Respiratory Physiology & Neurobiology 177 (2011) 41–46

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

No effect of glutamine ingestion on indices of oxidative metabolism in stable COPD

Simon Marwood[a,*], Sandy Jack[b], Mukhtar Patel[b], Paul Walker[b], Joanna Bowtell[c], Peter Calverley[b]

[a] Health & Applied Social Sciences, Liverpool Hope University, Liverpool L16 9JD, UK
[b] Unit of Inflammation Research, School of Clinical Sciences, The University of Liverpool, UK
[c] Sports and Exercise Science, London South Bank University, London SE1 0AA, UK

ARTICLE INFO

Article history:
Accepted 9 March 2011

Keywords:
Chronic obstructive pulmonary disease
Oxygen uptake kinetics
Tricarboxylic acid cycle
Glutamine
Glutamate

ABSTRACT

COPD patients have reduced muscle glutamate which may contribute to an impaired response of oxidative metabolism to exercise. We hypothesised that prior glutamine supplementation would enhance $\dot{V}_{O_2}$ peak, $\dot{V}_{O_2}$ at lactate threshold and speed pulmonary oxygen uptake kinetics in COPD. 13 patients (9 males, age 66 ± 5 years, mean ± SD) with severe COPD (mean FEV_1 0.88 ± 0.23 l, 33 ± 7% predicted) performed on separate days ramp cycle-ergometry (5–10 W min^{-1}) to volitional exhaustion and subsequently square-wave transitions to 80% estimated lactate threshold (LT) following consumption of either placebo (CON) or 0.125 g $kg\,bm^{-1}$ of glutamine (GLN) in 5 ml $kg\,bm^{-1}$ placebo. Oral glutamine had no effect on peak or $\dot{V}_{O_2}$ at LT, {$\dot{V}_{O_2}$ peak: CON = 0.70 ± 0.1 l min^{-1} vs. GLN = 0.73 ± 0.2 l min^{-1}; LT: CON = 0.57 ± 0.1 l min^{-1} vs. GLN = 0.54 ± 0.1 l min^{-1}} or $\dot{V}_{O_2}$ kinetics {tau: CON = 68 ± 22 s vs. GLN = 68 ± 16 s}. Ingestion of glutamine before exercise did not improve indices of oxidative metabolism in this patient group.

1. Introduction

During exercise, patients suffering from chronic obstructive pulmonary disease (COPD) show an enhanced production of lactate, phosphocreatine breakdown and lower blood pH during exercise as compared to healthy age-matched controls (Engelen et al., 2000b; Kutsuzawa et al., 1995; Maltais et al., 1998). The skeletal muscles of such patients are characterised by a muscle fibre type shift toward a greater proportion of fast twitch fibres (Gosker et al., 2002; Maltais et al., 1999), a reduction in key oxidative enzymes such as citrate synthase (Maltais et al., 2000), adenine nucleotide loss (Steiner et al., 2005), enhanced muscle lactate concentration even at rest (Engelen et al., 2000b) and slowed pulmonary oxygen uptake kinetics during recovery from exercise (Okamoto et al., 2003). Furthermore, an acute intervention to relieve intracellular metabolic inertia (dichloroacetate infusion), independent of oxygen delivery resulted in significant improvements in $\dot{V}_{O_2}$ peak and peak work rate during incremental exercise (Calvert et al., 2008). Taken together, these data suggest that in addition to the dyspnoea associated with COPD, impaired oxidative metabolism (and thus increased reliance upon anaerobic metabolism) in the ambulatory muscles can help explain the exercise intolerance found with this disease.

One explanation for this increased reliance upon anaerobic metabolism in COPD is the augmented delay (~300 s) in the attainment of a steady state of oxygen uptake during the transition from rest to exercise (Chiappa et al., 2008; Puente-Maestu et al., 2001; Somfay et al., 2002) as compared to ~180 s in healthy, untrained controls (Somfay et al., 2002). The extent of this "metabolic inertia" dictates the magnitude of the oxygen deficit produced during the rest–exercise transition, which in turn determines the degree of reliance upon anaerobic metabolism and the production of potentially fatiguing milieu, i.e. increased inorganic phosphate and ADP, reduced ATP and phosphocreatine (Allen et al., 2008). Indeed, an inverse relationship ($r = -0.7$) has previously been shown between the time constant of the kinetics of pulmonary oxygen uptake and the time to exhaustion at a constant workload in COPD (Chiappa et al., 2008).

Candidate sites within the oxidative respiratory chain that might contribute to this metabolic inertia include the pyruvate dehyrogenase complex (PDC), electron transport chain, oxygen delivery and the tricarboxylic acid (TCA) cycle. In COPD, a lag in the activation of the PDC (Calvert et al., 2008) and delivery of oxygen (Calvert et al., 2008; Chiappa et al., 2008, 2009; Laveneziana et al., 2009; Palange et al., 1995) have both been shown to be limiting to oxidative metabolism during the transition from rest to exercise. The tricarboxylic (TCA) cycle and its intermediates (TCAi) do not seem to limit oxidative metabolism during the transition from rest to exercise in healthy populations, (Bruce et al., 2001), however there is evidence that the TCAi may be a limiting factor for oxidative metabolism in COPD.

* Corresponding author. Tel.: +44 0151 291 3629; fax: +44 0151 291 3414.
E-mail address: marwoos@hope.ac.uk (S. Marwood).

1569-9048/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.resp.2011.03.010

During the transition from rest to exercise in healthy subjects there is a ~300% increase in the concentration of the TCAi within the first 5 min. This *anaplerosis* of the TCA cycle is primarily determined by increased flux through the alanine aminotransferase reaction (Gibala et al., 1997), which requires glutamate and pyruvate, producing alanine and entering the TCA cycle at the level of α-ketoglutarate. In healthy populations, oxidative metabolism does not appear to be limited by TCAi pool size (Bruce et al., 2001); however TCAi pool expansion is impaired in COPD patients (Engelen et al., 2001) and is associated with a low concentration of glutamate in the resting muscle compared to healthy age-matched controls (Engelen et al., 2000a,b, 2001). Glutamate concentration determines flux through the alanine aminotransferase reaction (Bruce et al., 2001; Gibala et al., 2002; Gibala and Saltin, 1999). It is therefore feasible that the reduced muscle glutamate concentration in COPD inhibits flux through the alanine aminotransferase reaction, and thus anaplerosis, resulting in significant metabolic inertia and a consequent retarding of the rate of increase of oxidative metabolism during the transition to exercise.

Ingested glutamine is rapidly absorbed causing a sharp rise in plasma glutamine concentration (Marwood and Bowtell, 2007). In apparent contrast to glutamate ingestion (Darmaun et al., 1986; Matthews et al., 1993; Rutten et al., 2008), circulating glutamine is readily taken up by the skeletal muscle (Mittendorfer et al., 2001) and can be converted to glutamate via the enzyme glutaminase. Therefore if low muscle glutamate concentration is inhibiting flux through the alanine aminotransferase reaction in COPD, increasing glutamine ingestion may reverse this effect. Indeed glutamine ingestion has previously been shown to enhance the exercise-induced increase in the TCAi pool during the transition from rest to exercise in healthy subjects (Bruce et al., 2001). However, in these healthy subjects for whom TCAi pool size is not limiting, additional expansion of the TCAi pool did not enhance oxidative metabolism. In contrast, COPD patients experience significant metabolic inertia and blunted TCAi pool expansion during exercise associated with reduced glutamate availability. We therefore hypothesise that glutamine ingestion and the resultant augmented TCAi pool expansion at the onset of exercise will enhance oxidative metabolism in COPD patients. Therefore the aim of the present study was to determine whether prior glutamine ingestion enhances $\dot{V}_{O_2}$ peak and $\dot{V}_{O_2}$ at lactate threshold during incremental exercise and speeds pulmonary oxygen uptake kinetics during constant load exercise.

2. Method

2.1. Patients

We recruited 13 COPD patients (9 males) with clinically and physiologically confirmed moderate to severe COPD, all of which completed the study. All patients were ex-smokers, clinically stable (free from exacerbations for at least 3 months prior to the study) and not taking any oral steroids. All patients gave written informed consent and the protocol was approved by the South Sefton Research Ethics Committee.

2.2. Lung function

One week prior to the first exercise tests, all patients underwent spirometry, measurement of lung volumes and measurement of lung diffusion (whole-body plethysmography, ZAN560, Oberthulba, Germany). The equipment was calibrated according to the manufacturer's instructions. Subjects were asked to omit short-acting bronchodilators for 8 h, long-acting beta-agonists for 12 h and long-acting anti-cholinergics for 24 h before testing. Subjects were asked not to consume caffeine containing drinks on the day of testing. All tests were performed in accordance with ATS/ERS testing criteria.

2.3. Experimental design

Patients visited the laboratory on 4–6 occasions, completing a single exercise test each time on a cycle ergometer (Lode Corival, Groningen, The Netherlands). Prior to each visit to the laboratory, patients were requested to maintain an overnight fast and to consume their habitual diet and refrain from strenuous physical activity and consuming alcohol or caffeine in the 48 h prior to each experiment. One hour before exercise subjects consumed either 5 ml kg^{-1} of weak lemon flavoured cordial (ASDA, Leeds, UK) as a placebo (CON) or the same volume of placebo in which was dissolved 0.125 g kg^{-1} of glutamine (L-glutamine, Myprotein.com, UK), (GLN) in a double blind manner. During all exercise tests and in the 5 min following exercise cessation, heart activity (12-lead ECG), blood pressure (manual auscultation), perceived exertion (10-point scale for leg fatigue and breathlessness, (Jack et al., 2004)), oxygen saturation of blood (pulse oximetry, Nonin 8500, Nonin Medical, USA), ventilation and gas exchange (Zan 600, Oberthulba, Germany) were monitored. The Zan 600 metabolic cart uses a pneumotachometer for measuring ventilation, in combination with amperometric solid electrolyte oxygen sensor and infra-red carbon dioxide sensor. Capillary earlobe blood samples were also taken at rest, end-exercise and 4-min post exercise for determination of blood lactate concentration (Biosen C-line, EKF, Germany).

2.4. Part (a): incremental exercise

Patients completed two (one in each condition, the order randomly allocated) ramp incremental tests 3–7 days apart. Ramp rate was at 5 or 10 W min^{-1} (55–65 rpm) until volitional termination of exercise, the lower ramp rate being chosen for those patients with an FEV_1 < 1 l. The $\dot{V}_{O_2}$ at lactate threshold was estimated via the v-slope method with confirmatory data from the ventilatory equivalent and end-tidal pressure plots for oxygen uptake and carbon dioxide production (Beaver et al., 1986). $\dot{V}_{O_2}$ peak was defined as the highest 30-s average at any stage during the test.

2.5. Part (b): constant load exercise

One week following completion of part (a), patients completed a number of constant load exercise transitions of 6 min in duration instigated from a 3-min baseline of rest to a workload equivalent to 80% of the lactate threshold; cadence was maintained at 55–65 rpm each time. Patients completed 1 (six patients) or 2 (seven patients) transitions in each condition (the order of which was allocated randomly), with only one exercise transition per day. All testing for part (b) was completed within 10 days. Patients conducted an equal number of each type of exercise test in each condition (i.e. placebo or glutamine ingestion).

2.6. Oxygen uptake kinetics

Since exercise was undertaken at an intensity below the lactate threshold, pulmonary oxygen uptake kinetics during constant load exercise were assumed to proceed via single exponential term (fundamental phase) following a delay relative to the start of exercise:

$$\dot{V}_{O_{2(t)}} = \dot{V}_{O_{2(b)}} + A_{V_{O_2}} * (1 - e^{-(t-TD)/\tau_{V_{O_2}}})$$

The delay term accounts for the transit time of deoxygenated blood from the peripheral muscles to the lungs (cardiodynamic phase); data within this phase were removed from the dataset prior to the

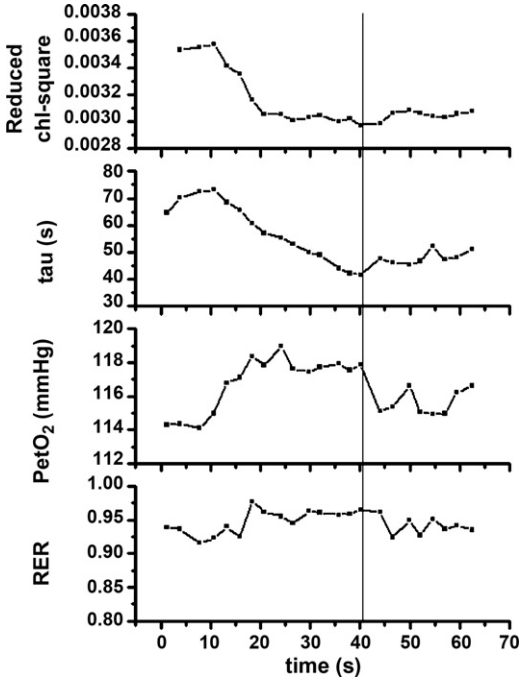

Fig. 1. Determination of the end of the cardiodynamic phase of pulmonary oxygen uptake kinetics. Vertical line represents the point in time where a sharp drop in RER and the end-tidal pressure of O_2 coincides with a plateau or minima for the value of τ and a minima for the reduced chi-square value.

modelling process. $\dot{V}_{O_{2(b)}}$ is the oxygen uptake measured in the last minute of rest, $A_{V_{O_2}}$ is the asymptotic amplitude of the fundamental component of the response, $\tau_{V_{O_2}}$ is the time constant of the fundamental component and *TD* is a time delay similar, but not equal to the cardiodynamic–fundamental phase transition time (which is defined as $TD_{V_{O_2}}$). Steady state oxygen uptake ($\dot{V}_{O_{2(ss)}}$) is therefore the sum of $\dot{V}_{O_{2(b)}}$ and $A_{V_{O_2}}$. To give an indication of the overall kinetic response to exercise, the mean response time ($MRT_{V_{O_2}}$) was calculated as $TD_{V_{O_2}} + \tau_{V_{O_2}}$.

To prepare the data for the modelling process, abnormal breaths due to coughs and swallows were first removed from the data so as not to skew the underlying response. The criterion for removal of these breaths was those that were different to the mean of the surrounding four data points by more than three times the standard deviation of those four points (Brittain et al., 2001). Data were then interpolated second-by-second; when more than one transition was completed for a given condition (i.e. placebo or glutamine) the datasets were ensemble averaged to yield a single response for that condition. The end of the cardiodynamic phase ($TD_{V_{O_2}}$) was evaluated by (i) observing the point at which there was a sharp fall in end-tidal pressure of O_2 and RER (Whipp and Ward, 1990) coincident with (ii) the time-point displaying a plateau or minima for the value of τ and a minima for the reduced chi-square value when the start of the fitting window was incrementally from 0 s up to 60 s. Custom written software in Microsoft Excel, using the Solver function, was utilised for all modelling processes, an example of which is provided in Fig. 1.

2.7. Statistics

All values are presented as mean ± standard deviation, with differences between conditions evaluated by a paired sample *t*-test. Relationships between variables were assessed via a Pearson correlation with statistical significance accepted at $P \leq 0.05$.

Table 1
Baseline characteristics of patients.

	Value	% predicted
Age (yrs)	66 ± 5	N/A
Height (m)	1.69 ± 7.0	N/A
Mass (kg)	74.8 ± 17.4	N/A
BMI	26.2 ± 5.3	N/A
FEV_1 (l)	0.88 ± 0.23	32.5 ± 6.9
FVC (l)	2.38 ± 0.64	70 ± 14
FEV_1/FVC (%)	40 ± 15	51 ± 20
TLC (l)	7.2 ± 1.1	119 ± 17
RV (l)	5.1 ± 1.0	221 ± 65
DLCO ($mmol^{-1}\,min^{-1}\,kPa^{-1}$)	3.1 ± 1.1	32.2 ± 9.4
KCO	0.77 ± 0.25	56 ± 20

DLCO: diffusing capacity of the lung for carbon monoxide; KCO: carbon monoxide transfer coefficient.

3. Results

3.1. Patients

Table 1 shows patient characteristics including resting lung function data. The static lung function results suggest that the patient group had significant airflow obstruction as shown by FEV_1 and FEV_1/FVC data, pulmonary hyperinflation as shown by total lung capacity (TLC) and residual volume (RV) data with evidence of emphysema as shown by the diffusing capacity of the lung for carbon monoxide (DLCO) and carbon monoxide transfer coefficient (KCO) data.

3.2. Part a: incremental exercise

Glutamine ingestion had no effect on the $\dot{V}_{O_2}$ at lactate threshold (CON: 0.57 ± 0.1 l min^{-1} vs GLN: 0.54 ± 0.1 l min^{-1}), $\dot{V}_{O_2}$ peak (CON: 0.70 ± 0.1 l min^{-1} vs GLN: 0.73 ± 0.2 l min^{-1}) or peak workrate achieved (CON: 39 ± 17 W vs GLN: 42 ± 18 W). There was also no effect of glutamine ingestion on resting (CON: 1.1 ± 0.3 mmol l^{-1} vs GLN: 1.0 ± 0.3 mmol l^{-1}), end- (CON: 2.3 ± 0.8 mmol l^{-1} vs GLN: 2.2 ± 0.9 mmol l^{-1}), or post-exercise (CON: 2.2 ± 0.8 mmol l^{-1} vs GLN: 2.6 ± 1.3 mmol l^{-1}), blood lactate concentration. At the termination of exercise, blood oxygen saturation (CON: 90 ± 4% vs GLN: 90 ± 4%), ratings of perceived leg exertion (CON: 5.5 ± 2.7 vs GLN: 5.4 ± 3.2) and ratings of perceived breathlessness (CON: 6.4 ± 1.9 vs GLN: 7.2 ± 2.4) were not different between trials.

3.3. Part b: constant load exercise

A representative plot of the oxygen uptake response to constant load exercise is shown in Fig. 2. The confidence interval of $\tau_{V_{O_2}}$ was 6.9 ± 2.0 s in CON and 7.4 ± 3.4 s in GLN (CON: 11.4 ± 4.9 vs 11.5 ± 4.8, expressed as % of $\tau_{V_{O_2}}$). When comparing the 6 patients who completed two transitions in each condition with the remaining 7 who completed just one, there did not appear to be any significant narrowing of these confidence intervals (CON: 6.9 ± 2.2 s vs 6.8 ± 2.0 s; GLN: 6.6 ± 2.0 s vs 8.1 ± 4.3 s or CON: 10.4 ± 2.7 vs 12.3 ± 6.4; GLN: 10.7 ± 3.2 vs GLN: 12.1 ± 6.1 expressed as % of $\tau_{V_{O_2}}$; two transition vs one transition, respectively). There was no relationship between markers of the extent of airflow obstruction, emphysema or pulmonary hyperinflation and $\tau_{V_{O_2}}$, its confidence interval or $MRT_{V_{O_2}}$. The only exception to this was a significant relationship between the absolute total lung capacity and the 95% confidence interval for $\tau_{V_{O_2}}$ ($r = 0.74$, $P < 0.01$) indicating that ~54% of the variation in the 95% confidence interval of $\tau_{V_{O_2}}$ could be explained by absolute total lung capacity.

Glutamine ingestion had no effect on either the time-constant or mean response time of oxygen uptake kinetics (Table 2) or sub-

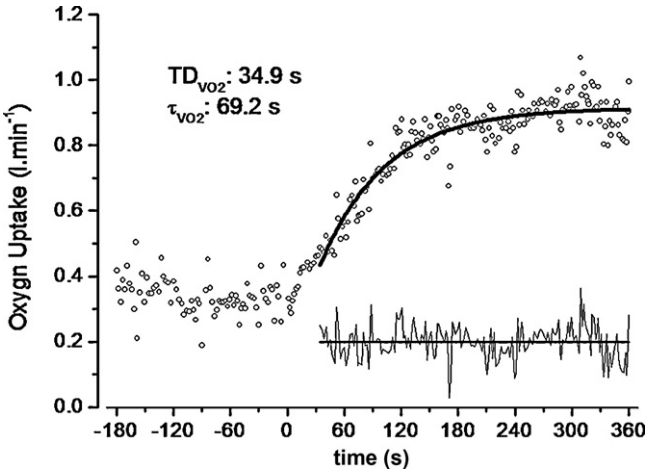

Fig. 2. Representative plot of pulmonary oxygen uptake kinetics with exponential curve fit and residuals shown; in this example a placebo trial is shown.

Table 2
Parameters of constant load exercise in the placebo (CON) and glutamine ingestion (GLN) conditions.

	CON	GLN
$V_{O_{2(b)}}$ (l min^{-1})	0.28 ± 0.05	0.29 ± 0.05
$A_{V_{O_2}}$ (l min^{-1})	0.36 ± 0.07	0.36 ± 0.10
$V_{O_{2(ss)}}$ (l min^{-1})	0.64 ± 0.09	0.66 ± 0.13
$TD_{V_{O_2}}$ (s)	34 ± 10	36.0 ± 6.1
$\tau_{V_{O_2}}$ (s)	68 ± 22	68 ± 16
$MRT_{V_{O_2}}$ (s)	102 ± 24	104 ± 16
Pre-ex blood [lactate] (mmol l^{-1})	1.01 ± 0.33	1.12 ± 0.23
End-ex blood [lactate] (mmol l^{-1})	2.05 ± 0.93	2.05 ± 0.93
Oxygen saturation (%)	92 ± 3	91 ± 3
Leg fatigue	2.7 ± 2.3	2.7 ± 2.1
Breathlessness	3.1 ± 1.6	3.1 ± 1.6

jective indicators of fatigue (Table 2). There was also no effect of glutamine ingestion on resting or end-exercise blood lactate concentration or blood oxygen saturation (Table 2).

4. Discussion

The main finding of the present study was that glutamine ingestion prior to exercise conferred no beneficial effect on oxidative metabolism or performance during incremental and constant load exercise in COPD. Peak work-rate, peak oxygen uptake, oxygen uptake at lactate threshold and oxygen uptake kinetics were all unchanged following prior glutamine ingestion as compared to placebo. Given these findings it is therefore unsurprising that blood lactate concentration and subjective indicators of fatigue were also unaffected by glutamine ingestion.

Potential sites within the oxidative respiratory chain that may contribute to metabolic inertia at the onset of exercise, in both health and disease include oxygen delivery, the electron transport chain, the PDC and the TCA cycle. Enhancing oxygen delivery through hyperoxia has been demonstrated to enhance performance during maximal incremental exercise in healthy subjects (Prieur et al., 2002), and during high intensity constant load exercise in both healthy subjects and COPD (Siqueira et al., 2010; Wilkerson et al., 2006). Whilst such interventions have no effect on the fundamental phase of pulmonary oxygen uptake kinetics in healthy subjects, (Hughson and Kowalchuk, 1995; Wilkerson et al., 2006), this has been shown to be speeded by hyperoxia and heliox in COPD (Chiappa et al., 2009; Palange et al., 1995). Infusion of L-NAME speeded pulmonary oxygen uptake kinetics at the onset of moderate and heavy exercise in healthy subjects, possibly by relieving inhibition of enzymes in the electron transport chain (Jones et al., 2003, 2004a); such studies have not however been replicated in COPD. Activation of the PDC prior to exercise via DCA infusion has on a number of occasions been shown to reduce the muscle lactate accumulation and phosphocreatine degradation during subsequent constant load exercise, suggesting a role of the PDC in determining intracellular metabolic inertia (Howlett et al., 1999; Timmons et al., 1998a,b). Despite these findings, a number of studies have found no effect of DCA infusion on the fundamental time constant of pulmonary oxygen uptake kinetics at the onset of exercise (Koppo et al., 2004; Jones et al., 2004b; Marwood et al., 2010; Rossiter et al., 2003). During incremental exercise, it was recently demonstrated that DCA infusion enhanced peak $\dot{V}_{O_2}$ and work rate in COPD (Calvert et al., 2008). Importantly, this effect of DCA infusion during incremental exercise was not replicated in healthy subjects (Wilkerson et al., 2009), demonstrating that the effects of a given intervention on intracellular inertia may be apparently small in healthy subjects, but significant in certain diseased populations. The present study is the first to examine the potential for the TCAi pool size to confer a limiting role in COPD patients. Whilst there is little evidence in healthy subjects to suggest that the rate and magnitude of the exercise-induced increase in the TCAi at the onset of exercise is limiting to oxidative metabolism (Bowtell et al., 2007), the above discussion demonstrates how findings in healthy subjects can rarely be applied to diseased populations such as COPD without experimental confirmation.

In contrast to healthy subjects, anaplerosis of the TCA cycle is apparently impaired in COPD during exercise since Engelen et al., (Engelen et al., 2001) showed no change in muscle succinate (one of the TCAi) concentration and a 5% *fall* in muscle alanine concentration (indicative of impaired flux through the primary anaplerotic reaction, alanine aminotransferase). The concentration of glutamate appears to be the most important factor in determining flux through the alanine aminotransferase reaction (Bruce et al., 2001; Gibala et al., 2002; Gibala and Saltin, 1999). The very low muscle glutamate content seen at rest in COPD as compared to healthy controls (Engelen et al., 2000a,b, 2001) therefore suggests impairment of TCA cycle anaplerosis in COPD due to low muscle glutamate concentration, and thus a potential mechanism by which oxidative metabolism is inhibited. It has previously been shown that glutamine ingestion prior to exercise results in an enhancement of the exercise induced increase in the TCAi pool (Bruce et al., 2001). In the present study, glutamine ingestion was therefore used as a means by which to mitigate a chronically low muscle glutamate concentration, and thus enhance the exercise-induced increase in the TCAi pool, in an attempt to reduce some of the significant metabolic inertia which exists in COPD. However as previously demonstrated in healthy subjects (Bruce et al., 2001; Marwood and Bowtell, 2008; Marwood and Bowtell, 2007), in the present study glutamine ingestion had no effect on the measured indices of oxidative metabolism suggesting either that glutamine ingestion did not impact upon TCA cycle anaplerosis or that the normal rate and magnitude of the increase of the TCAi during exercise is not limiting to oxidative metabolism in COPD.

Though we replicated the glutamine ingestion protocol of Bruce et al. (Bruce et al., 2001), the present study was limited by not confirming its intended effects either by measuring muscle TCAi (Bruce et al., 2001) or plasma (Marwood and Bowtell, 2007) glutamine concentrations. However, a previous study with a similar patient group showed no difference in the plasma glutamine response to glutamine ingestion as compared to healthy controls (Rutten et al., 2006). Therefore it seems unlikely that the sharp rise in plasma glutamine concentration as seen in previous, identical glutamine ingestion regimes (Marwood and Bowtell, 2007) did not occur. Since the Km of glutamine transport in human skeletal muscle is close to normal plasma glutamine concentration (Ahmed et al.,

1993), glutamine uptake by the muscle is primarily dependent upon extracellular glutamine concentration. There is no evidence to suggest that either the system Nm amino acid transporter or glutaminase activity are adversely affected in this patient group. However, it is plausible that intramuscular alanine aminotransferase activity is reduced in COPD since this has been shown in the sera of COPD as compared to healthy controls (Cepelak et al., 2006). Though unlikely, it is possible therefore that glutamine ingestion did not result in TCAi pool expansion as previously observed in controls (Bruce et al., 2001) since we were unable to experimentally confirm this.

Assuming that glutamine ingestion did result in an enhancement of the exercise-induced increase in the TCAi pool, then we can conclude that the normal rate and magnitude of the changes in the TCAi pool are not limiting oxidative metabolism during exercise in COPD. However any increase in the capacity for TCA cycle flux provided for by glutamine ingestion necessitates a concomitant increase in oxygen delivery to oxidise the additional NADH and $FADH_2$. Hence, manipulations of the TCAi alone, may serve only to exacerbate the oxygen delivery limitation that exists within this patient group, (Chiappa et al., 2008, 2009; Laveneziana et al., 2009; Palange et al., 1995), with no overall effect on oxidative metabolism. In other words, interventions to enhance oxidative metabolism may only be effective if they relieve inhibition at multiple limiting sites in the respiratory chain, e.g. glutamine ingestion combined with hyperoxia or heliox. In addition to this possibility, the methods used to assess oxidative metabolism deserve some discussion as to their suitability to detect alterations in oxidative metabolism due to glutamine ingestion.

The indirect methods used in the present study to measure the impact of glutamine ingestion on indices of oxidative metabolism have previously been shown to be sufficiently sensitive to detect changes reflecting improvements to oxidative metabolism in this patient group. For example, the lactate threshold, peak work rate, peak oxygen uptake and oxygen uptake kinetics have all been shown to be improved in COPD patients following both acute, e.g. dichloroacetate infusion (Calvert et al., 2008), heliox (Chiappa et al., 2009) and hyperoxia (Palange et al., 1995) and chronic, e.g. training (Casaburi et al., 1997; Coppoolse et al., 1999; Franssen et al., 2004; Ortega et al., 2002; Otsuka et al., 1997; Vogiatzis et al., 2005) interventions designed to enhance the capability of oxidative metabolism. Consequently the present data suggest no effect of glutamine ingestion on $\dot{V}_{O_2}$ peak, $\dot{V}_{O_2}$ at lactate threshold and pulmonary oxygen uptake kinetics in COPD. Based on the proposed effects of glutamine ingestion, we therefore suggest that the rate and magnitude of anaplerosis of the TCA cycle at the onset of exercise is not limiting to oxidative metabolism in COPD. Whether this remains the case in the presence of interventions which can enhance oxygen delivery, (e.g. hyperoxia, heliox), remains to be elucidated.

Perhaps the parameter around which most uncertainty can arise is that of the time constant of oxygen uptake kinetics. The confidence by which pulmonary oxygen uptake kinetics can be modelled is dependent on the amplitude of the response, number of exercise transitions and the "noise" in the breath-by-breath data (Lamarra et al., 1987). Hence type 2 errors can easily occur if wide confidence intervals do not allow discrimination between "real" effects of the imposed intervention. In the present study, the amplitude of the response was relatively low (~0.4 l), reflecting the low-work rate at lactate threshold, and only one or two transitions were completed in each condition. However, COPD patients do not possess greater breath-to-breath noise than healthy subjects (Puente-Maestu et al., 2002), suggesting that the severity of airflow does not impact upon the confidence of the modelled parameters; although in the present study there appeared to be a weak relationship between total lung capacity (an index of pulmonary hyperinflation) and the 95% confidence interval of the time constant of pulmonary oxygen uptake kinetics ($r = 0.74$). More importantly, the 95% confidence interval for the time constant of oxygen uptake kinetics we observed (~11–12% of the mean) is more than acceptable (Fawkner et al., 2002) and is an improvement on previous studies in COPD (Palange et al., 1995). We are therefore confident of the modelling process, and together with the similarity of the mean value of this parameter between conditions, it seems unlikely a type 2 error occurred; rather the oxygen uptake kinetic data supports the blood lactate and perceived exertion data during constant load exercise and the $\dot{V}_{O_2}$ peak, peak work-rate and $\dot{V}_{O_2}$ at lactate threshold data during incremental exercise, all showing no effect of glutamine ingestion on oxidative metabolism in COPD.

In summary, the present study showed no effect of glutamine ingestion on aerobic function during incremental or constant load exercise in COPD. We used glutamine ingestion as a means to mitigate the chronically low muscle glutamate concentration in this patient group and enhance flux through the alanine aminotransferase reaction, thus reducing metabolic inertia at the level of the TCA cycle. Based on this hypothesis the data therefore suggest that TCA cycle anaplerosis is not limiting for oxidative metabolism during exercise in COPD patients.

References

Ahmed, A., Maxwell, D.L., Taylor, P.M., Rennie, M.J., 1993. Glutamine transport in human skeletal muscle. Am. J. Physiol. 264, E993–E1000.

Allen, D.G., Lamb, G.D., Westerblad, H., 2008. Skeletal muscle fatigue: cellular mechanisms. Physiol. Rev. 88, 287–332.

Beaver, W.L., Wasserman, K., Whipp, B.J., 1986. A new method for detecting anaerobic threshold by gas exchange. J. Appl. Physiol. 60, 2020–2027.

Bowtell, J.L., Marwood, S., Bruce, M., Constantin-Teodosiu, D., Greenhaff, P.L., 2007. Tricarboxylic acid cycle intermediate pool size: functional importance for oxidative metabolism in exercising human skeletal muscle. Sports Med. 37, 1071–1088.

Brittain, C.J., Rossiter, H.B., Kowalchuk, J.M., Whipp, B.J., 2001. Effect of prior metabolic rate on the kinetics of oxygen uptake during moderate-intensity exercise. Eur. J. Appl. Physiol. 86, 125–134.

Bruce, M., Constantin-Teodosiu, D., Greenhaff, P.L., Boobis, L.H., Williams, C., Bowtell, J.L., 2001. Glutamine supplementation promotes anaplerosis but not oxidative energy delivery in human skeletal muscle. Am. J. Physiol Endocrinol. Metab. 280, E669–E675.

Calvert, L.D., Shelley, R., Singh, S.J., Greenhaff, P.L., Bankart, J., Morgan, M.D., Steiner, M.C., 2008. Dichloroacetate enhances performance and reduces blood lactate during maximal cycle exercise in chronic obstructive pulmonary disease. Am. J. Respir. Crit Care Med. 177, 1090–1094.

Casaburi, R., Porszasz, J., Burns, M.R., Carithers, E.R., Chang, R.S., Cooper, C.B., 1997. Physiologic benefits of exercise training in rehabilitation of patients with severe chronic obstructive pulmonary disease. Am. J. Respir. Crit Care Med. 155, 1541–1551.

Cepelak, I., Dodig, S., Romic, D., Ruljancic, N., Popovic-Grle, S., Malic, A., 2006. Enzyme catalytic activities in chronic obstructive pulmonary disease. Arch. Med. Res. 37, 624–629.

Chiappa, G.R., Borghi-Silva, A., Ferreira, L.F., Carrascosa, C., Oliveira, C.C., Maia, J., Gimenes, A.C., Queiroga Jr., F., Berton, D., Ferreira, E.M., Nery, L.E., Neder, J.A., 2008. Kinetics of muscle deoxygenation are accelerated at the onset of heavy-intensity exercise in patients with COPD: relationship to central cardiovascular dynamics. J. Appl. Physiol. 104, 1341–1350.

Chiappa, G.R., Queiroga Jr., F., Meda, E., Ferreira, L.F., Diefenthaeler, F., Nunes, M., Vaz, M.A., Machado, M.C., Nery, L.E., Neder, J.A., 2009. Heliox improves oxygen delivery and utilization during dynamic exercise in patients with chronic obstructive pulmonary disease. Am. J. Respir. Crit Care Med. 179, 1004–1010.

Coppoolse, R., Schols, A.M., Baarends, E.M., Mostert, R., Akkermans, M.A., Janssen, P.P., Wouters, E.F., 1999. Interval versus continuous training in patients with severe COPD: a randomized clinical trial. Eur. Respir. J. 14, 258–263.

Darmaun, D., Matthews, D.E., Bier, D.M., 1986. Glutamine and glutamate kinetics in humans. Am. J. Physiol. 251, E117–E126.

Engelen, M.P., Schols, A.M., Does, J.D., Deutz, N.E., Wouters, E.F., 2000a. Altered glutamate metabolism is associated with reduced muscle glutathione levels in patients with emphysema. Am. J. Respir. Crit Care Med. 161, 98–103.

Engelen, M.P., Schols, A.M., Does, J.D., Gosker, H.R., Deutz, N.E., Wouters, E.F., 2000b. Exercise-induced lactate increase in relation to muscle substrates in patients with chronic obstructive pulmonary disease. Am. J. Respir. Crit Care Med. 162, 1697–1704.

Engelen, M.P., Wouters, E.F., Deutz, N.E., Does, J.D., Schols, A.M., 2001. Effects of exercise on amino acid metabolism in patients with chronic obstructive pulmonary disease. Am. J. Respir. Crit Care Med. 163, 859–864.

Fawkner, S.G., Armstrong, N., Potter, C.R., Welsman, J.R., 2002. Oxygen uptake kinetics in children and adults after the onset of moderate-intensity exercise. J. Sports Sci. 20, 319–326.
Franssen, F.M., Broekhuizen, R., Janssen, P.P., Wouters, E.F., Schols, A.M., 2004. Effects of whole-body exercise training on body composition and functional capacity in normal-weight patients with COPD. Chest 125, 2021–2028.
Gibala, M.J., MacLean, D.A., Graham, T.E., Saltin, B., 1997. Anaplerotic processes in human skeletal muscle during brief dynamic exercise. J. Physiol. 502, 703–713.
Gibala, M.J., Saltin, B., 1999. PDH activation by dichloroacetate reduces TCA cycle intermediates at rest but not during exercise in humans. Am. J. Physiol. 277, E33–E38.
Gibala, M.J., Peirce, N., Constantin-Teodosiu, D., Greenhaff, P.L., 2002. Exercise with low muscle glycogen augments TCA cycle anaplerosis but impairs oxidative energy provision in humans. J. Physiol. 540, 1079–1086.
Gosker, H.R., van Mameren, H., van Dijk, P.J., Engelen, M.P., van der Vusse, G.J., Wouters, E.F., Schols, A.M., 2002. Skeletal muscle fibre-type shifting and metabolic profile in patients with chronic obstructive pulmonary disease. Eur. Respir. J. 19, 617–625.
Howlett, R.A., Heigenhauser, G.J., Hultman, E., Hollidge-Horvat, M.G., Spriet, L.L., 1999. Effects of dichloroacetate infusion on human skeletal muscle metabolism at the onset of exercise. Am. J. Physiol. 277, E18–E25.
Hughson, R.L., Kowalchuk, J.M., 1995. Kinetics of oxygen uptake for submaximal exercise in hyperoxia, normoxia, and hypoxia. Can. J. Appl. Physiol. 20, 198–210.
Jack, S., Rossiter, H.B., Pearson, M.G., Ward, S.A., Warburton, C.J., Whipp, B.J., 2004. Ventilatory responses to inhaled carbon dioxide, hypoxia, and exercise in idiopathic hyperventilation. Am. J. Respir. Crit Care Med. 170, 118–125.
Jones, A.M., Wilkerson, D.P., Koppo, K., Wilmshurst, S., Campbell, I.T., 2003. Inhibition of nitric oxide synthase by L-NAME speeds phase II pulmonary.VO_2 kinetics in the transition to moderate-intensity exercise in man. J. Physiol. 552, 265–272.
Jones, A.M., Wilkerson, D.P., Wilmshurst, S., Campbell, I.T., 2004a. Influence of L-NAME on pulmonary O_2 uptake kinetics during heavy-intensity cycle exercise. J. Appl. Physiol. 96, 1033–1038.
Jones, A.M., Koppo, K., Wilkerson, D.P., Wilmshurst, S., Campbell, I.T., 2004b. Dichloroacetate does not speed phase-II pulmonary VO_2 kinetics following the onset of heavy intensity cycle exercise. Pflugers Arch. 447, 867–874.
Koppo, K., Wilkerson, D.P., Bouckaert, J., Wilmshurst, S., Campbell, I.T., Jones, A.M., 2004. Influence of DCA on pulmonary $(\dot{V}O_2)$ kinetics during moderate-intensity cycle exercise. Med. Sci. Sports Exerc. 36, 1159–1164.
Kutsuzawa, T., Shioya, S., Kurita, D., Haida, M., Ohta, Y., Yamabayashi, H., 1995. Muscle energy metabolism and nutritional status in patients with chronic obstructive pulmonary disease. A 31P magnetic resonance study. Am. J. Respir. Crit Care Med. 152, 647–652.
Lamarra, N., Whipp, B.J., Ward, S.A., Wasserman, K., 1987. Effect of interbreath fluctuations on characterizing exercise gas exchange kinetics. J. Appl. Physiol. 62, 2003–2012.
Laveneziana, P., Palange, P., Ora, J., Martolini, D., O'Donnell, D.E., 2009. Bronchodilator effect on ventilatory, pulmonary gas exchange, and heart rate kinetics during high-intensity exercise in COPD. Eur. J. Appl. Physiol..
Maltais, F., Jobin, J., Sullivan, M.J., Bernard, S., Whittom, F., Killian, K.J., Desmeules, M., Belanger, M., Leblanc, P., 1998. Metabolic and hemodynamic responses of lower limb during exercise in patients with COPD. J. Appl. Physiol. 84, 1573–1580.
Maltais, F., Sullivan, M.J., Leblanc, P., Duscha, B.D., Schachat, F.H., Simard, C., Blank, J.M., Jobin, J., 1999. Altered expression of myosin heavy chain in the vastus lateralis muscle in patients with COPD. Eur. Respir. J. 13, 850–854.
Maltais, F., Leblanc, P., Whittom, F., Simard, C., Marquis, K., Belanger, M., Breton, M.J., Jobin, J., 2000. Oxidative enzyme activities of the vastus lateralis muscle and the functional status in patients with COPD. Thorax 55, 848–853.
Marwood, S., Bowtell, J.L., 2007. Effects of glutamine and hyperoxia on pulmonary oxygen uptake and muscle deoxygenation kinetics. Eur. J. Appl. Physiol. 99, 149–161.
Marwood, S., Bowtell, J., 2008. No effect of glutamine supplementation and hyperoxia on oxidative metabolism and performance during high-intensity exercise. J. Sports Sci. 26, 1081–1090.
Marwood, S., Constantin-Teodosiu, D., Casey, E., Whyte, M., Boobis, L., Bowtell, J., 2010. No acetyl group deficit is evident at the onset of exercise at 90% of maximal oxygen uptake in humans. J. Sports Sci. (January), 1–13.
Matthews, D.E., Marano, M.A., Campbell, R.G., 1993. Splanchnic bed utilization of glutamine and glutamic acid in humans. Am. J. Physiol. 264, E848–E854.
Mittendorfer, B., Volpi, E., Wolfe, R.R., 2001. Whole body and skeletal muscle glutamine metabolism in healthy subjects. Am. J. Physiol. Endocrinol. Metab. 280, E323–E333.
Okamoto, T., Kanazawa, H., Hirata, K., Yoshikawa, J., 2003. Evaluation of oxygen uptake kinetics and oxygen kinetics of peripheral skeletal muscle during recovery from exercise in patients with chronic obstructive pulmonary disease. Clin. Physiol. Funct. Imaging 23, 257–262.
Ortega, F., Toral, J., Cejudo, P., Villagomez, R., Sanchez, H., Castillo, J., Montemayor, T., 2002. Comparison of effects of strength and endurance training in patients with chronic obstructive pulmonary disease. Am. J. Respir. Crit Care Med. 166, 669–674.
Otsuka, T., Kurihara, N., Fujii, T., Fujimoto, S., Yoshikawa, J., 1997. Effect of exercise training and detraining on gas exchange kinetics in patients with chronic obstructive pulmonary disease. Clin. Physiol. 17, 287–297.
Palange, P., Galassetti, P., Mannix, E.T., Farber, M.O., Manfredi, F., Serra, P., Carlone, S., 1995. Oxygen effect on O_2 deficit and VO_2 kinetics during exercise in obstructive pulmonary disease. J. Appl. Physiol. 78, 2228–2234.
Prieur, F., Benoit, H., Busso, T., Castells, J., Geyssant, A., Denis, C., 2002. Effects of moderate hyperoxia on oxygen consumption during submaximal and maximal exercise. Eur. J. Appl. Physiol. 88, 235–242.
Puente-Maestu, L., Sanz, M.L., Sanz, P., Nunez, A., Gonzalez, F., Whipp, B.J., 2001. Reproducibility of the parameters of the on-transient cardiopulmonary responses during moderate exercise in patients with chronic obstructive pulmonary disease. Eur. J. Appl. Physiol. 85, 434–441.
Puente-Maestu, L., Abad, M.J., Godoy, R., Perez-Parra, J.M., Cubillo, J.M., Whipp, B.J., 2002. Breath-by-breath fluctuations of pulmonary gas exchange and ventilation in COPD patients. Eur. J. Appl. Physiol. 87, 535–541.
Rossiter, H.B., Ward, S.A., Howe, F.A., Wood, D.M., Kowalchuk, J.M., Griffiths, J.R., Whipp, B.J., 2003. Effects of dichloroacetate on VO_2 and intramuscular 31P metabolite kinetics during high-intensity exercise in humans. J. Appl. Physiol. 95, 1105–1115.
Rutten, E.P., Engelen, M.P., Wouters, E.F., Schols, A.M., Deutz, N.E., 2006. Metabolic effects of glutamine and glutamate ingestion in healthy subjects and in persons with chronic obstructive pulmonary disease. Am. J. Clin. Nutr. 83, 115–123.
Rutten, E.P., Engelen, M.P., Gosker, H., Bast, A., Cosemans, K., Vissers, Y.L., Wouters, E.F., Deutz, N.E., Schols, A.M., 2008. Metabolic and functional effects of glutamate intake in patients with chronic obstructive pulmonary disease (COPD). Clin. Nutr. 27, 408–415.
Siqueira, A.C., Borghi-Silva, A., Bravo, D.M., Ferreira, E.M., Chiappa, G.R., Neder, J.A., 2010. Effects of hyperoxia on the dynamics of skeletal muscle oxygenation at the onset of heavy-intensity exercise in patients with COPD. Respir. Physiol. Neurobiol. 172, 8–14.
Somfay, A., Porszasz, J., Lee, S.M., Casaburi, R., 2002. Effect of hyperoxia on gas exchange and lactate kinetics following exercise onset in nonhypoxemic COPD patients. Chest 121, 393–400.
Steiner, M.C., Evans, R., Deacon, S.J., Singh, S.J., Patel, P., Fox, J., Greenhaff, P.L., Morgan, M.D., 2005. Adenine nucleotide loss in the skeletal muscles during exercise in chronic obstructive pulmonary disease. Thorax 60, 932–936.
Timmons, J.A., Gustafsson, T., Sundberg, C.J., Jansson, E., Greenhaff, P.L., 1998a. Muscle acetyl group availability is a major determinant of oxygen deficit in humans during submaximal exercise. Am. J. Physiol. 274, E377–E380.
Timmons, J.A., Gustafsson, T., Sundberg, C.J., Jansson, E., Hultman, E., Kaijser, L., Chwalbinska-Moneta, J., Constantin-Teodosiu, D., Macdonald, I.A., Greenhaff, P.L., 1998b. Substrate availability limits human skeletal muscle oxidative ATP regeneration at the onset of ischemic exercise. J. Clin. Invest. 101, 79–85.
Vogiatzis, I., Terzis, G., Nanas, S., Stratakos, G., Simoes, D.C., Georgiadou, O., Zakynthinos, S., Roussos, C., 2005. Skeletal muscle adaptations to interval training in patients with advanced COPD. Chest 128, 3838–3845.
Whipp, B.J., Ward, S.A., 1990. Physiological determinants of pulmonary gas exchange kinetics during exercise. Med. Sci. Sports Exerc. 22, 62–71.
Wilkerson, D.P., Berger, N.J., Jones, A.M., 2006. Influence of hyperoxia on pulmonary O_2 uptake kinetics following the onset of exercise in humans. Respir. Physiol. Neurobiol. 153, 92–106.
Wilkerson, D.P., Campbell, I.T., Blackwell, J.R., Berger, N.J., Jones, A.M., 2009. Influence of dichloroacetate on pulmonary gas exchange and ventilation during incremental exercise in healthy humans. Respir. Physiol. Neurobiol. 168, 224–229.