Exercise causes blood glutathione oxidation in chronic obstructive pulmonary disease: prevention by O₂ therapy

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Exercise causes blood glutathione oxidation in chronic obstructive pulmonary disease: prevention by O₂ therapy. J. Appl. Physiol. 81(5): 2199–2202, 1996.—The aim of the present study was to determine whether glutathione oxidation occurs in chronic obstructive pulmonary disease (COPD) patients who perform exercise and whether this could be prevented. Blood glutathione red-ox ratio [oxidized-to-reduced glutathione (GSSG/GSH)] was significantly increased when patients performed exercise for a short period of time until exhaustion. Their resting blood GSSG/GSH was 0.039 ± 0.008 (SD) (n = 5), whereas after exercise it increased to 0.085 ± 0.019, P < 0.01. Glutathione oxidation associated with exercise was partially prevented by oxygen therapy (resting value: 0.037 ± 0.014, n = 5; after exercise: 0.047 ± 0.016, n = 5, P < 0.01). We conclude that light exercise causes an oxidation of glutathione in COPD patients, which can be partially prevented by oxygen therapy.

Free radicals; oxidative stress; hypoxia

Free radicals are formed in virtually all cells. Antioxidant mechanisms exist that protect against their harmful effects. Oxidative stress, which occurs when the balance between prooxidant and antioxidant mechanisms is shifted in favor of the former, is associated with various diseases such as coronary heart disease (7), cataract formation (18), or idiopathic pulmonary fibrosis (3). Identification of pathological processes in which free radicals are involved is important because it provides us with a rational test to test therapeutic interventions with antioxidants; however, great care should be taken in the evaluation of the results (15).

A major problem in defining the role of oxidants in human disease is the inadequacy of the methodology used to measure free radical reactions in whole animals (9). We have developed a new method to measure accurately glutathione oxidation in human blood (2, 19) and have used it to determine that oxidative stress occurs only when exercise is exhaustive (16).

Chronic obstructive pulmonary disease (COPD) patients may become exhausted daily in their life when they perform the light exercise necessary to carry out their ordinary activities. The aim of this work was to test whether oxidation of glutathione occurs in COPD patients when they perform submaximal exercise. We have found that such oxidation does, indeed, occur and that it can be partially reduced by oxygen therapy.

Methods

Patients. The study was carried out on nine men diagnosed with advanced hypoxemic COPD. All were ambulatory patients in stable clinical and functional state. Five of them were receiving long-term domiciliary oxygen therapy. Four more patients, also diagnosed with COPD but before they started oxygen therapy, also performed exercise. All nine patients performed exercise while breathing room air and, 1 day later, those who received oxygen therapy at home repeated the bout of exercise under the same conditions as before but while receiving oxygen therapy (see below).

Spirometry, lung volume measurements (body plethysmography), and mouth occlusion pressure (P₀.1) while the subjects were breathing air were accomplished by using standard techniques on a Masterlab module (Jeger, Wurzburg, Germany). Maximum inspiratory pressure was measured near residual volume by using a Siebelmed 163 electromanometer (Siebel, Barcelona, Spain) connected to an x-y Servogortz 731 recorder (Nuremberg, Germany).

All patients received a complete explanation of the purpose of the study. The ethical recommendations of the Declaration of Helsinki were followed. Informed consent was obtained from each patient.

Exercise. Subjects performed exercise sessions in two different situations: 1) as control, breathing room air [inspired O₂ fraction (FᵢO₂) 21%] and, a day later, 2) during oxygen therapy with nasal cannulas at an oxygen flow rate of 2–3 l/min. This represented 1 l/min more than the rate the patients were receiving at home, as recommended by the American Thoracic Society (1). Exercise was performed on a bicycle ergometer and consisted of pedaling at 50–60 revolutions/min at a constant workload of 40 W, which is the first step of a standardized cycloergometric protocol (17). We chose this workload to produce an energy expenditure of ~3 metabolic equivalents (MET; 1MET = 3.5 ml O₂ used per kg body wt and min), which is equivalent to the power output required to walk in their usual activity during their ordinary life (11). Exercise lasted until the patient felt limited by dyspnea, which occurred after ~10 min. Electrocardiogram was monitored and recorded by using a Marquette Case 15 stress-testing device. Arterial blood samples were taken from the radial artery before and immediately after (30–120 s) the exercise. When patients arrived at the laboratory, before the beginning of the exercise test, they rested for 30 min. Then the resting sample was taken.

Metabolite determinations. Reduced (GSH) and oxidized glutathione (GSSG) were determined by a new method we have recently developed to determine accurately glutathione status in blood (2, 19). Essentially, immediately after sampling, blood samples were treated with 6% perchloric acid containing 1 mM EDTA (1:1) to determine GSH or with 6% perchloric acid containing 50 mM N-ethylmaleimide and 1 mM EDTA to determine GSSG. The presence of N-ethylmaleimide prevented a significant formation of GSSG, which occurs when perchloric acid is used to extract GSSG. Then
samples were centrifuged for 10 min at 3,500 revolutions/min, and the acidic supernatants were neutralized and used for determination of metabolites. These acid samples are stable for at least 1 wk when kept at −20°C (2). Lactate was determined by a spectrophotometric method with lactate dehydrogenase and NAD. Arterial PO2, PCO2, pH, and hemoglobin saturation were determined by an ABL-3 (Radiometer, Copenhagen, Denmark).

Results are expressed as means ± SD for the number of observations in parentheses. Statistical analyses were performed by Student’s t-test for paired samples.

RESULTS AND DISCUSSION

Table 1 shows blood parameters and heart rate of the patients before and after exercise, both in the absence and in the presence of oxygen therapy. The age of the patients was 62 ± 4 yr (n = 5). Their pulmonary function test values were forced expiratory volume in 1 s (FEV1): 0.79 ± 0.07 liter; FEV1-to-forced vital capacity ratio: 49 ± 3%; residual volume-to-total lung capacity ratio: 47 ± 8%; maximum inspiratory pressure: 70 ± 12 cmH2O; P0.1: 3.6 ± 0.8 cmH2O (n = 5). Exercise lasted 9 ± 5 min when the patients were breathing room air and 12 ± 2 min under oxygen therapy. Their heart rate was 105 ± 5 beats/min at rest and 126 ± 6 beats/min after exercise while breathing room air and 100 ± 8 and 115 ± 7 beats/min after exercise with oxygen therapy (n = 5). Their dyspnea score (measured on an analogue 0–100 visual scale) was 10 ± 7 at rest and 47 ± 22 at the end of exercise while breathing room air and 6 ± 2 and 45 ± 24 at the end of exercise with oxygen therapy (n = 5).

Table 2 shows that submaximal exercise causes glutathione oxidation in COPD patients. This was evidenced by an increase in GSSG and a slight decrease in GSH levels in blood. The fact that changes in GSH after exercise are not statistically significant is due to the high interindividual variability found in blood GSH values, a fact extensively discussed by Mills et al. (13). It must be emphasized that the patients performed exercise (~40 W for up to 6 min), i.e., the kind of exercise a person is expected to perform in usual day-to-day activity. Thus blood glutathione is oxidized in these patients many times a day as they perform light exercise, which is, nevertheless, hard for them in the course of their ordinary life. This contrasts with the situation of healthy subjects who become exhausted only when they voluntarily perform strenuous exercise. In COPD patients, light exercise caused an increase in blood lactate (Table 2). We previously found that in healthy subjects strenuous exercise only causes glutathione oxidation when it is exhaustive and that changes in GSSG levels are proportional to changes in blood lactate levels (16). Glutathione oxidation only occurs after exercise and not at rest. Indeed, we reported in our earlier work (16) that blood GSH and GSSG levels in resting healthy subjects were, respectively, 800 ± 300 µmol/l (n = 19) and 29 ± 10 µmol/l (n = 15). Now we have found that in COPD patients resting GSH and GSSG levels are, respectively, 884 ± 166 µmol/l (n = 9) and 25.3 ± 12.7 µmol/l (n = 9). Thus there is no statistically significant difference between resting blood GSH and GSSG levels in healthy subjects (16) and in COPD patients (Table 2).

The increased formation of free radicals caused by exhaustive exercise (6) may have various causes such as an increased availability of iron from myoglobin (14), formation of xanthine oxidase from xanthine dehydrogenase (12), or increased formation of peroxynitrite (5) due to hypoxia. The mechanisms of oxidative stress associated with exercise have been recently reviewed (10). Glutathione oxidation in blood of healthy humans was first studied by Gohil et al. (8).

Table 1. Effect of physical exercise and oxygen therapy on PO2, PCO2, pH, and hemoglobin saturation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Oxygen Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Postexercise</td>
</tr>
<tr>
<td>PO2, Torr (mmHg)</td>
<td>56 ± 8</td>
<td>53 ± 8</td>
</tr>
<tr>
<td>PCO2, Torr (mmHg)</td>
<td>46 ± 8</td>
<td>46 ± 6</td>
</tr>
<tr>
<td>pH</td>
<td>7.41 ± 0.02</td>
<td>7.37 ± 0.02*</td>
</tr>
<tr>
<td>Sat Hb, %</td>
<td>88 ± 4</td>
<td>85 ± 6</td>
</tr>
</tbody>
</table>

Results are means ± SD for 5 chronic obstructive pulmonary disease (COPD) patients. Control means that patients were breathing room air. Sat Hb, hemoglobin saturation. Significance is expressed as follows: *P < 0.05, †P < 0.01 between rest and exercise; ‡P < 0.05, §P < 0.01 between control and oxygen therapy.

Table 2. Effect of physical exercise on glutathione and lactate levels in arterial blood

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Postexercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate, mM</td>
<td>1.1 ± 0.4</td>
<td>3.2 ± 0.8*</td>
</tr>
<tr>
<td>GSH, µM</td>
<td>884 ± 166</td>
<td>862 ± 325</td>
</tr>
<tr>
<td>GSSG, µM</td>
<td>25.3 ± 12.7</td>
<td>45.6 ± 21.4*</td>
</tr>
<tr>
<td>GSSG/GSH (×106)</td>
<td>30 ± 16</td>
<td>62 ± 34*</td>
</tr>
</tbody>
</table>

Results are means ± SD for 9 COPD patients. GSH, reduced glutathione; GSSG, oxidized glutathione. Significance is expressed as follows: *P < 0.01 between rest and exercise.

Fig. 1. Effect of exercise on oxidized-to-reduced glutathione ratio (GSSG/GSH) in arterial blood of chronic obstructive pulmonary disease patients under 2 conditions: control (breathing room air) and oxygen therapy (inspired O2 fraction 24–26% O2; flow rate 2–3 l/min). Results are means ± SD for 5 patients. Significance was determined by the Student’s t-test and is expressed as follows: *P < 0.05 between control and O2 therapy.
EXERCISE, GLUTATHIONE OXIDATION, AND COPD

Table 3. Effect of physical exercise on glutathione status and lactate levels in arterial blood

<table>
<thead>
<tr>
<th>Patient</th>
<th>Control Rest</th>
<th>Control Postexercise</th>
<th>Oxygen Therapy During Exercise Rest</th>
<th>Oxygen Therapy During Exercise Postexercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactate mM</td>
<td>GSH, µM</td>
<td>GSSG/GSH (×10³)</td>
<td>Lactate mM</td>
</tr>
<tr>
<td>1</td>
<td>1.03</td>
<td>720</td>
<td>33</td>
<td>2.62</td>
</tr>
<tr>
<td>2</td>
<td>1.38</td>
<td>780</td>
<td>40</td>
<td>3.28</td>
</tr>
<tr>
<td>3</td>
<td>1.17</td>
<td>880</td>
<td>39</td>
<td>3.81</td>
</tr>
<tr>
<td>4</td>
<td>1.61</td>
<td>760</td>
<td>32</td>
<td>2.86</td>
</tr>
<tr>
<td>5</td>
<td>1.17</td>
<td>740</td>
<td>53</td>
<td>3.45</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.27 ± 0.23</td>
<td>776 ± 62</td>
<td>39 ± 8</td>
<td>3.20 ± 0.47</td>
</tr>
</tbody>
</table>

Significance is expressed as follows: *P < 0.05, †P < 0.01 between rest and exercise.

We tested whether oxygen therapy (FIO₂ 24–26% O₂) protected against blood glutathione oxidation. Figure 1 is a histogram showing that exercise caused a significant increase in GSSG/GSH ratio in COPD patients. This is partially prevented by oxygen therapy (see Table 3 and Fig. 1).

Table 3 shows results of COPD patients receiving long-term domiciliary oxygen therapy when they performed exercise while breathing room air or with oxygen therapy (FIO₂ 24–26% O₂). It shows that oxygen therapy partially prevents glutathione oxidation associated with exercise in COPD patients. Blood GSSG/GSH changed from 33 at rest to 85 after exercise without oxygen but from 37 at rest to only 47 after exercise, when it was performed while the patients were receiving oxygen therapy (see Table 3).

A rational approach to prevent the oxidative stress associated with exercise in COPD patients might be the administration of antioxidants. In fact, oral administration of N-acetyl cysteine to COPD patients increases plasma levels of cysteine and GSH (4). We previously found that oral administration of antioxidants partially prevents the oxidative stress induced by exhaustive physical exercise in healthy subjects (16). The possible protection by oral administration of antioxidants in COPD patients remains to be established.

In conclusion, our results indicate that COPD patients show a blood glutathione oxidation when they perform the kind of low-output exercise they carry out in ordinary life. Oxygen therapy partially protects against such oxidation.

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