Plasma ammonia response to incremental cycling and walking tests in COPD

L.D. Calvert*, M.C. Steiner, M.D. Morgan, S.J. Singh

Department of Respiratory Medicine, Institute for Lung Health, University Hospitals of Leicester NHS Trust, Glenfield Hospital, Groby Road, Leicester, LE3, UK

Received 2 October 2009; accepted 23 November 2009
Available online 8 December 2009

Summary
Objective: It is well documented that plasma ammonia accumulates during exercise under conditions of metabolic stress. Metabolic stress (when skeletal muscle ATP supply fails to meet demand) occurs at low work rates during cycling in patients with COPD, but not described during walking. Walking is an important activity for many patients with COPD and is commonly prescribed in pragmatic outpatient pulmonary rehabilitation programmes. In this study we explored whether metabolic stress occurs during incremental walking at the low work rates these patients achieve.

Methods: Twenty-nine subjects with stable COPD [mean(SD) age 68(7) years, FEV1 50(19)% predicted] performed maximal cardiopulmonary exercise tests on a cycle ergometer and treadmill. Plasma ammonia concentration was measured at rest, 1 and 2 min of exercise, peak exercise and 2 min recovery.

Results: Subjects achieved mean(SD) cycle work rate of 57(20) W with VO2max 15.5(4.6) ml/min per kg, and treadmill distance 284(175) m with VO2peak 16.8(4.2) ml/min per kg. Plasma ammonia concentration rose significantly (p < 0.001) with walking [mean(SEM) change 24.7(3.8) mmol/l] and cycling [mean(SEM) change 35.2(4.3) mmol/l], but peak exercise ammonia was lower in walking (p < 0.01). In a subgroup of subjects (n = 7) plasma ammonia did not rise during either cycling or walking despite similar lactate rise and peak exercise indices.

Conclusion: Our data indicate that failure of muscle ATP re-synthesis to meet demand and development of metabolic stress can occur during walking in COPD patients at the low work rates these patients achieve. This may therefore be a factor contributing to exercise limitation independent of ventilatory limitation.

* Corresponding author. Department of Respiratory Medicine, Peterborough District Hospitals, Thorpe Road, Peterborough, PE3 6DA, UK. Tel.: +44 1733 875065; fax: +44 1733 875287.
E-mail address: lori.calvert@pbh-tr.nhs.uk (L.D. Calvert).

© 2009 Elsevier Ltd. All rights reserved.

0954-6111/S - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2009.11.012
Introduction

Exercise intolerance is a major factor limiting participation in activities of daily living in people with chronic obstructive pulmonary disease (COPD) and patients commonly complain of limited ability to perform walking exercise. Pulmonary rehabilitation programmes aim to improve exercise capacity, and in many centres walking is the most frequent mode of exercise employed.

It is now appreciated that impaired lower limb skeletal muscle function has a significant impact on exercise capacity in patients with COPD. The energy for muscular contraction is provided by the dephosphorylation of adenosine 5-triphosphate (ATP) to ADP. When ATP utilisation during exercise cannot be matched by its re-synthesis, ATP depletion occurs due to irreversible deamination to form IMP and ammonia. This is associated with a rise in plasma ammonia. These circumstances have been termed 'metabolic stress' because of the detrimental impact on muscle contraction and association with fatigue in healthy subjects. The accumulation of ammonia in the blood can be used as an indicator of ATP loss and the development of metabolic stress in exercising skeletal muscle.

Skeletal muscle ATP depletion and plasma ammonia accumulation has been demonstrated during cycle exercise in COPD patients. Data from the literature suggests that the metabolic and physiological responses to walking and cycling can differ significantly, and the degree to which walking exercise imposes skeletal muscle metabolic stress in COPD has not yet been established. However, understanding the magnitude of ATP mismatch and metabolic stress during walking may be important in making decisions on exercise prescription, for example during PR, in these patients.

In this study, plasma ammonia was used as a marker of skeletal muscle ATP depletion and metabolic stress. We hypothesised that metabolic stress would develop during walking despite the low work rates these patients achieve. In a cohort of COPD patients with significant exercise limitation, we examined firstly whether plasma ammonia accumulated during an incremental walking test, and secondly whether walking provoked a similar peak response to cycling.

In our previous work we observed, in a post hoc analysis, a subgroup of patients who did not demonstrate a significant rise in blood ammonia during cycling exercise despite showing a rise in blood lactate. We hypothesised that there would therefore be a subgroup of patients that would not develop plasma ammonia accumulation with either walking or cycling exercise tests.

Methods

Study population

Stable patients (n = 29) who met GOLD criteria for COPD were recruited from the pulmonary rehabilitation waiting list at Glenfield Hospital. Patients were excluded if taking maintenance oral corticosteroids, had significant cardiac dysfunction, or had undergone pulmonary rehabilitation within the last 2 years. Full approval was obtained from the Leicestershire Research Ethics Committee and all participants provided informed written consent.

Study design

Subjects attended an initial visit for baseline measurements [spirometry performed to ERS standards (Vitalograph Model R, Buckingham, UK); body mass index] and familiarisation tests on the cycle and treadmill. On a second visit at least 72 h later, subjects performed a maximal symptom-limited incremental exercise test on an electrically-braked cycle ergometer (Ergometric Er900; Ergoline GmbH, Bitz, Germany) with increments of 10 W/min, using a ramp protocol. Subjects re-attended after a further 72 h or more to perform a maximal symptom-limited treadmill exercise test (RAM 770CE Treadmill; RAM Medical and Industrial Instruments & Supplies, Padova, Italy). The incremental walking protocol mirrored the ISWT (initial speed 1.8 km/h, increasing every minute by 0.6 km/h, gradient 0°) because this field test is commonly used in clinical practice for assessment of exercise ability and as an outcome measure in pulmonary rehabilitation. Ventilation and gas exchange measurements were made throughout the exercise tests using a breath-by-breath computerised system (Zan-680 ErgoTest, Zan Messgeraete GmbH, Germany). End-exercise Borg breathlessness score and reason for termination were recorded.

Half an hour prior to the exercise test a 12 g retrograde cannula was inserted into a superficial lower forearm vein and placed inside a hand-warmers throughout exercise, warmed to 50–55 °C. Blood was sampled for ammonia and lactate concentration during cycle and treadmill exercise tests at rest, at 1 min and 2 min of exercise, at peak exercise, and at 2 min after exercise cessation (recovery). Timing for blood tests was determined from our previous studies examining pattern of ammonia rise in COPD patients. Whole blood lactate concentrations were measured in duplicate immediately following exercise (YSI 1500 sport l-lactate analyser, YSI Inc, USA). Blood for ammonia was centrifuged immediately, plasma stored at −196 °C in liquid nitrogen, and analysed in duplicate by a validated enzyme assay technique (Sigma–Aldrich Co. Ltd, UK) within 24 h as previously described.

Data analysis

Using data from our previous study we required 22 patients with an ammonia response to detect a 15 μmol/l within-group difference in peak exercise blood ammonia between the 2 exercise tests (80% power, p = 0.05). Normality of data was confirmed and significance was assumed at p < 0.05. Paired t-tests were used to evaluate differences between cycle and treadmill. Correlations between parameters were calculated with Pearson’s correlation tests (SPSS package version 15.0, SPSS Inc, Chicago, USA). Subgroups were defined a priori by exercise-induced ammonia rise with cycling above (group 1) or below (group 2) 15 μmol/l. This was based on the 95% limit of agreement for resting variability of repeat measures of plasma ammonia (unpublished data in healthy subjects) and on our previous post hoc analyses in cycle exercise demonstrating...
these two subgroups. Between-group comparisons were made using independent sample t-tests.

Results

Subject characteristics and exercise parameters

Twenty-nine subjects (21 male) with COPD completed the study. Mean(±SD) age was 68(7) years, FEV$_1$ 50(19)% predicted and 1.27(0.50) l, and BMI 28(7) kg/m$^2$. Twenty subjects were ex-smokers and nine were current smokers. All patients reported breathlessness on exertion [MRC breathlessness score 5 (n = 4), 4 (n = 7), 3 (n = 10) or 2 (n = 8)].

Exercise data is shown in Table 1. In the cycle exercise test, 11 subjects were deemed ventilatory limited [defined as >90% maximum voluntary ventilation (FEV$_1$ × 35)]. The main cause of symptom limitation during cycling was either dyspnoea (n = 10) or leg fatigue (n = 14). Two patients complained of maximal effort and three of uncomfortable mouthpiece. In the walking exercise test, 12 subjects were deemed ventilatory limited. Main cause of symptom limitation was dyspnoea (n = 12) with fewer subjects complaining of leg fatigue (n = 8). Other causes were maximal effort (n = 4), speed (n = 2) and uncomfortable mouthpiece (n = 3). There was no significant difference in reason for stopping in ventilatory limited patients and a similar number complained of dyspnoea or fatigue.

Ammonia and lactate response to exercise

Table 2 shows plasma ammonia and blood lactate responses to cycle and treadmill exercise. In the COPD group as a whole, there was a significant (p < 0.001) plasma ammonia and blood lactate rise with both cycling and treadmill walking. The pattern of plasma ammonia and blood lactate rise was similar for both exercise modalities, although peak exercise concentrations were statistically lower in treadmill walking (p < 0.01) (Fig. 1). The rise in ammonia concentration correlated with peak work on cycle (r = 0.66, p < 0.001) and distance on treadmill (r = 0.43, p = 0.02). There was correlation between cycle and treadmill tests in ammonia rise (r = 0.61, p < 0.001) and lactate rise (r = 0.74, p < 0.001). There were no differences between patients who did or did not reach ventilation limitation in peak exercise indices, or ammonia and lactate responses for both cycling and walking.

Subgroup analysis

Subjects who displayed an ammonia response to cycle exercise (group 1, n = 22) had a consistent response in treadmill walking except in three cases where exercise-induced ammonia change was below 15 μmol/l with walking. All 3 patients had BMI over 40 and one had exercise desaturation to 80% in walking but not cycling. All subjects without an ammonia rise with cycling (group 2, n = 7) similarly did not have a response with walking (Fig. 2). In group 2 peak work was significantly lower for cycling and walking distance was lower without reaching statistical significance. ISWT distance was significantly lower. There were no other significant differences in any measured demographic variable, medication, exercise parameter, ventilatory limitation or exercise-induced lactate rise between the 2 subgroups (Table 3). In group 1, 12 patients stopped cycling due to fatigue and 7 due to dyspnoea, compared with 2 and 3 patients respectively in group 2. During walking, 7 patients stopped due to fatigue and 10 patients due to dyspnoea in group 1, compared with 1 and 2 respectively in group 2.

Subgroup comparison of the ammonia and lactate response to exercise is shown in Fig. 3. The mean(SEM) ammonia rise from resting values in group 1 was 45.3(3.4) μmol/l (cycle) and 31.9(3.9) μmol/l (treadmill). In group 2 ammonia change with exercise was 3.5(1.3) μmol/l (bike) and 2.3(2.3) μmol/l (treadmill). Blood lactate accumulation with exercise was not significantly different between group 1 and group 2 for cycling [mean(SEM) 1.51(0.15) mmol/l and 1.17(0.22) mmol/l, p = 0.24], or walking [mean(SEM) 0.94(0.18) mmol/l and 0.70(0.12) mmol/l respectively, p = 0.47].

Discussion

In this study, we explored the plasma ammonia response to a maximal incremental walking exercise test, which mirrored the ISWT used in PR, in a cohort of patients with COPD. We observed a rise in plasma ammonia despite the low work rates these individuals achieved. These results suggest that muscle ATP depletion and metabolic stress occur during walking and may be a factor contributing to exercise limitation in patients with COPD. However, there was a subgroup of patients who did not have an ammonia rise during walking, and in whom a similar response was seen with cycling.

Metabolic stress develops in exercising skeletal muscle when ATP re-synthesis fails to meet demand, and the
accumulation of ammonia in the blood has been widely used as an indicator of this.\textsuperscript{2,6,7} In COPD patients, although there is evidence of altered skeletal muscle oxidative energy metabolism,\textsuperscript{16,17} it has been unclear whether sufficient exercise work rates can be achieved to cause significant metabolic stress, particularly in patients with evidence of a ventilatory limit to exercise. We have recently confirmed that adenine nucleotide loss and plasma ammonia accumulation does occur during cycle exercise in COPD patients and that its magnitude was comparable to that observed in similar-aged healthy subjects despite substantially lower absolute work rates in COPD patients.\textsuperscript{8,9} Walking is more functionally relevant to patients than cycling and understanding the magnitude of metabolic stress during walking may therefore be more clinically relevant. However, there are important differences between metabolic responses to walking and cycling and our previous findings may not be transferable. In the current study, significant ATP loss as indicated by a rise in plasma ammonia did occur during walking exercise, but at a lower magnitude to that seen during cycling. These findings suggest that the imbalance between ATP demand and re-synthesis and the consequent development of metabolic stress is occurring during incremental walking in these patients. There was little difference in ammonia response between patients defined as ventilatory limited and non-ventilatory limited, suggesting that metabolic stress develops in the ambulatory muscles even in patients with an apparent ventilatory limit to exercise.

### Table 2

Table 2  Plasma ammonia and blood lactate concentrations at rest and in response to incremental exercise in all COPD subjects (n = 29).

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>1 min exercise</th>
<th>2 min exercise</th>
<th>Peak exercise</th>
<th>2 min of recovery</th>
<th>Change over rest concentration\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ammonia concentration ((\mu\text{mol/l}))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycle</td>
<td>52.6 (14.6)</td>
<td>62.3 (17.9)\textsuperscript{*}</td>
<td>68.7 (18.6)\textsuperscript{**}</td>
<td>84.0 (23.5)\textsuperscript{*}</td>
<td>79.6 (23.3)\textsuperscript{**}</td>
<td>35.2 (4.27)\textsuperscript{**}</td>
</tr>
<tr>
<td>Treadmill</td>
<td>49.1 (16.3)</td>
<td>58.4 (21.5)\textsuperscript{*}</td>
<td>62.4 (21.3)\textsuperscript{**}</td>
<td>71.5 (25.1)\textsuperscript{**}</td>
<td>63.7 (24.7)\textsuperscript{**}</td>
<td>24.7 (3.83)\textsuperscript{**}</td>
</tr>
<tr>
<td><strong>Lactate concentration ((\mu\text{mol/l}))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycle</td>
<td>0.73 (0.15)</td>
<td>0.90 (0.21)\textsuperscript{*}</td>
<td>1.01 (0.26)\textsuperscript{**}</td>
<td>1.91 (0.67)\textsuperscript{*}</td>
<td>2.07 (0.76)\textsuperscript{*}</td>
<td>1.43 (0.12)\textsuperscript{*}</td>
</tr>
<tr>
<td>Treadmill</td>
<td>0.71 (0.19)</td>
<td>0.81 (0.26)\textsuperscript{*}</td>
<td>0.92 (0.32)\textsuperscript{**}</td>
<td>1.39 (0.68)\textsuperscript{**}</td>
<td>1.54 (0.81)\textsuperscript{**}</td>
<td>0.88 (0.14)\textsuperscript{**}</td>
</tr>
</tbody>
</table>

Mean (SD) values unless stated. \textsuperscript{*}p < 0.001 within-group, compared with resting value. \textsuperscript{1}p < 0.001, \textsuperscript{11}p < 0.01 between group cycle vs treadmill analysis.

a Mean (SEM).

---

**Figure 1**

Mean(SE) change from resting concentration for plasma ammonia (\(\mu\text{mol/l}\)) (A) and blood lactate (\(\text{mmol/l}\)) (B) during incremental exercise in COPD subjects (n = 29) with cycling (closed square) and treadmill walking (closed triangle). \textsuperscript{*}p < 0.01, \textsuperscript{**}p < 0.001, between cycle and treadmill analysis.

**Figure 2**

Scatter to show exercise-induced change in ammonia concentration for individual subjects (n = 29) achieved in cycling and treadmill exercise. The dotted line represents the threshold for definition of a significant ammonia response (see Methods section for more detail).
Troosters found no difference in incremental walking ammonia response in COPD 679 lactate rise and less contractile fatigue 13 with walking, protocols used. However, a consistent finding is lower blood differences are likely to be due to different exercise 10 gas exchange inefficiency with walking, cycling, but not metabolic stress. Whilst Palange and between the two exercise modailities. 19 Some of these differences cannot be determined from our data. One possibility is that patients without an ammonia response 22 patients with an ammonia response to cycle exercise (group 1, n = 22) and without an ammonia response to exercise (group 2, n = 7). Table 3 Baseline characteristics and exercise parameters for COPD subjects with an ammonia response to cycle exercise (group 1, n = 22) and without an ammonia response to exercise (group 2, n = 7). Group 1 Group 2

| Age (years) | 68 (6) | 69 (10) |
| FEV1 (% predicted) | 50 (19) | 52 (19) |
| FVC | 2.56 (0.73) | 2.39 (0.79) |
| FEV1/FVC (%) | 50 (11) | 51 (10) |
| BMI | 28 (6) | 29 (9) |
| FFMI (kg/m2) | 18 (2) | 19 (3) |
| Oxygen saturation at rest (%) | 96 (2) | 96 (1) |
| ISWT distance (m) | 307 (121) | 183 (118)* |
| ESWT distance (s) | 176 (55) | 151 (48) |
| Cycle VO2max (ml/min per kg) | 16.1 (4.8) | 13.6 (3.3) |
| Peak workload (W) | 61 (20) | 43 (14) |
| Peak VEmax (% MVV) | 86 (22) | 78 (28) |
| Heart rate (% predicted) | 73 (11) | 76 (10) |
| Lactate rise (mmol/l) | 1.51 (0.15) | 1.17 (0.22) |
| Treadmill VO2peak (ml/min per kg) | 17.0 (4.4) | 16.4 (3.5) |
| Distance (m) | 309 (184) | 205 (123) |
| Time (s) | 329 (124) | 248 (109) |
| VEmax (% MVV) | 85 (21) | 89 (21) |
| Heart rate (% predicted) | 72 (10) | 74 (6) |
| Lactate rise (mmol/l) | 0.94 (0.18) | 0.70 (0.12) |

Data expressed as mean (SD). FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; BMI, body mass index; FFMI, fat free mass index; VO2peak, peak oxygen uptake; VEmax, (MVV, maximum ventilation; MVV, maximum voluntary ventilation (calculated as FEV1 × 35); ISWT, incremental shuttle walk test; ESWT, endurance shuttle walk test. *p = 0.043; **p = 0.025.

There are a number of studies in the literature comparing ventilatory and metabolic responses to walking and cycling, but not metabolic stress. Whilst Palange and others have reported higher ventilatory demand and increased gas exchange inefficiency with walking,10–12,18 Troosters found no difference in VO2peak and blood gases between the two exercise modalities.19 Some of these differences are likely to be due to different exercise protocols used. However, a consistent finding is lower blood lactate rise and less contractile fatigue13 with walking, suggesting reduced metabolic response. Our results indicate that whilst important ventilatory and metabolic differences exist, both exercise modalities can lead to metabolic stress.

Whilst we observed a similar overall pattern of lactate and ammonia accumulation during exercise in walking and cycling, we reproduced our previous observation of disso- ciation of the ammonia and lactate response in some patients.9 In other words, some subjects did not demon- strate a blood ammonia rise during exercise despite showing a rise in blood lactate. This was broadly consistent between the two exercise platforms. The reasons for these differences cannot be determined from our data. One possibility is that patients without an ammonia response were unable to exercise to sufficient intensity to cause ATP loss. In support of this hypothesis, there was a trend towards lower exercise capacity and lactate accumulation in this group, although this was not statistically significant and larger sample size may be necessary to investigate this further. These individuals may have terminated exercise for other reasons such as motivation. Although we did not observe significantly higher peak ventilation rates or lower oxygen saturations in this group and the proportion of patients with ventilation limitation were similar between the two groups, we have not measured other ventilatory indices such as dynamic hyperinflation. Alternatively, this group may have had better preserved mitochondrial function or type I fibre proportions than those who did demonstrate a rise in ammonia. This would result in more efficient adenine nucleotide rephosphorylation and therefore less deamination and reduced ammonia production.5 Previous literature on COPD has suggested a shift in fibre composition towards a greater proportion of type II fibres although significant variability is present.20,21 The prepon- derance of type II fibres may explain the rise in ammonia at low absolute work rates in the overall COPD cohort whereas conversely, the absence of an ammonia rise may identify patients in whom type I fibres are better preserved. It is possible that this subgroup may respond differently to interventions targeting skeletal muscles. However, the clinical implications of the differential ammonia response are yet to be determined.

Ability to perform walking exercise is an important concern for many patients with COPD and is commonly applied in pragmatic outpatient pulmonary rehabilitation programmes. In this study, plasma ammonia rise occurred during walking in many individuals with COPD despite low work intensities. This is in contrast to studies in healthy subjects where ammonia accumulation is only seen at high exercise intensities,6,7,22 and suggests that ATP depletion may be a more frequent occurrence in COPD compared with healthy subjects. It is conceivable therefore from our results that normal activities of daily living and the regular high intensity walking exercise entailed in pulmonary rehabilitation represent repeated bouts of skeletal muscle metabolic stress for some COPD patients. Since the adenine nucleotide deamination pathway is a potential source of metabolite loss. In support of this hypothesis, there was a trend to lower exercise capacity and lactate accumulation in this group, although this was not statistically significant and larger sample size may be necessary to investigate this further. These individuals may have terminated exercise for other reasons such as motivation. Although we did not observe significantly higher peak ventilation rates or lower oxygen saturations in this group and the proportion of patients with ventilation limitation were similar between the two groups, we have not measured other ventilatory indices such as dynamic hyperinflation. Alternatively, this group may have had better preserved mitochondrial function or type I fibre proportions than those who did demonstrate a rise in ammonia. This would result in more efficient adenine nucleotide rephosphorylation and therefore less deamination and reduced ammonia production.5 Previous literature on COPD has suggested a shift in fibre composition towards a greater proportion of type II fibres although significant variability is present.20,21 The preponderance of type II fibres may explain the rise in ammonia at low absolute work rates in the overall COPD cohort whereas conversely, the absence of an ammonia rise may identify patients in whom type I fibres are better preserved. It is possible that this subgroup may respond differently to interventions targeting skeletal muscles. However, the clinical implications of the differential ammonia response are yet to be determined.

We acknowledge limitations to the interpretation of our data. To avoid the need for muscle biopsy immediately post-exercise (a technically challenging and invasive procedure), we did not measure adenine nucleotide loss directly, relying on the less invasive measurement of plasma ammonia. However, numerous previous investigations in healthy subjects have confirmed the relationship between AMP deamination and plasma ammonia accumula- tion1,4,22,24 and we have also demonstrated this in COPD patients.9 Although a control group has not been used, the ammonia response to incremental cycling and walking is extensively documented in healthy subjects and the aim of
this study was to determine whether metabolic stress was occurring in walking rather than making any comparison with response in healthy subjects. Our observations are limited to maximal incremental exercise performance and may not be transferable to endurance exercise. The ISWT was used in this study as it is a common clinical assessment tool and pulmonary rehabilitation outcome measure and exercise is prescribed from this measure. Sample size is small and further studies are required to fully evaluate the clinical usefulness of ammonia determination during exercise in COPD patients.

In summary, we have demonstrated that plasma ammonia concentration increases significantly during incremental treadmill walking in many subjects with COPD. Although the pattern of response was similar to cycling, the magnitude of rise was lower with walking. Our data indicate that failure of muscle ATP re-synthesis to meet demand and development of metabolic stress can occur during walking in this population, and this may therefore be a factor contributing to exercise limitation independent of ventilatory limitation to exercise.

References


