Original Article

Lactate Dehydrogenase Isozymes in Skeletal Muscle of Patients With Chronic Obstructive Pulmonary Disease

Sonia H. Torres,a,* María Montes de Oca,b Eduardo Loeb,b Priva Zabner-Oziel,c Valentina Wallis,c and Noelina Hernándeza

a  Sección de Adaptación Muscular, Instituto de Medicina Experimental, Universidad Central de Venezuela, Caracas, Venezuela
b  Servicio de Neumonología, Hospital Universitario de Caracas, Universidad Central de Venezuela, Caracas, Venezuela
c  Instituto de Oncología y Hematología, Ministerio del Poder Popular de la Salud, Caracas, Venezuela

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ABSTRACT

Introduction and Objectives: In patients with chronic obstructive pulmonary disease (COPD), lactate dehydrogenase (LDH) levels in skeletal muscles are normal or tend to be elevated; on exercise, these levels increase more rapidly than in individuals without COPD. As it is likely that concentrations of LDH isozymes LDH4 and LDH5 are elevated in such patients, we measured those isozymes in peripheral muscle of patients with COPD.

Patients and Methods: Eighteen patients with COPD and 10 healthy nonsmokers were included in the study. Spirometry and the 6-minute walk test were performed, and a biopsy of the quadriceps muscle was taken to measure levels of both total LDH and LDH isozymes by agarose gel electrophoresis and to classify the types of muscle fibers.

Results: Controls and patients had similar concentrations of total LDH (mean [SE], 130 [30] μmol/min/g vs 152 [50] μmol/min/g, respectively) and LDH isozymes. A subgroup of 5 patients showed increased levels of isozymes LDH1, LDH2, and LDH3, with decreased LDH 5 levels; these patients were women and had a lower oxygen saturation. The LDH 5 level was directly correlated with the 6-minute walk test and oxygen saturation. The percentage of type IIA fibers correlated directly with LDH3 and LDH4 concentrations whereas type IIX fibers were inversely correlated with LDH 3 concentration.

Conclusion: Measurement of LDH isozyme concentrations enabled a subgroup of patients to be identified with a higher concentration of cardiac isoenzymes and lower concentration of muscle isoenzymes, a situation which might indicate adaptation that favors aerobic metabolism.

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Isoenzimas de lactatodeshidrogenasa en el músculo esquelético de pacientes con EPOC

RESUMEN

Introducción y objetivos: En los pacientes con enfermedad pulmonar obstructiva crónica (EPOC), las cifras de la enzima lactatodeshidrogenasa (LDH) en los músculos esqueléticos son normales o tienen cierta tendencia a aumentar; cuando dichos pacientes hacen ejercicio, los valores se elevan más rápidamente que en personas sin la enfermedad. Es probable que las concentraciones de las isoenzimas 4 y 5 de la LDH puedan estar aumentadas en estos pacientes, por lo que se han determinado las isoenzimas de la LDH en el músculo periférico de pacientes con EPOC.

Pacientes y método: Se ha estudiado a 18 pacientes con EPOC y a 10 personas sanas no fumadoras. Se les realizaron pruebas de función pulmonar, la prueba de la marcha de 6 min y biopsia del músculo cuádriceps

Palabras clave:
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*Corresponding author
E-mail address: sonia.hecker@ucv.ve (S.H. Torres).
greater. In type II fibers, LDH4 and LDH5 are the predominant isozymes.

The oxidative enzymes citrate synthase and coenzyme A dehydrogenase are generally less active in these patients. However, analysis of the activity of the glycolytic enzyme LDH in muscle skeleton has yielded contradictory results. Some studies have reported similar total concentrations of LDH in patients with COPD and healthy individuals whereas others suggest that levels tend to be higher in COPD. Patients with COPD and contractile fatigue have also been shown to have higher concentrations of LDH in the quadriceps compared to patients without any such fatigue. Some authors have also reported early production of lactic acid during exercise in patients with COPD.

To our knowledge, no studies have assessed LDH isozymes in skeletal muscle of patients with COPD or, indeed, in that of suffers of other chronic diseases such as heart failure. The aim of the present study was to measure the concentrations of LDH and its isozymes in the vastus lateralis of the quadriceps of patients with COPD and to compare these concentrations with those of healthy individuals. We also aimed to investigate whether the LDH isozymes were related to the proportion of types of muscle fiber, spirometry values, oxygen saturation (SaO2), and functional capacity measured by the 6-minute walk test.

**Patients and Methods**

Eighteen patients from the Pulmonology Department of the Hospital Universitario de Caracas, Venezuela, with diagnosis of moderate-severe COPD were included in the study. The control group was formed of 10 age-matched healthy nonsmokers. All patients and controls signed the informed consent after receiving information about the study procedures. The protocol was approved by our institution's ethics committee.

Diagnosis of COPD was reached according to the internationally established criteria of the American Thoracic Society and the European Respiratory Society. On study entry, patients were clinically stable, receiving appropriate bronchodilator treatment (β2-agonists, anticholinergics, theophylline, and inhaled corticosteroids) and were not regular users of systemic corticosteroids.

Patients were eligible for inclusion if they showed significant response to bronchodilator administration, defined as an increase in forced expiratory volume in 1 second (FEV1) greater than 12% and 200 mL. Patients with concurrent diseases such as congestive heart failure, diabetes mellitus, ischemic heart disease, peripheral vascular disease, and neuromuscular disorders were excluded. All patients and controls were mixed race and reported leading a sedentary lifestyle.

**Lung Function**

Resting lung function was assessed with a spirometer (MedGraphics Cardio System, St Paul, Minnesota, USA). Forced vital capacity (FVC), FEV1, and FEV1/FVC were calculated according to the recommendations of the American Thoracic Society. Normal values were defined according to published reference values.

**Six-Minute Walk Test**

The 6-minute walk test was conducted in an obstacle-free passage 22 m long. The test was standardized according to international guidelines. We performed 2 tests for each patient with a 30-minute interval between each. The corresponding instructions were given to the patients, and they were encouraged to walk briskly for a period of 6 minutes, taking rests if necessary. In addition to determining the distance covered, SaO2 was also measured during the 6-minute duration of the test with a pulse oximeter (Respironics Inc, Model 950 Oximeter, Kennesaw, Georgia, USA). Also measured at rest and during peak exercise were heart rate and severity of dyspnea according to the Borg scale. The distance covered was measured in meters. For the purposes of analysis, the longer of the distances covered in the 2 tests was chosen. The theoretical distance that each patient should cover was determined using the Enright and Sherrill equation, as validated by Cote et al, and the percentage of the actual distance covered with respect to the theoretical distance was calculated.

**Muscle Biopsy**

After sterilization and administration of local anesthetic (lidocaine at 2%), samples were taken from the vastus lateralis of the quadriceps with a Bergstrom needle. The chosen biopsy site was half way between the greater trochanter and the patella, in the superficial part of the muscle. Part of the biopsy material was soaked in optimal cutting temperature compound and frozen in isopentane cooled with liquid nitrogen. The remaining material was frozen directly in liquid nitrogen. Both samples were stored at –70°C until processing.
fibers were classified using the first sample. Serial 10 mm slices were cut in a cryostat set to –20°C, and the adenosine triphosphatase reaction was carried out after preincubation at different pHs (10.3, 4.6, and 4.37).17 From the remaining sample, a homogenate was prepared in potassium phosphate buffer. Part of this homogenate was used for measuring LDH activity by fluorometric methods,18 and the result was expressed in μmol/min/g of wet tissue; the remainder was used for measuring isozyme concentration by agarose gel electrophoresis in accordance with the manufacturer’s instructions (Helena Titan Gel LD, Helena Laboratories, Beaumont, Texas, USA).

Statistical Analysis

Data are expressed as means (SD). The nonparametric Mann-Whitney U test was chosen to investigate group differences because of the sample size and because some of the study variables were not normally distributed, as indicated by the Kolmogorov-Smirnov test. To assess the relationship between LDH isozymes and anthropometric characteristics, lung function, 6-minute walk test, and type of fiber, the Spearman correlation coefficients were calculated. The statistics program used for the analysis was Statistica (Statsoft Inc, Tulsa, Oklahoma, USA). Statistical significance was established at a P value of less than .05.

Results

The mean (SD) values of the physical characteristics, lung function, SaO₂, and 6-minute walk test are presented in Table 1. The lung function results indicate that patients had severe airflow obstruction (FEV₁/FVC, 49% [2%]; FEV₁, 46% [4%]).

Total LDH concentration (130 [30] vs 152 [50] μmol/min/g of wet tissue) and isozyme concentrations, as well as their relative proportions, were similar in patients and controls (Figure 1). Patients showed no differences in the proportion of the types of fiber compared to controls (type I, 44% [2%] vs 48% [2%]; type IIA, 37% [2%] vs 32% [4%]; and type IIX, 19% [3%] vs 20% [4%], respectively). Inspection of the proportions of isozymes revealed that 5 patients had a different profile from controls and 13 had a similar one. Figure 2 shows the densitometry results from agarose gel electrophoresis of the muscle extract of a control subject (Figure 2A) and those corresponding to 2 patients who had different profiles to controls (Figures 2B and 2C). When attempting to characterize the 5 patients with a differing profile, we found that, although total LDH concentration was similar to that of the other patients, isozyme concentrations differed, with decreased LDH₅ and increased LDH₁, LDH₂, and LDH₃ (Figure 3).

Table 1 presents the anthropometric and functional characteristics of the patients with normal and displaced LDH isozyme profiles. Comparison of these characteristics showed that the group of patients with displaced profiles was formed exclusively of women. This would probably explain why the height and distance covered in

<table>
<thead>
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<th>Variable</th>
<th>COPD (n=18)</th>
<th>Controls (n=10)</th>
<th>P</th>
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<td>3/7</td>
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<td>57 (2)</td>
<td>NS</td>
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<td>Weight, kg</td>
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<td>65 (4)</td>
<td>NS</td>
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<tr>
<td>FVC, %</td>
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</tr>
<tr>
<td>FEV₁, %</td>
<td>46 (4)</td>
<td>111 (5)</td>
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<td>FEV₁/FVC, %</td>
<td>49 (2)</td>
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<tr>
<td>SaO₂, %</td>
<td>93 (2)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>6MWD, m</td>
<td>488 (39)</td>
<td>ND</td>
<td></td>
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</tbody>
</table>

Abbreviations: BMI, body mass index; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; ND, not determined; NS, not significant; SaO₂, oxygen saturation at rest; 6MWD, distance covered in the 6-minute walk test.

A Data are expressed as means (SD).
the 6-minute walk test were significantly lower than those of the patients with normal profiles. These patients also had lower SaO2 than those with normal isozyme profiles. In the group with displaced profiles, the proportion of type IIX fibers was lower (4% [2%] vs 22%

\[3\%\], respectively). Given that all patients with a displaced profile were women, the differences were analyzed by sex. Women, as expected, were shorter and covered a shorter distance than men (Table 3). Their LDH1 and LDH4 profiles were different to those of men (for women vs men, 35.2 [6] vs 19.2 [2] μmol/min/g for LDH1 [P<0.01], and 47.4 [8] vs 81.1 [9] μmol/min/g for LDH4 [P<0.05]). Although the numbers were small, the 5 women with displaced isozyme profile were compared with other women with a normal profile, and it was also found that they had different levels of LDH1 and LDH4; those with a displaced profile had higher LDH1 (46.7 [11] vs 23.6 [2] μmol/min/g; P<0.03) and lower LDH4 (25.0 [3] vs 70.0 [6] mmol/min/g; P<0.01) than women with normal isozyme profiles. The women with displaced profiles tended to have a smaller proportion of type IIX fibers. However, distance covered in the 6-minute walk test was similar.

In patients, the distance covered in the 6-minute walk test was directly correlated with the concentration of LDH1 (r=0.70; P<0.02) and inversely correlated with that of LDH4 (r=−0.63; P<0.05). SaO2 at rest showed an inverse correlation with the proportion of isozymes LDH1, LDH2, and LDH4 and LDH5: those with a displaced profile had higher LDH1 (46.7 [11] vs 23.6 [2] μmol/min/g; P<0.03) and lower LDH4 (25.0 [3] vs 70.0 [6] mmol/min/g; P<0.01) than women with normal isozyme profiles. The concentration of LDH2 was also directly correlated with SaO2 at rest (r=0.84; P<0.002).

Figure 4 shows the correlations between the percentages of types of muscle fiber and the concentration of isozyme LDH5 in patients with COPD. The proportion of type IIA fibers was significantly and
directly correlated with concentrations of LDH1 (Figure 4A) and LDH4 (figure not shown; r=0.65; P<0.03) and the proportion of type IIX fibers showed a significant inverse correlation with LDH4 concentration (Figure 4B). Likewise, there was an inverse correlation between the proportion of type IIA fibers and concentrations of LDH2 (r=−0.62; P<0.05), in addition to a trend towards a direct correlation between the proportion of type IIX fibers and LDH4 concentration (r=0.57; P<0.07).

Discussion

To our knowledge, this is the first time that LDH isozymes have been measured in skeletal muscle of patients with COPD. The most relevant findings of this study were as follows: a) similar concentrations of total LDH in patients and controls; b) similar concentrations and proportions of LDH isozymes in patients with COPD and in controls; c) in this particular group of patients with COPD, no differences in the types of skeletal muscle fibers on comparison with the control group; d) differentiation of a subgroup of patients, all of whom were women and who had a lower SaO2, at rest than other patients, from other patients through a lower proportion and concentration of LDH4, with increased cardiac forms of muscle fiber; and e) higher concentrations of LDH4 and lower concentrations of LDH1 in women compared to men.

In the present study, concentrations of LDH were similar in patients with COPD and controls, coinciding with the findings reported in patients with mild-moderate COPD (FEV1, 65% [8%])14 and in those with similar FEV1, to our study group (40% [9%]).6 A trend towards higher levels of LDH has been reported in patients with more severe COPD (FEV1, 25%).4 These observations might indicate an increase in total LDH levels in peripheral muscle as the disease becomes more severe.

The profile of the types of fiber did not show any differences between patients with COPD and controls, in contrast to other studies of COPD in which an increase in the proportion of type II fibers has been reported.1,19,20 This may be due to the fact that airflow obstruction in our patients (FEV1, 46% [15%]) was not as marked as forms with predominance of muscular polypeptide. It has been reported in those with similar FEV1 to our study group (40% [9%]).6 A trend showed a significant inverse correlation with LDH3 concentration (figure not shown; r=−0.57; P<0.05) and the proportion of type IIX fibers, which was more marked in this subgroup of patients, and might represent a form of adaptation that can reduce the extent of conversion towards anaerobic metabolism.

Women had higher concentrations of LDH4 and lower concentrations of LDH1 than men, as well as a higher proportion of type IIA fibers. However, in the group with a displaced profile, these characteristics were even more marked, whether due to genetic or adaptive reasons.

In conclusion, patients with COPD had concentrations and proportions of LDH isozymes similar to controls. However, a subgroup of patients showed a decrease both in the proportion and the concentration of LDH1, with an increase in LDH4, and LDH2, with corresponding increase in the proportion of type IIA muscle fibers. This change may be related to a state of moderate hypoxia, which was more marked in this subgroup of patients, and might represent a form of adaptation that can reduce the extent of conversion towards anaerobic metabolism.

References

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