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Original Article

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

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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 LDH_4 and LDH_5 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 LDH_1, LDH_2, and LDH_3, 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 LDH_3 and LDH_4 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.

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

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para medir tanto la LDH total como las isoenzimas de la LDH por electroforesis en gel de agarosa, así como para clasificar los tipos de fibras.

Resultados: Las concentraciones de LDH total (media ± error estándar: 130 ± 30 frente a 152 ± 50 μmol/min/g) y sus isoenzimas fueron similares en controles y pacientes. Un subgrupo de 5 pacientes mostró un aumento de las isoenzimas LDH_1, LDH_2 y LDH_3, con disminución de la LDH_5; estos pacientes eran mujeres y tenían menor saturación de oxígeno. El valor de la LDH_5 se relacionó directamente con la prueba de la marcha de 6 min y la saturación de oxígeno. El porcentaje de fibras IIA mostró una correlación directa con la concentración de LDH_3 y LDH_4, y las fibras IIX se relacionaron inversamente con la LDH_3.

Conclusión: A partir de las concentraciones de las isoenzimas de la LDH se pudo identificar a un subgrupo de pacientes con una concentración mayor de las isoenzimas cardíacas y menor de las musculares, lo que eventualmente podría representar una adaptación que favorezca el metabolismo aeróbico.

Introduction

Lactate dehydrogenase (LDH) is a tetramer formed from 2 types of polypeptide denoted M (for muscle) and H (for heart). These units can combine to form 5 different isozymes: 4 H for LDH_1, 3 H and 1 M for LDH_2, 2 H and 2 M for LDH_3, 1 H and 3 M for LDH_4, and 4 M for LDH_5. LDH_1 and LDH_2 are the predominant isozymes present in the heart and it is believed they favor the formation of pyruvate from lactate, whereas LDH_4 and LDH_5 predominate in the liver and muscle, where they may be able to reverse the reaction to produce lactate.[1]

Although type I skeletal muscle fibers have lower concentrations of LDH than type II fibers, the relative concentration of LDH_1 and LDH_2 is greater.[2] In type II fibers, LDH_4 and LDH_5 are the predominant isozymes. Several studies have reported an increase in the proportion of type II fibers in patients with chronic obstructive pulmonary disease (COPD),[3] suggesting that LDH_4 and LDH_5 concentrations might be elevated. Decreased activity of oxidative enzymes in patients with COPD compared to healthy individuals has been reported in a number of studies.[4-6] The oxidative enzymes citrate synthase and β-hydroxyacyl-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[6,7] whereas others suggest that levels tend to be higher in COPD.[4] 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.[8] Some authors have also reported early production of lactic acid during exercise in patients with COPD.[6,9]

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 (SaO_2), 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.[10] On study entry, patients were clinically stable, receiving appropriate bronchodilator treatment (β_2-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 (FEV_1) 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$_2$ System, St Paul, Minnesota, USA). Forced vital capacity (FVC), FEV_1, and FEV_1/FVC were calculated according to the recommendations of the American Thoracic Society.[11] Normal values were defined according to published reference values.[12]

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.[13] 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, SaO_2 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.[14] 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 Sherill equation, as validated by Cote et al,[15] 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 Bergström needle.[16] 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. Muscle

Table 1
Anthropometric and Functional Characteristics of the Patients With Chronic Obstructive Pulmonary Disease (COPD) and the Control Subjectsa

Variable	COPD (n=18)	Controls (n=10)	*P*
Sex: male/female	8/10	3/7	
Age, y	62 (2)	57 (2)	NS
Weight, kg	58 (3)	65 (4)	NS
BMI, kg/cm²	24.0 (1.5)	29.0 (2.6)	NS
FVC, %	74 (4)	114 (5)	<.0001
FEV₁, %	46 (4)	111 (5)	<.0001
FEV₁/FVC, %	49 (2)	81 (2)	<.0001
SaO₂, %	93 (2)	ND	
6MWD, m	488 (39)	ND	

Abbreviations: BMI, body mass index; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; ND, not determined; NS, not significant; SaO_2, oxygen saturation at rest; 6MWD, distance covered in the 6-minute walk test.
aData are expressed as means (SD).

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, SaO_2, and 6-minute walk test are presented in Table 1. The lung

function results indicate that patients had severe airflow obstruction (FEV_1/FVC, 49% [2%]; FEV_1, 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 proportions, we found that, although total LDH concentration was similar to that of the other patients, isozyme concentrations differed, with decreased LDH_5 and increased LDH_3, LDH_2, and LDH_1 (Figure 3).

Table 2 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

Figure 2. Profile of the lactate dehydrogenase (LDH) isozymes in agarose gel: healthy individual (panel A) and 2 patients with profiles differing from that of controls (panel B and panel C).
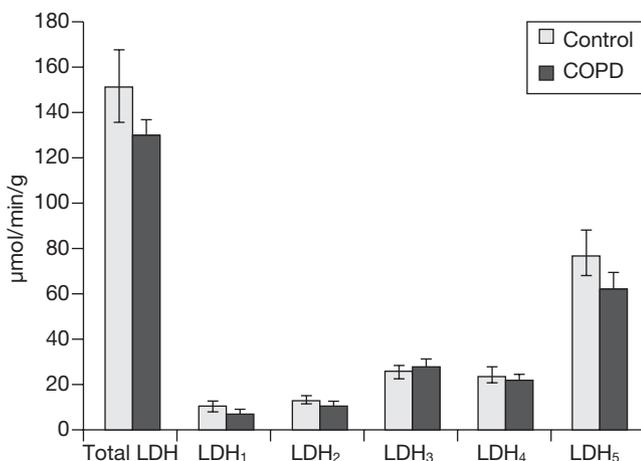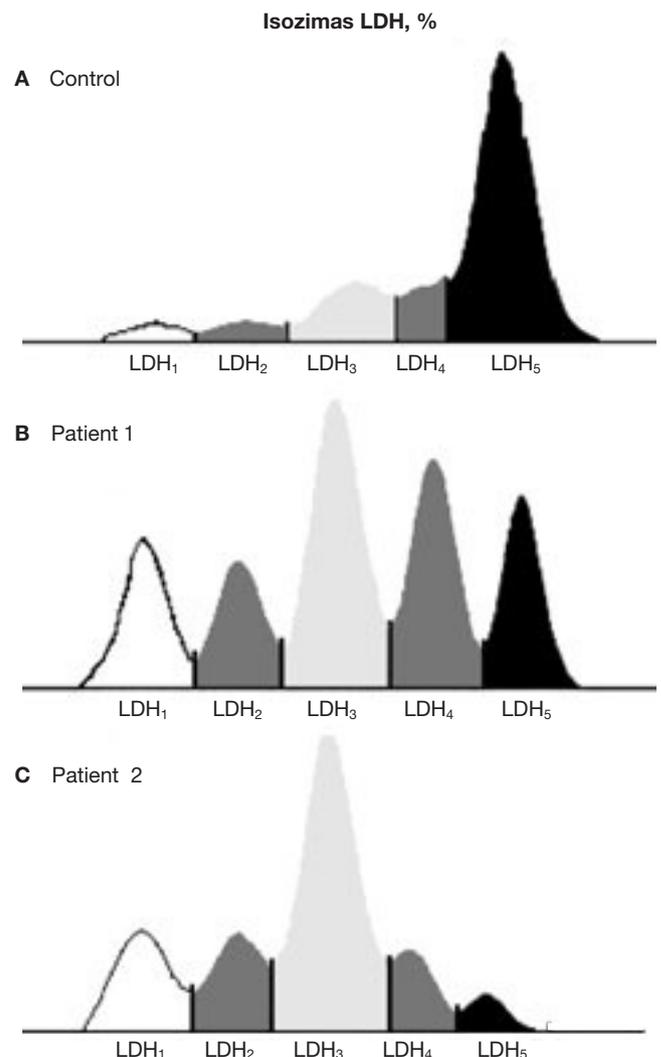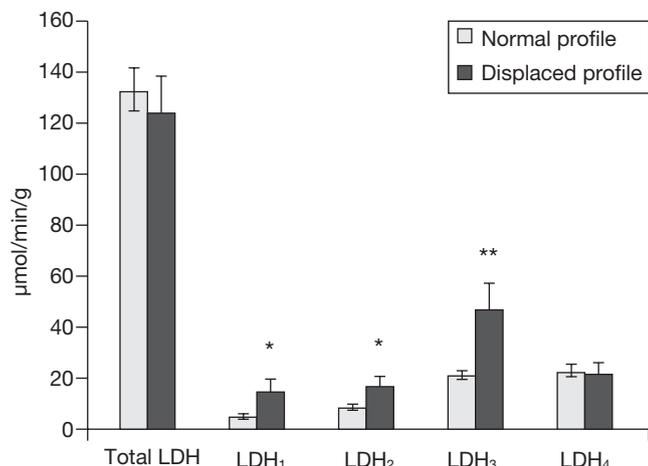

Figure 1. Concentrations of total lactate dehydrogenase (LDH) and its isozymes in the control group and in patients with chronic obstructive pulmonary disease (COPD).

Figure 3. Concentrations of total lactate dehydrogenase (LDH) and its isozymes in patients with a normal profile and in those with a displaced profile. [a]P<.05. [b]P<.001.

Table 2

Anthropometric and Functional Characteristics of Patients with Chronic Obstructive Pulmonary Disease, According to Whether the Lactate Dehydrogenase Isozyme Profile Was Normal or Displaced[a]

Variable	Normal Profile (n=13)	Displaced Profile (n=5)	P
Male/female	8/5	0/5	
Age, y	62 (3)	62 (2)	NS
Weight, kg	60 (4)	53 (5)	NS
Height, cm	160 (12)	147 (5)	<.05
BMI, kg/m^2	23.9 (2.0)	24.3 (1.8)	NS
FVC, %	73 (4)	75 (11)	NS
FEV$_1$, %	45 (4)	49 (8)	NS
FEV$_1$/FVC, %	45 (2)	52 (3)	NS
SaO$_2$, %	96 (0.3)	89 (3)	<.05
6MWD, m	557 (55)	405 (25)	<.05
6MWD, %[b]	109 (7)	83 (9)	<.05

Abbreviations: BMI, body mass index; FEV$_1$, forced expiratory volume in 1 second; FVC, forced vital capacity; NS, not significant; SaO$_2$, oxygen saturation at rest; 6MWD, distance covered in the 6-minute walk test.

[a]Results are expressed as means (SD).

[b]Percentage of theoretical value calculated from the individual characteristics.

Table 3

Anthropometric and Functional Characteristics of the Patients With Chronic Obstructive Pulmonary Disease by Sex[a]

Variable	Women (n=10)	Men (n=8)	P
Age, y	61 (2)	63 (4)	NS
Weight, kg	53 (3)	65 (6)	<.06, NS
Height, m	147 (2)	168 (3)	<.0001
BMI, kg/m^2	23.0 (1.3)	25.4 (3.1)	NS
FVC, %	73 (4)	75 (11)	NS
FEV$_1$, %	45 (4)	49 (1)	NS
FEV$_1$/FVC, %	45 (2)	52 (3)	NS
SaO$_2$, %	90 (3)	96 (0.4)	NS
6MWD, %[b]	85 (7)	111 (7)	<.05

Abbreviations: BMI, body mass index; FEV$_1$, forced expiratory volume in 1 second; FVC, forced vital capacity; NS, not significant; SaO$_2$, oxygen saturation at rest; 6MWD, distance covered in the 6-minute walk test.

[a]Results are expressed as means (SD).

[b]Percentage of theoretical calculated according to the individual characteristics.

the 6-minute walk test were significantly lower than those of the patients with normal profiles. These patients also had lower SaO$_2$ than those with normal isozyme profiles. In the group with displaced profiles, the proportion of type IIA fibers was greater than that of the group with normal profiles (48% [2%] vs 34% [2%], respectively) whereas 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 LDH$_3$ and LDH$_5$ profiles were different to those of men (for women vs men, 35.2 [6] vs 19.2 [2] μmol/min/g for LDH$_3$ [P<.01], and 47.4 [8] vs 81.1 [9] μmol/min/g for LDH$_5$ [P<.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 LDH$_3$ and LDH$_5$: those with a displaced profile had higher LDH$_3$ (46.7 [11] vs 23.6 [2] μmol/min/g; P<.03) and lower LDH$_5$ (25.0 [3] vs 70.0 [6] mmol/min/g; P<.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 LDH$_5$ (r=0.70; P<.02) and inversely correlated with that of LDH$_3$ (r=−0.63; P<.05). SaO$_2$ at rest showed an inverse correlation with the proportion of isozymes LDH$_1$, LDH$_2$, and LDH$_3$ (r=−0.61, P<.05; r=−0.64, P<.05; and r=−0.68, P<.05, respectively) and a direct correlation with LDH$_5$ (r=0.66; P<.05). The concentration of LDH$_5$ was also directly correlated with SaO$_2$ at rest (r=0.84; P<.002).

Figure 4 shows the correlations between the percentages of types of muscle fiber and the concentration of isozyme LDH$_3$ in patients with COPD. The proportion of type IIA fibers was significantly and

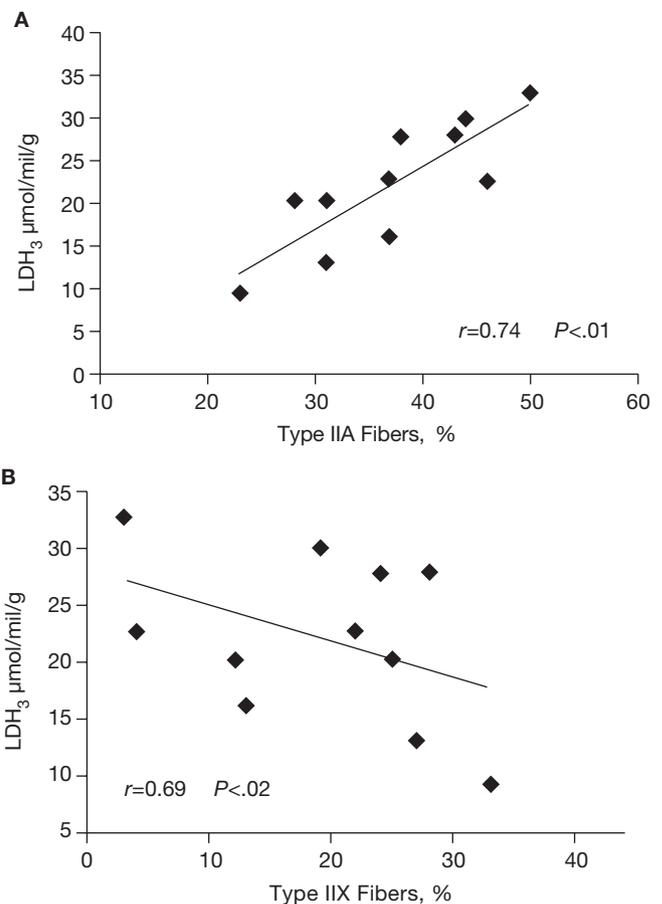**Figure 4.** A, Direct correlation between the proportion of type IIA fibers and the concentration of the isozyme of lactate dehydrogenase (LDH) LDH$_3$ in the vastus lateralis of patients with chronic obstructive pulmonary disease. B, correlation between the proportion of type IIX fibers and the concentration of the lactate dehydrogenase 3 (LDH$_3$) isozyme in the vastus lateralis of patients with chronic obstructive pulmonary disease.

directly correlated with concentrations of LDH_3 (Figure 4A) and LDH_4 (figure not shown; $r=0.65$; $P<.03$) and the proportion of type IIX fibers showed a significant inverse correlation with LDH_3 concentration (Figure 4B). Likewise, there was an inverse correlation between the proportion of type IIA fibers and concentrations of LDH_5 ($r=-0.62$; $P<.05$), in addition to a trend towards a direct correlation between the proportion of type IIX fibers and LDH_5 concentration ($r=0.57$; $P<.07$).

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 SaO_2 at rest than other patients, from other patients through a lower proportion and concentration of LDH_5, with increased cardiac forms of muscle fiber; and *e)* higher concentrations of LDH_3 and lower concentrations of LDH_5 in women compared to men.

In the present study, concentrations of LHD were similar in patients with COPD and controls, coinciding with the findings reported in patients with mild-moderate COPD (FEV_1, 65% [8%])[7] and in those with similar FEV_1 to our study group (40% [9%]).[6] A trend towards higher levels of LDH has been reported in patients with more severe COPD (FEV_1, 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.[3,19,20] This may be due to the fact that airflow obstruction in our patients (FEV_1, 46% [15%]) was not as marked as that of other studies, in which FEV_1 was 37% (11%)[19] and 32% (9%).[20] In patients with COPD, a process of transformation from type I to type IIX fibers has been reported.[21] It may be that this transformation is not marked in patients with moderate COPD. In addition, in the control group of the present study, perhaps because of the small number of individuals included, the proportion of type IIX fibers was greater than that normally found in control subjects. The fact that no differences were found in the proportion of types of fiber among patients and controls is in line with the similar concentrations of LDH and its isozymes found in the 2 groups.

It is noteworthy that an inverse correlation was found between the proportions of type IIA fibers and LDH_5, whereas a direct correlation was found for LDH_4 and LDH_3. This suggests that, since more fibers with greater glycolytic capacity are present, LDH is displaced towards forms with predominance of muscular polypeptide. It has been reported that the M subunit of LDH predominates in type II fibers.[22,23] We were unable to find previous studies that analyze the profile of LDH isozymes in type IIA and IIX fibers. We did not find a relationship between the proportion of type I fibers and isozymes with H subunit predominance in patients with COPD, as has been described in healthy individuals.[22,23]

In mice exposed to mild hypoxia, the proportion of LDH isozymes shifted towards the LDH_1 and LDH_2 forms in several tissues while LDH_5 decreased.[24] In addition, in rats submitted to high-altitude hypoxia, the LDH muscle gene was not upregulated.[25] The findings of the present study suggest that in the group of patients who had a displaced isozyme profile, hypoxia—as manifest by lower SaO_2—may have been related to an increased proportion of LDH_1, LDH_2, and LDH_3, as indicated by the correlation between these variables. An attractive explanation would be that the displacement of the profile

towards H isoforms could allow the LDH to function in the lactate-pyruvate direction, thereby favoring uptake and oxidation of lactate. In a study of uptake and release of lactate labeled with carbon 14 in rabbits, it was found that the net release of lactate was greater in muscle where type IIB fibers predominated and minimal in the preparation comprising mainly type I fibers.[26] The presence of a lactate shuttle that, in oxidative conditions, drives the formation of pyruvate from lactate is still the subject of debate[2,27]; however, displacement of the LDH profile towards LDH_1, LDH_2, and LDH_3 may benefit patients in whom it occurs, whether for genetic reasons or as a result of an adaptive mechanism to compensate the hypoxia. This might allow such patients to make greater use of lactate because the reaction would revert to the lactate-pyruvate direction.

Women had higher concentrations of LDH_3 and lower concentrations of LDH_5 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 LDH_5, with an increase in LDH_1, LDH_2, and LDH_3, 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.

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