to determine $\dot{V}O_2$ kinetics and exercise performance?
5) What is the influence of dietary supplementation with the NOS substrate, L-arginine, on $\dot{V}O_2$ kinetics and exercise tolerance?
6) What is the influence of dietary nitrate supplementation on $\dot{V}O_2$ kinetics and exercise tolerance?
7) Does dietary nitrate supplementation (a substrate for NO synthesis) influence muscle bioenergetics?
8) Does administration of the antioxidant, N-acetylcysteine, influence NO bioavailability, $\dot{V}O_2$ kinetics and exercise tolerance?

Summary of the Main Findings

The Influence of Whole Body Exercise Training

Chapter 4 sought to determine whether RST was more effective for provoking adaptations in $\dot{V}O_2$ kinetics and exercise tolerance compared to work-matched continuous endurance training (ET). It was demonstrated that phase II $\dot{V}O_2$ kinetics was speeded during both moderate (25%) and severe (21%) exercise with the $\dot{V}O_2$ slow component also reduced (23%) during severe exercise following RST. These parameters were not significantly different following ET. The use of NIRS in this investigation allowed inferences to be made regarding the mechanistic bases for the changes in the $\dot{V}O_2$ kinetics. The changes in $\dot{V}O_2$ kinetics following RST were accompanied by changes in the NIRS derived [HHb] signal, a proxy for muscle O_2 extraction, whereas following ET, no significant changes in [HHb] kinetics were noted. These RST-induced changes in [HHb] kinetics were significantly related to the RST-induced changes in $\dot{V}O_2$ kinetics. These data were considered to be supportive of the metabolic inertia hypothesis for the control of $\dot{V}O_2$ kinetics. With regards to the research question of this thesis, the key finding from this investigation was that severe exercise tolerance was significantly enhanced following RST (53%; Table 12.1), which improved parameters of $\dot{V}O_2$ kinetics, whereas exercise tolerance was not significantly improved by work-matched ET, which did not elicit significant adaptations to $\dot{V}O_2$ kinetics. These data suggested that the extent to which a training intervention is ergogenic may be related, at least in part, to improved $\dot{V}O_2$ kinetics.

The Influence of Inspiratory Muscle Training

It was demonstrated in chapter 4 that whole body exercise training could improve exercise tolerance through modulating $\dot{V}O_2$ kinetics and that these changes in $\dot{V}O_2$ kinetics were related to on changes in muscle O_2 extraction. While it has been demonstrated that specific

training of the inspiratory muscles improves exercise performance, an effect that has been postulated to result from an increased muscle blood flow, it is unclear if this is linked to improved $\dot{V}O_2$ kinetics. The purpose of chapter 5 was therefore to determine whether IMT could improve exercise tolerance through modifying aspects of the dynamic $\dot{V}O_2$ response during exercise. IMT improved $\dot{V}O_2$ kinetics during severe exercise (8% increase in the fundamental amplitude, 12% reduction in the $\dot{V}O_2$ slow component and a 21% speeding in overall $\dot{V}O_2$ kinetics) and maximal exercise (8% increase in the fundamental amplitude and a 36% reduction in the $\dot{V}O_2$ slow component), whereas during moderate exercise, which did not cause inspiratory muscle fatigue, IMT did not improve $\dot{V}O_2$ kinetics. The SHAM control intervention did not elicit adaptations to $\dot{V}O_2$ kinetics and did not influence the tolerable duration of severe or extreme exercise. However, following the IMT intervention, where changes in $\dot{V}O_2$ dynamics were observed, severe and extreme exercise tolerance were improved by 39% and 18%, respectively (Table 12.1). It was speculated that IMT improved muscle perfusion, resulting in a modification of the $\dot{V}O_2$ response amplitudes and a subsequent improvement in exercise tolerance.

The Optimal Prior Exercise/Recovery Protocol

It was demonstrated in chapter 5 that changing the $\dot{V}O_2$ response amplitudes without changing the phase II τ conferred performance benefits. These specific changes in $\dot{V}O_2$ kinetics are also consistently observed during supra-GET exercise following a prior bout of supra-GET exercise; however, this priming-induced $\dot{V}O_2$ profile is not always accompanied by enhanced exercise tolerance. Thus, in chapter 6 a variety of different prior exercise permutations were investigated where both the prior exercise intensity (either 40 or 70% Δ) and the recovery duration separating the priming and criterion exercise bouts (3, 9 or 20 min) were manipulated. This approach was used to establish the optimal combination of

these variables required to maximise performance gains in the criterion exercise bout. It was of interest to observe the $\dot{V}O_2$ profile that accompanied this optimal priming condition and how it differed from sub optimal priming permutations. The findings of chapter 6 revealed that exercise tolerance was: not significantly influenced by 40% Δ priming exercise, irrespective of the intervening recovery duration; compromised following 70% Δ priming with 3 minutes recovery (-16%); improved following 70% Δ priming with 9 minutes recovery (+ 15%); and maximised following 70% Δ priming with 20 minutes recovery (+ 30%; Table 12.1). However, improved $\dot{V}O_2$ kinetics were observed following 40% Δ priming with 3 minutes recovery, which did not improve exercise tolerance. Moreover, $\dot{V}O_2$ kinetics was enhanced to the greatest extent following 70% Δ priming with 3 minutes recovery, where exercise tolerance was compromised, whereas following 70% Δ priming with 20 minutes recovery, where exercise tolerance was optimised, the changes in $\dot{V}O_2$ kinetics was smaller than those observed following 70% Δ priming with both 3 and 9 minutes of recovery. These data demonstrated that, in the context of prior exercise, $\dot{V}O_2$ kinetics does not explain changes in exercise tolerance, at least in isolation. It was interesting to note that performance was best when $\dot{V}O_2$ kinetics was enhanced, the blood [lactate] had returned to ~ 3mM, and the baseline $\dot{V}O_2$ (prior to the onset of the criterion bout) had been restored to the prior exercise baseline $\dot{V}O_2$. In contrast, performance was compromised, despite improved $\dot{V}O_2$ kinetics, when the blood [lactate] and baseline $\dot{V}O_2$ were considerably elevated (in the 70-3-80 condition). Assuming an elevated baseline $\dot{V}O_2$ reflects an incomplete PCr resynthesis (Rossiter *et al*., 1999), these data suggested that $\dot{V}O_2$ kinetics may interact with metabolite accumulation and the depletion of the finite anaerobic reserves (i.e., the metabolic determinants of the W′) to determine exercise tolerance.

The Interaction between Pacing Strategy and Event Duration

The pacing strategy adopted during short-duration high-intensity exercise has implications for both the dynamics of the $\dot{V}O_2$ response and exercise performance. As the event duration is extended beyond 3 min, the influence of pacing strategy on exercise performance is controversial and its influence on $\dot{V}O_2$ kinetics has yet to be investigated. Chapter 7 was therefore concerned with determining whether pacing strategy influenced short duration exercise performance through improving $\dot{V}O_2$ kinetics and if pacing strategy influenced $\dot{V}O_2$ kinetics and exercise performance in a similar manner as the event duration was extended. The key findings from chapter 7 were that $\dot{V}O_2$ kinetics was fastest following a FS, slowest following a SS and intermediate following an ES for both the 3 and 6 minute exercise trials. In the 3 minutes trials, exercise performance was optimised with a FS but was similar with SS and ES strategies, whereas in the 6 minute trials exercise performance was similar across the three pacing strategies despite significant differences in the $\dot{V}O_2$ kinetics. If $\dot{V}O_2$ kinetics was the sole factor determining exercise performance then, compared to ES, exercise performance would have been compromised in the SS trial and improved in the FS trial for both the 3 and 6 minute conditions. That this was not the case implies that factors other than, or in conjunction with, $\dot{V}O_2$ kinetics determines exercise tolerance. It is noteworthy in this regard that in the 6 min trials the $\dot{V}O_{2max}$ was attained in all cases and exercise performance was unaffected. However, in the 3 min trials, a FS resulted in the attainment of the $\dot{V}O_{2max}$ and an improved exercise performance (Table 12.1), whereas the $\dot{V}O_{2max}$ was not attained in the SS and ES conditions and exercise performance was unaffected. These findings are therefore consistent with the findings of chapter 6 and indicate that $\dot{V}O_2$ kinetics alone does not determine exercise performance, but through interaction with other physiological parameters; in this case the $\dot{V}O_{2max}$.

Influence of L-arginine Supplementation

The remaining experimental chapters of this thesis were focused on further exploring the influence of NO on $\dot{V}O_2$ kinetics and, specifically, how potential changes to parameters of $\dot{V}O_2$ kinetics that accompanied an increased NO synthesis/bioavailability might alter high-intensity exercise tolerance. Acute dietary supplementation with the NOS substrate, L-arginine, was investigated in chapter 8. In line with an elevated NO production, L-arginine supplementation increased the plasma [NO_2^-] and reduced systolic blood pressure. During severe exercise, the fundamental $\dot{V}O_2$ amplitude was increased (+ 7%), the $\dot{V}O_2$ slow component was reduced (-24%) and exercise tolerance was enhanced (+ 20%; Table 12.1) following L-arginine supplementation. Also, remarkably, acute L-arginine supplementation reduced the O_2 cost of moderate-intensity cycling exercise. These data suggested that an increased NO synthesis from the NOS pathway may enhance exercise efficiency.

Influence of Dietary Nitrate Supplementation

Chapter 8 demonstrated that increasing NO production through the conventional NOS pathway was ergogenic and that this effect was accompanied by improved $\dot{V}O_2$ kinetics. An additional NO generating pathway has also been identified, whereby NO_3^- can be reduced to NO_2^- and thence NO. Chapter 9 investigated whether similar effects to those observed following L-arginine supplementation were manifest following dietary NO_3^- supplementation, administered as NO_3^- -rich beetroot juice. Consistent with chapter 8, dietary NO_3^- increased markers of NO synthesis, increased the fundamental $\dot{V}O_2$ amplitude (+ 7%), reduced the $\dot{V}O_2$ slow component (-23%) and enhanced exercise tolerance (+ 16%; Table 12.1). Furthermore, dietary NO_3^- supplementation also reduced the O_2 cost of moderate-intensity cycling exercise. These data suggested that the effects of increased NO on the parameters of $\dot{V}O_2$ kinetics and exercise tolerance are similar irrespective of its

pathway of generation and are supportive of a link between $\dot{V}O_2$ kinetics and exercise tolerance.

Influence of Nitric Oxide on Muscle Metabolism

The mechanistic bases for the changes in exercise efficiency observed in chapters 8 and 9 were unclear but could be consequent to a reduced O_2 cost of ATP resynthesis (increased mitochondrial P/O ratio), a reduced ATP cost of force production, an impaired mitochondrial function with a compensatory increased in anaerobic energy liberation, or some combination thereof. Accordingly, chapter 10 sought to investigate the mechanistic bases for the NO mediated changes in $\dot{V}O_2$ kinetics and exercise tolerance. Dietary NO_3^- supplementation reduced both the pulmonary $\dot{V}O_2$ (-25%) and muscle PCr (-35%) amplitudes during low-intensity exercise, and the pulmonary $\dot{V}O_2$ (-52%) and muscle PCr slow component (-59%; Table 12.1) amplitudes during high-intensity exercise. These data suggested that dietary NO_3^- supplementation reduced the O_2 cost of low- and high-intensity exercise, predominantly, by reducing the ATP cost of muscle force production. In addition NO_3^- supplementation improved high-intensity exercise tolerance by 25% and this was accompanied by a reduced accumulation of ADP and P_i and a retarded rate of muscle PCr depletion. These data suggested that NO_3^- supplementation improved $\dot{V}O_2$ dynamics by reducing PCr hydrolysis and improved exercise tolerance by reducing the degree of intramuscular metabolic perturbation.

Influence of Antioxidant Administration

Hypothetically, administration of an antioxidant has the potential to increase NO synthesis during exercise through preserving the catalytic activity of the NOS enzymes and increasing NO bioavailability through scavenging the potent NO scavenger, O_2^-. To investigate this issue the powerful antioxidant, NAC, was administered via intravenous infusion in chapter 11. However, unlike NO_3^- and L-arginine supplementation, which increased markers of NO synthesis, plasma [NO_2^-] was not significantly altered with NAC infusion. Pulmonary $\dot{V}O_2$ kinetics and exercise tolerance were also not significantly different following NAC administration. It was concluded that antioxidant administration does not improve NO synthesis and/or bioavailability, $\dot{V}O_2$ kinetics or exercise tolerance in healthy adult humans. Despite these seemingly negative findings from this particular study, there were a couple of noteworthy observations: 1) NO synthesis and/or bioavailability was not significantly different and neither was $\dot{V}O_2$ kinetics, 2) $\dot{V}O_2$ kinetics was not significantly different and neither was exercise tolerance. These findings therefore do not oppose the notion that NO is an important determinant of $\dot{V}O_2$ kinetics and that $\dot{V}O_2$ kinetics is an important determinant of exercise tolerance.

$\dot{V}O_2$ Kinetics as a Determinant of Exercise Tolerance

The main focus of this series of experiments was to investigate how $\dot{V}O_2$ kinetics influenced exercise tolerance/performance. To this end, a number of interventions that have been demonstrated to alter $\dot{V}O_2$ kinetics were used to assess how these changes in $\dot{V}O_2$ kinetics influenced the ability to tolerate intense exercise (Table 12.1). The first intervention that was investigated was whole-body exercise training. Although a number of longitudinal studies have demonstrated that a period of exercise training can provoke improved $\dot{V}O_2$ kinetics (Hickson *et al*., 1978; Hagberg *et al*., 1980; Phillips *et al*., 1995; Womack *et al*., 1995; Carter *et al*., 2000; Demarle *et al*., 2001; Billat *et al*., 2002; Saunders *et al*., 2003; Berger *et al*., 2006b; Daussin *et al*., 2008; Mckay *et al*., 2009), only a limited number of these investigations attempted to link the improvements in $\dot{V}O_2$ kinetics to the changes in exercise tolerance (Demarle *et al*., 2001; Billat *et al*., 2002; Daussin *et al*., 2008; Mckay *et al*., 2009). In chapter 4, just six sessions of RST resulted in a 21% speeding in the phase II τ, a 24% reduction in the $\dot{V}O_2$ slow component and a 13% speeding in overall $\dot{V}O_2$ kinetics, and these changes in $\dot{V}O_2$ kinetics were accompanied by a 53% improvement in severe exercise tolerance (Table 12.1). Work-matched ET did not significantly alter $\dot{V}O_2$ kinetics and exercise tolerance was not significantly altered. These data suggest that the improved exercise tolerance that is manifest following a period of exercise training is linked, at least in part, to the improved $\dot{V}O_2$ kinetics.

Table 12.1: Summary of the changes in V_{O_2} kinetics and exercise performance reported in the experimental chapters.

Chapter number	Intervention	Comparison	Intensity	Percentage change in V_{O_2} kinetics	Percentage change in exercise performance
Four	RST	RST pre *vs*. RST post	Severe	• Phase II τ (21% speeding) • V_{O_2} slow component (23% reduction)	• 59% increase in time-to-exhaustion at 70% Δ
Five	IMT	IMT pre *vs*. IMT post	Severe	• Fundamental amplitude (8% increase) • V_{O_2} slow component (12% reduction) • Overall V_{O_2} kinetics (21% speeding)	• 39% increase in time-to-exhaustion at 60% Δ
			Extreme	• Fundamental amplitude (8% increase) • V_{O_2} slow component (36% reduction)	• 16% increase in time-to-exhaustion at $V_{O_{2max}}$

Table 12.1: Summary of the changes in V_{O_2} kinetics and exercise performance reported in the experimental chapters.

Chapter number	Intervention	Comparison	Intensity	Percentage change in V_{O_2} kinetics	Percentage change in exercise performance
Six	Prior exercise	70-3-80 *vs*. Control	Severe	• Fundamental amplitude (73% increase) • V_{O_2} slow component (54% reduction) • Overall V_{O_2} kinetics (41% speeding)	• 16% reduction in time-to-exhaustion at 80% Δ
		70-9-80 *vs*. Control		• Fundamental amplitude (73% increase) • V_{O_2} slow component (40% reduction) • Overall V_{O_2} kinetics (32% speeding)	• 15% improvement in time-to-exhaustion at 80% Δ
		70-20-80 *vs*. Control		• Fundamental amplitude (55% increase) • V_{O_2} slow component (26% reduction) • Overall V_{O_2} kinetics (23% speeding)	• 30% improvement in time-to-exhaustion at 80% Δ

Table 12.1: Summary of the changes in V_{O_2} kinetics and exercise performance reported in the experimental chapters.

Chapter number	Intervention	Comparison	Intensity	Percentage change in V_{O_2} kinetics	Percentage change in exercise performance
Seven	Pacing	3-FS *vs*. 3-ES	Extreme	• Overall V_{O_2} kinetics (24% speeding) • O_2 deficit (12% reduction)	• 7% increase in total sprint work done
		3-FS *vs*. 3-SS		• Overall V_{O_2} kinetics (44% speeding) • O_2 deficit (38% reduction)	• 10% increase in total sprint work done
Eight	L-arginine	L-arginine *vs*. placebo	Severe	• Fundamental amplitude (8% increase) • V_{O_2} slow component (24% reduction) • Phase II τ (21% slowing)	• 20% increase in time-to-exhaustion at 70% Δ

Table 12.1: Summary of the changes in V_{O_2} kinetics and exercise performance reported in the experimental chapters.

Chapter number	Intervention	Comparison	Intensity	Percentage change in V_{O_2} kinetics	Percentage change in exercise performance
Nine	Dietary Nitrate	Dietary nitrate *vs*. placebo	Severe	• Fundamental amplitude (7% increase) • V_{O_2} slow component (23% reduction)	• 16% increase in time-to-exhaustion at 70% Δ
Ten	Dietary Nitrate	Dietary nitrate *vs*. placebo	High-intensity	• V_{O_2} slow component (52% reduction)	• 25% increase in time-to-exhaustion at 35% MVC

The viewpoint that $\dot{V}O_2$ kinetics is a direct determinant of exercise tolerance was also supported by the findings reported in chapter 5. Specifically, following a period of IMT, the fundamental $\dot{V}O_2$ amplitude was increased (+ 9%), with no significant change in the phase II τ, the $\dot{V}O_2$ slow component reduced (- 12%), the overall τ speeded (-21%) and severe exercise tolerance was extended (+ 39%; Table 12.1). The improved tolerance of extreme exercise (+ 18%) following IMT was also accompanied by changes in the fundamental (+ 8%) and slow component (- 36%) $\dot{V}O_2$ amplitudes. However, when parameters of $\dot{V}O_2$ kinetics were not significantly influenced, in the SHAM condition, exercise tolerance was not significantly different. These findings corroborate the findings of chapter 4 in demonstrating that $\dot{V}O_2$ kinetics is an important determinant of exercise tolerance, and extend these findings by demonstrating that exercise tolerance can be enhanced, albeit by a smaller magnitude, even without changes in phase II τ. Based on these findings, the improved exercise performance that has often been observed following specific training of the respiratory muscles (Voliantis *et al*., 2001; Romer *et al*., 2002; McConnell and Romer, 2004; Griffiths and McConnell, 2007) appears to be linked to improved $\dot{V}O_2$ kinetics.

These initial findings implied that the link between $\dot{V}O_2$ kinetics and exercise tolerance was straightforward insofar as interventions that improved $\dot{V}O_2$ kinetics also improved exercise tolerance and the magnitude of improvement in exercise tolerance appeared to be linked to the magnitude of improvement in $\dot{V}O_2$ kinetics. In chapter 6, however, prior exercise at 70% Δ with 3, 9 and 20 minutes recovery speeded overall $\dot{V}O_2$ kinetics by 41%, 32% and 23%, respectively, when compared to an unprimed control condition, whereas exercise tolerance was compromised (- 16%), enhanced (+ 15%) and optimised (+ 30%; Table 12.1) in the respective prior exercise condition. Exercise tolerance was also not significantly

different following 40% Δ priming with 3 minutes recovery despite the fact that overall $\dot{V}O_2$ kinetics was speeded. If $\dot{V}O_2$ kinetics was the sole determinant of exercise tolerance then exercise tolerance would have been expected to be optimised following 70% Δ prior exercise with 3 minutes recovery, where the greatest speeding in overall $\dot{V}O_2$ kinetics was observed, not following 70% Δ prior exercise with 20 minutes recovery, where comparatively smaller changes in $\dot{V}O_2$ kinetics was observed. Moreover, exercise tolerance would have been expected to be enhanced following 40% Δ priming with 3 minutes recovery where $\dot{V}O_2$ kinetics was speeded. These data demonstrate that $\dot{V}O_2$ kinetics and exercise tolerance can be dissociated under certain conditions. In addition, these data are also consistent with the disparate findings in the prior exercise literature with reports of enhanced (Jones *et al*., 2003a; Burnley *et al*., 2005; Carter *et al*., 2005), compromised (Wilkerson *et al*., 2004b; Ferguson *et al*., 2007) and unchanged (Koppo and Bouckaert, 2002; Burnley *et al*., 2005; Carter *et al*., 2005) exercise performance despite manifestation of the characteristic effects of prior exercise on $\dot{V}O_2$ kinetics. Based on these data, if $\dot{V}O_2$ kinetics does influence exercise tolerance then it must do so through interacting with other physiological parameters.

The complexity of the interplay between $\dot{V}O_2$ kinetics and exercise tolerance was also exemplified by the findings presented in chapter 7. It was demonstrated that $\dot{V}O_2$ kinetics was faster, relative to SS, in the ES and FS starting strategies, and in FS compared to ES, in both the 3 and 6 minute exercise trials. In the 3 minute trial initiated with FS pacing strategy, where $\dot{V}O_2$ kinetics was fastest, exercise performance was enhanced compared to the ES and SS conditions (Table 12.1); however, despite the fact that $\dot{V}O_2$ kinetics was slower in SS compared to ES, exercise performance was not significantly different between these two conditions. Moreover, in spite of similar changes in $\dot{V}O_2$ kinetics in the 3 and 6

minute exercise trials, exercise performance was only enhanced in FS, relative to ES and SS, in the 3 minute trials. Therefore, while this study shows that the enhanced exercise performance that has been observed during short duration exercise initiated with a FS pacing strategy is linked to improved $\dot{V}O_2$ kinetics (Ariyoshi *et al*., 1979; Bishop *et al*., 2002; Jones *et al*., 2008; Aisbett *et al*., 2009a, b; Hettinga *et al*., 2009), it is clear that the pacing-induced changes in $\dot{V}O_2$ kinetics do not directly influence exercise performance in longer duration events. It remains unclear why exercise performance is not compromised with SS pacing strategies where $\dot{V}O_2$ kinetics is slowest.

While NO production has a potent influence on $\dot{V}O_2$ kinetics (Jones *et al*., 2003b, 2004b; Wilkerson *et al*., 2004a), the influence of NO on muscle force production (Kobzick *et al*., 1994; Murrant *et al*., 1994; Murrant and Barclay, 1995; Morrison *et al*., 1996; Murrant *et al*., 1997; Reid, 1998; Ferreira and Reid, 2008) and exercise performance (Colombani *et al*., 1999; Stevens *et al*., 2000; Buford and Koch, 2004; Campbell *et al*., 2006; Liu *et al*., 2009; Rassaf et al., 2007; Allen *et al*., 2010; Dreissigacker *et al*., 2010) is controversial. In chapter 8, where NO production was increased by the administration of L-arginine, the fundamental $\dot{V}O_2$ amplitude was increased (+ 8%), with a trend for a slower phase II τ (+ 15%), the $\dot{V}O_2$ slow component was reduced (- 24%), and severe exercise tolerance was extended (+ 20%; Table 12.1). These data suggest that potentiating NO production improves exercise tolerance (allowing the same sub maximal force production to be maintained for a greater duration). This change in exercise tolerance was observed when some of the parameters of $\dot{V}O_2$ kinetics were improved. These findings were essentially corroborated by the findings presented in chapter 9 where NO production was increased by dietary NO_3^- supplementation. Indeed, with a NO_3^- enriched diet exercise tolerance was enhanced by 16%, the fundamental $\dot{V}O_2$ amplitude was increased (+ 7%), a slower phase II

τ (+ 21%) was observed and the $\dot{V}O_2$ slow component was reduced (- 23%; Table 12.1). A novel finding from this investigation was that despite the slower phase II τ, exercise tolerance was enhanced with NO_3^- supplementation as the $\dot{V}O_2$ slow component was reduced. These data indicate that the $\dot{V}O_2$ slow component may be a more important determinant of exercise tolerance than the phase II τ during severe exercise.

Using ^{31}P-MRS, the muscle metabolic milieu following NO_3^- supplementation was examined in chapter 10. It was revealed that the improved $\dot{V}O_2$ kinetics invoked by NO_3^- supplementation was accompanied by a reduction in the accumulation of ADP and P_i, metabolites that have been implicated in the process of muscle fatigue (Allen *et al*., 2008) and a retarded rate of hydrolysis of the finite muscle PCr reserves. These data are important as they identify the metabolic corollaries of improved $\dot{V}O_2$ kinetics and help elucidate how interventions that improve $\dot{V}O_2$ kinetics may be ergogenic. Indeed, it appears that NO_3^- supplementation is ergogenic as it offsets the attainment of critically high levels of fatigue-inducing metabolites and critically low levels of the finite PCr and glycogen substrates facilitating a greater tolerable duration of intense exercise. Based on these data interventions that attenuate the degree of metabolic perturbation appear to alter the stimulation of $\dot{V}O_2$ and reduce fatigue facilitating an enhanced exercise tolerance.

In the final experimental chapter it was found that administration of the pharmacological antioxidant, NAC, did not significantly impact on $\dot{V}O_2$ kinetics and exercise tolerance. While it would clearly be too simplistic to conclude that NAC was not ergogenic as it failed to improve $\dot{V}O_2$ kinetics, within the context of this thesis it supports the notion that interventions that fail to improve $\dot{V}O_2$ kinetics are less likely to be ergogenic.

In summary, this thesis has demonstrated that $\dot{V}O_2$ kinetics has an important influence on exercise tolerance. Nonetheless, scenarios have been identified in the experimental chapters whereby the changes in $\dot{V}O_2$ kinetics do not correspond to the changes in exercise tolerance. Accordingly, it appears that other parameters must interact with $\dot{V}O_2$ kinetics to elicit performance changes. The potential candidates in this regard are discussed in the following section.

Parameters that Interact with $\dot{V}O_2$ Kinetics to Influence Exercise Tolerance

$\dot{V}O_{2max}$

During severe exercise, the $\dot{V}O_{2max}$ dictates the extent to which the $\dot{V}O_2$ slow component can develop and the rate of development of the $\dot{V}O_2$ slow component determines how rapidly the $\dot{V}O_{2max}$ will be attained (Burnley and Jones, 2007); thus, there is a clear interdependence between these two physiological parameters. That is, when one of these parameters is modulated it has direct consequences for the other parameter and the interaction between these two parameters also has direct functional implications for exercise tolerance (Berger *et al*., 2006a; Burnley *et al*., 2006). Indeed, interventions that increase the $\dot{V}O_{2max}$ increase both the $\dot{V}O_2$ slow component and the tolerable duration of severe exercise (Berger *et al*., 2006a), while interventions that reduce the $\dot{V}O_{2max}$ reduced both the $\dot{V}O_2$ slow component and severe exercise tolerance (Burnley *et al*., 2006). The interactive influence of these parameters in determining exercise tolerance is also supported by findings presented in the current thesis. Specifically, severe exercise tolerance was enhanced to a greater extent following RST, which increased the $\dot{V}O_{2max}$ and reduced the $\dot{V}O_2$ slow component, when compared to IMT and L-arginine and NO_3^- supplementation, which reduced the $\dot{V}O_2$ slow component with no change in the $\dot{V}O_{2max}$. An intervention which increases the $\dot{V}O_{2max}$ and reduces the $\dot{V}O_2$ slow component would enhance exercise tolerance to a greater extent than

would be elicited by the exclusive improvement of one of these parameters, through delaying the point of rapid depletion of the finite anaerobic reserves and accumulation of fatiguing metabolites.

The findings of this thesis also demonstrate that an interaction between $\dot{V}_{O_2}$ kinetics and the $\dot{V}_{O_{2max}}$ is evident during extreme exercise. In chapter 5, a period of IMT, which increased the fundamental $\dot{V}_{O_2}$ amplitude and reduced the $\dot{V}_{O_2}$ slow component, permitted the attainment of the $\dot{V}_{O_{2max}}$ (indicative of severe exercise) and improved exercise tolerance, whereas fatigue ensued before the attainment of the $\dot{V}_{O_{2max}}$ prior to IMT (indicative of extreme exercise). Therefore, the improved exercise tolerance with IMT was consequent, at least in part, to enhanced $\dot{V}_{O_2}$ kinetics, which facilitated the attainment of the $\dot{V}_{O_{2max}}$, resulting in a greater total energy yield from oxidative metabolism.

Support for the postulate that $\dot{V}_{O_2}$ kinetics interacts with the $\dot{V}_{O_{2max}}$ to determine extreme exercise tolerance is also found in the findings of chapter 7. It was shown that when high-intensity short-duration exercise was initiated with a FS pacing strategy, overall $\dot{V}_{O_2}$ kinetics was speeded compared to exercise initiated with ES and SS strategies. Importantly, the faster $\dot{V}_{O_2}$ kinetics with FS resulted in the attainment of the $\dot{V}_{O_{2max}}$ and exercise performance was enhanced, while the comparatively slower $\dot{V}_{O_2}$ kinetics in ES and SS prevented the attainment of the $\dot{V}_{O_{2max}}$ and exercise performance was compromised, relative to FS. The improved $\dot{V}_{O_2}$ kinetics invoked by a FS resulted in an intensity-domain shift in the $\dot{V}_{O_2}$ response such that extreme-intensity exercise was transformed into severe-intensity exercise with a corresponding improvement in exercise performance. Further support for the interactive influence of $\dot{V}_{O_2}$ kinetics and the $\dot{V}_{O_{2max}}$ in determining exercise performance can be found in the observations made during the 6 minute trials. Here, the

$\dot{V}_{O_{2max}}$ was attained with all of the pacing strategies investigated and the faster $\dot{V}_{O_2}$ kinetics with FS did not elicit performance enhancement. These data demonstrated that faster $\dot{V}_{O_2}$ kinetics, *per se*, did not improve exercise performance. Rather, when the faster $\dot{V}_{O_2}$ kinetics allowed the attainment of the $\dot{V}_{O_{2max}}$, and thus an additional oxidative energy yield, performance was enhanced. Therefore, this thesis provides firm support for the hypothesis that the interaction between $\dot{V}_{O_2}$ kinetics and the $\dot{V}_{O_{2max}}$ is an important determinant of endurance exercise performance, as hypothesised by Burnley and Jones (2007) and Jones and Burnley (2009).

W′

During severe exercise performed above the CP, the tolerable duration of exercise is a function of the curvature constant of the hyperbolic power-duration relationship (W′; Monod and Scherrer, 1965; Poole *et al*., 1988; Jones *et al*., 2010). The W′ represents a fixed and finite amount of mechanical work that can be completed above the CP before task failure during severe exercise. Accordingly, interventions that influence the size, or rate of utilization, of the W′ would be expected to have predictable ramifications for exercise tolerance, provided that the CP was not also altered. Although not precisely known, the determinant(s) of the W′ have been hypothesised to be either the potential for ATP yield from substrate-level phosphorylation and/or the accumulation of fatigue-related metabolites (Monod and Scherrer, 1965; Moritani *et al*., 1981 Poole *et al*., 1988; Fukuba *et al*., 2003; Jones *et al*., 2008). Assuming this to be true, an increase in substrate-level phosphorylation and metabolite accumulation would increase the depletion of the W′ and fatigue would be attained more rapidly. Conversely, fatigue development would be retarded by interventions that attenuate substrate-level phosphorylation and metabolite accumulation owing to a reduced rate of W′ utilisation. Through influencing the magnitude

of the O_2 deficit, which is indicative of the extent of PCr and glycogen depletion and metabolite accumulation (Jones and Poole, 2005), the parameters of $\dot{V}O_2$ kinetics have an important role in characterising the rate at which the W′ is depleted. A number of findings in this thesis can be forwarded to support the postulate that exercise tolerance is determined through the interaction between the $\dot{V}O_2$ kinetics and the W′ (Burnley and Jones, 2007, Jones and Burnley, 2009).

The first evidence in this thesis that can be cited to support the interaction between $\dot{V}O_2$ kinetics and the W′ in determining exercise tolerance is provided in chapter 4. RST speeded phase II $\dot{V}O_2$ kinetics and reduced the $\dot{V}O_2$ slow component and this resulted in a reduction in blood lactate accumulation (suggestive of a reduction in anaerobic glycolysis and muscle acidosis) and an improved severe exercise tolerance. A period of RST has also been shown to increase the W′ (Jenkins and Quigley, 1993) and muscle [glycogen] (Gibala *et al*., 2006); to reduce muscle lactate accumulation (Burgomaster *et al*., 2006), glycogen utilisation (Burgomaster *et al*., 2006, 2008) and PCr hydrolysis (Burgomaster *et al*., 2006, 2008); and to increase muscle buffering capacity (Gibala *et al*., 2006). Collectively, these data indicate that RST can increase the W′ and, through eliciting adaptations to $\dot{V}O_2$ kinetics (as evidenced in chapter 4), retard the rate of utilization of the W′, which provides a logical explanation for the RST-induced improvement in exercise tolerance.

An increase in the W′ has also been reported following training of the respiratory muscles (Johnson *et al*., 2007). In chapter 5, IMT improved $\dot{V}O_2$ kinetics during severe exercise and this was accompanied by a reduction in blood lactate accumulation, which would be expected to indicate a reduced depletion of the W′, and an improved exercise tolerance. Extreme exercise tolerance was also enhanced following IMT and this occurred in concert

with improved $\dot{V}O_2$ kinetics. Therefore, these data suggest that both RST and IMT improve exercise tolerance through improving $\dot{V}O_2$ kinetics and the W′.

In chapter 6, it was demonstrated that the prior exercise condition that provoked the greatest speeding in overall $\dot{V}O_2$ kinetics, but also the greatest metabolic perturbation prior to the criterion exercise bout (as evidenced by the highest blood [lactate] and $\dot{V}O_2$ in the baseline preceding the criterion bout), resulted in an impaired exercise tolerance. However, exercise tolerance was optimised when the recovery duration separating the prior and criterion bouts was elongated as overall $\dot{V}O_2$ kinetics remained speeded, albeit to a smaller extent, the baseline $\dot{V}O_2$ was not significantly elevated and the blood [lactate] was only mildly elevated. Assuming that the baseline $\dot{V}O_2$ is indicative of muscle [PCr] (Rossiter *et al*., 2002) and using blood [lactate] as an indicator of muscle [lactate]/pH, these data imply that the degree of metabolic perturbation prior to the criterion exercise bout has direct implications for the magnitude of W′ that can be expended in that exercise bout. When the W′ available was reduced, as inferred by the considerable elevations in both blood [lactate] and $\dot{V}O_2$, exercise tolerance was compromised despite the greatest speeding of overall $\dot{V}O_2$ kinetics (in the 70-3-80 condition). On the other hand, exercise tolerance was optimised when overall $\dot{V}O_2$ kinetics remained speeded but the inferred W′ had recovered to near baseline values (in the 70-20-80 condition). The findings of this study provide evidence that it is the interaction of $\dot{V}O_2$ kinetics and W′ that explains the changes in exercise tolerance rather than the influence of $\dot{V}O_2$ kinetics, *per se*.

Interaction between $\dot{V}O_{2max}$ and W′

While the previous sections have focused on the interaction between $\dot{V}O_2$ kinetics and the $\dot{V}O_{2max}$ and $\dot{V}O_2$ kinetics and the W′, there is also evidence to link the attainment of the

$\dot{V}O_{2max}$ with the complete utilisation of the W′ (Jones *et al*., 2003a). Indeed, Jones *et al*. (2003a) showed a strong trend for the W′ to be increased following prior supra-GET exercise which facilitated the attainment of the $\dot{V}O_{2max}$ compared to the control condition where the $\dot{V}O_{2max}$ was not attained (Figure 12.1). Importantly, this priming-induced attainment of the $\dot{V}O_{2max}$ and increase in the W′ was accompanied by an improved exercise tolerance. These data are essential as they intricately link $\dot{V}O_2$ kinetics, the $\dot{V}O_{2max}$ and the W′ and help establish a 'triad' of parameters that interact to determine exercise tolerance (Burnley and Jones, 2007).

Application of the Triad Model

Given that the original findings presented in this thesis can be accounted for by the interaction of three physiological parameters, namely, $\dot{V}O_2$ kinetics, the $\dot{V}O_{2max}$ and the W′, it is justifiable to propose a model to explain exercise tolerance. This 'triad model' (Figure 12.2) represents an important addition to this field as existing models of fatigue do not adequately explain fatigue in the severe and extreme exercise intensity domains.

Firstly, let us consider the interventions that elevated NO synthesis in chapters 8 and 9. While there is currently no evidence that an increased NO production alters the W′, and it has been shown that short term dietary interventions (< 6 days) that increase NO production do not increase the $\dot{V}O_{2max}$ (Vanhatalo *et al*. 2010), this thesis has shown that the $\dot{V}O_2$ slow component is reduced by interventions that increase NO production (chapters 8-10). Provided that the W′ is unaltered with an elevated NO, these data indicate that the 16-20% improvements in exercise tolerance observed in these studies can be attributed to the improved $\dot{V}O_2$ kinetics, which would retard the utilization of the W′ and delay the attainment of the $\dot{V}O_{2max}$. However, following the RST intervention, which improved $\dot{V}O_2$

kinetics (speeded phase II τ and reduced $\dot{V}_{O_2}$ slow component) and increased the $\dot{V}_{O_{2max}}$, and has been shown to increase the W′ (Jenkins and Quigley, 1993), exercise tolerance was improved by 53%. The greater magnitude of performance enhancement with RST may be attributed to the abundant physiological adaptations provoked by this intervention that, in turn, had a greater impact on the triad determinants of exercise tolerance and thus the tolerable duration of exercise. Indeed, the elevated W′ would permit more supra-CP work to be completed prior to fatigue and when coupled with the improved $\dot{V}_{O_2}$ kinetics, this larger W′ would be depleted less rapidly further augmenting the tolerable duration of exercise. The greater $\dot{V}_{O_{2max}}$, combined with the reduced $\dot{V}_{O_2}$ slow component, would also delay the attainment of the $\dot{V}_{O_{2max}}$ and retard the depletion of W′ (Burnley and Jones, 2007). Using the integrated triad model, it is obvious why RST resulted in a greater improvement in exercise tolerance than did IMT.

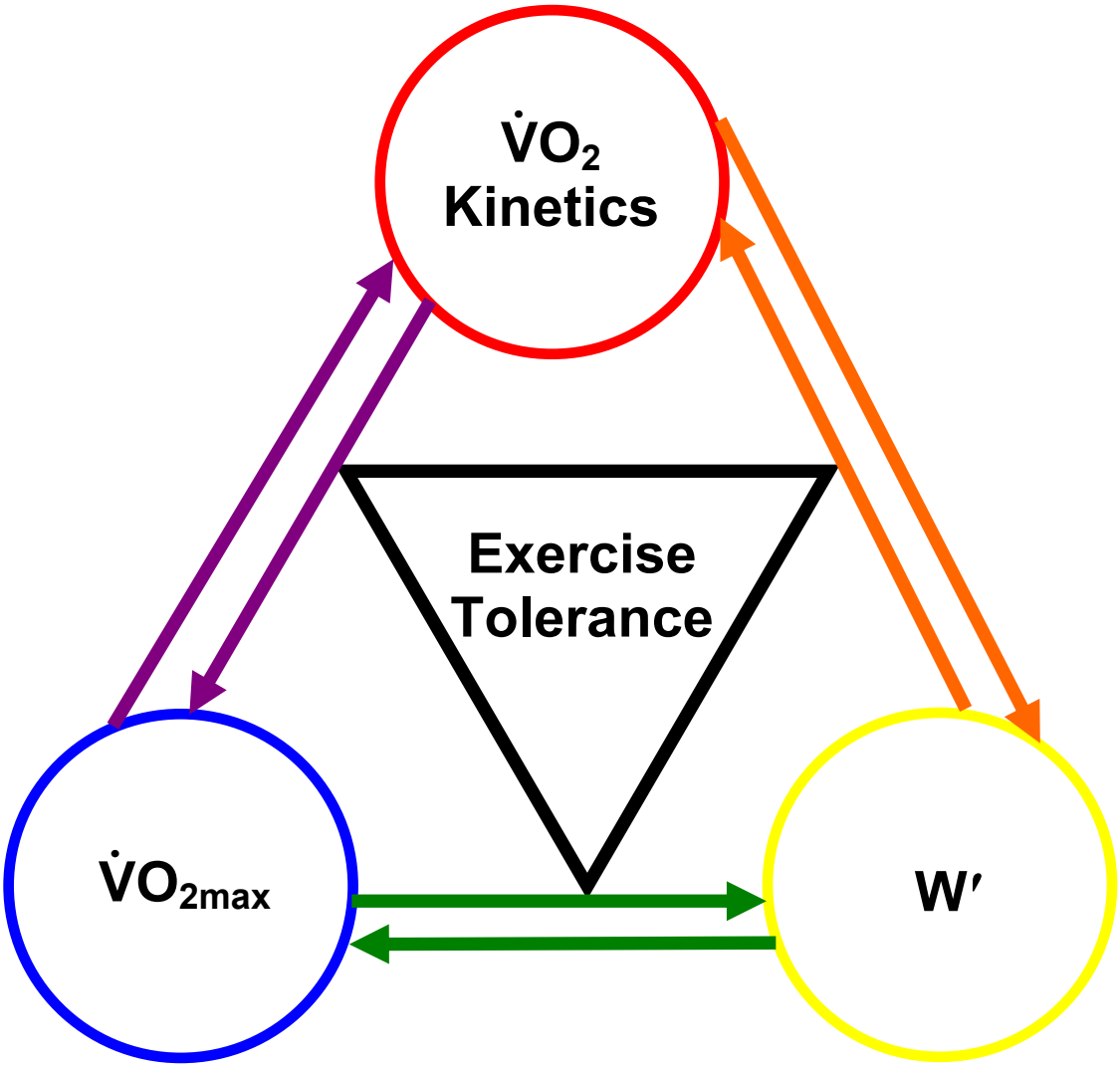

Figure 12.1: Schematic of the triad model of exercise tolerance. This model shows that $\dot{V}_{O_2}$ kinetics, the $\dot{V}_{O_{2max}}$ and the W′ interact with one another to determine the tolerable duration of exercise performed above the critical power. See text for further details.

By considering the interaction of these three key physiological parameters, rather than assessing each parameter individually, this model can also be used to explain the apparently complex findings presented in chapters 6 and 7. When prior exercise speeded overall $\dot{V}_{O_2}$ kinetics, exercise tolerance was only enhanced when the W′, inferred from measurement of $\dot{V}_{O_2}$ and blood [lactate] in the baseline, was not depleted (70-9-80 and 70-20-80). Where the W′ was depleted (in the 70-3-80 condition), the speeding in overall $\dot{V}_{O_2}$ kinetics was unable to compensate and exercise tolerance was compromised. When the recovery

duration was extended to 9 minutes to allow a partial reconstitution of the W′, exercise tolerance was enhanced despite the fact that the magnitude of speeding in overall $\dot{V}_{O_2}$ kinetics was reduced compared to 70-3-80. However, when the recovery duration was extended to 20 minutes to allow a near complete restoration of the W′ and a preservation of the faster overall $\dot{V}_{O_2}$ kinetics relative to the control condition, exercise tolerance was maximised. Therefore, this performance enhancement was manifest when the largest portion of the W′ was available, when $\dot{V}_{O_2}$ kinetics was still enhanced and when the attainment of the $\dot{V}_{O_{2max}}$ was delayed. Without reference to the triad concept, it might have been concluded, erroneously, that $\dot{V}_{O_2}$ kinetics is not an important determinant of exercise tolerance.

The observation that exercise performance was enhanced during a 3 minute trial, but not during a 6 minute trial initiated with a FS strategy, despite the observation that $\dot{V}_{O_2}$ kinetics was faster with FS in both duration trials, also indicates that $\dot{V}_{O_2}$ kinetics, alone, cannot regulate exercise performance. Upon closer inspection of the data, it was shown that the $\dot{V}_{O_{2max}}$ was attained in all the 6 minute trials but that the $\dot{V}_{O_{2max}}$ was only achieved in the 3 minute trial initiated with a FS. Thus, the 3 minute FS trial was ergogenic, relative to 3-ES and 3-SS, because the faster $\dot{V}_{O_2}$ kinetics increased the oxidative energy yield by permitting the attainment of the $\dot{V}_{O_{2max}}$ and this would also be expected to enable the complete utilisation of the W′ (Jones *et al*., 2003a) thereby increasing the total energy liberation in this condition. In the 6 minute FS trial, however, the duration for which the FS power output had to be maintained was doubled and this would be expected to result in a significant consumption of the W′. Therefore, it is likely that performance enhancement was not observed in the 6 minute FS trial as $\dot{V}_{O_{2max}}$ was achieved in all conditions and the

faster $\dot{V}O_2$ kinetics was negated by a greater initial W′ depletion. These data provide further support for the triad concept as a model to explain exercise performance (Figure 12.2).

In summary and in line with the aims set out at the end of the literature review, this thesis has examined the extent to which $\dot{V}O_2$ kinetics is a determinant of exercise tolerance and how $\dot{V}O_2$ kinetics interacts with other physiological parameters to influence exercise tolerance. The findings of this thesis demonstrate that for $\dot{V}O_2$ kinetics to be considered as an important determinant of exercise tolerance/performance it must be considered as a component of the triad model as it cannot unilaterally account for changes in exercise tolerance in all experimental scenarios (Figure 12.2).

Limitations

Constant Work Rate Tests to Exhaustion

The purpose of this thesis was to investigate $\dot{V}O_2$ kinetics as a determinant of exercise tolerance and as such an accurate and valid determination of these parameters is essential for a robust investigation of this question. In order to compare the interaction between $\dot{V}O_2$ kinetics and exercise tolerance, it was necessary to assess these parameters during the same exercise test. The conventional approach to assess pulmonary $\dot{V}O_2$ kinetics requires the administration of a 'step' exercise test whereby the work rate is abruptly increased from an 'unloaded' baseline to a target work rate. However, while this approach enhances the validity of the investigation of $\dot{V}O_2$ kinetics as a determinant of exercise tolerance, it limits the generalisation of these findings to 'true' endurance sports performance where success is not determined by the ability to sustain a given sub maximal power output for the longest possible time. Accordingly, time-to-exhaustion tests have a lower ecological validity and are less reliable compared to time-trial (where the aim is to cover a given distance in the

fastest time possible) or distance/power-trial (where the aim is to cover the greatest distance or generate the highest power possible over a set time period) performance tests (e.g., Jeukendrup *et al*., 1996; Laursen *et al*., 2007). Importantly, however, Amann and colleagues (2008) recently demonstrated that time-to-exhaustion and time-trial tests had a similar sensitivity in detecting a change in exercise performance with different levels of arterial hypoxaemia. The authors contended that while the error of measurement is higher with time-to-exhaustion trials, the magnitude of change is also far greater such that the sensitivity of these performance tests is similar in detecting a change in exercise performance with an intervention. Taken together with the benefits for the collection and interpretation of the $\dot{V}O_2$ kinetics data, a constant work rate time-to-exhaustion test seemed most appropriate for use in this thesis.

Is Pulmonary $\dot{V}O_2$ an Accurate Proxy for Muscle $\dot{V}O_2$?

Given the purpose of this thesis, it is essential that the pulmonary $\dot{V}O_2$ signal is reflective of the muscle $\dot{V}O_2$ signal. As discussed in the general methods, a limitation associated with the use of pulmonary $\dot{V}O_2$ is the breath-by-breath variability in the response. In an attempt to circumvent this limitation subjects completed repeat transitions and the individual transitions were filtered to remove errant breaths (those lying more than 4 standard deviations outside the local mean) and then averaged in order to increase the signal-to-noise ratio and enhance the confidence in the parameters of interest. When these procedures have been adopted it has been shown that pulmonary $\dot{V}O_2$ kinetics is a close proxy of muscle $\dot{V}O_2$ kinetics (Barstow *et al*., 1990; Poole *et al*., 1991 Grassi *et al*., 1996; Krustrup *et al*., 2009). It is also important to note that pulmonary $\dot{V}O_2$ indicates the whole-body O_2 deficit which may be more important during whole body exercise.

Is Plasma [Nitrite] Reflective of Muscle [Nitrite]?

While it is recognised that the plasma [NO_2^-] is a sensitive marker of NO synthesis (Lauer *et al*., 2001, 2002; Rassaf *et al*., 2007), it is unclear if plasma [NO_2^-] is indicative of muscle [NO_2^-]. In chapters 9 and 10, where dietary NO_3^- was administered to increase NO_2^- and facilitate an increased NO production, there was an improvement in muscle contractile efficiency and this was accompanied by an increase in plasma [NO_2^-]. That a similar response was observed in chapter 8 with L-arginine demonstrates that these effects can be ascribed to an increase in NO production. It has been shown that NO_2^- reduction to NO occurs predominantly in the tissues and not in the blood (Li *et al*., 2008) and as such the extent of NO synthesis following dietary NO_3^- supplementation would be more accurately revealed by assessing the muscle [NO_2^-]. The extent to which plasma [NO_2^-] reflects muscle [NO_2^-] is currently unclear (Bryan *et al*., 2005; Bryan, 2006) and as such caution must be exercised when making inferences of muscle [NO_2^-] based on plasma measures of [NO_2^-].

Is Electromyographic Data Reflective of Muscle Activation?

In chapters 6 and 10, iEMG was used to infer the patterns of muscle activation. While this technique is informative as it quantifies the gross electrical activity of the muscles, there are a number of limitations with this technique that requires consideration when interpreting the data derived from iEMG. The limitations of the techniques used in chapters 6 and 10 include: surface EMG amplitude cancellation; the iEMG was not normalised to an M wave; the inability to discern an increase in motor unit recruitment from an increased firing rate of the already recruited muscle fibres; a fatigue related reflex inhibition; and spectral compression due to changes in muscle fibre conduction velocity

(Weir *et al*., 2006). It is therefore clear that there are a number of potential limitations with this technique that must be acknowledged.

Is the NIRS Derived [HHb] Signal Reflective of Muscle O_2 Extraction?

The NIRS technique is a useful non-invasive technique to assess muscle oxygenation and has been used to infer muscle O_2 extraction. However, there are limitations with the use of this technique that must be acknowledged. One of the limitations with the NIRS measurements made in chapters 4 and 6-8 was that a one channel device was used. This is problematic as it has been shown that there is considerable spatial heterogeneity in the NIRS response at different measurement sites on the quadriceps muscle during cycle exercise (Koga *et al.*, 2008). In addition, given that NIRS has only recently been used to investigate muscle oxygenation, it is unclear if the optical parameters are influenced during exercise which may compromise their accuracy. Finally, while the NIRS signal is predominantly reflective of the oxygenation of haemoglobin, the signal is also contaminated by light absorption from myoglobin and cytochrome oxidase (Boushel *et al*., 2001) which may influence the inferences drawn from the parameters.

Applications

Notwithstanding the limitations identified above, the findings of this thesis may have important implications for our understanding of the factors that can elicit enhanced exercise performance. While these data were gathered in recreationally active adults and will have implications for exercise performance in this population, it is possible that these findings will also be considered for performance enhancement in trained athletes and for improving the functional capacity in elderly and patient populations. These data demonstrate that when an intervention optimises the parameters that comprise the triad model, then exercise

performance is enhanced. Accordingly, these data would be informative to coaches and clinicians and would help them design interventions, either nutritional or physical, aimed at enhancing these physiological parameters for a subsequent improvement in exercise tolerance/performance. In addition, while exercise performance in this thesis was assessed by the time-to-exhaustion test, every 16% improvement in this test would be expected to correspond to a 1% enhancement in actual race performance (Hopkins *et al*., 1999). Therefore, the improved $\dot{V}_{O_2}$ kinetics provoked by the interventions imposed in this thesis would be expected to correspond to an improvement in actual exercise performance in the range of 1-4% (Table 12.1). This would represent a highly meaningful improvement in exercise performance and would be expected to translate to finishing in a higher position in a race.

The findings that an increased NO production confers physiological and performance benefits, as presented in chapters 8-10, may also have a number of important applications. Not only did the increase in NO reduce blood pressure, which would benefit cardiovascular health, but it also improved exercise efficiency and exercise tolerance. These studies are also useful as they identify practical, cost-effective and short-term dietary interventions that can increase NO production. These dietary approaches could be readily adopted by members of the general public or elite athletes to elicit the aforementioned benefits.

Although not the main focus of this thesis, the improved efficiency during moderate-intensity exercise observed following these NO enhancing dietary interventions is potentially a very exciting finding. It is possible that this intervention may enhance the functional capacity in the elderly and diseased. These populations have a pronounced reduction in the $\dot{V}_{O_{2max}}$ such that a given moderate work bout may represent a larger

fraction of the $\dot{V}_{O2max}$ leading to a greater degree of metabolic perturbation and effort sensation and the early termination of exercise. Through reducing the O_2 cost of moderate-intensity exercise, dietary interventions that increase NO production would be expected to attenuate the above impediments allowing these populations to more readily complete the activities of daily living, enhancing quality of life. These suggestions are substantiated by the recent data of Lansley *et al*. (2011) who demonstrated that dietary NO_3^- supplementation reduced the O_2 cost of walking at 4 $km \cdot h^{-1}$. Therefore, dietary interventions that increase NO production have important implications for improving cardiovascular health, exercise performance in the healthy, and the functional capacity of the elderly and diseased.

Topics for Further Research

The Optimal Experimental Approach

It has recently been contended that for the true extent of functional improvement conferred by an intervention to be appreciated then characterisation of the derivatives of the hyperbolic power-duration relationship, CP and W′, must be calculated (Whipp and Ward, 2009). Additional research that would benefit this topic would impose exercise tests whereby the initial portion of the test comprised a step increment to a given severe constant work rate bout for a fixed period (6 minutes), for accurate characterisation of $\dot{V}_{O2}$ kinetics, followed by a final 'performance' stage where subject would be instructed to complete as much work as possible in a 2 minute period. Not only would this test allow for the appropriate assessment of $\dot{V}_{O2}$ kinetics but it would also provide an ecologically valid performance test. This design would strengthen the ability to detect links between $\dot{V}_{O2}$ kinetics and exercise tolerance. A similar test has already been investigated (Burnley *et al*., 2005) and this test may be considered as more reliable than a time-to-exhaustion test.

Utilisation of Invasive Techniques

Mechanistically, some of the most exciting and novel findings of this thesis were presented in chapters 9 and 10 where dietary NO_3^- supplementation was investigated. It was shown that exercise efficiency was increased and exercise tolerance enhanced following NO_3^- supplementation and that these effects were associated with a reduced rate of total ATP turnover. While these findings are remarkable, there are a number of questions that still require attention to advance this emerging area of research. Firstly, if the NO-mediated improvement in exercise efficiency can be predominantly ascribed to a reduced ATP cost of force production, then what are the mechanisms that elicit this adaptation? It is known that the total myocyte ATP turnover rate is essentially a function of the ATP turnover rates related to Ca^{2+} cycling (determined by Ca^{2+}-ATPase) and the interaction between actin and myosin (determined by actomyosin-ATPase; Barclay *et al*., 2007). Therefore, it is likely that NO exerts its influence on exercise efficiency by influencing the activity of one, or both, of the Ca^{2+}-ATPase and actomyosin-ATPase enzymes. Further research using invasive techniques would allow the activities of these Ca^{2+}-ATPase and actomyosin-ATPase enzymes to be assessed *in vitro* to ascertain the mechanistic bases for this reduced ATP cost of force production.

Although the findings presented in chapter 10 suggest that the improved exercise efficiency with NO_3^- supplementation occurs consequent to a reduced ATP cost of force production, it is not possible to refute an increase in the mitochondrial P/O ratio (reduced O_2 cost of ATP resynthesis) until this parameter is measured following NO_3^- supplementation. Again, this would require the use of invasive techniques to obtain muscle biopsies followed by isolation of the mitochondria for the *in vitro* determination of the mitochondrial P/O ratio (Tonkonogi and Sahlin, 1997). A parameter related to the mitochondrial P/O ratio is the

mitochondrial UCP3 and the expression of this protein, along with the proportion of type I muscle fibres, has been reported to be related to cycling efficiency in humans (Mogensen *et al*., 2006). It would therefore be informative to assess muscle fibre recruitment patterns directly and to assess the expression of UCP3 as potential contributors to the improved exercise efficiency measured following by NO_3^- supplementation.

Given the indirect nature of the measurements used to estimate $\dot{V}_{O_2}$ and efficiency in chapters 8-10, further research is necessary to determine how NO_3^- supplementation impacts on muscle $\dot{V}_{O_2}$, through use of the direct Fick technique (Bangsbo *et al*., 2000), and muscle efficiency, by determining muscle heat production (González-Alonso *et al*., 2000). Likewise, it is unclear if dietary NO_3^- supplementation increases the muscle $[NO_2^-]$ and this warrants further research attention.

The Controversy Surrounding NO and Muscle Force Production

The improved exercise tolerance that was observed following dietary interventions that increased NO in this thesis occurred when the exercise duration was in the range of 6-15 minutes. To what extent an increased NO production influences exercise tolerance during short duration high-intensity exercise (i.e., sprint or maximal type exercise) or long duration low-intensity exercise (i.e., marathon type exercise) is presently unclear. The influence of NO on fatigue is controversial with demonstrations that fatigue is exacerbated (e.g., Grassi *et al*., 2005), attenuated (e.g., Zhu *et al*., 2003) or unaffected (e.g., Zhu *et al*., 2006) by NO. It is possible, therefore, that NO could compromise performance in power based sports if maximal force production is impaired. However, given the finding of a reduced ATP cost of sub maximal force production in chapter 10, it is possible that NO_3^- supplementation could result in a greater maximal force production for the same maximal

ATP turnover rate or an increase in both maximal force production and energy yield. If confirmed this would be expected to predispose to an improved performance in power based sports. Improvements in cycling efficiency (Coyle, 2005) and running economy (Jones, 2006) have important implications for improvement in performance during prolonged endurance events. NO_3^- supplementation may therefore be conducive for long endurance performance. In short, additional research is required to determine the extent of performance enhancement conferred by NO_3^- supplementation.

Translation to Elderly and Pathological Populations

A final area that is worthy of further research would be to extend the findings of the present thesis to elderly and patient populations. Given that $\dot{V}O_2$ kinetics is significantly slower in these populations (Barstow and Scheuermann, 2005; Poole *et al*., 2005) and that phase II $\dot{V}O_2$ kinetics appear amenable to speeding by interventions that increase muscle O_2 availability (DeLorey *et al*., 2004), it is possible that the interventions investigated in this thesis would elicit greater improvements in $\dot{V}O_2$ kinetics and exercise tolerance in these populations. It is also necessary to determine if the triad model is as effective in explaining exercise tolerance in these populations as it appears to be in the subjects who participated in the studies of this thesis.

Conclusion

The kinetics of $\dot{V}O_2$ influences the magnitude of the O_2 deficit and therefore the rate of ATP resynthesis from substrate level phosphorylation and the extent of metabolite accumulation. Improving $\dot{V}O_2$ kinetics reduces the O_2 deficit, with attendant reductions in PCr and glycogen utilisation and fatigue-related metabolite accumulation, and this enhanced $\dot{V}O_2$ response has been accompanied by an enhanced exercise tolerance (e.g., Hagberg *et al*.,

1980). This thesis has confirmed the hypothetical model proposed by Burnley and Jones (2007) by demonstrating that $\dot{V}_{O_2}$ kinetics must interact with other physiological parameters in order to influence exercise tolerance. Based on the findings presented in this thesis, $\dot{V}_{O_2}$ kinetics interacts with the $\dot{V}_{O_{2max}}$ and the W′ to determine the tolerable duration of exercise.

That exercise tolerance is determined by the interaction between these three parameters was substantiated in chapter 6 where it was shown that exercise tolerance was optimised, not by the prior exercise protocol that enhanced $\dot{V}_{O_2}$ kinetics to the greatest extent, but when the prior exercise regime achieved an appropriate balance between the speeding in overall $\dot{V}_{O_2}$ kinetics, the reconstitution of the W′ and the time at which the $\dot{V}_{O_{2max}}$ was attained. Further support for this contention was provided in chapter 7. Here, during a 3 minute exercise trial, the faster $\dot{V}_{O_2}$ kinetics invoked by a FS pacing strategy allowed the $\dot{V}_{O_{2max}}$ to be attained, which would be expected to result in the complete utilisation of the W′ (Jones *et al*., 2003a), and exercise performance was enhanced compared to ES and SS, where $\dot{V}_{O_2}$ kinetics was slower and the $\dot{V}_{O_{2max}}$ was not attained. When the same pacing strategies were applied to a 6 minute exercise trial, $\dot{V}_{O_2}$ kinetics was again fastest with a FS; however, the $\dot{V}_{O_{2max}}$ was achieved in all conditions and exercise performance was not significantly different between the variously paced trials. Collectively, these studies provide clear support for the notion that the performance enhancement afforded by enhanced $\dot{V}_{O_2}$ kinetics is a function of its interaction with the $\dot{V}_{O_{2max}}$ and the W′. That is, if an intervention has adverse effects on the $\dot{V}_{O_{2max}}$ and/or the W′ then exercise performance may be compromised even if $\dot{V}_{O_2}$ kinetics is enhanced (as supported by the findings in chapters 6 and 7), whereas an intervention that augments the $\dot{V}_{O_{2max}}$ and/or the W′ in conjunction with improved $\dot{V}_{O_2}$ kinetics would elicit greater performance gains than if only $\dot{V}_{O_2}$ kinetics was

improved (as demonstrated by the greater improvements in exercise tolerance in chapter 4 compared to chapters 8-9).

In summary, this thesis has presented a triad model to explain exercise tolerance during exercise performed above CP. When considered in isolation, changes in $\dot{V}_{O_2}$ kinetics cannot account for all the changes in exercise performance reported in this thesis. The triad model, on the other hand, is able to explain the changes in exercise performance provoked by the various interventions that were imposed. When evaluating the extent to which $\dot{V}_{O_2}$ kinetics is a determinant of exercise tolerance, the findings of this thesis advocate an integrated rather than an isolated approach.

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