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Inhibition of nitric oxide synthase by L-NAME speeds phase II pulmonary $\dot{V}_{O_2}$ kinetics in the transition to moderate-intensity exercise in man

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There is evidence that the rate at which oxygen uptake ($\dot{V}_{O_2}$) rises at the transition to higher metabolic rates within the moderate exercise intensity domain is modulated by oxidative enzyme inertia, and also that nitric oxide regulates mitochondrial function through competitive inhibition of cytochrome *c* oxidase in the electron transport chain. We therefore hypothesised that inhibition of nitric oxide synthase (NOS) by nitro-L-arginine methyl ester (L-NAME) would alleviate the inhibition of mitochondrial $\dot{V}_{O_2}$ by nitric oxide and result in a speeding of $\dot{V}_{O_2}$ kinetics at the onset of moderate-intensity exercise. Seven males performed square-wave transitions from unloaded cycling to a work rate requiring 90 % of predetermined gas exchange threshold with and without prior intravenous infusion of L-NAME (4 mg kg^{-1} in 50 ml saline over 60 min). Pulmonary gas exchange was measured breath-by-breath and $\dot{V}_{O_2}$ kinetics were determined from the averaged response to four exercise bouts performed in each condition using a mono-exponential function following elimination of the phase I response. There were no significant differences between the control and L-NAME conditions for baseline $\dot{V}_{O_2}$ (means ± S.E.M. 797 ± 32 *vs.* 794 ± 29), the duration of phase I (15.4 ± 0.8 *vs.* 17.2 ± 0.6), or the steady-state increment in $\dot{V}_{O_2}$ above baseline (1000 ± 83 *vs.* 990 ± 85 ml min^{-1}), respectively. However, the phase II time constant of the $\dot{V}_{O_2}$ response was significantly smaller following L-NAME infusion (22.1 ± 2.4 *vs.* 17.9 ± 2.3; $P < 0.05$). These data indicate that inhibition of NOS by L-NAME results in a significant (19 %) speeding of pulmonary $\dot{V}_{O_2}$ kinetics in the transition to moderate-intensity cycle exercise in man. At least part of the intrinsic inertia to oxidative metabolism at the onset of moderate-intensity exercise may result from competitive inhibition of mitochondrial $\dot{V}_{O_2}$ by nitric oxide at cytochrome *c* oxidase, although other mechanisms for the effect of L-NAME on $\dot{V}_{O_2}$ kinetics remain to be explored.

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In the transition to a higher metabolic rate, there is an inherent 'lag' in the rate at which oxygen uptake ($\dot{V}_{O_2}$) rises to meet the increased energetic requirement. There is debate over whether this lag is caused by a limitation in the rate at which O_2 is delivered to working muscle or by the rate at which mitochondria adjust oxidative ATP supply to meet demand (Whipp & Mahler, 1980; Grassi *et al.* 1998; Tschakovsky & Hughson, 1999; Grassi *et al.* 2000). While the $\dot{V}_{O_2}$ kinetics are inevitably influenced by the interaction of both metabolic substrate and O_2 availability (Tschakovsky & Hughson, 1999), there is evidence that the kinetic adjustment of $\dot{V}_{O_2}$ in the transition to a higher work rate within the moderate exercise intensity domain (i.e. exercise that does not cause a significant accumulation of muscle or blood lactate), is predominantly limited by an oxidative enzyme inertia rather than by muscle O_2 supply (Whipp & Mahler, 1980; Yoshida & Whipp, 1994; Grassi *et al.* 1996,1998). However, the precise locus of this oxidative enzyme inertia remains to be firmly established.

Nitric oxide has been implicated in a wide range of physiological functions including neurotransmission, platelet aggregation and the regulation of vasodilatation (Joyner & Dietz, 1997). It has also been demonstrated that nitric oxide may reversibly inhibit mitochondrial respiration, by binding to the O_2-binding site at cytochrome *c* oxidase in the electron transport chain (Cleeter *et al.* 1994; Shen *et al.* 1994; Brown, 2000, 2001). Recently, Kindig *et al.* (2002) reported that inhibition of nitric oxide synthase (NOS) by the L-arginine analogue nitro-L-arginine methyl ester (L-NAME) caused a 32 % reduction in the time constant of the primary $\dot{V}_{O_2}$ response to moderate-intensity treadmill running in the thoroughbred horse, with no change in the steady-state $\dot{V}_{O_2}$. In humans, Frandsen *et al.* (2001) established that muscle $\dot{V}_{O_2}$ after 10 and 20 min of sub-maximal exercise was not affected by infusion of L-NAME but they did not investigate the $\dot{V}_{O_2}$ kinetic response at exercise onset. It therefore remains to be established whether L-NAME

infusion causes a speeding of $\dot{V}_{O_2}$ kinetics in man. We therefore hypothesised that if nitric oxide is partly responsible for determining the rate of the $\dot{V}_{O_2}$ adjustment at the onset of moderate-intensity exercise in man, then NOS inhibition by L-NAME should lead to a significant speeding of the $\dot{V}_{O_2}$ kinetics.

METHODS

Participants

Seven healthy males (mean ± S.D. age 25 ± 3 years, body mass 77.7 ± 8.3 kg) volunteered to participate in this study. All participants were informed of the experimental procedures, the potential risks and discomfort, and that they could withdraw from the study at any time. All participants gave their written informed consent. The experiments were approved by the Manchester Metropolitan University and South Cheshire Local Research Ethics Committees and conformed to the declaration of Helsinki.

Procedures

The participants were required to visit the laboratory on five occasions. On the first visit to the laboratory, the participants completed an incremental exercise test in order to determine the gas exchange threshold (GET) and $\dot{V}_{O_2peak}$ (see below). The remaining four visits to the laboratory were used to complete the experimentation. On each of these visits, the participants completed two bouts of moderate-intensity cycle exercise. On two occasions, the exercise bouts were preceded by infusion of L-NAME (see below). The participants therefore performed a total of four bouts of moderate-intensity exercise in each condition (i.e. two exercise bouts on each of two days). The conditions were presented in random order, and were separated by at least 48 h. All exercise tests were performed on an electronically braked cycle ergometer (Jaeger Ergoline E800, Germany).

Incremental exercise test

Following 3 min of 'unloaded' cycling, the work rate was increased by 5 W every 10 s (i.e. 30 W min^{-1}) until the participant was unable to continue. The participants cycled at a self-selected pedal rate (60–90 rev min^{-1}) and this pedal rate and the saddle and handlebar height and configuration were recorded and reproduced in subsequent tests. Pulmonary gas exchange was measured on a breath-by-breath basis (see below). The $\dot{V}_{O_2peak}$ was determined as the highest value recorded in any 30 s period before the participant's volitional termination of the test. The GET was determined as the first disproportionate increase in $\dot{V}_{CO_2}$ from visual inspection of individual plots of $\dot{V}_{CO_2}$ *versus* $\dot{V}_{O_2}$ by an experienced reviewer, and the work rate that would require 90 % of the $\dot{V}_{O_2}$ at GET was calculated. In the present study, it was important that the work rate was below the GET in order that the results were not complicated by possible changes in O_2 delivery resulting from NOS inhibition, since $\dot{V}_{O_2}$ kinetics above the GET may be determined, in part, by O_2 delivery (Tschakovsky & Hughson, 1999).

Square-wave exercise tests

The moderate-intensity exercise bouts were performed with and without prior infusion of L-NAME. Our protocol for the L-NAME infusion was based on that described by Frandsen *et al.* (2001) who demonstrated that this infusion protocol resulted in a 67 % reduction in NOS activity in skeletal muscle. Participants first rested for a 20 min period, before a cannula was placed in a hand vein and L-NAME (4 mg (kg body mass)$^{-1}$ in 50 ml saline) was infused over 60 min. Throughout the infusion, blood pressure and heart rate were monitored. Following an additional 30 min rest, the participants mounted the cycle ergometer and the exercise protocol commenced. The exercise protocol began with 3 min of baseline pedalling at 20 W (the lowest available work rate on the cycle ergometer), followed by an abrupt transition to the 90 % GET work rate for 6 min. Following 3 min rest, this procedure (3 min of baseline pedalling and 6 min exercise at 90 % GET) was repeated. This 'double square-wave' protocol was completed on four separate days, twice with and twice without prior infusion of L-NAME.

Pulmonary gas exchange was measured breath-by-breath and heart rate was monitored by short-range telemetry (Polar Electro Oy, Kempele, Finland) throughout all exercise tests. Subjects wore a nose-clip and breathed through a low dead space, low resistance mouthpiece and volume sensor assembly. Pulmonary gas exchange was measured with a mass spectrometer and volume turbine system (Morgan EX670, Morgan Medical Limited, Gillingham, Kent). The system was calibrated prior to each test with gases of known concentration. A fingertip blood sample was collected into a capillary tube immediately before and after one of the exercise bouts in each condition and subsequently analysed for blood [lactate] (YSI 1500 Sport lactate analyser, Yellow Springs Instruments, Ohio, USA).

Analysis of $\dot{V}_{O_2}$ kinetics

The breath-by-breath $\dot{V}_{O_2}$ data for each transition were interpolated to give second-by-second values and time-aligned to the start of exercise. For each participant and each condition, the four repeat transitions were then averaged in order to enhance the underlying response characteristics. The baseline $\dot{V}_{O_2}$ was defined as the average $\dot{V}_{O_2}$ measured during unloaded cycling between 160 and 20 s before the start of exercise. The cardio-dynamic component (phase I) was ignored by eliminating the first 20 s of data after the onset of exercise. Subsequently, non-linear regression techniques were used to fit the remaining $\dot{V}_{O_2}$ data with an exponential function:

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2baseline} + A(1 - e^{-(t - TD)/\tau}).$$

This model includes an amplitude (A), a time constant (τ) and a delay time (TD). An iterative process was used in order to minimise the sum of squared error between the fitted function and the observed values.

To provide an indication of the overall rate of $\dot{V}_{O_2}$ adaptation, the 'mean response time' (MRT; the sum of the time constant and time delay values) and the O_2 deficit (product of the MRT and the steady-state amplitude of the $\dot{V}_{O_2}$ response above baseline) were calculated.

Statistics

Paired-samples t tests were used to test for significant differences in the $\dot{V}_{O_2}$ kinetic parameters between the control and L-NAME conditions with significance declared when $P < 0.05$. Results are reported as means ± S.E.M. unless otherwise stated.

RESULTS

The participants' mean ± S.D. $\dot{V}_{O_2peak}$ was 49.4 ± 5.6 ml kg^{-1} min^{-1} with GET occurring at 51 ± 8 % $\dot{V}_{O_2peak}$. The mean ± S.D. increase in work rate above baseline cycling (20 W) to 90 % GET was 104 ± 26 W.

As would be expected for exercise in the moderate-intensity domain, blood [lactate] did not rise significantly

Table 1. Heart rate (HR) and blood [lactate] responses following infusion of L-NAME compared to control

Subject number	Baseline HR		End-exercise HR		Δ blood [lactate]	
	Control	L-NAME	Control	L-NAME	Control	L-NAME
	(beats min^{-1})		(beats min^{-1})		(mM)	
1	94	73	115	108	0.7	0.6
2	66	65	107	113	0.1	0.1
3	76	65	113	107	0.5	0.3
4	100	72	127	109	0.0	−0.2
5	88	75	125	115	0.1	−0.1
6	87	67	109	105	0.3	0.4
7	84	77	109	107	−0.2	−0.5
Mean	85	71*	115	109	0.2	0.1
S.E.M.	4	2	3	1	0.1	0.1

*Significantly different from control condition ($P < 0.01$).

above pre-exercise values either in the control or L-NAME conditions (Δ[lactate] 0.2 ± 0.1 mM and 0.1 ± 0.1 mM respectively; Table 1). Furthermore, the $\dot{V}_{O_2}$ data were well fitted by a mono-exponential function with delay, i.e. the exercise did not elicit the $\dot{V}_{O_2}$ 'slow component' phenomenon that is evident during exercise above the GET.

At rest, during the L-NAME infusion, blood pressure was significantly higher, and heart rate significantly lower compared to the control condition ($P < 0.05$). Compared to control, heart rate was lower following L-NAME infusion during unloaded cycling (84 ± 4 *vs.* 71 ± 2 beats min^{-1}; $P < 0.01$) while the end-exercise heart rate tended to be lower (115 ± 3 *vs.* 109 ± 1 beats min^{-1}), (Table 1).

The $\dot{V}_{O_2}$ kinetic response data are presented in Table 2, and the response of a typical subject is shown in Fig. 1. Infusion of L-NAME did not significantly affect baseline $\dot{V}_{O_2}$, the steady-state $\dot{V}_{O_2}$ amplitude, or the time delay. However, L-NAME infusion resulted in a significant speeding of the $\dot{V}_{O_2}$ kinetic response (τ reduced from 22.1 ± 2.4 to

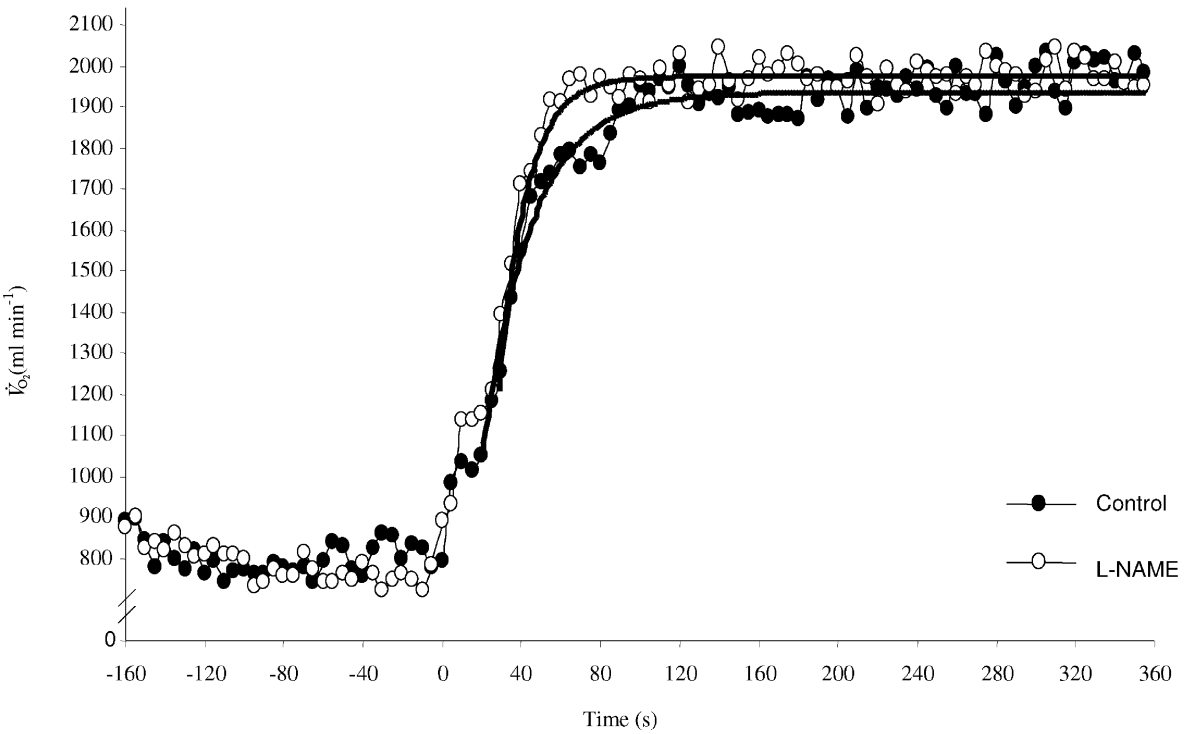

Figure 1. $\dot{V}_{O_2}$ kinetic response in the transition to moderate intensity exercise following infusion of L-NAME (○) and in control condition (●)

Notice the faster adjustment in the L-NAME condition. Continuous lines represent mono-exponential curve fits to the data.

Table 2. $\dot{V}_{O_2}$ kinetics in the transition to moderate intensity exercise following infusion of L-NAME compared to control

Subject number	Baseline $\dot{V}_{O_2}$		$\dot{V}_{O_2}$ amplitude		End-exercise $\dot{V}_{O_2}$		Time delay		$\dot{V}_{O_2}$ time constant	
	Control	L-NAME	Control	L-NAME	Control	L-NAME	Control	L-NAME	Control	L-NAME
	(ml min^{-1})		(ml min^{-1})		(ml min^{-1})		(s)		(s)	
1	773	804	1098	1016	1891	1820	17.0	19.6	28.5	26.1
2	737	720	840	796	1577	1516	13.3	16.1	13.4	8.1
3	789	795	1212	1195	2001	1990	12.7	17.0	25.0	14.7
4	940	892	1128	1184	2068	2076	15.9	16.0	22.0	21.5
5	844	854	1232	1245	2076	2099	17.8	15.0	16.9	17.3
6	673	668	685	714	1358	1382	13.6	17.6	30.8	22.8
7	825	828	808	782	1633	1610	17.8	18.8	18.0	14.9
Mean	797	794	1000	990	1801	1785	15.4	17.2	22.1	17.9*
S.E.M.	32	29	83	85	106	108	0.8	0.6	2.4	2.3

* Significantly different from control condition ($P < 0.05$).

17.9 ± 2.3 s; $P < 0.05$). The MRT of the response (from 37.5 ± 2.4 s to 35.1 ± 2.6 s; $P < 0.05$) and the O_2 deficit (617 ± 60 *vs.* 588 ± 69 ml; $P < 0.05$) were significantly reduced in the L-NAME condition.

DISCUSSION

We have demonstrated that infusion of L-NAME leads to a 19 % speeding of the phase II pulmonary $\dot{V}_{O_2}$ kinetic response in the transition to a higher metabolic rate within the moderate exercise intensity domain in man. To our knowledge, this is the only intervention that has ever been shown to cause a speeding of pulmonary $\dot{V}_{O_2}$ kinetics during moderate intensity upright cycle exercise in young, healthy participants. The mean response time was also significantly reduced by L-NAME despite the fact that the duration of phase I (reflected in the time delay term) was increased. These results extend the earlier findings of Kindig *et al.* (2001) who demonstrated that infusion of L-NAME resulted in a 32 % speeding of $\dot{V}_{O_2}$ kinetics during moderate intensity treadmill running in the thoroughbred horse. Collectively, these studies indicate that reducing nitric oxide inhibition of cytochrome *c* oxidase may remove some of the inertia to mitochondrial respiration and allow an acceleration of $\dot{V}_{O_2}$ kinetics in the transition to a higher metabolic rate within the moderate exercise intensity domain.

It has been reported that nitric oxide activates soluble guanylate cyclase in vascular smooth muscle resulting in vascular relaxation and increased tissue blood flow (Moncada *et al.* 1991). However, it is presently unclear whether NOS inhibition results in a reduction in skeletal muscle blood flow during exercise. In a number of species, there is evidence that cardiac output and/or muscle blood flow are reduced during exercise, and that O_2 extraction at the muscle is correspondingly increased following NOS inhibition (Hirai *et al.* 1994; O'Leary *et al.* 1994; Kindig *et al.* 2000; Shen *et al.* 2000). In contrast, several studies in humans have reported no difference in skeletal muscle blood flow during exercise, at least in the steady state, between NOS-inhibited and control conditions (Bradley *et al.* 1999; Radegran & Saltin, 1999; Frandsen *et al.* 2001). Although one study with human volunteers indicated that NOS inhibition reduced skeletal muscle blood flow (Dyke *et al.* 1995), it should be noted that blood flow was estimated using plethysmography during pauses in contraction in that study. This is consistent with evidence that NOS inhibition might indeed reduce skeletal muscle blood flow during recovery from exercise (Frandsen *et al.* 2001). We did not measure muscle blood flow in the present study. However, the ~13 beats min^{-1} lower heart rate at the onset of exercise in the L-NAME condition suggests that there might have been a slightly lower cardiac output and muscle blood flow early in the transition from rest to exercise. The longer duration of phase I, while not statistically significant, is also consistent with there having been a more sluggish cardiac output response at exercise onset. It is important to emphasise here that despite the increase in blood pressure that is noted following L-NAME infusion, our results cannot be explained by an increased bulk muscle blood flow or O_2 delivery (Frandsen *et al.* 2001). Therefore, NOS inhibition speeded the pulmonary $\dot{V}_{O_2}$ kinetics in phase II despite the probability that there was no change in (or even a slightly lower and/or slower) bulk O_2 delivery to the working muscle in the transition from unloaded pedalling to moderate intensity exercise. It is possible, however, that the lower heart rate in the L-NAME condition, presumably resulting from baroreceptor-mediated sympathetic withdrawal, reflected a reduction in blood flow to areas with low metabolic activity due to vasoconstriction in the vascular beds of these tissues, and that blood flow to active muscle was preserved. Although very unlikely (see Frandsen *et al.* 2001), it is possible that blood flow to active muscle

was greater and/or faster following L-NAME infusion. However, even if this were true, an increased muscle blood flow and/or O_2 availability would not be expected to result in faster $\dot{V}_{O_2}$ kinetics during exercise of this type (Gerbino *et al.* 1996; MacDonald *et al.* 1997; Grassi *et al.* 1998; Burnley *et al.* 2000).

It is pertinent here to consider the extent to which the measurement of $\dot{V}_{O_2}$ kinetics at the lung reflects the kinetics of O_2 consumption at the muscle. Where there are no perturbations to muscle blood flow, there is evidence that pulmonary $\dot{V}_{O_2}$ kinetics in phase II provide a close estimate (to within ~10 %) of muscle $\dot{V}_{O_2}$ kinetics (Barstow *et al.* 1990; Grassi *et al.* 1996; Rossiter *et al.* 1999). However, this relationship becomes more complicated where there are changes in the cardiovascular response to exercise (Barstow *et al.* 1990; Essfeld *et al.* 1991), as may be the case following NOS inhibition. Using a mathematical modelling approach, Barstow *et al.* (1990) predicted that for the same muscle $\dot{V}_{O_2}$ kinetics, slower cardiac output kinetics would result in a lengthening of phase I and apparently faster phase II pulmonary $\dot{V}_{O_2}$ kinetics (it is important to note, here, however, that these effects were quantitatively rather small and cannot fully explain the difference we observed between the L-NAME and control conditions in the present study). Interestingly, when the relationship between muscle and pulmonary $\dot{V}_{O_2}$ kinetics was distorted by changes in cardiac output kinetics, the calculated O_2 deficit was unaffected (Barstow *et al.* 1990) suggesting that in these conditions the O_2 deficit might still be used to infer changes in muscle $\dot{V}_{O_2}$ kinetics. In the present study, the O_2 deficit was significantly reduced following L-NAME infusion which, given that there were no differences in either baseline or steady-state $\dot{V}_{O_2}$, may be taken as evidence that muscle $\dot{V}_{O_2}$ kinetics were speeded if it is assumed that: (1) steady-state blood flow, and (2) changes in venous O_2 stores, were similar between conditions (Barstow *et al.* 1990). The first of these assumptions seems reasonable (Frandsen *et al.* 2001), but the likelihood of there being a reduction in venous volume owing to vasoconstriction in non-working vascular beds following L-NAME infusion makes it difficult to estimate the latter. However, a large reduction in venous volume would be expected to result in a reduction in the duration of phase I (which we did not observe) and to have relatively little impact on the phase II time constant (where we observed a significant speeding), (Barstow *et al.* 1990). In the present study, it can be estimated ($\Delta[O_2$ stores$] = \Delta\dot{V}_{O_2}$ ss (MRT $- \tau$); Whipp & Ward, 1982) that there was relatively little difference between the change in O_2 stores in the control condition (257 ml O_2) and the L-NAME condition (284 ml O_2). Therefore, we believe that the changes in pulmonary $\dot{V}_{O_2}$ kinetics that we observed following L-NAME infusion – which included reductions in the phase II time constant, mean response time, and O_2 deficit – reflected a speeding of muscle $\dot{V}_{O_2}$ kinetics. However, further studies involving direct measures of muscle blood flow and O_2 extraction across a working muscle are required to confirm this.

There was considerable inter-individual variability in the effect of L-NAME on $\dot{V}_{O_2}$ kinetics (0–41 % speeding; Table 2). The reason for the lack of effect in two of our seven participants is unclear. One possibility is that L-NAME did not cause a substantial inhibition of NOS in these participants. It is interesting to note, however, that the two participants whose $\dot{V}_{O_2}$ kinetics were not speeded by L-NAME had the greatest reductions in exercise heart rate (numbers 4 and 5; see Tables 1 and 2). It is therefore possible that the effect of NOS inhibition on relieving mitochondrial inertia was masked by a substantial reduction in O_2 delivery to skeletal muscle in these participants. On the other hand, as discussed above, the lower heart rate following L-NAME infusion might also be related to a reduction in the requirement for blood flow because of an increased O_2 extraction at the muscle or as a consequence of peripheral vasoconstriction in tissue with relatively low metabolic activity.

It is well known that muscle P_{O_2} can modulate tissue respiratory control so that an appropriate $\dot{V}_{O_2}$ is attained during submaximal exercise (Wilson *et al.* 1977). However, at least during moderate intensity exercise, it appears that muscle P_{O_2} does not become sufficiently low that muscle $\dot{V}_{O_2}$ is compromised. Therefore, O_2 availability cannot be considered limiting in this situation. Indeed, there is evidence that, under normal control conditions, cardiac output and/or muscle blood flow kinetics are faster than muscle $\dot{V}_{O_2}$ kinetics, and that O_2 delivery is maintained such that it is likely in excess of muscle O_2 requirement at any point in the transition to moderate intensity exercise (Yoshida & Whipp, 1994; Grassi *et al.* 1996; MacDonald *et al.* 1998; see also Bangsbo *et al.* 2000 for higher intensity exercise). Consistent with this, it has been shown that eliminating any delay in O_2 delivery by pump-perfusing canine gastrocnemius muscle at the required steady-state blood flow across the rest-to-exercise transition did not significantly influence $\dot{V}_{O_2}$ kinetics (Grassi *et al.* 1998). Furthermore, increasing O_2 delivery and/or blood flow to muscle through the performance of prior heavy exercise (Gerbino *et al.* 1996; Burnley *et al.* 2000) or the inspiration of hyperoxic gas mixtures (MacDonald *et al.* 1997) do not result in a speeding of phase II $\dot{V}_{O_2}$ kinetics during moderate intensity cycle exercise in man. It therefore appears that, under the conditions of the present study, the speed of the $\dot{V}_{O_2}$ adjustment in the transition to a higher metabolic rate is primarily limited by an inertia within the mitochondrial oxidative machinery.

The exact location of the intra-muscular limitation to $\dot{V}_{O_2}$ kinetics during moderate intensity exercise remains to be firmly established. Significant research attention has been directed to the possible role of pyruvate dehydrogenase

complex (PDC) activation and/or the availability of acetyl groups in limiting mitochondrial ATP production at exercise onset (Timmons *et al.* 1998; Howlett *et al.* 1999). These studies demonstrated that prior activation of the PDC by infusion of dichloroacetate (DCA) resulted in a marked attenuation of substrate-level phosphorylation, presumably due to an increased mitochondrial ATP production. However, a number of recent studies have reported that DCA infusion did not affect muscle $\dot{V}_{O_2}$ kinetics or substrate-level phosphorylation during exercise. In dogs, Grassi *et al.* (2002) reported that $\dot{V}_{O_2}$ kinetics were unchanged despite significant accumulation of acetyl groups before muscle contraction at ~65 % $\dot{V}_{O_2peak}$. In humans, DCA infusion did not reduce substrate-level phosphorylation (Bangsbo *et al.* 2002; Savasi *et al.* 2002) nor alter muscle $\dot{V}_{O_2}$ kinetics (Bangsbo *et al.* 2002) during maximal intensity exercise. It has recently been suggested that the PDC and/or acetyl group availability may only limit mitochondrial respiration at high intensities of submaximal exercise (~65–90 % $\dot{V}_{O_2peak}$) where there is a mismatch between the demands of the TCA cycle for substrate and the degree of PDC activation (Roberts *et al.* 2002). While the influence of PDC activation on $\dot{V}_{O_2}$ kinetics during moderate intensity exercise in humans requires further attention, it is likely that acetyl group availability is not limiting during low to moderate intensity exercise (< 50 % $\dot{V}_{O_2peak}$) such as that used in the present study (Evans *et al.* 2001; Watt *et al.* 2002).

There is a growing body of evidence that nitric oxide regulates mitochondrial function by inhibiting cytochrome *c* oxidase, the terminal enzyme in the electron transport chain which is responsible for virtually all O_2 consumption in mammals (Shen *et al.* 1994, 2000; Brown, 2000). Nitric oxide has a high affinity for the O_2-binding site of cytochrome *c* oxidase when this site is reduced and therefore competes with O_2 for the binding site. This competitive inhibition of $\dot{V}_{O_2}$ by nitric oxide might serve to reduce the reliance on muscle O_2 extraction in meeting the O_2 requirement during exercise, therefore allowing maintenance of a higher intra-muscular P_{O_2} (Shen *et al.* 2000). Indeed, it has been suggested that the competition between nitric oxide and O_2 at cytochrome *c* oxidase might increase the sensitivity of mitochondrial respiration to O_2 concentration because of an increase in the Michaelis-Menten constant (K_m) of cytochrome *c* oxidase for O_2 (Brown, 1995). It has also been suggested that nitric oxide might extend the zone of effective tissue respiration by increasing the O_2 gradient away from the blood vessel (Thomas *et al.* 2001). Nitric oxide synthase (NOS), the enzyme responsible for synthesis of nitric oxide, is located within the skeletal muscle vascular endothelium as well as within myocytes (Frandsen *et al.* 1996). It is known that nitric oxide synthesis is greatly increased at the onset of exercise (Balon & Nadler, 1994) and that this may be attenuated with the use of L-arginine analogues such as L-NAME. The procedure we used in the present study for L-NAME infusion (4 mg (kg body mass)$^{-1}$ infused over 60 min) has been shown to result in a 67 ± 8 % reduction in muscle NOS activity (Frandsen *et al.* 2001), and we therefore postulate that the speeding of $\dot{V}_{O_2}$ kinetics that we observed resulted from a reduction of nitric oxide inhibition of mitochondrial $\dot{V}_{O_2}$ at cytochrome *c* oxidase.

Interestingly, although the speed of the $\dot{V}_{O_2}$ adaptation to exercise was greater in the L-NAME condition, the amplitude of the $\dot{V}_{O_2}$ response above baseline was not different to the control condition. Our finding that the steady-state $\dot{V}_{O_2}$ (established within ~2 min) was not affected is consistent with other studies that have infused NOS inhibitors before exercise in humans (Radegran & Saltin, 1999; Frandsen *et al.* 2001) and other species (O'Leary *et al.* 1994; Mills *et al.* 1999; Kindig *et al.* 2002). For example, in the Frandsen *et al.* (2001) study, leg oxygen uptake was not significantly different between the L-NAME and control conditions after either 10 or 20 min of submaximal exercise. In horses, the steady-state $\dot{V}_{O_2}$ amplitude was unaltered by L-NAME despite significantly faster $\dot{V}_{O_2}$ kinetics (Kindig *et al.* 2002). It therefore appears that the inhibitory effect of nitric oxide on muscle $\dot{V}_{O_2}$ might only be evident in the transition to a higher metabolic rate, and that regulation of muscle blood flow and O_2 extraction enable the same steady-state $\dot{V}_{O_2}$ to be attained. One report that the steady-state muscle $\dot{V}_{O_2}$ was significantly increased during submaximal exercise in conscious dogs following NOS inhibition (Shen *et al.* 2000) is difficult to accept unless muscle efficiency is somehow influenced by nitric oxide in this species.

Although we favour the relief of nitric oxide inhibition of cytochrome *c* oxidase as an explanation for our results since the pernicious influence of nitric oxide at this site has been well documented (Shen *et al.* 1994; Brown, 2000), other possible effects of L-NAME should also be considered. Nitric oxide and its derivatives (reactive nitrogen species) inhibit a number of other enzymes that are involved in energy transduction including creatine kinase, glyceraldehyde-3-phosphate dehydrogenase and aconitase (Zhang & Snyder, 1995; Kaasik *et al.* 1999), as well as respiratory complexes I, II, III, and IV (Cassina & Radi, 1996; Brown, 1999). The inhibition of NOS by L-NAME therefore has the potential to influence exercise $\dot{V}_{O_2}$ by a number of mechanisms. It should also be emphasised that although L-NAME infusion caused a substantial speeding of $\dot{V}_{O_2}$ kinetics in the present study, the majority of the inertia in mitochondrial respiration in the transition to higher metabolic rates remains to be explained.

In conclusion, we have shown that inhibition of nitric oxide synthase by L-NAME resulted in a significant

speeding of pulmonary $\dot{V}_{O_2}$ kinetics in the transition to moderate intensity cycle exercise. This is the first study to demonstrate a speeding of pulmonary $\dot{V}_{O_2}$ kinetics in young healthy subjects performing moderate-intensity upright cycle exercise. These data indicate that inhibition of mitochondrial $\dot{V}_{O_2}$ by nitric oxide (possibly at cytochrome *c* oxidase) might contribute, in part, to the inertia in oxidative metabolism at the onset of moderate-intensity exercise.

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