Oxidative enzyme activities of the vastus lateralis muscle and the functional status in patients with COPD

François Maltais, Pierre LeBlanc, François Whittom, Clermont Simard, Karine Marquis, Marthe Bélanger, Marie-Josée Breton, Jean Jobin

Abstract

Background—Enzymatic and biochemical abnormalities of the peripheral muscle may play a role in exercise intolerance in patients with chronic obstructive pulmonary disease (COPD). A study was undertaken to measure the mitochondrial enzyme activity of the vastus lateralis muscle in patients with COPD and to evaluate the relationship between enzyme activities and functional status.

Methods—Fifty seven patients with COPD of mean (SD) age 66 (7) years with forced expiratory volume in one second (FEV1) 39 (15)% predicted and peak oxygen uptake (Vo2) of 14 (4) ml/min/kg and 15 normal subjects of similar age were included in the study. Each subject performed a stepwise exercise test up to maximal capacity during which five-breath averages of Vo2 were measured. Muscle specimens were obtained by percutaneous needle biopsy of the vastus lateralis muscle and the activity of two mitochondrial enzymes (citrate synthase (CS) and 3-hydroxyacyl CoA dehydrogenase (HADH)) was measured. The functional status of the patients was classified according to peak Vo2.

Results—CS and HADH activities were markedly reduced in patients with COPD compared with normal subjects (22.3 (2.7) versus 29.5 (7.3) µmol/min/g muscle (p<0.0001) and 5.1 (2.0) versus 6.7 (1.9) µmol/min/g muscle (p<0.005), respectively). The activity of CS decreased progressively with the deterioration in the functional status while that of HADH was not related to functional status. Using a stepwise regression analysis, percentage predicted functional residual capacity (FRC), the activity of CS, oxygen desaturation during exercise, age, and inspiratory capacity (% pred) were found to be significant discriminators of peak Vo2. The regression model explained 59% of the variance in peak Vo2 (p<0.0001).

Conclusions—The oxidative capacity of the vastus lateralis muscle is reduced in patients with moderate to severe COPD compared with normal subjects of similar age. In these individuals the activity of CS correlated significantly with peak exercise capacity and independently of lung function impairment.

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Keywords: chronic obstructive pulmonary disease; exercise; oxidative enzymes
Muscle oxidative capacity and functional status in COPD

Table 1 Characteristics of study subjects, pulmonary function tests, and peak exercise data

<table>
<thead>
<tr>
<th></th>
<th>Normal subjects (n=15)</th>
<th>COPD (n=57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62 (5)</td>
<td>66 (7)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26 (4)</td>
<td>25 (5)</td>
</tr>
<tr>
<td>FEV₁ (l)</td>
<td>2.88 (0.28)</td>
<td>1.04 (0.41)</td>
</tr>
<tr>
<td>FEV₁ (% pred)</td>
<td>106 (99)</td>
<td>39 (15)</td>
</tr>
<tr>
<td>FVC (l)</td>
<td>3.68 (0.40)</td>
<td>2.57 (0.70)</td>
</tr>
<tr>
<td>FVC (% pred)</td>
<td>94 (8)</td>
<td>64 (16)</td>
</tr>
<tr>
<td>TLC (% pred)</td>
<td>–</td>
<td>117 (18)</td>
</tr>
<tr>
<td>TLCO (% pred)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>V̇E (l/min)</td>
<td>75 (19)</td>
<td>36 (13)</td>
</tr>
<tr>
<td>V̇E (% max pred)</td>
<td>74 (18)</td>
<td>90 (23)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>156 (10^)</td>
<td>131 (19)</td>
</tr>
<tr>
<td>Heart rate (% max pred)</td>
<td>92 (7)^</td>
<td>79 (11)^</td>
</tr>
<tr>
<td>Borg scores</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>5.0 (3.1)</td>
<td>7.3 (2.1)</td>
</tr>
<tr>
<td>Leg fatigue</td>
<td>5.2 (3.5)</td>
<td>5.7 (3.0)</td>
</tr>
<tr>
<td>Rest</td>
<td>0.98 (0.01)</td>
<td>0.96 (0.04)</td>
</tr>
<tr>
<td>Peak exercise</td>
<td>0.98 (0.01)</td>
<td>0.90 (0.07)</td>
</tr>
<tr>
<td>PaCO₂ (kPa)</td>
<td>5.2 (0.5)</td>
<td>5.6 (0.9)</td>
</tr>
<tr>
<td>Peak exercise</td>
<td>4.9 (0.7)</td>
<td>6.4 (1.2)</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>0.75 (0.40)</td>
<td>0.98 (0.63)</td>
</tr>
<tr>
<td>Rest</td>
<td>8.06 (2.38)</td>
<td>3.91 (2.02)</td>
</tr>
</tbody>
</table>

Exercise test

After the insertion of an arterial cannula in a radial artery, subjects were seated on an electrically braked cycle ergometer (Quinton Corival 400, A-H Robins, Seattle, WA, USA) and connected to the expired gas analysis circuit through a mouthpiece. Five-breath averages of oxygen uptake (V̇O₂) and minute ventilation (V̇E) were measured by an automated system equipped with a pneumotachograph, O₂ and CO₂ analysers, and a mixing chamber (Quinton Qplex, A-H Robins, Seattle, WA, USA). After 3–5 minutes of rest a progressive symptom limited stepwise exercise test was performed. Each exercise step lasted one minute and increments of 10 and 20 watts were used in COPD and normal subjects, respectively. Dyspnoea and leg fatigue perception were rated on a modified Borg scale at one minute intervals during exercise. Arterial blood was analysed at rest and at maximal exercise to determine blood lactate concentrations and gas tensions. During exercise blood samples were placed on ice and centrifuged at room temperature immediately after termination of the exercise test. Lactate concentrations were measured on plasma with an enzymatic technique (Kit lactate, Boehringer Mannheim, Mannheim, Germany).
In patients with COPD the functional status was graded according to peak VO₂ using the approach previously used in chronic heart failure by Weber et al.¹ where >20 ml/kg/min = mild to no impairment (class A, n = 4), >16 and ≤20 ml/kg/min = mild to moderate impairment (class B, n = 15), >10 and ≤16 ml/kg/min = moderate to severe impairment (class C, n = 28), and >6 and ≤10 ml/kg/min = severe impairment (class D, n = 10).

### Results

Anthropometric characteristics, pulmonary function, peak exercise data, and blood gas tensions for normal subjects and patients with COPD are shown in table 1. Group mean values for age and body mass index were comparable for each group. On average, patients had moderate to severe airflow obstruction and hyperinflation. As expected, exercise tolerance was markedly reduced in patients compared with normal subjects. In patients, exercise was accompanied by a rapid increase in symptom scores, absent or reduced ventilatory reserve, O₂ desaturation, and CO₂ retention. The pharmacological treatment profile was similar for the four groups.

Individual values for the activity of CS and HADH are shown in fig 1. CS and HADH were significantly reduced in patients with COPD compared with normal subjects (p<0.0001 and p<0.005, respectively).

### Statistical analysis

Values are reported as mean (SD). The activity of CS and HADH was significantly decreased in patients with COPD compared with normal subjects (p<0.0001 and p<0.005, respectively).

![Figure 1](http://thorax.bmj.com/)

**Table 2 Characteristics of study subjects in relation to the functional status**

<table>
<thead>
<tr>
<th>Class</th>
<th>N (n=10)</th>
<th>Class</th>
<th>N (n=28)</th>
<th>Class</th>
<th>N (n=15)</th>
<th>Class</th>
<th>N (n=4)</th>
</tr>
</thead>
</table>
| Age (years) | 65 (8) | 69 (8) | 62 (8) | 56 (9) |<br>Sex (M/F) | 7/3 | 24/4 | 14/1 | 4/0 |<br>BM (kg/m²) | 28.0 (7.7) | 23.5 (3.9) | 25.8 (4.7) | 22.2 (2.8) |<br>FVC (l) | 0.68 (0.5) | 1.02 (0.36) | 1.20 (0.30) | 1.56 (0.76) |<br>FRC (l) | 28.0 (7.7) | 23.5 (3.9) | 25.8 (4.7) | 22.2 (2.8) |<br>FEV1/FVC (%) | 41 (11) | 38 (10) | 43 (14) | 47 (13) |<br>PO2 (kPa) | 9.6 (2.3) | 10.2 (1.6) | 11.2 (1.5) | 11.7 (0.5) |<br>PCO2 (kPa) | 6.5 (1.1) | 5.5 (0.6) | 5.4 (0.9) | 4.9 (0.3) |<br>Lactate (mmol/l) | 1.06 (0.70) | 1.02 (0.70) | 0.80 (0.41) | 0.95 (0.22) |<br>Enzyme activities were measured at 25°C and expressed in µmol/min/g muscle. The activities of citrate synthase (CS, EC 4.1.3.7) and of 4-hydroxacyl CoA dehydrogenase (HADH) in normal subjects (N) and patients with chronic obstructive pulmonary disease (COPD). The horizontal lines represent group mean values. The activity of CS and HADH was significantly decreased in patients with COPD compared with normal subjects (p<0.0001 and p<0.005, respectively).

**Values are mean (SD). Values followed by different superscript letters are significantly different.**

**BMI** = body mass index; **FEV1** = forced expiratory volume in one second; **FVC** = forced vital capacity; **TLCO** = carbon monoxide transfer factor; **PO2,PCO2** = carbon monoxide and oxygen gas tensions; **FRC** = functional residual capacity; **IC** = inspiratory capacity; **TLC** = total lung capacity; **FRC**, **IC**, and **TLC** % predicted, the activity of CS and HADH, and oxygen desaturation during exercise (resting SaO₂ – peak exercise SaO₂) as independent variables. A value of p < 0.05 was considered statistically significant.

**Figure 1 Individual values for the activity of (A) citrate synthase (CS) and (B) 3-hydroxacyl CoA dehydrogenase (HADH) in normal subjects (N) and patients with COPD (COPD) compared with normal subjects (p<0.0001 and p<0.005, respectively).**

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with COPD, a much larger number of patients was included in the present study and the control group was older and less fit. These methodological improvements strengthen the conclusion that a low oxidative capacity of the skeletal muscle is a frequent finding in patients with moderate to severe COPD. However, the decrease in the activity of CS, which is involved in the citric acid cycle, was greater than that of HADH which regulates the β oxidation of fatty acids. In addition, there was a larger overlap between normal subjects and patients with COPD for the activity of HADH than for CS. This discordance in expression in the activity of CS and HADH is intriguing since, in most situations, the activity of all mitochondrial enzymes varies in a parallel and proportional fashion. A differential response in the activity of the enzymes of the citric acid cycle and of β oxidation has previously been observed after intense interval training in normal subjects. The significance of this finding in our patients is unclear. Conceivably, the β oxidation pathway could be relatively preserved in order to facilitate the metabolism of blood fatty acids which are increased in a proportion of patients with COPD. This would advantageously decrease CO₂ production and ventilatory requirements, because the lipid metabolism leads to lower CO₂ production than carbohydrate. The presence of a discordance expression of mitochondrial enzymes in patients with COPD is also suggested by a study showing a higher activity of cytochrome oxidase, an enzyme of the electron transport chain, in the skeletal muscle of patients with COPD compared with healthy subjects. This uncoupling between the different mitochondrial enzymes will need to be explored since it may provide some interesting clues as to the mechanisms of the development of skeletal muscle dysfunction in COPD.

Because a large number of patients with a wide range of peak VO₂ (6–25 ml/min/kg) were included in the present study, the determinants of peak exercise capacity in COPD could be evaluated. In the stepwise regression analysis it was found that peak exercise capacity in patients with COPD could be best explained by a combination of several factors including % predicted FRC, the activity of CS, oxygen desaturation during exercise, age, and the % predicted IC. The activity of CS was a significant correlate of peak VO₂ independent of the impairment in lung function, and its decrease was accompanied by a progressive worsening in the functional status. Although correlation analysis never proves a causal relationship, these findings and other studies strongly suggest that alteration in the function of peripheral skeletal muscle contributes to exercise intolerance in COPD. Poor skeletal muscle oxidative capacity probably influences exercise capacity through modifications of the muscle metabolism which increase lactate and CO₂ production and ventilatory needs for a given exercise level. Impaired muscle metabolism is also associated with premature muscle acidosis, a contributory factor to muscle fatigue and early termination of exercise.
Several physiological mechanisms may explain why FRC and IC were significantly correlated with peak VO₂. At a higher FRC the inspiratory muscles, especially the diaphragm, will be mechanically disadvantaged and part of the tidal breathing probably occurs in the alinear portion of the pressure-volume curve of the respiratory system, thus increasing the mechanical load on the already disadvantaged respiratory muscles. In exercising flow limited patients with COPD, the increase in tidal volume and thus ventilation can only occur at the expense of the inspiratory reserve volume since the end expiratory lung volume does not decrease below FRC during exercise as is the case in normal subjects. For this reason, the ability to increase tidal volume and minute ventilation should be more closely related to the IC than to VC. Lastly, both the level of hyperinflation and the reduction in IC are closely related to the development of dyspnoea during exercise. Consistent with these notions, IC was found to explain approximately 70% of the variance in peak exercise work rate in 25 patients with obstructive lung disease. The much higher contribution of IC to peak exercise capacity in that study compared with the present one is probably due to the use of the absolute value of IC rather than % predicted to predict peak exercise work rate. Since body size is an important determinant of both variables, the relationship between IC in litres and work capacity in watts will be stronger than the relationship between IC % predicted and peak exercise capacity normalised for body weight. The regression model explained 59% of the variance in peak VO₂. The individual tolerance to the discomfort of exercise is likely to be one factor contributing to peak VO₂ that was not explored in the present study. As others, we found that symptom scores at peak exercise tended to decrease with the deterioration in functional status from class A to class D. A likely explanation for this is that patients with poor functional status are less tolerant of the uncomfortable sensations of exercise than more fit patients. The physiological profile at peak exercise of class D patients with a lower heart rate, VO₂/MVV ratio, and lactate level than patients with a better functional status also exemplified the potential impact of symptom tolerance on peak VO₂. It should be pointed out, however, that the steep increase in dyspnoea and the early rises in Pco₂ and lactate in class D patients are indicative of severe physiological abnormalities and that exercise could not have been tolerated much longer even with a greater tolerance to dyspnoea and leg fatigue. In conclusion, the present study confirms that the oxidative capacity of the vastus lateralis muscle is reduced in patients with COPD and that the activity of CS is correlated with their functional status. The clinical implication of this study is that exercise intolerance in patients with COPD is of multifactorial origins and that the relative contributions of airflow obstruction, hyperinflation, O₂ desaturation, and peripheral muscle dysfunction to exercise intolerance should be addressed to optimise the functional status of a given patient with COPD.

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