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The plasma ammonia response to cycle exercise in chronic obstructive pulmonary disease

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ABSTRACT

We examined plasma ammonia response to exercise in COPD and explored the relationship between plasma ammonia concentration and muscle adenine nucleotide metabolism.

Twenty-five stable COPD patients and 13 similar-aged controls underwent incremental and constant workrate cycle exercise tests. Arterialised-venous blood was sampled at rest, 1-minute intervals during, and up to 5minutes after exercise for ammonia and lactate concentration.

Peak incremental workrate was significantly less in COPD subjects (67[21]W) than similar-aged controls (156[46]W),$p<0.001$. In COPD and control subjects, plasma ammonia concentration increased during incremental exercise ($p<0.001$) until 2minutes post-exercise, then declined by 5minutes post-exercise. However, two distinct patterns were seen in COPD subjects. In one group (n=16), ammonia increased [42.8(3.3)umol/l] by a similar magnitude as controls [55.5(7.0)umol/l], $p=0.12$. In a second COPD group (n=9) no ammonia increase was observed despite a similar lactate increase. Ammonia change with incremental and constant workrate exercise strongly correlated in COPD subjects ($r=0.88$,$p<0.001$). Plasma ammonia increase correlated with muscle IMP formation after constant workrate exercise ($r = 0.61$,$p=0.029$).

Plasma ammonia concentration increases during incremental and constant workrate cycle exercise in COPD subjects at lower absolute workrates compared with similar-aged controls. The plasma ammonia response may provide useful information about adenine nucleotide metabolism and therefore muscle fatigue during exercise in patients with COPD.

Keywords: adenine nucleotides, chronic obstructive pulmonary disease, metabolic response, skeletal muscle dysfunction

Abstract word count: 200words

INTRODUCTION

Abnormal peripheral muscle function has been identified as an important contributor to exercise intolerance in patients with chronic obstructive pulmonary disease (COPD). This is independent of disease severity and linked to disability, poor quality of life and survival(1). Quadriceps muscle samples, taken from COPD patients at rest, show reductions in oxidative enzyme activity and the proportion of type I fibres compared with age-matched healthy controls(2-4). An accelerated rise in blood lactate during exercise in COPD has been reported compared to control subjects, and is associated with a reduction in quadriceps muscle mitochondrial enzyme activity(5). This implies that resynthesis of adenosine 5–triphosphate (ATP) during muscle contraction from oxidative sources is impaired in COPD with a consequent increase in non-oxidative metabolism and presumably fatigue during exercise. Importantly, this impairment in skeletal muscle energy metabolism may be a remediable feature of an otherwise largely irreversible pulmonary disease.

We have previously shown in COPD that ATP degradation and inosine 5–monophosphate (IMP) accumulation in skeletal muscle during exercise occurs despite the significantly lower absolute workrates that these individuals can achieve(6). This suggests that metabolic stress occurs in patients with COPD at these low absolute workrates that may be relevant to their activities of daily living. However, there was significant inter-individual variability in the magnitude of the metabolic response, and further understanding of the characteristics and mechanisms underlying the skeletal muscle metabolic response to exercise is currently required. Measurement of metabolic events during exercise may be an important investigational tool, but obtaining muscle biopsies during exercise is technically difficult in this frail elderly population and therefore not practical for larger clinical trials. Alternative methods for studying the metabolic response are therefore needed.

During intense exercise, ATP degradation occurs when oxidative and non-oxidative ATP re-synthesis fail to meet ATP demand. This is associated with accumulation of IMP as a result of irreversible deamination of adenosine 5-monophosphate (AMP) in exercising skeletal muscle. This process has been described in the literature as metabolic stress(7;8). During this reaction, ammonia is produced in stochiometry with IMP and released into the bloodstream. In young healthy adults blood ammonia concentration has been shown to increase during incremental exercise only when high intensities are reached(9;10) and this has been implicated in development of fatigue and physical exhaustion(11). Although plasma ammonia has been shown to closely reflect muscle adenine nucleotide metabolism in healthy subjects(11), the ammonia response to exercise in subjects with COPD has not been reported.

We hypothesised that, in COPD subjects, changes in ammonia concentration during exercise would reflect adenine nucleotide metabolism within skeletal muscle, and provide a useful marker of skeletal muscle metabolism that is less invasive than obtaining a muscle biopsy. In this study we have examined the plasma ammonia response to both incremental and constant workrate (WR) cycle exercise in COPD, and explored the relationship between plasma ammonia concentration and skeletal muscle adenine nucleotide metabolism.

METHODS

Stable patients with COPD (aged 50-85 years, n=25) who met GOLD criteria(12) were recruited from outpatient clinics. Patients were excluded if taking maintenance oral corticosteroids, were unable to perform exercise tests, demonstrated exercise desaturation (SaO_2 < 85%), had significant cardiac dysfunction, an exacerbation of COPD within the previous 6 weeks or pulmonary rehabilitation within the last year. Similar-aged healthy controls (n=13) were recruited by local advertisement and screened for abnormal lung function and significant cardiac or respiratory

disease. Full approval was obtained from the Leicestershire Research Ethics Committee and all participants provided informed written consent.

Study design

Participants attended an initial visit to collect baseline data and familiarise with the exercise test. On a subsequent visit at least 72 hours later, subjects performed a maximal (symptom-limited) incremental exercise test on an electrically braked cycle ergometer. A week later subjects performed a constant WR exercise challenge.

Baseline measurements: Spirometry was performed to ERS standards on three occasions in the seated position (Vitallograph Model R, Buckingham, UK)(13). Body mass index (BMI) was calculated from height (measured by wall mounted stadiometer to the nearest 0.1cm) and weight (measured in light clothing to the nearest 0.1 kg (SECA, UK)). FFM (Kg) was estimated with subjects semi-supine using bioelectrical impedance, and calculated using disease-specific equations(14). Isometric quadriceps force was evaluated using the Cybex II Norm dynamometer (CYBEX NORM^{TM} Testing and Rehabilitation System, CYBEX$^{®}$ International, New York) with subjects seated at 70^{o} knee flexion. Physical activity was assessed using a physical activity questionnaire adapted for the elderly(15) and validated in subjects with COPD(16). The questionnaire consists of scores for household activities, sport activities and leisure activities, resulting in an overall activity score.

Exercise challenge: In the incremental test, WR was increased by 10 watts every minute (COPD) or 20 watts every minute (healthy) using a ramp protocol to determine peak exercise work capacity (Ergometric Er900 (Ergoline GmbH, Germany). Participants cycled at a cadence of 40-45rpm and were encouraged to continue cycling at the required rate for as long as possible. Ventilation and gas

exchange measurements were made throughout the test using a breath-by-breath computerised system (Zan-680 ErgoTest, Zan Messgeraete GmbH, Germany). Peak ventilation was expressed as a percentage of maximum voluntary ventilation (MVV) and patients with COPD were deemed ventilatory limited if peak ventilation exceeded 90% MVV(17). In the constant WR test work increased over 1 minute, then subjects cycled at constant WR until symptom-limited. The intensity for this test was set at 80% of the peak work achieved during the incremental test.

Blood/biopsy analysis: Half an hour prior to the exercise test a 12g retrograde cannula was inserted into a superficial lower forearm vein and placed inside a hand-warmer, warmed to 50-55°C. The hand-warmer enables arterialised-venous blood to be collected, which is representative of arterial blood and is therefore not contaminated by ammonia generated by the hand and forearm muscles. The method used has been previously validated and used for plasma ammonia measurements in healthy subjects(18;19). Arterialised-venous blood samples were taken at rest (subjects rested on couch for 30 minutes), every minute during exercise to peak exercise, and at two and five minutes after exercise, and placed immediately on ice. Blood for ammonia analysis was centrifuged immediately following the exercise test, plasma stored at –196^{0}C in liquid nitrogen, and analysed in duplicate usually immediately, but always within 24 hours, using a validated enzyme assay technique (Sigma-Aldrich Co. Ltd, UK). The coefficient of variation for ammonia determined from standards was 5%. Whole blood lactate concentrations were analysed immediately following exercise using a bench-top analyser (YSI 1500 sport l-lactate analyser, YSI Inc, USA). The coefficient of variation for lactate determined from standards was 2%.

Muscle biopsies of the vastus lateralis (Bergstrom technique(20)) were taken at rest (subjects rested on a couch for 30 minutes) and immediately post-exercise (within 10 seconds of peak exercise with subject seated on bike) following the constant WR challenge. This meant that muscle samples were taken following an exercise challenge of the same relative intensity for all subjects. Muscle samples

were frozen and stored immediately in liquid nitrogen. Following subsequent freeze-drying, powdering and extraction, samples were analysed for phosphocreatine (PCr) and creatine concentrations using the spectrophotometric method of Harris et al(21). Adenine nucleotides (ATP, ADP and AMP) and their breakdown derivatives (IMP, inosine and xanthine) were measured using high pressure liquid chromatography (HPLC)(22). Total creatine concentration was calculated as the sum of PCr and creatine. All measurements were corrected for non-muscle constituents using total creatine(23).

Data analysis: Between-group comparisons were made using the Student's unpaired t-test or Mann Whitney-U test when not-normally distributed. Within-group comparisons are made using paired t-tests. Correlations between parameters were calculated with Pearson's correlation tests. Data were analysed using SPSS package version 14.0 (SPSS Inc Chicago, USA). Significance was assumed at $p<0.05$.

RESULTS

Patient characteristics

A total of 25 patients with COPD and 13 similar-aged controls were included. One COPD patient dropped out after familiarisation and was not included in analyses. Missing data in the incremental test were due to equipment failure (2 control, 2 COPD) and insufficient blood for accurate analysis (1 control). Missing data in the constant WR test were due to equipment failure (1 control, 3 COPD) intolerance of procedure (2 COPD), and insufficient biopsy material (3 control, 7 COPD). Baseline characteristics for COPD and control subjects are shown in Table 1A and are presented as mean when normally distributed and median when not. Demographically the groups were well matched apart from FEV_1 and physical activity score, which were expected.

Table 1: Baseline characteristics (A) and exercise data from incremental cycle test (B) for similar-aged control subjects and COPD subjects

A		
	Controls n=13	COPD n=25
Age	68 (7)	69 (7)
Gender	10 m	20 m
FEV_1 (% pred)	101 (16)	47 (12)*
FEV_1	2.88 (0.71)	1.21 (0.29) *
Isometric quadriceps strength (Nm)	155 (52)	130 (47)
FFMI (Kg/m^2)	19 (3)	18 (2)
BMI (Kg/m^2)	26 (4)	27 (4)
Physical activity score‡	15.7 (10.3)	5.7 (5.8)*
B		
Heart rate (% pred)	93 (7)	73 (10)*
Peak WR (watts)	156 (46)	67 (20)*
$VO_{2\ peak}$ (ml/kg/min)	28.50 (8.25)	17.21 (3.41)*
Peak V_E (L/min)	74 (21)	37 (10)*
Peak V_E (% MVV)	66 (16)	79 (20)†
Peak RQ	1.14 (0.15)	0.96 (0.07)
Peak PE‡	16 (6)	16 (9)

Expressed as mean (SD) unless stated: ‡ Median (IQR)
† $p<0.05$, * $p<0.001$ compared with controls

BMI= body mass index; FFMI= fat free mass index; $VO_{2\ peak}$= peak oxygen uptake; Peak V_E = peak ventilation; Peak RQ= peak respiratory quotient; Peak PE= perceived exertion at peak exercise, MVV= maximum voluntary ventilation (calculated as FEV_1 x 40), WR = workrate.

Incremental exercise

Data from the incremental exercise test is shown in Table 1B. Peak WR [mean (SD)] was significantly lower in subjects with COPD [67.2(20.5)Watts] than similar-aged controls [156.2(45.7)Watts], $p<0.001$. Peak ventilation was significantly increased in subjects with COPD compared with similar-aged controls, and 5 COPD subjects were ventilatory limited.

Tables 2A and 2B show mean (SD) plasma ammonia and blood lactate responses to incremental cycle exercise. Resting plasma ammonia concentrations were similar for COPD and similar-aged subjects and within published ranges(7;9;24). Plasma ammonia concentration increased during exercise in subjects with COPD ($p<0.001$) and similar-aged subjects ($p<0.001$) and continued to increase at 2 minutes after exercise before declining towards baseline at 5 minutes after exercise.

Table 2: Plasma ammonia and blood lactate concentrations at rest and in response to incremental exercise in all COPD subjects (n=24) and age-matched controls (n=12) [A] and in COPD subjects with [group 1:n=15, peak work 69(21) Watts] and without [group 2:n=9, peak work 63(19) Watts] an ammonia increase with exercise [B].

A

		Rest	Peak exercise	2min recovery	5min recovery	Peak change
Ammonia Mean (SD) umol/l	Control	63.7 (16.5)	106.2 (30.9)*	109.3(26.7)*	95.8(26.6) *	55.5 (7.0)
	All COPD	56.5 (13.4)	80.4 (21.3)*	81.9(21.8)*	70.0(18.1) *	28.7(4.3) $^{+}$
Lactate Mean (SD) mmol/l	Control	0.64 (0.16)	2.96 (0.73)*	3.67(0.83)*	3.44(1.2) *	3.17 (0.27)
	All COPD	0.72 (0.25)	1.94(0.83)*	2.28(0.91)*	2.05(0.84) *	1.64(0.82) $^{+}$

B

Ammonia Mean (SD) umol/l	COPD group 1	55.9 (12.5)	91.3 (18.4)*	93.0 (19.2)*	77.1(18.7) *	42.8 (3.3)
	COPD group 2	60.8 (14.4)	62.1 (10.5)	64.5 (12.2)	58.2(8.7)	5.1 (1.1) $^{+\dagger}$
Lactate Mean (SD) mmol/l	COPD group 1	0.70 (0.23)	2.00 (0.89)*	2.36 (0.97)*	2.18(0.91) *	1.78 (0.21) $^{+}$
	COPD group 2	0.77 (0.30)	1.84 (0.73)**	2.16 (0.88)*	1.81(0.68) *	1.39 (0.30) $^{+}$

Expressed as mean (SD). Group 1= COPD subjects with ammonia response during exercise, Group 2= COPD subjects without ammonia response during exercise
*p<0.001 **p<0.01 Within group, compared with resting
$^{+}$p<0.001Between COPD and control group analysis of peak change
†p<0.001 Between COPD group1 and group2 analysis of peak change

In COPD subjects two distinct patterns of response appeared when the plasma ammonia increase with incremental exercise was plotted against peak oxygen uptake [Figure 1A]. In one group of COPD subjects plasma ammonia increased significantly with exercise, and change in ammonia concentration correlated with peak oxygen uptake [Pearson correlation r=0.56, p=0.03]. The ammonia increase in control subjects also correlated with oxygen consumption [r= 0.56, p=0.07]. In a second group of subjects with COPD, ammonia did not rise with exercise despite subjects achieving similar peak oxygen uptake [mean(SD) 17.1(4.2)ml/kg/min vs 17.3(3.0)ml/kg/min (p=0.88) in group with ammonia rise] and peak WR [63(19)Watts vs 69(21)Watts (p=0.45) in group with ammonia rise]. However, no such differential response was seen with blood lactate [Figure 1B], and the change in blood lactate during exercise was not significantly different between the two groups of COPD subjects (p=0.30) [Table 2B]. The magnitude of blood lactate accumulation was correlated with peak oxygen uptake as expected [r= 0.55, p=0.007]. The differential ammonia response in COPD subjects could not be predicted from demographic variables, medication or exercise parameters, including limitations to exercise and ventilatory limitation, which were not significantly different between the two groups.

Figure 2 shows the pattern of change in plasma ammonia from resting concentration to peak WR during incremental exercise for all control subjects, COPD subjects with an ammonia rise and COPD subjects with no ammonia rise. In age-matched controls, plasma ammonia concentration remained near resting concentration at low WRs. At higher intensity exercise, ammonia concentration increased with increasing WR. In COPD subjects with an ammonia response, plasma ammonia concentration increased from the onset of exercise and continued to rise with increasing WR.

The increase in plasma ammonia concentration during exercise [mean (SE) 42.8(3.3)umol/l] in COPD subjects with a response (n=15) was not significantly different to similar-aged controls [mean (SE) 55.5(7.0)umol/l] (p =0.12), despite the significantly lower absolute peak WR achieved [Tables 2A and 2B]. However, the increase in blood lactate in these COPD subjects was significantly lower than control subjects (p<0.001). In the COPD group with no measurable ammonia increase (n=9), the change in plasma ammonia concentration was within repeatability of the measurement. Unlike our findings in similar-aged subjects, where there was a linear relationship between peak ammonia and lactate concentrations [r = 0.61, p = 0.046], there was no relationship between plasma ammonia and blood lactate concentration in all subjects with COPD [r = 0.02, p = 0.938].

Constant WR exercise

Subjects with or without a plasma ammonia increase in the incremental exercise challenge had a consistent response in the constant WR exercise challenge. Change in plasma ammonia concentration during incremental exercise strongly correlated with plasma ammonia change during constant WR exercise in all subjects with COPD [Figure 3: r= 0.88, p<0.001].

Biopsy data: ATP degradation and IMP accumulation occurred in skeletal muscle during constant WR exercise in all COPD subjects [n=14 mean (SD) change -3.11(1.41)mmol/kg dry weight, p= 0.046 and 0.58(0.23)mmol/kg dry weight, p=0.029, respectively] and similar-aged controls [n=9, mean(SD) change -4.44(1.42)mmol/kg dry weight, p=0.019, and 2.86 (0.81)mmol/kg dry weight, p= 0.01, respectively]. The absolute WRs were significantly different between COPD subjects and similar-aged controls [mean (SD) 52(17)Watts and 128(38)Watts respectively, p<0.001]. PCr and PCR/Cr ratio fell significantly and to a similar extent in COPD subjects and controls. PCr concentrations pre and post exercise were 72.1(12.8)mmol/kg and 53.4(18.3)mmol/kg dry weight

respectively in COPD subjects and 70.9(7.4)mmol/kg and 40.0(10.3)mmol/kg dry weight respectively in controls. PCr/Cr ratios pre and post exercise were 1.36(0.33) and 0.80(0.36) respectively in COPD subjects and 1.43(0.23) and 0.53(0.21) respectively in controls. Insufficient tissue was available for analysis of other purine nucleotide derivatives. No statistically significant differences were seen in the exercise-induced change in muscle metabolites between the two COPD subgroups (with and without an ammonia response), although because of missing biopsy data, numbers were small.

Correlation existed between muscle IMP accumulation and plasma ammonia increase in subjects with COPD [$r = 0.61$, $p = 0.029$] and similar-aged controls [$r = 0.66$, $p = 0.055$]. Figures 4A and 4B demonstrate these correlations graphically. No correlation was found between plasma ammonia increase and muscle ATP degradation or between muscle IMP accumulation and ATP degradation in either COPD or control subjects.

DISCUSSION

This study is the first to describe the plasma ammonia response to cycle exercise in COPD. Overall, we found a significant exercise-induced increase in plasma ammonia concentration, which began early in exercise and peaked 2 minutes after exercise. Similar-aged controls displayed a curvilinear ammonia response to incremental exercise, which was similar to findings documented in the literature for young healthy subjects(9;10). However, there was a differential ammonia response to exercise in the COPD cohort. In one subgroup, the increase in plasma ammonia from rest to end-exercise was similar to controls despite significantly lower peak WRs. The other subgroup of COPD subjects did not demonstrate an increase in plasma ammonia concentration despite having a rise in blood lactate concentration.

Failure of energy delivery by oxidative and anaerobic ATP resynthesis to meet the demands of muscle force generation results in an increase in ADP and AMP, and activation of AMP deaminase. The irreversible deamination of AMP leads to accumulation of IMP in exercising muscles and release of ammonia into the bloodstream. In the short term, by preventing excessive accumulation of ADP and AMP, this increases the phosphorylation potential of the adenine nucleotide pool allowing the adenylate kinase reaction and contraction to continue. Such a situation is not sustainable because of the resulting accumulation of ADP and decline in ATP availability. Substantial metabolic stress is said to have occurred under these conditions(6;7) and is associated with fatigue in healthy subjects(25;26). We have previously shown that ATP loss and IMP accumulation in muscle occurs at significantly lower absolute WRs in subjects with COPD than healthy subjects(6). Data from the present study supports these findings and demonstrates a rise in ammonia with exercise at lower absolute WRs compared with similar-aged control subjects. This data supports our previous observations that skeletal muscles in subjects with COPD are working under conditions of metabolic stress at low absolute work intensities similar to those required for activities of daily living(6). The increase in plasma ammonia correlated with muscle IMP accumulation in constant WR exercise supporting our hypothesis that plasma ammonia may be a useful marker of the nucleotide metabolic response within skeletal muscle.

The main source of plasma ammonia produced in skeletal muscles during intense exercise is from deamination of AMP, which constitutes part of the purine nucleotide cycle (PNC). It has been demonstrated in humans that the activity of the PNC and blood ammonia production is predominantly a reflection of fast twitch (type II) fibre activity during short-term intense exercise(27). Dudley and co-workers reported an inverse relationship between the proportion of slow twitch (type I) fibres of the vastus lateralis muscle and ammonia increase during intense exercise in healthy subjects(28). Our findings of an early increase in plasma ammonia in incremental exercise in subjects with COPD suggest that fast twitch fibre recruitment is occurring at

low WRs in this population. Atrophy of type I fibres and an increased proportion of type II fibres in skeletal muscle samples taken at rest in patients with COPD is well recognised(2;3).

A reduction in oxidative enzyme concentrations in muscles of subjects with COPD has been demonstrated at rest(4;5). This implies either reduced oxidative ATP metabolism and/or increased reliance on anaerobic ATP resynthesis during exercise, or a preferential atrophy of oxidative muscle fibres. Increased reliance on glycolytic metabolism has recently been associated with contractile fatigue following cycle exercise(29). Further support for this is provided by Maltais and colleagues, who showed an early and accelerated blood lactate accumulation during incremental exercise in severe COPD(5). Although blood lactate concentration can be used as a marker of metabolic response to exercise, plasma ammonia may more closely reflect changes in adenine nucleotide metabolism occurring under conditions of metabolic stress.

It was unclear from our data why some subjects with COPD failed to display an ammonia response during exercise. We were unable to identify differences in demographics, disease severity or the pattern of exercise response between these patients and those who did show a rise in ammonia. Missing biopsy samples rendered interpretation of exercise-induced metabolite changes difficult between the two COPD subgroups because of the small samples size. However, an increase in blood lactate does not necessarily have to be matched by an increase in plasma ammonia. One possibility is that in patients without a rise in ammonia, the ATP demands of contraction were being met, and fatigue was attributable to another factor not associated with the failure of energy delivery. An alternative explanation is that these patients may have differed in muscle fibre composition, such that considerably less ammonia was generated. In this respect, human slow twitch muscle fibres are known to have considerably less deamination of AMP to IMP, and therefore less ammonia generation. Previous literature in COPD has suggested a shift in fibre composition towards a greater proportion of type II fibres(2;3) but this phenomenon may vary considerably across the COPD population and it is possible that patients not showing a rise in ammonia were

those with better preservation of type I (slow twitch) fibres. This was a post-hoc analysis and as such does need to be confirmed in future studies together with measurements of muscle fibre composition, blood flow and oxidative enzyme concentrations to explain these observations.

A number of limitations to the current study are acknowledged, particularly in interpreting the muscle biopsy data. As we have found in previously studies, tissue from biopsies taken immediately post-exercise was small and in some cases inadequate for complete analysis. This highlights the technical difficulties and limitations in using muscle biopsies to investigate the metabolic response to exercise in COPD. Biopsies were taken following constant WR exercise, which we have shown in previous work(6) to induce skeletal muscle metabolic stress. Because the WR for the constant WR test was determined by performance during the incremental test, the metabolic response to exercise measured in the muscles will have been influenced by the limit to maximal performance. This highlights some of the problems with standardising sub-maximal exercise tests for studies. However, an intensity of 80% work achieved in the maximal incremental test was felt to be appropriate for several reasons. It reflects the intensity at which we ask patients to perform endurance training in rehabilitation and therefore has some practical relevance. In addition, similar work intensities have been demonstrated to stimulate an ammonia response to sub-maximal exercise in healthy subjects(27). Although we did not measure muscle ammonia directly in this study, a strong relationship has been shown to exist between muscle adenine nucleotide loss and plasma ammonia accumulation(30). Measurements from forearm arterialised blood may be less sensitive than those taken from femoral venous blood as this directly drains the exercising muscles, but the aim of this study was to evaluate less invasive measurements of ammonia that may be practical as an investigational tool in clinical studies. For this reason we did not undertake femoral venous cannulation. Finally, whilst our control group were not physically well trained and were representative of the healthy elderly population, they were significantly less active than the COPD

group. Thus we are unable to distinguish the effects of loss of fitness due to inactivity from other aspects of the disease on the aetiology of our observations.

COPD is a leading cause of disability worldwide and places an increasing burden on healthcare resources(12). Peripheral muscle dysfunction and in particular impaired energy metabolism may prove an important remediable source of exercise intolerance in this population despite largely irreversible lung impairment. In healthy subjects training increases ammonia workload threshold(31) and a reduction in blood ammonia concentration appears to delay onset of fatigue and increase duration of intense exercise(11;31). It is feasible that similar results with training could be achieved in COPD patients who have an observed ammonia increase with exercise. Plasma ammonia may be a marker of metabolic stress in the skeletal muscles and therefore could be used as an outcome when assessing the impact of interventions targeting skeletal muscle energy metabolism such as pulmonary rehabilitation.

In conclusion, we have shown that plasma ammonia concentration increases during incremental and constant WR cycle exercise in subjects with COPD. Compared with similar-aged controls, similar peak exercise ammonia concentrations are reached despite significantly lower peak WRs. The observed differential ammonia response to cycle exercise appears to be distinct from the lactate response and may provide a useful clinical marker for investigating differences in skeletal muscle energy metabolism during exercise in COPD patients.

FIGURE LEGENDS

Figure 1. Plot of peak oxygen uptake (VO_2 in ml/kg/min expressed as % predicted) and maximum plasma ammonia change (umol/l) [1A] or maximum blood lactate change (mmol/l) [1B] following maximal incremental cycle exercise in COPD subjects (n=24). Subjects with an ammonia response are indicated by open circles (n=15); subjects without an ammonia response are indicated by closed circles (n=9).

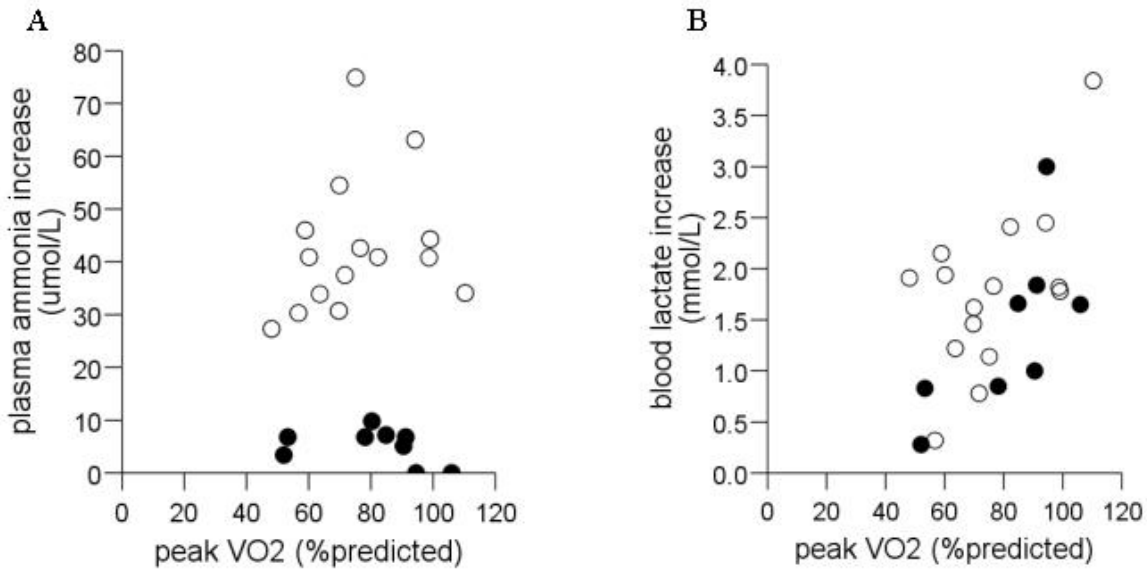


Figure 2. Mean (SD) change in plasma ammonia concentration (umol/l) from resting concentration during incremental exercise in COPD subjects with ammonia increase (closed circle, n=15), COPD subjects without ammonia increase (closed square, n=9) and similar-aged controls (closed diamond, n=12)

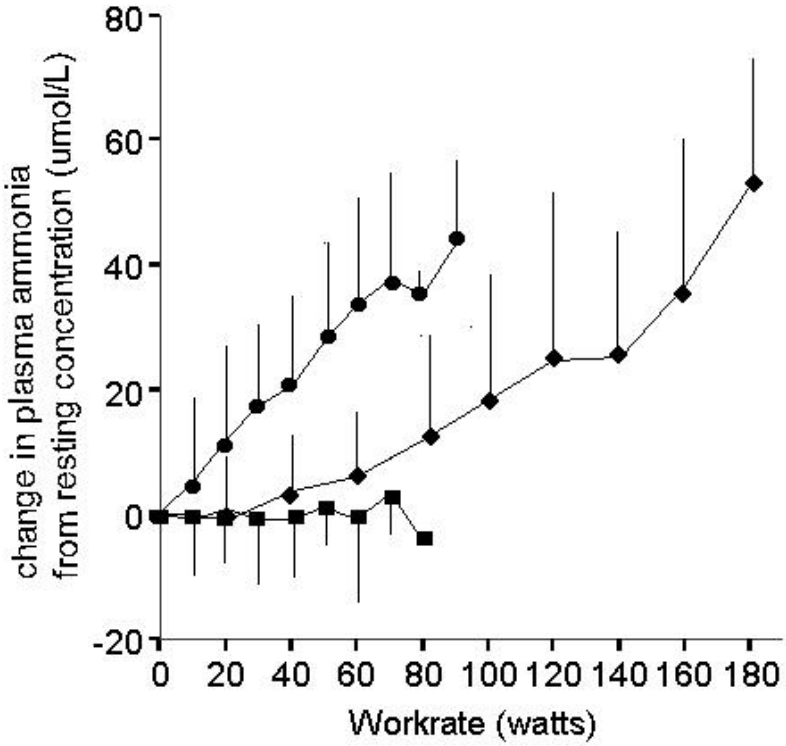


Figure 3. Scatter to show correlation between maximum plasma ammonia change (umol/l) during maximal incremental and sub-maximal constant WR cycle exercise in COPD subjects (n=21); R^2=0.766. Open circles indicate COPD subjects with ammonia response (n=12) closed circles indicate COPD subjects without ammonia response (n=9)

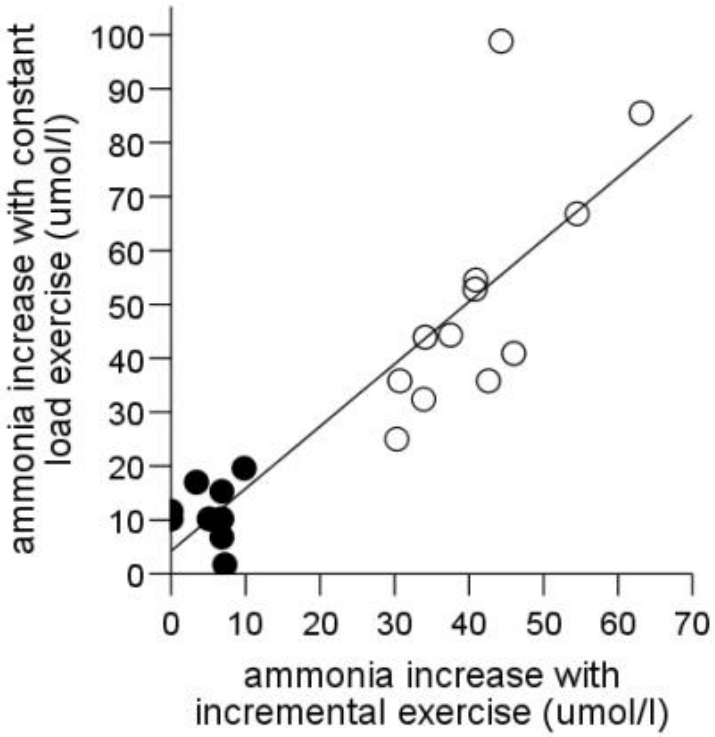
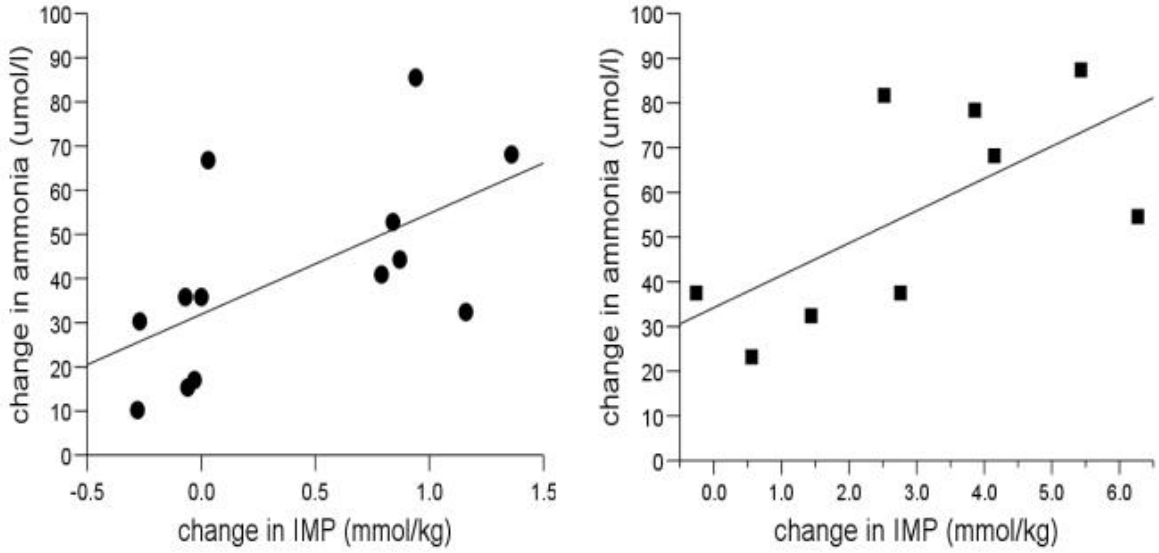


Figure 4. Correlation between change in plasma ammonia concentration and quadriceps muscle IMP accumulation in response to sub-maximal constant WR cycle exercise: Figure 4A: COPD subjects (n=13), r = 0.61, p = 0.029. Figure 4B: Similar-aged control subjects (n=9), r = 0.66, p = 0.055.



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