No acetyl group deficit evident at the onset of exercise at 90% $\dot{V}o_2$ max in humans

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Running title: Dichloroacetate infusion and exercise at 90%VO$_2$max

Abstract

The existence of an acetyl group deficit at ≥90% $\dot{V}o_2$ max has proved controversial, with contradictory results likely relating to limitations in previous research. The purpose of the present study was therefore to test whether or not the 'acetyl group deficit' occurs at the start of exercise at 90% $\dot{V}o_2$ max in a well controlled study. Eight male participants (age: 33.6 ± 2.0 yrs; $\dot{V}o_2$ max: 3.60 ± 0.21 l.min^{-1}) completed two exercise bouts at 90% $\dot{V}o_2$ max for 3-min following either 30-min saline (control) or dichloroacetate (50 mg.kg^{-1}bm) infusion, ending 15-min before exercise. Muscle biopsies were obtained immediately before and following exercise while continuous non-invasive measures of pulmonary oxygen uptake and muscle deoxygenation were made. Muscle pyruvate dehydrogenase activity was significantly higher prior to exercise following dichloroacetate infusion (control: 2.67 ± 0.98) vs dichloroacetate: 17.9 ± 1.1 mmol acetyl-CoA·min^{-1}·mg^{-1} protein, *P*=0.01) and resulted in higher pre- and post-exercise muscle acetylcarnitine (pre ex control: 3.3 ± 0.95 vs pre-ex dichloroacetate: 8.0 ± 0.88 vs post-ex control: 11.9 ± 1.1 vs post-ex dichloroacetate: 17.2 ± 1.1; mmol·kg^{-1} dm, *P*<0.05), yet substrate level phosphorylation (control: 125.0 ± 20 vs dichloroacetate: 113.0 ± 13 mmol adenosine triphosphate·kg^{-1}dm) and $\dot{V}o_2$ kinetics (τ_{Vo_2}), (control: 19.2 ± 2.2 vs dichloroacetate: 22.8 ± 2.5 s), were unaltered. Additionally, dichloroacetate infusion blunted the slow component of $\dot{V}o_2$ & muscle deoxygenation and slowed muscle deoxygenation kinetics, possibly by enhancing oxygen delivery during exercise. These data support the hypothesis that the 'acetyl group deficit' does not occur at ≥90%$\dot{V}o_2$ max.

Introduction

The rise of [adenosine diphosphate] in the exercising muscle is a key stimulant of mitochondrial oxidative phosphorylation at the onset of exercise. However, substrate level phosphorylation (phosphocreatine hydrolysis and lactate accumulation), which meets the shortfall of adenosine triphosphate resynthesis from oxidative phosphorylation at the onset of exercise, buffers the rise of [adenosine diphosphate] in the exercising muscle and thus, counter intuitively, prevents a more rapid activation of oxidative phosphorylation (Kindig, Howlett, Stary, Walsh, & Hogan, 2005). In order to account for the relative stability of [adenosine diphosphate] in the face of large increases in oxidative phosphorylation during the rest-to-work transition, it has been suggested (Korzeniewski & Zoladz, 2004) that direct activation of enzymes in the oxidative respiratory chain (adenosine triphosphate supply pathways) is also required (Hochachka & Matheson, 1992). It was further demonstrated that enhanced activation of adenosine triphosphate supply pathways (e.g. by training or pharmacological intervention) reduced the adenosine diphosphate concentration, and thus phosphocreatine degradation & lactate accumulation, required to stimulate a given increase in oxygen consumption (Korzeniewski & Zoladz 2004). Hence prior activation of key enzymes in the oxidative respiratory chain should speed muscle oxygen consumption kinetics and be mirrored by reductions in substrate level phosphorylation (Korzeniewski & Zoladz 2004).

A number of studies have demonstrated that the pyruvate dehydrogenase complex is a key enzyme, which limits adenosine triphosphate supply pathways at the onset of at least during moderate intensity exercise, since activation of the pyruvate dehydrogenase complex via dichloroacetate infusion prior to exercise has repeatedly been shown to reduce reliance on non-oxidative adenosine triphosphate provision, at the onset of sub-maximal intensity exercise (Durkot, De Garavilla, Caretti, & Francesconi, 1995; Timmons *et al.*, 1996; Timmons, Poucher, Constantin-Teodosiu, Macdonald, & Greenhaff, 1997; Timmons, Gustafsson, Sundberg, Jansson, & Greenhaff, 1998a; Timmons *et al.*, 1998b; Howlett, Heigenhauser, Hultman, Hollidge-Horvat, & Spriet, 1999; Parolin *et al.*, 2000; Roberts, Loxham, Poucher, Constantin-Teodosiu, & Greenhaff, 2002; Howlett & Hogan,

2003). The pyruvate dehydrogenase complex catalyses the irreversible conversion of pyruvate to acetyl-CoA, through which carbohydrates are committed to oxidative metabolism in the tricarboxylic acid cycle and electron transport chain. Intravenous infusion of dichloroacetate promotes dephosphorylation of the pyruvate dehydrogenase complex via inhibition of pyruvate dehydrogenase kinase, resulting in near maximal activation of the pyruvate dehydrogenase complex and a concomitant stockpiling of acetyl groups in resting muscle (Constantin-Teodosiu, Simpson, & Greenhaff, 1999). Hence, Greenhaff et al. (Greenhaff *et al.*, 2002) coined the term "acetyl group deficit" to describe the lag in pyruvate dehydrogenase complex activation and thus acetyl-group delivery to the tricarboxylic acid cycle at the onset of exercise. However, Roberts et al. (Roberts *et al.* 2002) suggested that the acetyl group deficit exists within only a limited range of exercise intensities (65%-90% $\dot{V}o_2$ max). At higher exercise intensities (>90% $\dot{V}o_2$ max), these authors suggested that pyruvate dehydrogenase complex flux and acetyl group availability would not limit tricarboxylic acid cycle flux due to rapid activation of the pyruvate dehydrogenase complex at the onset of muscle contraction, probably mediated via increased [Ca^{2+}]. In contrast, during lower intensity exercise (<65% $\dot{V}o_2$ max), the magnitude of metabolic inertia would be less, since the rate of adenosine triphosphate demand would be lower and therefore better matched by the existing rate of pyruvate dehydrogenase complex activation and flux at the onset of exercise. Nevertheless, the existence or otherwise of an 'acetyl group deficit' at exercise intensities ≥90% $\dot{V}o_2$ max remains controversial since the results of previous studies examining this issue have not been consistent. This may in part be due to the variety of exercise modes and intensities employed; however, the collection of insufficient data and use of confounding methodologies in some studies have generated inconsistent conclusions regarding the acetyl group deficit.

Previous research on this issue has shown that dichloroacetate infusion reduced both blood lactate accumulation and the absolute degradation of phosphocreatine (measured via ^{31}P-MRS) during high intensity knee-extensor exercise (Rossiter *et al.*, 2003),

although pulmonary $\dot{V}o_2$ kinetics were unaffected. In addition, Timmons et al. (Timmons, Constantin-Teodosiu, Poucher, & Greenhaff, 2004) showed that phosphocreatine degradation was reduced during the first minute of intense tetanic contraction in canine skeletal muscle following dichloroacetate infusion. In contrast, other studies (Bangsbo, Gibala, Krustrup, Gonzalez-Alonso, & Saltin, 2002; Savasi, Evans, Heigenhauser, & Spriet, 2002) found no effect of prior dichloroacetate infusion on non-oxidative adenosine triphosphate provision at the onset of cycle exercise at 90% $\dot{V}o_2$ max and leg kicking at 110% of peak thigh $\dot{V}o_2$ max, respectively. Furthermore, there was no effect of dichloroacetate infusion prior to cycle exercise at 80% $\dot{V}o_2$ peak on pulmonary oxygen uptake kinetics (Jones, Koppo, Wilkerson, Wilmshurst, & Campbell, 2004).

These contradictory data likely relate to a number of differences between and limitations within some of the previous research. The studies by Bangsbo et al. (Bangsbo *et al.* 2002), Rossiter et al. (Rossiter *et al.* 2003) and Jones et al. (Jones *et al.* 2004) failed to make concurrent measurement of both acetyl group availability and pyruvate dehydrogenase complex activation status before and during exercise. Accurate measures of both of these factors are necessary when evaluating the acetyl group deficit (Roberts *et al.* 2002; Roberts, Loxham, Poucher, Constantin-Teodosiu, & Greenhaff, 2005). In addition, Savasi et al. (Savasi *et al.* 2002) and Jones et al. (Jones *et al.* 2004) utilised intermittent exercise protocols, which are likely to blunt the effects of dichloroacetate infusion, since prior exercise causes activation of the pyruvate dehydrogenase complex. Furthermore, it is not clear how well the data from different species, e.g. canine muscle, (Timmons *et al.* 2004), and small muscle mass exercise (Bangsbo *et al.* 2002; Rossiter *et al.* 2003) compare to upright large muscle mass exercise in humans. Indeed determination of pulmonary $\dot{V}o_2$ kinetics in the latter study was limited by the relatively small amplitude of the response to exercise ($\sim$ 0.8 l·min^{-1})

In an attempt to resolve some of the present conflict, the purpose of the present study was therefore to determine whether the acetyl group deficit was evident at the start of exercise at 90% $\dot{V}o_2$ max. To achieve this, pyruvate dehydrogenase complex activation status, muscle acetylcarnitine and substrate level phosphorylation were measured from muscle samples extracted prior to and immediately following exercise with prior infusion of saline or dichloroacetate. In a novel approach, these measures were taken in combination with continuous, non-invasive measurements of pulmonary oxygen uptake and muscle deoxygenation (via near infrared spectroscopy). We hypothesised that activation of the pyruvate dehydrogenase complex and accumulation of acetylcarnitine prior to exercise via dichloroacetate infusion would not be effective in enhancing the rate of increase of oxidative metabolism during the transition to exercise at this intensity.

Materials and Methods

Ethical Approval

The study was approved by the ethical committee of the Guy's & St Thomas' Hospital Trust and all procedures were conducted in accordance with the Declaration of Helsinki.

Participants

Eight healthy and active male non-smokers (age: 33.6 ± 2.0 yrs; mass: 74.7 ± 3.0 kg; $\dot{V}o_2$ max: 3.60 ± 0.2 l·min^{-1}) completed the two-trial study. Before taking part in the study, all participants underwent routine medical screening and completed a general health questionnaire. All gave their written informed consent to take part in the study and were aware that they were free to withdraw from the experiment at any point.

Preliminary testing

Participants performed two preliminary exercise tests and one familiarisation trial in the 2 weeks before the first experimental trial. A submaximal test, to determine the relationship between pulmonary oxygen uptake and power output, and a maximal ramp test, to determine $\dot{V}o_2$ max, were completed. From these tests and a number of familiarisation trials, a workload which elicited 90% $\dot{V}o_2$ max after 3 min of exercise was calculated (301 ± 14 W), in a similar manner to the procedures of Savasi et al. (2002).

Pre-experimental procedures

Every effort was made to control factors such as diet and training status and participants were fully familiarised with the laboratory conditions and procedures having completed numerous preliminary trials. Participants were therefore instructed to consume their habitual diet and refrain from strenuous physical activity, smoking and consuming alcohol in the 48 h prior to each trial. Participants were also asked to record food consumed during the 48 h prior to the first trial and to eat the same foods during the same period prior to the second trial.

Dichloroacetate

dichloroacetate (monosodium salt) was obtained from Sigma Aldrich and prepared under sterile conditions by the pharmacy at St Thomas' Hospital (London) at a concentration of 25 $mg^.ml^{-1}$. Participants received 50 $mg^.kg^{-1}$ body mass (bm) dichloroacetate over the 30 min infusion period, which ended 15 min before exercise.

Experimental protocol

On arrival at the laboratory (am) overnight fasted participants rested in an upright, seated position for ~30 min before a cannula was inserted into an antecubital vein and a resting blood sample obtained. A 30 min infusion (either 0.9% saline or dichloroacetate) was then started, ending 15 min before the start of exercise. Participants received a total volume of 2 $ml^.kg^{-1}$bm at an infusion rate of 4 $ml^.kg^{-1} bm^.h^{-1}$. Approximately 10 min before the onset of exercise, the skin and fascia over the anterior aspect of one thigh was anaesthetised (2-3 ml of 2% w:v lignocaine, Antigen Pharmaceuticals, Ireland) and two small incisions were made in skin, subcutaneous layers and fascia to allow extraction of muscle samples using the percutaneous needle biopsy technique with suction applied. Alternate legs were used for the two trials. Immediately before exercise a muscle biopsy was extracted from the vastus lateralis of the leg. Participants then positioned themselves on the ergometer and breathed at rest through a mouthpiece connected to a unit, which calculated breath-by-breath oxygen consumption (MedGraphics CPX/D, Minnesota, USA). Continuous, non-invasive measurements of muscle deoxygenation status were made via near infrared spectroscopy measurements on the non-biopsied leg (see below). Exercise commenced from rest (stationary, so as not to blunt the effect of dichloroacetate infusion) and consisted of cycling for 3 s at 20 W (in order to overcome the inertia of the ergometer flywheel) and then 177 s at 90% $\dot{V}o_2$ max. It was not possible to mechanically accelerate the flywheel prior to exercise onset. Immediately upon completion of exercise a second biopsy was extracted from the second incision while the participants were seated on the bike. The participants then remained seated on the ergometer for 12 min before completing a

further 5 min of exercise at 100 W; after which a tourniquet was immediately inflated around the leg (non-biopsied leg, interrogated by near infrared spectroscopy) to 260 mmHg for 1 min and then quickly deflated. This allowed for collection of post-exercise blood samples and normalisation of the near infrared spectroscopy data to the physiological range – see *near infrared spectroscopy*. After 14 days the participants repeated the above protocol, but having the other solution infused this time.

Near infrared spectroscopy

A near infrared spectrometer (NIRO-500, Hamamatsu, Japan) was used to examine relative changes in the muscle oxygenation status of the exercising muscle. Two laser optodes were set into a custom designed holder that cut out ambient light, eliminated relative movement and maintained an inter-optode spacing of 40mm. A differential path length factor of 4.94cm was used (Duncan *et al.*, 1995); therefore the total path length (near infrared spectroscopy * differential path length factor) was set at 19.8cm. The optode holder was attached to the flat portion of the subject's vastus lateralis with tape and the participants' cycling shorts pulled down over the top. Before exercise commenced, the subject extended the interrogated leg in a relaxed and still state to record a stable zero baseline at rest. The NIRO-500 laser was set to fire twice a second. Changes in the deoxygenated haemoglobin signal (Hb) received from near infrared spectroscopy are dependent on the balance between oxygen supply and utilisation in the field of near infrared spectroscopy interrogation; the application and release of an ischaemic tourniquet following 5 min of exercise at 100W (see above) produces maximum and minimum changes in Hb and allowed normalisation of the near infrared spectroscopy data to the physiological range (*%Hb*), (Marwood & Bowtell, 2007). To allow for individual differences and some day-to-day variability relating to the placement of the optodes, this procedure was conducted for each individual trial. Since near infrared spectroscopy measures changes in oxy/deoxy-haemoglobin concentration in the volume of interrogated tissue, shifts in water from the extracellular to intracellular space will not affect near infrared spectroscopy measurements due to haemoconcentration of the blood. However, subsequent alterations in muscle cell

volume may increase the ratio of muscle cell to capillary volume in the area of near infrared spectroscopy interrogation possibly blunting the near infrared signal. Whilst this effect may impact upon the absolute value attained during exercise it seems unlikely to affect the kinetic response.

Pulmonary gas exchange

Pulmonary gas exchange was measured breath by breath using an automated system (MedGraphics CPX/D). Participants wore a nose-clip and breathed through a mouthpiece connected to a pneumotach (manufactured by MedGraphics). The system was calibrated prior to each trial using two gases of known concentration spanning the range of anticipated fractional gas concentrations (low O_2 / high CO_2 and high O_2 / zero CO_2). A previous study has demonstrated validity of the MedGraphics CPX/D system during simulated exercise testing in both normoxia and hyperoxia (Prieur *et al.*, 1998).

$\dot{V}o_2$ & near infrared spectroscopy *kinetic analysis*

Abnormal breaths due to coughs and swallows were first removed from the $\dot{V}o_2$ data to prevent skewing of the underlying response. The criterion for removal of these breaths was those that were different to the mean of the adjacent four data points by more than three times the standard deviation of those four points. The $\dot{V}o_2$ data were then interpolated second-by-second between 0 – 180 s. The first 20 s of $\dot{V}o_2$ data were not included in the fitting processes. From 21 s onward, the data were fitted to a mono-exponential curve with a delay relative to the onset of exercise of the forms:

$$\dot{V}o_{2(t)} = \dot{V}o_{2(0)} + A_{1Vo_2} * (1 - e^{-(t - TD_{1Vo_2})/\tau_{Vo_2}})$$

Where $\dot{V}o_{2(0)}$ is the pulmonary oxygen consumption measured at the onset of exercise (Rossiter *et al.*, 1999), A_{1Vo_2} is the asymptotic amplitude of the phase II response, τ_{Vo_2} is the time constant for the phase II component of the response and TD_{1Vo_2} is a time delay similar, but not equal to the phase I – phase II transition time (Rossiter *et al.*

1999). The absolute oxygen uptake of the phase II (fundamental) component of the response ($\dot{V}o_{2(\phi2)}$) is therefore the sum of $\dot{V}o_{2(0)}$ and A_{1Vo_2}. The fitting strategy was designed to identify the onset of the "slow component" of the response to exercise (TD_{2Vo_2}). Using custom written software and the "Solver" function in Microsoft Excel, the fitting window was widened by 1 s intervals from 60 s until the end of exercise with the time constant of the curve of best fit for each time window plotted against time (Marwood & Bowtell 2007). The goodness of fit was determined by the maintenance of a flat profile for the residual plot and minimisation of the sum of the error squared data (Solver, Microsoft Excel). The onset of the slow component could then be identified as the point at which a plateau in the value of τ was followed by a progressive increase, as its value becomes affected by the slow component (Marwood & Bowtell 2007). The time at which this occurred was used as the optimal fitting window with which to estimate the kinetics of the phase II component. The amplitude of the slow component (A_{2Vo_2}) was calculated as the difference between this and the mean $\dot{V}o_2$ during the final 30 s of exercise ($\dot{V}o_{2\,(180\text{-}150s)}$). To provide an indication of the overall kinetic response to exercise, the mean response time (MRT_{VO_2}) was calculated as $TD_1 + \tau$. Total oxygen uptake during exercise was calculated via integration of the $\dot{V}o_2$ - time curve and estimated accumulated oxygen deficit was calculated as this value minus ($\dot{V}o_{2\,(180\text{-}150s)}$ * 3 min).

Near infrared spectroscopy kinetic analysis

%Hb kinetics were analysed in a similar manner to $\dot{V}o_2$ kinetics. Data prior to the minimum points which occurred shortly after the onset of exercise (*%Hb_min*, see figure 4) were removed from the dataset. Although it is not certain whether the processes underlying the *%Hb* response are exponential in nature, visual inspection of the data and reference to previous literature (DeLorey, Kowalchuk, & Paterson, 2005) suggests that a monoexponential decay model of the form below provides a reasonable estimate

of the time course of muscle deoxygenation during the "primary" phase of the %Hb response:

$$\%Hb(t) = \%Hb_{min} + A_{\%Hb} * (1 - e^{(t-TD_{1\%Hb})/\tau_{\%Hb}})$$

$TD_{1\%Hb}$ is the time delay (relative to the start of exercise) of this "primary" component of %Hb and is derived during the fitting process, whereas $A_{\%Hb}$ and $\tau_{\%Hb}$ are the amplitude and the time constant of this response. The overall amplitude of this "primary" response of %Hb ($\%Hb_p$) is therefore the sum of $\%Hb_{min}$ and $A_{\%Hb}$.

By implication of the model used, a plateau occured in the %Hb data. However, similar to $\dot{V}o_2$ kinetics during heavy and very heavy exercise intensities, this plateau was only transient. Hence the fitting strategy utilised was similar to that for $\dot{V}o_2$ kinetics whereby an iterative process aids determination of the optimum fitting window for the "primary" response of %Hb kinetics. Specifically, utilising the same routines, the fitting window was widened by 0.5 s intervals from 30 s until the end of exercise with the time constant of the curve of best fit for each time window plotted against time; identification of the optimum window for fitting %Hb kinetics and the goodness of fit determined as for $\dot{V}o_2$ kinetics. The degree of drift in the %Hb data from the "primary" %Hb response to end exercise ($A_{2\%Hb}$, akin to a "slow component" of %Hb) was calculated as the difference relative to the final 30 s of exercise ($\%Hb_{180-150s}$). To give an indication of the overall kinetic response to exercise, the mean response time ($MRT_{\%Hb}$) was calculated as TD_1 + τ.

Blood analysis

Venous blood samples were taken at rest (-45 min), immediately post infusion (-15 min), immediately prior to exercise (0 min) and 3, 6 and 12 min following the termination of exercise (6, 9, 15 min respectively). Venous blood samples were immediately centrifuged at 13,000 rpm for 2 min, the supernatant collected, snap frozen in liquid

nitrogen and stored at -80°C. Plasma samples were later analysed for lactate concentration by an automated colorimetric assay (Cobas Mira).

Muscle analysis

After extraction, muscle samples were immediately immersed in liquid nitrogen, removed from the needle while muscle still frozen and stored in liquid nitrogen until freeze dried and stored at -80ºC. All samples were divided into two pieces under liquid nitrogen. One portion was freeze dried, dissected free of visible blood and connective tissue, powdered and washed twice with petroleum ether to remove any fat. Aliquots of the powdered muscle were extracted with 0.5 M perchloric acid (containing 1 mM EDTA), and after centrifugation, the supernatant was neutralized with 2.1 M $KHCO_3$. Extracts were assayed enzymatically for lactate, adenosine triphosphate, adenosine diphosphate, phosphocreatine and creatine using a fluorometer (Hitachi F2000 fluorescence spectrophotometer, Hitachi Instruments, Japan) and for acetylcarnitine and free carnitine by enzymatic assays that made use of radioisotopic substrate (Cederblad, Carlin, Constantin-Teodosiu, Harper, & Hultman, 1990). Intramuscular metabolite contents (adenosine triphosphate, phosphocreatine, creatine) were normalised for each individual's highest total creatine content across each of the four samples. The non-oxidative adenosine triphosphate provision or substrate level phosphorylation was calculated as 2 * Δ[adenosine triphosphate] + 1.5 * Δ[Lactate] + Δ[phosphocreatine]. The remaining portion of frozen wet muscle was used to assess pyruvate dehydrogenase complex activation (Constantin-Teodosiu, Cederblad, & Hultman, 1991) with a correction made for total alkaline protein content (Sahlin, Katz, & Broberg, 1990).

Statistics

All values are presented as mean ± standard deviation with an *n* of 8, unless otherwise indicated. Normality of distribution of all datasets was confirmed via one sample Shapiro-Wilk normality tests. Time course dependent data were analysed by a two way ANOVA for repeated measures {treatment (dichloroacetate vs saline) by time}. The specific location of any effect was identified using a pairwise analysis (t-test) corrected

by a Sidak-based Holm step-down procedure for multiple comparisons. All other data were analysed by a two-tailed paired t-test. Significance was set at the $P<0.05$ level of confidence.

Results

Pyruvate dehydrogenase complex activation

Both at rest and after exercise, pyruvate dehydrogenase complex activity was significantly higher following dichloroacetate infusion compared to control (P=0.01) and exercise resulted in a significant increase in pyruvate dehydrogenase complex activity in control only (P=0.001). There was no significant difference in pyruvate dehydrogenase complex activity between trials following exercise, {pre-ex: 2.7 ± 2.2 (control) vs 17.9 ± 2.4 (dichloroacetate); post-ex: 11.9 ± 4.9 (control) vs 17.2 ± 3.0 (dichloroacetate) mmol acetyl-CoA $min^{-1.}mg^{-1}$ protein, Fig 1}. Post-exercise muscle pyruvate dehydrogenase complex activity data are available for only 5 participants due to the extraction of insufficient muscle.

Muscle metabolites

Muscle adenosine triphosphate, phosphocreatine, lactate, creatine and total creatine concentrations were unaffected by dichloroacetate infusion compared to saline at rest or following exercise (Table 1). Substrate level phosphorylation was also not different following dichloroacetate infusion compared to saline {125 ± 57 (control) vs 113 ± 35 (dichloroacetate) mmol adenosine triphosphate $^.kg^{-1}$dm}.

Muscle acetylcarnitine concentration was significantly higher following dichloroacetate infusion compared to saline both prior to and after exercise; and significantly higher post-exercise compared to pre-exercise in both trials, (main effect time, P<0.001; main effect dichloroacetate, P=0.006; Fig. 2). The percentage increase in acetylcarnitine from rest to exercise was significantly higher following saline infusion compared to dichloroacetate {480 ± 393 (control) vs 114 ± 51 (dichloroacetate) %, main effect dichloroacetate, P=0.03, Fig. 2}, but the absolute increase in acetylcarnitine content from pre-exercise to post-exercise was not different between conditions {8.3 ± 4.1 (control) vs 8.3 ± 3.0 (dichloroacetate) $\Delta mmol^.kg^{-1}$dm}. Muscle free carnitine concentration showed the inverse of these effects. Total muscle carnitine concentration was unchanged by exercise or dichloroacetate infusion {pre-ex: 23.2 ± 3.3 (control) vs

21.2 ± 4.3 (dichloroacetate) mmol·kg⁻¹dm; post-ex: 21.1 ± 4.0 (control) vs 21.8 ± 3.9 (dichloroacetate) mmol·kg⁻¹dm}.

Pulmonary gas exchange

The time constant of the phase II kinetics of pulmonary oxygen uptake was not different between trials (Table 2). However, MRT_{VO_2} was greater following dichloroacetate infusion compared to control (P=0.04, Table 2}. In contrast, total oxygen consumption {7.95 ± 0.28 (control) vs 7.64 ± 0.29 (dichloroacetate) litres O_2} and estimated accumulated oxygen deficit {1.85 ± 0.23 (control) vs 1.77 ± 0.13 (dichloroacetate) litres O_2} were unchanged between trials (P=0.5, P=0.4, respectively). The onset of the slow component of oxygen consumption was also greater following dichloroacetate infusion compared to control (P=0.05, Table 2}. There was also a tendency for the absolute amplitude of the fundamental response (phase III) to be higher following dichloroacetate infusion (P=0.09) with a concomitant tendency for the amplitude of the slow component to be lower (P=0.07, Table 2). The average 95% confidence interval of the fitted time-constant parameter τ_{Vo_2} was 4.3 ± 0.8 and 3.1 ± 0.3) s in control and dichloroacetate trials, respectively. Figure 3 shows representative plots for control and dichloroacetate trials with the phase II kinetic curves and residual plots shown. End-exercise oxygen consumption represented 90.3 and 88.0 % $\dot{V}o_2$ max for the control and dichloroacetate trials respectively (P=0.3).

Near infrared spectroscopy

The time constant of the kinetics of *%Hb* was greater following dichloroacetate infusion compared to control (P=0.004, Table 3). Furthermore, $TD_{\%Hbmin}$ was also significantly greater following dichloroacetate infusion compared to control (P=0.04, Table 3). Consequently, $MRT_{\%Hb}$ was also greater following dichloroacetate infusion (P=0.003, Table 3). The average 95% confidence interval of the fitted parameter $\tau_{\%Hb}$ was 0.4(0.1) and 0.5(0.1) s for the control and dichloroacetate trials respectively. Figure 4 shows

representative plots for control and dichloroacetate trials with the "primary" component

kinetic curves and residual plots shown.

Plasma metabolites

Plasma lactate concentration was unchanged between trials; the post-exercise rise in

plasma lactate concentration (measured 3 min following the termination of exercise) was

also not different between trials {control: 5.9 ± 2.5 vs dichloroacetate: 6.6 ± 2.8 mmol$\cdot$l^{-1},

n.s.; Fig. 5}.

Discussion

The present investigation clearly demonstrates that prior dichloroacetate infusion, which resulted in activation of the pyruvate dehydrogenase complex and accumulation of acetyl groups had no effect on non-oxidative adenosine triphosphate provision following 3 min of cycle exercise at $90\% \dot{V}o_2$ max. Secondly, and in line with the metabolic findings, prior dichloroacetate infusion had no effect on the time constant of phase II pulmonary oxygen kinetics during subsequent severe intensity exercise.

Hochachka & Matheson (Hochachka & Matheson 1992) and recent theoretical studies (Korzeniewski & Zoladz 2004) suggest that direct activation of key enzymes in the oxidative respiratory chain (in addition to the stimulus provided by the rise in [adenosine diphosphate]) are required to adequately explain the time course of phosphocreatine, adenosine diphosphate and muscle oxygen consumption at the onset of exercise. In this regard, a large body of evidence suggests that a lag in pyruvate dehydrogenase complex activation and flux determines a significant proportion of the "metabolic inertia" inherent within skeletal muscle at the onset of exercise (Timmons *et al.* 1998a; Roberts *et al.* 2002; Greenhaff *et al.* 2002). Consequently, pyruvate dehydrogenase complex activation and stockpiling of acetyl groups in resting muscle prior to contraction via dichloroacetate infusion has been shown to reduce inertia in oxidative adenosine triphosphate provision at the onset of subsequent contraction (Roberts *et al.* 2002; Greenhaff *et al.* 2002). However, Roberts et al. (Roberts *et al.* 2002) suggested that the acetyl group deficit exists only within a finite range of exercise intensities, i.e. ~ 65–$90\% \dot{V}o_2$ max, outside of which the non-oxidative adenosine triphosphate provision will not be reduced by increasing resting acetyl group availability. The present data support the hypothesis of Roberts et al. (Roberts *et al.* 2002) that 90% of $\dot{V}o_2$ max is an upper limit to the range at which the acetyl group deficit exists. The present data bear similarity with previous studies (Savasi *et al.* 2002) that demonstrated no effect of dichloroacetate infusion on non-oxidative adenosine triphosphate provision following the transition to exercise at 110% max leg-kicking capacity (Bangsbo *et al.* 2002) and cycle

exercise at 90% $\dot{V}o_2$ max (Savasi *et al.* 2002). Conversely, the present data seem to be at odds with a recent paper (Timmons *et al.* 2004) where following dichloroacetate infusion phosphocreatine degradation in canine skeletal muscle was lower in the first minute of intense tetanic stimulation compared to control. However, aside from the obvious muscle fibre type difference, it is likely that these two datasets arise from distinct exercise intensity domains. A meta-analysis conducted by Timmons et al. (Timmons *et al.* 2004) suggested that an 'acetyl group deficit' exists only when the average rate of phosphocreatine degradation during the first minute of exercise is less than 1 mmol kg dm^{-1} s^{-1}, as was the case in the study by Timmons *et al.* 2004. Due to the timing of muscle samples in the present study (i.e. 3 min apart) it is not possible to accurately evaluate the initial phosphocreatine utilisation rate. However, the exercise intensity utilised in the present study has previously been demonstrated to result in an initial phosphocreatine utilisation rate > 1 mmol kg dm^{-1} s^{-1} (~ 1.2 mmol kg dm^{-1} s^{-1}) (Savasi *et al.* 2002). Given the negligible change in phosphocreatine from 2 min of exercise onwards even during moderate intensity exercise (Howlett *et al.* 1999), the reduction of phosphocreatine by ~ 45 mmol kg dm^{-1} during 3 min exercise in the present study is line with the data of Savasi et al. (Savasi *et al.* 2002) who demonstrated a reduction of ~ 43 mmol kg dm^{-1} across the total 90 s exercise period. Hence we are confident that the initial phosphocreatine utilisation rate was greater than the target level 1 mmol kg dm^{-1} s^{-1}.

A reasonable explanation for the lack of any beneficial effect of priming the muscle with acetyl groups prior to high intensity exercise could be as follows. The magnitude of pyruvate dehydrogenase complex activation is central to the control of acetyl-CoA delivery to the tricarboxylic acid cycle and carbohydrate oxidation in contracting skeletal muscle. As such, pyruvate dehydrogenase complex activity increases, from its relatively low activation status at rest, in parallel with exercise intensity up to an intensity of ~90% $\dot{V}o_2$ max as the rate of carbohydrate oxidation becomes maximal (Constantin-Teodosiu, Carlin, Cederblad, Harris, & Hultman, 1991; Howlett *et al.*, 1998). This would suggest that there is an upper workload intensity above which the rate of acetyl group

delivery is no longer limiting towards tricarboxylic acid cycle demand since (i) above this exercise intensity the pyruvate dehydrogenase complex is working maximally and (ii) any further gains in aerobic contribution would become disproportionately insignificant when reported relative to the total energy requirement.

In the present study, dichloroacetate infusion resulted in a significant enhancement of pre-exercise pyruvate dehydrogenase complex activation relative to control (2.7 vs 17.9 nmol acetyl-CoA $min^{-1} \cdot mg^{-1}$ protein), resulting in a several fold accumulation of acetylcarnitine prior to exercise (relative to control). However, despite this increased acetyl group availability accumulation of acetylcarnitine continued during the transition from rest to exercise following dichloroacetate infusion, a phenomenon not associated with those exercise intensities demonstrating an acetyl group deficit, (Timmons *et al.* 1996; Timmons, Poucher, Constantin-Teodosiu, Macdonald, & Greenhaff, 1998; Timmons *et al.* 1998a; Howlett *et al.* 1999; Roberts *et al.* 2002; Timmons *et al.* 2004). This suggests that a further limitation exists downstream of the pyruvate dehydrogenase complex reaction during the transition to exercise at this intensity.

Pulmonary oxygen uptake & muscle deoxygenation kinetics

Speeding of pulmonary oxygen uptake kinetics can be considered indicative of an increased rate of muscle oxygen consumption as the time constant of pulmonary $\dot{V}o_2$ kinetics is suggested to be a proxy for muscle oxygen consumption kinetics (Rossiter *et al.* 1999). To improve the signal-to-noise ratio of breath-by-breath pulmonary $\dot{V}o_2$ measurements multiple exercise transitions are often utilised and ensemble averaged. This reduces the 95% confidence interval (CI) of the estimation of $\tau_{\dot{V}o_2}$ during calculation of pulmonary $\dot{V}o_2$ kinetics (Lamarra, Whipp, Ward, & Wasserman, 1987; Fawkner, Armstrong, Potter, & Welsman, 2002). Unfortunately due to ethical committee concerns over the infusate, we were unable to conduct more than one exercise transition. However, despite the use of only a single transition for each condition in the present study, the large amplitude of the pulmonary $\dot{V}o_2$ and the familiarisation of the

participants with breathing through a mouthpiece resulted in average 95% CI for $\tau_{V_{O_2}}$ of 4.3 ± 0.8 and 3.1 ± 0.3 s for the CON and dichloroacetate trials, respectively. Fawkner et al (Fawkner *et al.* 2002) suggested that 95% CI of ±5 for $\tau_{V_{O_2}}$ is an appropriate target. Hence the $\tau_{V_{O_2}}$ data provide support for the muscle metabolite data, demonstrating no effect of dichloroacetate infusion prior to exercise at 90% $\dot{V}_{O_2}$ max.

In contrast to muscle metabolite and $\tau_{V_{O_2}}$ data, the mean response time of pulmonary oxygen uptake kinetics was slower following dichloroacetate infusion, one interpretation of which being that the rate of increase of oxidative metabolism was slower following dichloroacetate infusion. However, given the biopsy and $\tau_{V_{O_2}}$ data from the present study, and the finding of no difference in either estimated accumulated oxygen deficit or total oxygen uptake, this appears unlikely. The greater mean response time following dichloroacetate infusion appears to be primarily due to the tendency for a greater time delay of the phase I – phase II transition point of the fundamental component (*P*=0.1). Little physiological meaning has generally been attributed to this time-delay. In the face of no alteration in a number of parameters indicative of oxidative metabolism, the weight of the available evidence would suggest that dichloroacetate had no effect on the rate of increase of oxidative metabolism at the present exercise intensity.

The slow component of oxygen uptake is characterised by an additional oxygen uptake in excess of the phase II (fundamental) component which is typically presented ~ 90 – 180 s following the onset of exercise above the lactate threshold. In the present and a previous (24) study, the onset of the slow component was delayed following dichloroacetate infusion (111 vs 141 s). In the present study, this was associated with a blunting of the amplitude of the slow component as expressed after 3 min of exercise (*P*=0.07) as also demonstrated by Rossiter et al. (Rossiter *et al.* 2003). However in contrast to Rossiter et al (Rossiter *et al.* 2003), we also found tendencies for an increase in the absolute value of the fundamental component (i.e. phase III, *P*=0.09) in the

present study. There was no difference between trials in the end-exercise oxygen uptake. Taken together, these effects of dichloroacetate infusion on pulmonary oxygen uptake kinetics (no effect on the phase II time constant, blunted slow component and enhancement of the absolute value of the fundamental amplitude) are similar to that demonstrated by priming exercise (for review see Jones *et al.* 2003, (Jones, Koppo, & Burnley, 2003)). Priming exercise may exert its effect on subsequent exercise via a number of potential mechanisms. However the primary candidates would appear to be improvements in oxygen availability to the exercising muscle (via enhanced convection and diffusion, (Jones, Berger, Wilkerson, & Roberts, 2006) and increases in muscle fibre recruitment in all fibre types, (Burnley, Doust, Ball, & Jones, 2002).

Although, an enhancement in muscle fibre recruitment might best explain the tendency for the absolute amplitude of the fundamental component of pulmonary oxygen uptake kinetics to be higher following dichloroacetate infusion there is certainly no direct evidence to suggest that dichloroacetate has the capacity to alter muscle fibre recruitment. On the other hand, some (Macdonald, Pedersen, & Hughson, 1997), but not all (Wilkerson, Berger, & Jones, 2006) studies have encountered a similar phenomenon during exercise in hyperoxia where oxygen delivery is presumably enhanced. Therefore, evidence for an effect of dichloroacetate on oxygen delivery although debatable has thus far been based on inferences from pulmonary oxygen uptake kinetics during high intensity exercise (Rossiter *et al.* 2003; Jones *et al.* 2004) and present study. Nevertheless, the near infrared spectroscopy data from the present study provide further evidence that the above described effects of dichloroacetate infusion on pulmonary oxygen uptake kinetics occurred via an enhancement of oxygen delivery to the exercising muscle although the mechanism is unclear.

No previous study has utilised interrogation of exercising human muscle via near infrared spectroscopy following dichloroacetate infusion. Given previous data (Howlett & Hogan 2003) where dichloroacetate administration resulted in a faster rate of decrease in intracellular oxygen pressure at the onset of contractions in single, isolated muscle

fibres, one might have expected that the kinetics of *%Hb* during the transition from rest to exercise would be faster following dichloroacetate infusion. Alternatively, given the absence of any effect of dichloroacetate infusion on non-oxidative adenosine triphosphate provision in the present study, one might predict that dichloroacetate infusion would have no effect on *%Hb* kinetics. Therefore, the finding of *slower %Hb* kinetics (time constant and mean response time) during the transition from rest to exercise was unexpected. Slower *%Hb* kinetics are indicative of a slower rate of increase of fractional oxygen extraction by the muscle. This could be due to either (i) a slower rate of increase of oxidative or mitochondrial adenosine triphosphate provision or (ii) enhanced oxygen delivery throughout the exercise transition. The former possibility can be excluded on the basis of equivalent non-oxidative adenosine triphosphate provision in both trials. Therefore, it appears that dichloroacetate infusion could have promoted enhanced oxygen delivery in the exercising muscle such that the rate of increase of deoxygenation of the muscle microvasculature was slowed. Additionally, the amplitude of the drift in *%Hb* (similar to the slow component of pulmonary oxygen uptake) was also blunted by dichloroacetate infusion which is similar to that previously demonstrated in hyperoxia (Marwood & Bowtell 2007) further supporting the notion that oxygen delivery was enhanced. So far, there is little *direct* evidence available to support the idea that dichloroacetate enhances muscle oxygen delivery. However, it has been shown that oxygen delivery to canine hypoxic skeletal muscle was enhanced following dichloroacetate infusion (Graf, Leach, & Arieff, 1985). Furthermore, when human volunteers were given the same amount of dichloroacetate to the present study (50 mg$^.$kg^{-1}) in a single bolus, there was a significant decrease in peripheral resistance and a concomitant improvement in oxygen availability (Ludvik, Peer, Berzlanovich, Stifter, & Graf, 1991). The precise mechanism(s) behind these effects are not clear, but it has been suggested (Graf *et al.* 1985) that it could involve the binding of oxygen to haemoglobin. Whether this is related to an increase in haemoglobin oxygen saturation, an enhanced unloading of oxygen at the site of gas exchange or an interaction with nitric oxide release (reduced peripheral resistance and thus enhanced blood flow) is as yet unclear.

A large number of studies have demonstrated a reduction in non-oxidative adenosine triphosphate provision during the transition from rest to exercise following dichloroacetate infusion (Durkot *et al.* 1995; Timmons *et al.* 1996; Timmons *et al.* 1997; Timmons *et al.* 1998a; Timmons *et al.* 1998b; Howlett *et al.* 1999; Parolin *et al.* 2000; Roberts *et al.* 2002; Howlett & Hogan 2003). The mechanism by which dichloroacetate exerts this effect has been shown to be via activation of the pyruvate dehydrogenase complex and resultant reduction in the acetyl group deficit (Durkot *et al.* 1995; Timmons *et al.* 1996; Timmons *et al.* 1997; Timmons *et al.* 1998a; Timmons *et al.* 1998b; Howlett *et al.* 1999; Parolin *et al.* 2000; Roberts *et al.* 2002; Howlett & Hogan 2003). However given the present $\dot{V}o_2$ and *%Hb* kinetic data, the effects of prior dichloroacetate infusion on muscle metabolism at these lower exercise intensities (relative to the present study) may also in part be due to improvements in muscle oxygen delivery. In other words, improvements in oxygen delivery via dichloroacetate infusion may act in combination with the reduction in the acetyl group deficit and enhancement of pyruvate dehydrogenase complex activation to achieve the reduced reliance on non-oxidative adenosine triphosphate provision during subsequent moderate intensity exercise. Such an effect would however need to be confirmed in future studies.

The present study investigated the effect of prior dichloroacetate infusion on oxidative metabolism during subsequent exercise at 90% $\dot{V}o_2$ max. In contrast to some previous studies, the expected effects of dichloroacetate on pyruvate dehydrogenase complex activity and acetylcarnitine accumulation were directly assessed, and we utilised a combination of both invasive (muscle metabolite analysis) and non-invasive (pulmonary oxygen uptake and muscle deoxygenation kinetics) methods to evaluate the effect of dichloroacetate on oxidative metabolism. The results clearly demonstrate that despite the accumulation of muscle acetyl groups and activation of the pyruvate dehydrogenase complex induced by dichloroacetate, non-oxidative adenosine triphosphate provision during a 3 min bout of exercise at 90% $\dot{V}o_2$ max was not affected. Secondly, and in line

with the metabolic findings, the present study also demonstrated that prior dichloroacetate infusion had no effect on the phase II time component of pulmonary oxygen uptake. This absence of acetyl group deficit at the onset of exercise at $90\%\dot{V}o_2$ max, suggests an upper limit to the exercise intensity range at which the acetyl group deficit occurs beyond which further downstream limitations may be present. Other novel findings of the present study were that muscle deoxygenation kinetics were slowed and the slow component of pulmonary oxygen consumption and muscle deoxygenation was blunted following dichloroacetate infusion, indicating that dichloroacetate infusion enhanced oxygen delivery to the exercising muscle. The significance of these latter findings may be that prior infusion of dichloroacetate exerts its beneficial effects on non-oxidative adenosine triphosphate provision during moderate intensity exercise via a combination of reduction in the acetyl group deficit, activation of pyruvate dehydrogenase complex *and* an improvement in oxygen delivery.

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Tables

Table 1 Muscle adenosine triphosphate (ATP), phosphocreatine (PCr), lactate, glycogen, creatine (Cr) and total creatine (TCr) concentrations before and after 3 min bout of exercise at 90% $\dot{V}o_2$ max, following i.v. infusion of 0.9% saline (CON) or dichloroacetate (DCA)

	CON			DCA		
	Pre-ex	**Post-ex**	**Δ**	**Pre-ex**	**Post-ex**	**Δ**
ATP	25.0 ± 2.2	22.4 ± 3.2	-2.6 ± 3.3	24.1 ± 4.0	25.4 ± 9.1	1.3 ± 6.5
PCr*	77.2 ± 8.4	33 ± 13	-45 ± 18	72 ± 15	29.3 ± 8.0	-42.9 ± 7.0
Lactate*	3.2 ± 1.0	53 ± 30	50 ± 30	2.5 ± 0.7	51 ± 20	48 ± 20
Cr*	45 ± 11	99 ± 15	53 ± 21	46 ± 13	82 ± 26	36 ± 27
TCr	132 ± 11	136 ± 15	5 ± 15	130 ± 19	125 ± 16	5 ± 14

Values represent means ± SD and are expressed as mmol·kg^{-1} dry muscle (*n*=8). Δ change from pre-exercise to post-exercise; *Time main effect from pre-exercise to post-exercise within treatment groups (*P*<0.001).

Table 2 Pulmonary oxygen consumption parameters during a 3 min bout of exercise at 90% $\dot{V}o_2$ max, following i.v. infusion of 0.9% saline (CON) or dichloroacetate (DCA)

	CON	DCA
VO2$_{(b)}$ (l.min^{-1})	0.52 ± 0.03	0.48 ± 0.03
VO2$_{(0)}$ (l.min^{-1})	1.12 ± 0.31	0.82 ± 0.42
A$_{1VO2}$ (l.min^{-1})	1.89 ± 0.25	2.20 ± 0.40
VO$_{2(ss)}$ (l.min^{-1})	3.0 ± 1.4	3.0 ± 1.1
TD$_{1VO2}$ (s)*	4 ± 11	16.7 ± 3.7
τ_{1VO2} (s)	21.3 ± 5.4	22.9 ± 6.2
MRT$_{VO2}$ (s)*	25.4 ± 8.5	39.6 ± 6.2
TD$_{2VO2}$ (s)*	113 ± 25	151 ± 31
A$_{2VO2}$ (l.min^{-1})	0.25 ± 0.14	0.12 ± 0.20

Values represent mean ± SD (*n*=8). *Treatment (DCA infusion) main effect (*P*<0.05). $\dot{V}o_{2(0)}$: oxygen uptake at the onset of exercise; A_{1Vo_2}: asymptotic amplitude of the phase II response $\dot{V}o_{2(\phi2)}$: absolute value of fundamental response; TD_{1Vo_2}: time delay (relative to the onset of exercise) similar but not equal to the phase I – phase II transition; τ_{Vo_2}: time constant of the phase II response; MRT_{VO_2}: mean response time of phase II; TD_{2Vo_2}: time delay of the slow component; A_{2Vo_2}: amplitude of the slow component; $\dot{V}o_{2(180s-150s)}$: mean oxygen uptake during the last 30 s of exercise

Table 3 Summary of the %Hb kinetic parameters during a 3 min bout of exercise at 90% $\dot{V}o_2$ max, following i.v. infusion of 0.9% saline (CON) or dichloroacetate (DCA)

	CON	DCA
%Hb$_{min}$	10.7 ± 8.1	7.9 ± 5.9
A$_{\%Hb}$	57 ± 11	64 ± 11
%Hb(ss)	68 ± 12	72 ± 11
%Hb(150-180)	75 ± 12	76 ± 11
TD$_{\%Hbmin}$ (s)*	4.6 ± 1.4	6.2 ± 1.9
TD$_{\%Hb}$ (s)	4.5 ± 1.4	6.4 ± 2.7
$\tau_{\%Hb}$ (s)*	6.6 ± 3.1	9.7 ± 1.9
MRT$_{\%Hb}$ (s)*	11.4 ± 2.7	17.5 ± 2.7
TD$_{2\%Hb}$ (s)	100 ± 33	102 ± 47
A$_{2\%Hb}$*	7.2 ± 5.7	3.5 ± 4.3

Table 3. Values that represent mean ± SD (*n*=8). *Treatment (DCA infusion) main effect (*P*<0.05).   *%Hb$_{min}$*: minimum point achieved shortly after onset of exercise; *A$_{\%Hb}$*: amplitude of the "phase II" (primary) response; *%Hb$_{(ss)}$*: absolute value of the "phase II" (primary) response; TD$_{\%Hbmin}$: time delay of *%Hb$_{min}$*; TD$_{1\%Hb}$: time delay of the "phase II" (primary) response (derived during the fitting process); $\tau_{\%Hb}$: time constant of the "phase II" (primary) response; *MRT$_{\%Hb}$*: mean response time; *TD$_{2\%Hb}$*: time delay of the "slow component"; *A$_{2\%Hb}$*: amplitude of the "slow component"; *%Hb$_{(180-150s)}$*: mean during the final 30 s of exercise.

Figure legends

Figure 1. Muscle pyruvate dehydrogenase complex activation (PDCa) before and after a 3 min bout of exercise at 90% $\dot{V}o_2$ max following i.v. infusion of 0.9% saline (CON) or dichloroacetate (DCA). Values are expressed as nmol acetyl-CoA min^{-1} mg^{-1} protein (n=5). *Significantly different to resting value (pairwise analysis, P<0.05); $^{\$}$Significantly different to CON (pairwise analysis, P<0.05).

Figure 2. Muscle acetylcarnitine concentration before and after a 3 min bout of exercise at 90% $\dot{V}o_2$ max following i.v. infusion of 0.9% saline (CON) or dichloroacetate (DCA). There was a main effect of time (P<0.001) and of DCA infusion (P<0.01). *Significantly different to resting value (pairwise analysis, P<0.05); $^{\$}$Significantly different to CON (pairwise analysis, P<0.05).

Figure 3. Representative plots for pulmonary $\dot{V}o_2$ kinetics for CON (open squares) and DCA (closed squares) with the phase II kinetics curve and residuals shown.

Figure 4. Representative plots for pulmonary %Hb kinetics for CON (open squares) and DCA (closed squares) with the phase II kinetics curve and residuals shown.

Figure 5. Plasma lactate concentration before and after a 3 min bout of exercise at 90% $\dot{V}o_2$ max following i.v. infusion of 0.9% saline (CON) or dichloroacetate (DCA). *Significantly different to resting values (pairwise analysis, P<0.05).