Quadriceps metabolism during constant workrate cycling exercise in chronic obstructive pulmonary disease

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Saey D, Lemire BB, Gagnon P, Bombardier É, Tupling AR, Debigaré R, Côté CH, Maltais F. Quadriceps metabolism during constant workrate cycling exercise in chronic obstructive pulmonary disease. J Appl Physiol 110: 116–124, 2011. First published October 21, 2010; doi:10.1152/japplphysiol.00153.2010.—Impaired resting metabolism in peripheral muscles potentially contributes to exercise intolerance in chronic obstructive pulmonary disease (COPD). This study investigated the cytosolic energy metabolism of the quadriceps, from glycogen degradation to lactate accumulation, in exercising patients with COPD, in comparison to healthy controls. We measured, in 12 patients with COPD and 10 control subjects, resting and post-cycling exercise quadriceps levels of 1) energy substrates and end products of glycolysis (glycogen, glucose, pyruvate, and lactate) and intermediate markers of glycolysis (glucose-6-phosphate, glucose-1-phosphate, fructose-6-phosphate) and 2) the activity of key enzymes involved in the regulation of glycolysis (phosphofructokinase, lactate dehydrogenase). Exercise intensity (P < 0.01), duration (P = 0.049), and total work (P < 0.01) were reduced in patients with COPD. The variations in energy substrates and end products of glycolysis after cycling exercise were of similar magnitude in patients with COPD and controls. Glucose-6-phosphate (P = 0.036) and fructose-6-phosphate (P = 0.042) were significantly elevated in patients with COPD after exercise. Phosphofructokinase (P < 0.01) and lactate dehydrogenase (P = 0.02) activities were greater in COPD. Muscle glycogen utilization (P = 0.022) and lactate utilization (P = 0.025) per unit of work were greater in COPD. We conclude that cycling exercise induced changes in quadriceps metabolism in patients with COPD that were of similar magnitude to those of healthy controls. These intramuscular events required a much lower exercise work load and time to occur in COPD. Our data suggest a greater reliance on glycolysis during exercise in COPD, which may contribute to exercise intolerance in COPD.

cycle ergometer; muscle metabolism; muscle biopsy

EXERCISE LIMITATION IS A HALLMARK feature of chronic obstructive pulmonary disease (COPD). The contribution of peripheral muscle dysfunction to exercise intolerance in COPD is still debated (11). While appreciating the complex nature of exercise intolerance in COPD (34), our group has reported that peripheral muscle fatigue reduces exercise capacity in this disease (13, 39). We showed that the occurrence of quadriceps fatigue prevents bronchodilation to translate into improved exercise capacity (39) and that the preinduction of quadriceps fatigue reduces the endurance during constant workrate exercise (13). Susceptibility to muscle fatigue in COPD is assumed to be related to the reduction in the proportion of fatigue-resistant slow-twitch muscle fibers and in oxidative enzyme activity (16, 48). These phenotypic and enzymatic muscle changes modify muscle energy metabolism during exercise, resulting in premature muscle acidosis, lactate accumulation, and fatigue (9, 29, 49).

Under resting conditions, the peripheral muscle metabolic profile of patients with COPD shows low concentration of high-energy phosphates such as ATP and creatine phosphate as well as lower aerobic enzyme activity such as citrate synthase (CS) compared with aged-matched healthy controls (12, 19, 23). In addition, intermediate markers of glycolysis, namely, glucose-6-phosphate, glyceraldehyde-3-phosphate, fructose-6-phosphate as well as phosphofructokinase (PFK), and lactate dehydrogenase (LDH) activities were shown to be elevated in resting COPD muscle (19, 23). Together, these findings suggest an impaired muscle oxidative capacity with a reduction in phosphorylation potential and a greater reliance on glycolysis at rest that may well contribute to the exercise intolerance in COPD.

In healthy humans, a greater reliance on glycolysis during exercise is associated with premature lactate accumulation (47), muscle acidosis (41) ultimately translating into early exercise cessation. In this regard, little is known about muscle metabolism in response to exercise in patients with COPD. Studies using nuclear magnetic resonance spectroscopy on the forearm and calf muscles reported greater decline in muscle intracellular pH and in the creatine phosphate-to-inorganic phosphate ratio, suggesting an impaired aerobic capacity during exercise in patients with COPD (26, 45, 49). Steiner et al. (44) observed that adenine nucleotides as well as lactate and creatine phosphate were modulated in a similar fashion in patients with COPD compared with aged-matched healthy controls during constant workrate cycling exercise of similar duration but of lower intensity. Although this study provided good insight into the local muscle metabolic stress experienced by patients with COPD during cycling exercise, the metabolic abnormalities that might contribute to exercise limitation in COPD were not explored. In fact, no data are yet available on the extent of the metabolic perturbations occurring in the quadriceps of patients with COPD during exercise done until exhaustion.

The aim of the present study was to investigate, in a comprehensive fashion, the behavior of the quadriceps cytosolic energy metabolism, from glycogen degradation to lactate accumulation, in exercising patients with COPD, in comparison to healthy controls of similar age. To reach this objective, we measured in the quadriceps 1) several energy substrates and...
end products of glycolysis (glycogen, glucose, pyruvate, and lactate) and intermediate markers of glycolysis (glucose-6-
phosphate, glucose-1-phosphate, and fructose-6-phosphate) and 2) the activity of key enzymes involved in the regulation of
glycolysis [LDH, PFK, hexokinase (HK)] and of the citric acid
cycle [CS, 3-hydroxyacyl CoA dehydrogenase (HADH)] at
rest and immediately following cycling exercise in patients
with COPD and healthy controls. Because impaired energy
metabolism could compromise ATP resynthesis, we also eval-
uated the changes in intramuscular high-energy phosphate
compounds (ATP, creatine phosphate, creatine, and inorganic
phosphate) during exercise. To provide a clinically meaningful
interpretation of these intramuscular processes, we also ob-
tained detailed physiological responses during exercise in these
individuals.

We reasoned that if muscle metabolic abnormalities were
important contributors to exercise tolerance in COPD, the
exercise-induced production of glycolytic end products such as
lactate and pyruvate and intermediate markers of glycolysis
such as glucose-6-phosphate, glucose-1-phosphate, and fruc-
tose-6-phosphate as well as glycogen utilization should be
equal or greater in patients with COPD despite lower exercise
intensity, duration, and total work in comparison with healthy
controls. Conversely, if exercise was primarily limited by
ventilation and dyspnea, the degree of metabolic stress occur-
rning during exercise should be less in patients with COPD.

METHODS

Subjects

Twelve patients with moderate to severe COPD and ten healthy
aged-matched controls participated in this study. The diagnosis of
COPD was based on spirometry showing moderate to severe irrevers-
able airflow obstruction [postbronchodilator forced expiratory volume
in 1 s (FEV1) < 80% predicted value, and FEV1/forced vital capacity <
70%] (36) and current or past smoking history of at least 20 pack-yr.
In both groups, subjects were excluded if they presented any medical
condition, other than COPD, likely to influence muscle and exercise
testing (i.e., cardiovascular, neurological, musculoskeletal, locomotor,
or other respiratory diseases). Only men were included in the present
study to minimize measurement variability. The research protocol was
approved by the institutional ethics committee, and signed informed
consent was obtained from each subject.

Study Design

After reviewing medical history and familiarization with the study
procedures, subjects filled out a physical activity questionnaire (46).
Anthropometric measurements, pulmonary function testing, midhigh
cross-sectional area (MTCSA) by computed tomography, and a symp-
tom-limited incremental cycle exercise test were performed. Within 1
wk, subjects returned to the laboratory for a constant workrate cycle
exercise bout performed at 80% of their peak workrate until they
reached the point of exhaustion. Vastus lateralis biopsies were ob-
tained before and exactly 1 min after constant workrate cycle exercise.
After the postexercise biopsy, patients sat down to recuperate. Quad-
riceps force was then measured 15 min postexercise to quantify the
degree of muscle fatigue occurring with exercise.

Pulmonary Function Testing

Standard pulmonary function tests including spirometry, lung vol-
umes, and carbon monoxide diffusion capacity were obtained in all
subjects during the initial evaluation according to previously de-
scribed guidelines (4). Results were related to previously published
normal values (15, 25). Maximum voluntary ventilation (MVV) was
estimated by multiplying FEV1 by 35 (14).

Physical Activity Score

The level of physical activity in daily living was assessed with the
activity questionnaire described by Baeeke et al. (5) adapted for
elderly individuals (46) and used in patients with COPD (43). The
questionnaire attributes a score for household, sport, and other leisure-
time physical activities, together resulting in a global physical activity
score (Table 1). A score of 9–16 indicates a moderate level of daily
physical activity, whereas a score <9 depicts low levels of daily
physical activity and is associated with a sedentary lifestyle.

Incremental Exercise Testing

Patients were seated on an electrically braked ergocycle (Quinton
Corval 400; A. H. Robins, Seattle, WA) and connected to the
respiratory circuit through a mouthpiece. During exercise, a 12-lead
electrocardiogram was recorded continuously and arterial pressure
was monitored using an automatic arm blood pressure monitor (Quin-
ton Automated BP model 410; A. H. Robins). Heart rate was compu-
ted from standard ECG leads (42). The respiratory circuit consisted of
a pneumotachograph, O2 and CO2 analyzers, and mixing chamber
(Sensor Medics, Vmax Legacy, Yorba Linda, CA). After 3 min of
rest, subjects performed an incremental exercise test at a workrate of
50% of their peak workrate for 5 min, followed by 5 min of rest.
Minute ventilation (VE), oxygen uptake (VO2), and carbon dioxide production (VCO2) were measured at
rest and during exercise, on a breath-by-breath basis.

Constant Workrate Cycle Exercise

After 5 min of rest and 1 min of warm-up with unloaded pedaling,
a constant workrate cycle exercise bout was performed until exhaus-
tion at an intensity corresponding to 80% of the peak workrate
achieved during the incremental exercise test. Patients were asked to
pedal at 60 rpm, and standardized encouragement was provided
during exercise. Similarly to the incremental test, subjects were
connected to the respiratory circuit through a mouthpiece and wore a
nose clip during the test. VE, VO2, and VCO2 were measured at rest and
during exercise on a breath-by-breath basis. Heart rate was monitored
by an electrocardiograph (Cardiosoft program-Corima), and blood
pressure was monitored by an automated blood pressure monitor
(Quinton 410, Quinton, Botmhell, WA). Oxygen pulse saturation
(SpO2) was measured by a pulse oximeter (OSM2 Hexoximeter,
Radiometer, Copenhagen, Denmark). The perception of dyspnea and
leg fatigue were assessed at end-exercise using the modified 10-point
Borg scale (8).

Quadriceps Strength Measurements

Muscle strength was measured during maximal voluntary contrac-
tion of the quadriceps and potentiated quadriceps twitch force as
previously reported by our group (13). Subjects were seated in a
recumbent chair (N-K 330 Exercise Table, N-K Products, Elsinore,
CA) with 90° knee flexion and right ankle attached to a strain gauge
(Hewlett-Packard). The strain gauge was systematically adjusted per-
cordally measured 3 s after the end of the maximal voluntary contraction
maneuver. The femoral nerve was stimulated through a 70-mm
figure-of-eight coil powered by a double Magstim stimulator (Mags-
tim, Whitland, Dyed, Wales, UK). For analysis, the mean of the two highest values was calculated. To ensure that the magnetic stimulation was supramaximal, a quadriceps twitch force/power output relationship was obtained as previously published ensuring that a plateau in quadriceps twitch force was obtained in each subject (39).

Measurement of Mid thigh Muscle Area

A computed tomography (2) of the right thigh halfway between the pubic symphysis and the inferior condyle of the femur was performed using a fourth-generation Toshiba Scanner 900S (Toshiba) as routinely done in our laboratory (38). Each image was 5 mm thick and was taken at 120 kV and 200 mA with a scanning time of 1 s while the subject was lying in the supine position. The MTCSA was obtained by measuring the surface area of the tissue with a density of 40 to 100 Hounsfield units. This range of density was chosen because it corresponds to the density of muscle tissue (24). Computed tomography was used to estimate muscle mass because it is more specific than anthropometric measurements. For instance, the age-related fat infiltration of the muscles, which cannot be detected with anthropometric measurements, can be identified by computed tomography because of the fat-specific density.

Muscle biopsy

Needle biopsies of the vastus lateralis were performed as described by Bergström (6) and routinely done in our laboratory (30). The postexercise biopsy was performed through the same incision as the preexercise biopsy, allowing a fast access to the muscle. Immediately following exercise, the leg of the subject was quickly stabilized on a footstool and the postexercise biopsy was then taken while the patient was still seated on the cycle ergometer. This method allowed the postexercise biopsy to be obtained at 1 min postexercise in each subject. Muscle specimens were frozen in liquid nitrogen exactly 1 min after the end of exercise and stored at −80°C for future analysis. Quadriceps fiber typing and enzymatic activities associated with oxidative capacity and glycolysis were analyzed from preexercise biopsies only.

Fiber typing and surface areas. Muscle sections were stained to detect myofibrillar adenosine triphosphatase activity according to the single-step ethanol-modified technique (28). The medium contained 20 mM Na barbital, 10 mM CaCl2, 5 mM MgCl2, 5 mM Na2HPO4, 1.0 mM ATP at 21°C, 17%-18% ethanol and pH 9.4. At 37°C, ethanol concentrations were reduced to 7.5%-8.5%. The incubation time was 40 to 60 min at room temperature. The proportion of types I (nonstained), IIA (lightly stained), and IIX (darkly stained) fibers was assessed and calculated as the number of fibers of each type divided by the total number of muscle fibers. The mean cross-sectional area for each subject was measured by averaging the cross-sectional areas of 120 randomly selected fibers (7).

Enzymatic activity. Quadriceps muscle activity of LDH, PFK, HK, CS, and HADH was assessed using spectrophotometric techniques as previously described (30, 30). The reaction media used to measure enzymatic activities were 15 mM Tris-HCl (pH 8.0) containing 0.016 mM DTNB, 0.1 M acetyl-CoA, and 0.038 mM sodium oxaloacetate for citrate synthase and 100 mM triethanolamine (pH 7.0) containing 5 mM EDTA, 0.17 mM NADH, 1 mM KCN, and 0.1 M acetyl-CoA for HADH. Each assay was done in duplicate, and the average of the two values is reported. The coefficient of variation between duplicate measurements of enzymatic activities varied from 1.6% to 13.1%.

Metabolite and substrate measurements. From pre- and postexercise biopsies, following subsequent freeze drying, powdering, and extraction (20, 21), samples were analyzed for glycogen, glucose, pyruvate, and lactate as well as intermediate markers of glycolysis such as glucose-6-phosphate, glucose-1-phosphate, and fructose-6-phosphate. We also analyzed tissue samples for adenosine triphosphate, creatine phosphate, creatine, and inorganic phosphate. For the HPLC ATP assay, ~5 mg of freeze-dried tissue were extracted in perchloric acid (0.5 M) and neutralized by the addition of 2.3 M K2HPO4. The neutralized sample was centrifuged, and an aliquot was used for HPLC analysis. A Waters Alliance system was used consisting of a Waters 2690 Separations Module for solvent delivery and auto sampling and a Waters 996 Photo Diode Array Detector. The detector wavelength for monitoring the eluate was 254 nm. All measurements were conducted at room temperature by means of a reverse-phase column (Supelcosil LC-18-T 2 cm × 4.6 mm). The solvent system consisted of two buffers: buffer A (150 mM potassium dihydrogen orthophosphate, 150 mM potassium chloride, pH 6.0, was filtered through a 0.45-m filter) and buffer B (80% buffer A, 15% methanol, 5% acetonitrile). During the total 20-min run time, a gradient was applied at a constant flow of 1 ml/min starting with 100% buffer A and increasing buffer B to a maximum of 40% over the first 6 min and returning to 0% by 12 min. All standards were obtained from Sigma Chemical and dissolved in ultrapure water. Nucleotide concentrations were detected from the peak height of the chromatogram. All nucleotide values are expressed in millimoles per kilogram of dry weight. All other measurements were accomplished using fluorometric procedures (27) as described in detail previously (22). The coefficient of variation between duplicate measurements for the energy substrates and end products of glycolysis (glycogen, glucose, pyruvate, and lactate) and intermediate markers of glycolysis (glucose-6-phosphate, glucose-1-phosphate, and fructose-6-phosphate) varied from 2% to 12%. The coefficient of variation between duplicate measurements for the high-energy phosphate compounds (adenosine triphosphate, creatine phosphate, creatine, and inorganic phosphate) varied from 1% to 3%.

Statistical Analysis

Data are expressed as means ± SE and were analyzed using the statistical package program SAS (version 9.01). A P value of < 0.05 was considered statistically significant, while a P value between 0.05 and 0.10 was considered to show a statistical trend. Glycogen, glucose, pyruvate, lactate, glucose-6-phosphate, glucose-1-phosphate, and fructose-6-phosphate values were log transformed to obtain normal distribution for analysis. Student’s unpaired t-test was performed to compare patient characteristics as well as resting intramuscular data. The effects of exercise on energy substrates, intermediate markers of glycolysis, and high-energy phosphate compounds were compared using a mixed-model analysis of variance on the percentage changes with adjustment for baseline levels. To allow comparison of the glycolytic substrates variation in response to exercise between groups cycling for different duration and at different absolute intensities, the changes in glycogen, glucose, and lactate were corrected for the total cycle work that was performed during constant workrate exercise (44). Pearson correlation coefficients were used to evaluate relationships between MTCSA and the mean fiber cross-sectional area.

RESULTS

Subject Characteristics

Anthropometric characteristics and pulmonary function are provided in Table 1. Patients with COPD had moderate-to-severe airflow obstruction. Both groups were well matched for body mass index and levels of physical activity in daily living as assessed by the Voorrips scores.

Exercise Capacity

End-exercise values for the incremental and constant workrate cycling exercises are presented in Table 2. Exercise intensity, duration, and total cycle work were significantly lower in COPD. The perception of dyspnea and leg fatigue at
the end of incremental and constant workrate exercises were similar between both groups. The physiological measurements during constant workrate exercise are depicted in Fig. 1. As a result of a greater mean exercise intensity, \( V_{E1}, V_{O2}, \) respiratory exchange ratio, and lactate were greater \((P < 0.05)\) in controls from the 25\% duration of exercise until the end of exercise. When expressed as a percentage of maximum voluntary ventilation, \( V_{E1} \) was larger in patients with COPD who also exhibit a lower inspiratory capacity throughout exercise than controls. In addition, despite a greater fall in \( O_{2} \) induced by exercise in COPD compared with healthy subjects \( (3\% vs. 1\%) \), no difference was observed between the two groups for the decrease in \( P_{aO2} \) \( (-7.41 \pm 11.78 vs. -5.29 \pm 23.32 \text{mmHg}) \).

**Quadriceps Fatigue**

The fall in muscle voluntary contraction and potentiated quadriceps twitch force during exercise, expressed as a percentage of baseline values, was significant and of similar magnitude between both groups (Fig. 2).

**Muscle Characteristics and Metabolism**

Muscle characteristics and resting energy substrates data are provided in Table 3. Patients with COPD displayed a smaller mid thigh muscle cross-sectional area and proportion of type I fibers. The mean fiber cross-sectional areas averaged 4,489 \pm 239 \mu m^2 in patients with COPD and 5,281 \pm 344 \mu m^2 in healthy controls. Although this difference did not reach statistical significance \((P = 0.13)\), the 15\% reduction in mean fiber size in COPD is consistent with the 20\% reduction in MTCSA. Also, the mean fiber size significantly correlated with MTCSA \((r = 0.73, P < 0.01)\). PFK and LDH enzyme activities were greater in COPD, while oxidative enzyme activities were similar between COPD and controls. Resting levels of glycogen and lactate were significantly greater in COPD, while glucose and creatine phosphate tended to be greater in COPD.

**Quadriceps Metabolism at the End of Constant Workrate Cycle Exercise**

The influence of exercise on energy substrates and end products of glycolysis in the quadriceps expressed in percent difference from baseline values was of similar magnitude between both groups (Fig. 3A). However, the exercise-induced increase in glucose-6-phosphate and fructose-6-phosphate, two intermediate markers of glycolysis, was significantly greater in COPD (Fig. 3B). Exercise-induced changes in high-energy phosphate compounds were also of similar magnitude between both groups (Fig. 3C). These conclusions remained essentially unchanged when the absolute changes in the different variables were used in the analyses instead of the relative changes except for the absolute differences in glucose-6-phosphate and fructose-6-phosphate between COPD and healthy controls that were of borderline statistical significance \((P = 0.09\text{ and } P = 0.12)\, for \text{glucose-6-phosphate and fructose-6-phosphate, respectively}) . To account for the difference in total cycle work between COPD patients and controls, the exercise-induced changes in glycogen, glucose, and lactate were adjusted by dividing them by total cycle work performed (Fig. 3D). A greater utilization in muscle glycogen and greater accumulation of lactate were found in COPD compared with controls while glucose did not differ statistically between groups \((P = 0.224)\).

**DISCUSSION**

The main findings of this study are that the extent of changes in quadriceps metabolism and falls in quadriceps force-generating capacity after a constant workrate exercise done until exhaustion were similar in patients with COPD and age-matched controls despite a much lower total work performed by the former group. These intramuscular events were reached an average of 2.27 min faster and for 468 W/min lesser total work in COPD compared with controls. Taking into account the amount of work performed, our data show an increased utilization of glycogen and accumulation of lactate in

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**Table 1. Participant characteristics**

<table>
<thead>
<tr>
<th></th>
<th>COPD ((n = 12))</th>
<th>Controls ((n = 10))</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>65 ± 2</td>
<td>69 ± 2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74 ± 4</td>
<td>77 ± 4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>168 ± 1</td>
<td>170 ± 2</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26 ± 2</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>Smoking history, pack-yr</td>
<td>45 ± 1*</td>
<td>18 ± 7</td>
</tr>
<tr>
<td>Voorrips score</td>
<td>8.7 ± 1.2</td>
<td>9.0 ± 1.4</td>
</tr>
<tr>
<td>FEV₁, liters</td>
<td>1.3 ± 0.1*</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td>FVC, % predicted</td>
<td>45 ± 3*</td>
<td>103 ± 4</td>
</tr>
<tr>
<td>FVC, %</td>
<td>79 ± 5*</td>
<td>107 ± 5</td>
</tr>
<tr>
<td>FEV₁/FVC, %</td>
<td>46 ± 3*</td>
<td>74 ± 2</td>
</tr>
<tr>
<td>TLC, % predicted</td>
<td>105 ± 4</td>
<td>99 ± 6</td>
</tr>
<tr>
<td>RV, % predicted</td>
<td>127 ± 15*</td>
<td>93 ± 8</td>
</tr>
<tr>
<td>DLCO, % predicted</td>
<td>65.5 ± 4.1*</td>
<td>85.7 ± 6.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. COPD, chronic obstructive pulmonary disease; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity; DLCO, diffusing capacity for carbon monoxide. *\(P < 0.05\) vs. controls.

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**Table 2. End-exercise values for the incremental and constant work cycle exercises**

<table>
<thead>
<tr>
<th></th>
<th>COPD ((n = 12))</th>
<th>Controls ((n = 10))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle time, min</td>
<td>11.6 ± 0.5*</td>
<td>5.6 ± 0.3*</td>
</tr>
<tr>
<td>Work rate, W</td>
<td>106 ± 5*</td>
<td>85 ± 4*</td>
</tr>
<tr>
<td>Total cycle work, W/min</td>
<td>1,258 ± 10*</td>
<td>484 ± 42*</td>
</tr>
<tr>
<td>Dyspnea (Borg scale)</td>
<td>7.9 ± 0.4</td>
<td>7.3 ± 0.2</td>
</tr>
<tr>
<td>Leg fatigue (Borg scale)</td>
<td>7.1 ± 0.5</td>
<td>7.6 ± 0.2</td>
</tr>
<tr>
<td>(SPO_2), %</td>
<td>96.3 ± 0.5</td>
<td>94.1 ± 0.8*</td>
</tr>
<tr>
<td>(P_{aO2}), mmHg</td>
<td>79.5 ± 7.7*</td>
<td>101.2 ± 13.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. \(SPO_2\), oxygen pulse saturation; \(P_{aO2}\), partial pressure of oxygen in arterial blood. *\(P < 0.05\) vs. controls.
COPD patients. Moreover, key intermediate markers of glycolysis in the quadriceps of patients with COPD show significant increases after exercise. These results support the greater reliance on glycolysis during exercise in patients with COPD. The fact that patients with COPD reached similar metabolic perturbations and exhibited similar levels of quadriceps fatigue compared with controls suggests that the metabolic events taking place within the contracting muscles are tightly regulated during exercise.

The present study permitted investigation of whether cycle exercise done until exhaustion would be sufficient to stress the contractile limb muscles of patients with COPD. In the presence of classical evidence of ventilatory limitation, such as high dyspnea scores, a $\dot{V}E/\dot{V}MV > 1$, and dynamic hyperinflation, it is remarkable that patients with COPD experienced similar decreases in quadriceps force postexercise concurrently with similar intramuscular accumulation of inorganic phosphate and lactate compared with healthy controls. These observations are consistent with the notion that the degree of peripheral muscle fatigue and metabolic perturbations occurring during exercise are tightly regulated (1) and may contribute to exercise limitation in health and chronic diseases such as COPD (13). According to this theory, feedback signals originating in the fatigued muscles inhibit motor cortical output, thus preventing subsequent locomotor recruitment and the development of dangerous and potentially irreversible fatigue.

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**Fig. 1.** Physiological measurements during constant workrate exercise in patients with chronic obstructive pulmonary disease (COPD) and controls. The following were measured: ventilation ($\dot{V}E$), ventilation/maximal voluntary ventilation ($\dot{V}E/\dot{MVV}$), oxygen consumption ($\dot{V}O_2$), respiratory exchange ratio (RER), respiratory rate (RR), inspiratory capacity (IC), blood lactate, and heart rate (HR). Values are means ± SE. *$P < 0.05$ vs. controls.
In contrast with our initial hypothesis, glucose accumulation per workload was not increased in patients with COPD (Fig. 3D). Two nonmutually exclusive avenues could explain this observation: 1) the reported lower concentration of muscle GLUT4 transporters in COPD (19) could lessen the amount of blood glucose entering the exercising muscle and thus explain the similar accumulation of muscle glucose per workload between COPD patients and controls; 2) the utilization of muscle glucose could possibly be greater in COPD given the higher glycolytic activity in patients with COPD.

In contrast to previous studies (12, 19, 23, 35), there were no major differences in resting metabolites between patients with COPD and controls. The nonsignificant difference in resting ATP between the two groups is in accordance with Wuyam et al. (49). In contrast to previous studies showing that resting phosphagen energy content was slightly decreased in COPD (12, 19, 23), we, and previous investigators (44), found no differences in high-energy phosphate between COPD and controls, suggesting that the greater resting muscle glycogen, glucose, and some intermediate markers of glycolysis in COPD could serve to preserve resting ATP levels in patients with COPD.

The investigation of the mechanisms responsible for the current muscle metabolic findings was beyond the scope of this study. Although physical inactivity is often quoted as an important mechanism for the peripheral muscle abnormalities in COPD, the level of daily life activities as assessed by a

| Table 3. Midthigh cross-sectional area, enzymatic activity, fiber type distribution, energy substrates, end products of glycolysis, and high-energy-phosphate compounds |
|-------------------------------------------------|-----------------|
| COPD (n = 12) | Controls (n = 10) |
| MTCSA, cm² | 79 ± 3* | 99 ± 7 |
| Glycolytic activity | | |
| LDH, μmol·min⁻¹·g muscle⁻¹ | 74.4 ± 2.5* | 43.7 ± 4.2 |
| PFK, μmol·min⁻¹·g muscle⁻¹ | 48.9 ± 1.1* | 23.1 ± 1.0 |
| HK, μmol·min⁻¹·g muscle⁻¹ | 0.7 ± 0.1* | 0.9 ± 0.1 |
| Oxidative activity | | |
| CS, μmol·min⁻¹·g muscle⁻¹ | 12.6 ± 0.3 | 13.3 ± 0.6 |
| HADH, μmol·min⁻¹·g muscle⁻¹ | 4.8 ± 0.2 | 4.7 ± 0.3 |
| Fiber type distribution | | |
| Type I fibers, % total fibers | 39 ± 2* | 56 ± 5 |
| Type IIa fibers, % total fibers | 38 ± 3* | 25 ± 5 |
| Type IIb fibers, % total fibers | 23 ± 3 | 19 ± 4 |
| Energy substrates, end products of glycolysis, and high-energy-phosphate compounds | | |
| Gly, mmol/kg dry wt | 254.6 ± 20.8* | 178.7 ± 11.7 |
| Glu, mmol/kg dry wt | 3.1 ± 0.4| 1.9 ± 1.3 |
| Py, mmol/kg dry wt | 0.2 ± 0.02 | 0.3 ± 0.03 |
| La, mmol/kg dry wt | 10.2 ± 1.3* | 6.2 ± 5.6 |
| ATP, mmol/kg dry wt | 19.9 ± 0.6 | 18.9 ± 1.2 |
| CP, mmol/kg dry wt | 75.8 ± 5.9* | 60.3 ± 7.7 |
| CR, mmol/kg dry wt | 49.7 ± 3.5 | 54.7 ± 5.8 |
| Pi, mmol/kg dry wt | 32.1 ± 1.6 | 33.7 ± 4.7 |

Values are means ± SE. MTCSA; midthigh muscle cross-sectional area; LDH, lactate dehydrogenase; PFK, phosphofructokinase; HK, hexokinase; CS, citrate synthase; HADH, hydroxyacyl-coenzyme A dehydrogenase; Gly, glycogen; Glu, glucose; Py, pyruvate; La, lactate; ATP, adenosine triphosphate; CP, creatine phosphate; CR, creatine; Pi, inorganic phosphate. *P < 0.05 vs. controls; †P = 0.06 vs. controls; ‡P = 0.05 vs. controls.
Physical activity questionnaire was similar between patients and healthy controls. The greater reliance on glycolysis in COPD could, in part, be explained by the increase type IIa fiber proportion in these individuals. These fibers are characterized by a greater potential for high-energy phosphate and glycolysis than type I muscle fibers (18). Moreover, this glycolytic reliance could possibly involve the lactate and glucose transporters whose activity can be altered in COPD (19). Since these transporters are crucial for lactate removal and glucose entry in skeletal muscle, they could influence the relative contribution of the glycolytic pathway to the total energy expenditure during exercise. Targeting these transporters may be of great interest in future rehabilitation studies.

Apart from these intrinsic muscle changes, we cannot exclude that impairment in muscle perfusion and oxygenation could have influenced muscle metabolism during exercise in COPD (31). In one study, a 25% increase in quadriceps work capacity was observed in patients with COPD and modest exercise-induced oxygen desaturation breathing 100% oxygen in comparison to room air during localized knee extensor exercise (38). More recently, the effects of respiratory muscle unloading and of supplemental oxygen on the degree of quadriceps fatigue during cycling exercise were evaluated in patients with COPD whose end-exercise SpO2 averaged 87% (3). By reducing the work of breathing and improving oxygenation with respiratory muscle unloading or supplemental oxygen during exercise, a 30% reduction in the amount of quadriceps fatigue occurring during exercise was reported in comparison to the same exercise without respiratory assistance. Although limb blood flow was not measured in this study, this attenuation of muscle fatigue with ventilatory support and improved oxygenation could be due to a redistribution of blood flow and oxygen from the respiratory muscles to the limb muscles. The authors concluded that quadriceps fatigue in exercising patients with COPD is in part related to insufficient O2 transport to the limb muscles but that the bulk of fatigue is related to intrinsic muscles changes. Our data do not allow making inference about the proportion of the metabolic changes occurring during exercise within the COPD quadriceps that could be ascribed to possible impairments in muscle perfusion and oxygenation. Additional experiments involving manipulations in oxygen delivery to muscles would be required to address this issue.

Methodological Considerations

Constant workrate cycle exercise was performed at 80% of peak incremental workload to reach exhaustion within 8–10 min in most subjects. The end-exercise results showed similar Borg scale rating for leg fatigue and dyspnea in both groups. Moreover, similar heart rate, VO2, and VE values at the end of exercise were observed in both groups.
the constant workrate cycle exercise and incremental exercise indicate that the effort was maximal/near maximal in both groups.

The reductions in MTCSA and in the proportion of type I fibers found in patients with COPD were consistent with the results of larger studies (17, 32), suggesting that this small patient population was representative of the expected degree of muscle atrophy and morphometric changes for a moderate to severe COPD population. The observation of a similar CS and HADH activity between COPD and healthy controls was somewhat unexpected given the bulk of data showing that the quadriceps oxidative capacity is reduced in patients with COPD (23, 30). Since these enzyme activities are markedly influenced by the level of physical activity, one possible explanation for this finding is the fact that both groups were well matched for daily physical activity levels.

Potential Clinical Implications

Greater reliance on glycolytic metabolism during submaximal exercise leads to accumulation of lactate (47) and decrease in plasma pH levels (41), which ultimately translates into early cessation of exercise (40). Although the relative exercise intensity at which this study was performed was relatively high, patients with COPD frequently engage in daily life activities as stair climbing or fast walking, the absolute intensities of which are similar to those used in this study (~85 W). Thus, the observation that the quadriceps undergoes important metabolic perturbations reported in COPD may be clinically relevant because they may occur in daily life, potentially contributing to exercise limitation in this disease.

Our data also support the relevance for the implementation of therapeutic strategies such as aerobic-based activities, to reduce early variations in muscle metabolites and reliance on glycolysis while improving the muscle oxidative capacities (i.e., β-oxidation and oxidative phosphorylation) during submaximal exercise, ultimately resulting in improvements in functional capacities of COPD patients.

Conclusion

We conclude that constant work cycle exercise performed until exhaustion induced significant changes in quadriceps metabolites and energy substrates in patients with COPD. These intramuscular events were of similar magnitude to those occurring in age- and activity level-matched healthy controls but they required a much lower exercise workload and time to take place in patients with COPD. Taking into account the greater glycogen utilization and lactate accumulation per unit of work, the increases of intermediate markers of glycolysis, and the increased glycolytic enzyme activity, our data suggest a greater reliance on glycolysis during exercise in COPD which may contribute to exercise intolerance and impair the activity of daily living in COPD.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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