**31P-Nuclear magnetic resonance evidence of abnormal skeletal muscle metabolism in patients with chronic lung disease and congestive heart failure**


**ABSTRACT:** The development of 31P-nuclear magnetic resonance (NMR) has enabled direct and non-invasive measurements of muscle metabolism. Serial measurements of the phosphocreatine/inorganic phosphate (PCr/Pi) ratio, which is closely related to the adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio and pH during and after forearm exercise were performed in 11 patients with chronic lung disease (CLD), nine patients with chronic heart failure (CHF) and eight control subjects. As compared with control subjects, the PCr/Pi ratio in the patients with CLD or CHF was lower during the recovery period and significantly lower at three and 4 min exercise. The pH values after exercise were lower in patients with CLD or CHF compared to control subjects.

The PCr/Pi ratio at 4 min after exercise in the patients with CLD or CHF did not correlate with parameters of cardiac function or arterial and mixed venous oxygen tension. The arterial oxygen content and output in patients with CLD and CHF were significantly lower than that of control subjects. Nutritional parameters were not statistically different among the three groups.

These observations suggest that metabolic abnormalities may be present in the skeletal muscles of patients with CLD and CHF that are not due to undernutrition. These may result from reduced arterial oxygen output and, partially, from physical detraining.

Patients with chronic lung disease (CLD) or chronic heart failure (CHF) are forced to limit daily exercise. Numerous reports have found poor correlations between exercise tolerance and measurements of pulmonary and cardiac function [1, 2]. Intrinsic muscle abnormality may play an important role in exercise capacity of patients with CHF [3]. It has been reported that intrinsic muscle abnormality might result from the effects of exercise deconditioning and undernutrition [4-6]. A number of studies have investigated correlations between exercise tolerance and indices of cardiopulmonary function in patients with CLD [7], but there have been no reports focusing on the role of peripheral factors as determinants of exercise limitation.

Skeletal muscle metabolism has been studied with the use of either venous blood samples or muscle biopsy specimens. The development of 31P-nuclear magnetic resonance (NMR) has enabled direct and non-invasive measurements of muscle metabolism at rest and during different forms of exercise metabolic changes being assessed by measurement of high-energy phosphates and intracellular pH. A number of studies have suggested that alterations in muscle metabolism may represent one of many factors that explain the marked heterogeneity of symptoms and exercise intolerance of patients with CHF [8, 9].

The purpose of the present study was to investigate skeletal muscle metabolism during exercise in patients with CLD and CHF and the relationship of metabolic variables to cardiac haemodynamics.

**Methods**

**Subjects**

Eleven male patients with stable CLD were studied; mean age 69±6 yrs (±sd). The cause of CLD was chronic obstructive pulmonary disease (COPD) in
seven patients and restrictive pulmonary disease (old pulmonary tuberculosis) in four patients. The duration of CLD ranged from 2–23 yrs. The degree of dyspnoea in patients 2, 3, 5 and 1 was Hugh-Jones class II, III, IV and V, respectively. For comparison, a group of eight age-matched (65 ± 7.1 yrs) sedentary male control subjects (control) and nine age-matched (62 ± 6.8 yrs) male patients with CHF were studied. The degree of heart failure in patients 5, 3 and 1 was functional class II, III and IV (New York Heart Association), respectively. Control subjects complained of anterior chest discomfort and were admitted for the purpose of cardiac catheterization. They showed no cardiopulmonary abnormalities on physical examination, chest X-p, spirometry, electrocardiogram or two-dimensional echocardiography, but exercise-loaded electrocardiogram revealed mild ST-T abnormality, although not clearly significant, and all of them had no significant stenosis of the coronary arteries.

All CLD patients complained of dyspnoea, the degree of which was more than could be expected from chest X-p, spirometry and arterial blood gas analysis. Therefore, Swan-Ganz catheterization was performed, and the effects of some vaso-active agents (O_2, nitrates, Ca^{2+} blocker) on the cardiopulmonary system were investigated. Patients with CLD had no history of cardiac disease or abnormalities. Patients with CHF had no history of pulmonary disease or abnormalities. After cardiac catheterization, all of the subjects were thoroughly informed of the aims of the research and gave their consent.

Nuclear magnetic resonance procedure

NMR spectroscopy was performed with a 2.0 tesla, 30 cm bore superconducting magnet interfaced with a spectrometer operating at frequencies of 35.3 and 87.1 MHz for ^31P and ^1H, respectively, (BEM 250/80, Otsuka Electric, Osaka, Japan). The subject sat beside the magnet positioned so that the flexor digitorum superficialis muscle of the dominant arm rested on a coil with a surface diameter of 2.0 cm. The magnetic field was adjusted for homogeneity so that the line width at half maximum of the water proton was < 40 Hz. ^31P spectra were obtained with the use of a 30 degree excitation pulse with a 2 s repetition rate. Sixty free-induction decays were summed for each measurement. NMR spectroscopy was performed at rest (Pre), during exercise (E_1, E_2) and recovery (R_1, R_2). Exercise and recovery scans were recorded every minute for 4 min. To assess the changes in intracellular pH, we determined the chemical shift of the pH-dependent inorganic phosphate (Pi) peak (oA) relative to the pH-independent phosphocreatine (PCr) peak. Intracellular pH was calculated from the chemical shift data and Pi titration curve by the following equation [10]:

\[ \text{pH} = \text{pK} \cdot \log 10 \left( \frac{\partial \text{A}}{\partial \text{B}} \right) / \left( \frac{\partial \text{A}}{\partial \text{oA}} - \frac{\partial \text{B}}{\partial \text{oB}} \right) \]

where \( \partial \text{A} = 3.290 \), \( \partial \text{B} = 5.805 \), and \( \text{pK} = 6.90 \).

Exercise protocol

After positioning the forearm within the magnet, a 3 min resting NMR spectrum was obtained. The exercise routine consisted of repetitive finger flexor pulling of a lever with the three distal phalanges at a rate of 60 min⁻¹ (fig. 1). The lever was attached through a pulley system to a mass that was lifted a fixed distance of 0.05 m with each repetition. Exercise was commenced with an initial weight of 1.0 kg, which was maintained for 2 min, then with a weight of 1.25 kg, which was maintained for another 2 min. The total work was 132.3 J (= mass (1 kg or 1.25 kg) \times force of gravity (9.8 m s⁻²) \times distance (0.05 m) \times 60 (times-min⁻¹) \times 4 min). The work rate during the initial 2 min of exercise and during the last 2 min of exercise was 0.49 J per repetition (at repetition per 1 s; average power output, 0.49 W) and 0.61 J per repetition (at repetition per 1 s; average output, 0.61 W), respectively.

Spectra analysis

Quantification of metabolic components was obtained from the Fourier-transformed NMR spectra signal-amplitude analysis. PCr and Pi peak levels were measured and used to calculate the PCr/Pi ratio.

Examination of cardiopulmonary functions

Spirometry, blood gas measurements and Swan-Ganz catheterization were performed on all subjects. The
evaluations included forced expiratory volume in one second (FEV\(_1\)), the FEV\(_1\)-forced vital capacity ratio (FEV\(_1\)/% VC), forced vital capacity (FVC), arterial and mixed venous (pulmonary arterial) carbon dioxide tension (Paco\(_2\) and Pvco\(_2\)), oxygen tension (Pao\(_2\) and Pvo\(_2\)), pH (pHa and pHv), heart rate (HR), mean aortic pressure (AoPmean), mean pulmonary pressure (PAPmean), mean pulmonary capillary wedge pressure (PCWPmean), mean right atrial pressure (RAPmean), cardiac index (CI), arterial oxygen content (CaO\(_2\); CaO\(_2\)=Hb \times 0.0134 \times\) arterial oxygen saturation (SaO\(_2\)) + Pao\(_2\) \times 0.003), and arterial oxygen output (AOO; AOO = cardiac output \times CaO\(_2\)). Mean left ventricular ejection fractions (EF) were measured by either two-dimensional echocardiography or radionuclide angiography. All above mentioned examinations were accomplished within three days prior to NMR study.

**Nutritional and metabolic parameters**

Body mass index (kg\(\cdot\)m\(^{-2}\)) was calculated from body weight (kg) divided by height\(^2\) (m\(^2\)). Triceps skinfold thickness was measured with Lange skinfold calipers. Arm muscle circumference and creatinine height indices were calculated according to the methods of Blackburn and Harvey [11]. We measured maximal grasp power and forearm muscle circumference (FMC) in the region where the surface coil was placed. FMC was calculated from the forearm circumference (FC) and skinfold (FSF) in the region where the surface coil was placed (FMC (cm)= FC (cm)-(0.314 \times FSF(mm)).

Serum chemistries obtained routinely at initial evaluation included magnesium, creatinine, albumin, total cholesterol, haematocrit, leucocyte count, absolute lymphocyte count and iron.

**Statistical analysis**

All data were expressed as the mean\(\pm\)SD. Statistical analysis was done by one-way analysis of variance (ANOVA) with the Bonferroni simultaneous multiple comparison method to test the significance of the differences among the means in the three groups. Statistical analysis of the PCr/Pi ratio and pH was initially analysed using two-way ANOVA. When group differences were found, one-way ANOVA with the Bonferroni simultaneous multiple comparison method was used. A probability of <5% was considered significant.

**Results**

**Forearm energy metabolism**

Figure 2 shows representative spectra of \(^{31}\)P-NMR obtained at rest (Pre), during exercise (E\(_{1-4}\)), and recovery (R\(_{1-4}\)) from the control (a), chronic lung disease (b) and chronic heart failure (c) groups. Pi: inorganic phosphate; PCr: phosphocreatine; ATP: adenosine triphosphate; ppm: parts per million; NMR: nuclear magnetic resonance.

Phosphocreatine (PCr) and inorganic phosphate (Pi) level in the control, CLD and CHF group did not significantly differ under rest conditions (control vs CLD vs CHF: Pi: 62.8\(\pm\)28.3 vs 58.4\(\pm\)21.5 vs 60.6\(\pm\)19.9; PCr: 405.5\(\pm\)136.2 vs 351.4\(\pm\)118.9 vs 381.3\(\pm\)170.8; PCr/Pi: 5.81\(\pm\)2.03 vs 6.52\(\pm\)2.88 vs 5.91\(\pm\)2.48). The values indicate integrated areas for that peak in absolute units.
Exercise induced a progressive decrease in PCr and an increase in Pi. PCr and Pi tended to recover after terminating exercise in all three groups. Figure 3 shows the changes in PCr/Pi during exercise and during recovery. In the CLD and CHF groups, there was a tendency for the PCr/Pi ratio during exercise to be lower than the control group, but there were no statistical differences. Furthermore, the PCr/Pi ratio in the CLD and CHF groups poorly recovered to the pre-exercise levels at three (R3) and four (R4) minutes after exercise, a significantly lower value than that of the control group (p<0.05 or p<0.001).

The relationship between the PCr/Pi ratio at 4 min after exercise and several variables

We investigated the relationship between the PCr/Pi ratio at 4 min after exercise and several variables

Figure 4 shows the changes in pH during exercise and recovery. The pH at rest and during exercise were similar in all three groups. However, recovery of pH after terminating exercise was delayed in the CLD and CHD groups, without significant difference from the control group. The pH at 4 min after exercise (E4) in the control, CLD and CHF groups was 6.91±0.44, 6.61±0.18 and 6.58±0.30, respectively.

The relationship between the PCr/Pi ratio at 4 min after exercise and several variables

We investigated the relationship between the PCr/Pi ratio at 4 min after exercise and several variables
Steady-state pulmonary function, blood gases and haemodynamic data

Table 1 shows steady-state pulmonary function, blood gases and haemodynamic data. In the CLD group, FEV₁, FEV₁% and FVC were significantly lower than those in the other groups. Furthermore, in the CLD group Pao₂ was significantly lower and Paco₂ higher compared to both control and CHF groups. Pvo₂ was slightly lower in the CHF and CLD groups, but there was no statistical difference among the three groups. Arterial blood gas data measured before and just after forearm exercise in a few of the patients with CLD were similar (data not shown).

<table>
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<th>Lung function</th>
<th>Control</th>
<th>CHF</th>
<th>CLD</th>
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<tr>
<td>FEV₁ l</td>
<td>2.47±0.73</td>
<td>2.26±0.21</td>
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<tr>
<td>FEV₁%</td>
<td>75.0±9.1</td>
<td>75.4±2.0</td>
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<td>FVC l</td>
<td>3.33±0.94</td>
<td>3.07±0.33</td>
<td>1.99±0.74*</td>
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<tr>
<td>FVC % pred</td>
<td>100.8±26.1</td>
<td>79.7±28.4</td>
<td>62.9±28.4*</td>
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<tr>
<td>pHa</td>
<td>7.41±0.04</td>
<td>7.42±0.02</td>
<td>7.40±0.03</td>
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<tr>
<td>Pao₂ mmHg</td>
<td>89.3±11.5</td>
<td>89.5±3.6</td>
<td>67.8±8.4*</td>
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<tr>
<td>kPa</td>
<td>(11.9±1.5)</td>
<td>(11.9±0.5)</td>
<td>(9.1±1.1)</td>
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<tr>
<td>Paco₂ mmHg</td>
<td>36.4±3.4</td>
<td>35.3±5.3</td>
<td>45.5±5.7*</td>
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<td>kPa</td>
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<td>pHv</td>
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<td>Pvo₂ mmHg</td>
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<td>Pvc₀₂ mmHg</td>
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<td>HR beats·min⁻¹</td>
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<td>AoPmean mmHg</td>
<td>90.3±6.8</td>
<td>84.9±8.5</td>
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<td>PAPmean mmHg</td>
<td>14.0±2.1</td>
<td>18.4±5.7</td>
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<td>CI l·min⁻¹·m⁻²</td>
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<td>EF %</td>
<td>77.5±8.5</td>
<td>55.3±24.4*</td>
<td>69.8±11.5</td>
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<td>Cao₂ vol %</td>
<td>18.0±0.9</td>
<td>15.9±2.1*</td>
<td>16.0±1.6*</td>
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<tr>
<td>AO₂ vol % Cao₂</td>
<td>99.2±19.9</td>
<td>71.3±12.8*</td>
<td>72.6±18.7*</td>
</tr>
</tbody>
</table>

Values are mean±so. *: significant difference between control and CHF; †: significant difference between control and CLD; ‡: significant difference between CHF and CLD. FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; % pred: percentage predicted; pHa: arterial pH; Pao₂ and Paco₂: arterial oxygen and carbon dioxide tension, respectively; pHv: mixed venous pH; Pvo₂ and Pvc₀₂: mixed venous oxygen and carbon dioxide tension, respectively; HR: heart rate; AoP: aortic pressure; PAP: pulmonary artery pressure; PCWP: pulmonary capillary wedge pressure; RAP: right arterial pressure; CI: cardiac index; EF: ejection fraction; Cao₂: arterial oxygen content; AO₂: arterial oxygen output; CHF: chronic heart failure; CLD: chronic lung disease.

The HR and AoPmean were similar among the three groups. The PAPmean was slightly higher in the CHF and CLD group, and in the CLD group it was significantly higher than that in the control group. The mean value of EF in the CHF group was significantly lower than it was in the other two groups. The Cao₂ in the CLD and CHF group was significantly lower than that in the control group (p<0.05). The AO₂ in the CLD and CHF group was also significantly lower than that in the control group (p<0.01).

Nutritional and metabolic parameters

Body mass index, creatinine height index, triceps skinfold and arm muscle circumference did not differ significantly among the three groups (data not shown). Other variables (magnesium, creatinine, total protein, albumin, total cholesterol, haematomict, white blood cell count, absolute lymphocyte count and iron) also did not differ significantly among the three groups (data not shown). The maximal grasp power in the control, CHF and CLD groups was 38.2±4.5, 35.6±5.7 and 36.2±6.0 kg, respectively, showing no significant differences among the three groups. The FMC in the control, CHF and CLD groups was 21.2±1.8, 22.7±2.5 and 20.4±1.5 cm, respectively, showing no significant differences among the three groups.

Discussion

The results of the present study demonstrated that skeletal muscle metabolism during exercise, as assessed by 31P-NMR, is abnormal in patients with CLD and CHF. The severity of respiratory failure and pulmonary hypertension in the patients with CLD in the present study was relatively mild. Nevertheless, compared with age-matched control subjects, the PCR/Pi ratio decreased at a faster rate during exercise despite performing almost the same amount of external work and its recovery was more delayed. The pH was lower during the recovery period in the patients with CLD. The phosphorylation potential is an important measure of the energy status of the cell and can be used to determine the adequacy of energy reserves for vital cell functions. The PCR/Pi ratio is thought to be closely related to the ATP/ADP ratio, and a reduction of PCR/Pi reflects an impairment in oxidative metabolism of the muscle [12, 13]. Therefore, we measured the changes in PCR/Pi ratio at rest, during exercise and during recovery.

Abnormalities of PCR/Pi and pH might be explained by differences in muscle size. Since NMR samples a fixed volume of muscle, patients with a reduced muscle mass performing the same amount of external work would have greater energy requirements per gram of muscle examined. While we were unable to measure muscle mass directly, body mass index, maximal grasp power, arm muscle circumference and forearm muscle circumference in the region where the surface coil was placed were similar in three groups. Therefore, the differences in skeletal muscle metabolism cannot be explained by reduced muscle mass.
The most plausible explanation for the differences in muscle metabolism between normal subjects and patients with CLD and CHD may be reduced blood flow. Several earlier reports demonstrated that plethysmographic forearm blood flow is reduced in the patients with CHF [14, 15] at rest and in the supine position. On the other hand, in the upright or sitting-posture forearm blood flow at rest and during exercise did not show a significant difference between the control subjects and the patients with CHF [8, 16]. We may have studied patients with less severe circulatory dysfunction than the subjects studied by previous investigators; Leith et al. [17] showed that resting forearm blood flow is directly related to the level of circulatory dysfunction. Following that, metabolic abnormalities observed in the patients with CHF in the present study cannot be explained by impaired blood flow. Unfortunately, there are no reports concerning forearm blood flow in patients with CLD. The patients with CLD in the present study, however, may have less severe circulatory dysfunction and we found no remarkable difference in blood flow between control subjects and patients with CLD.

The fact that abnormal PCr/Pi and pH cannot be explained by impaired blood flow or muscle atrophy lets us consider that there is an abnormality of energy production, or reduced efficiency of contractions in the CLD and CHF groups examined.

Firstly, an impaired oxygen delivery might be considered. In our study, the Cao2 in the CLD and CHF groups was significantly lower than that in the control group, and the AOO was also significantly decreased in both groups. Decreased Cao2 and reduced cardiac output could give reduced AOO in both groups, and reduced AOO might reduce efficiency of contraction and cause abnormal PCr/Pi and pH during exercise.

Secondly, an abnormality of energy production, such as reduced mitochondrial oxidative capacity or altered metabolic control with a metabolic shift toward dependence on glycolysis might be a mechanism. This could reflect a change in fibre-type predominance or in the pattern of fibre recruitment.

Finally, a reduced efficiency of contraction could necessitate increased adenosine triphosphate (ATP) hydrolysis. In fact, several reports have demonstrated that type IIb fibre, which was characterized by low oxidative capacity, fewer mitochondria and easy fatigue, increased in percentage of composition, and oxidative enzyme capacity decreased in patients with CHF [3, 18]. An increased percentage of type IIb fibre is considered to cause reduced PCr/Pi during exercise and delayed recovery of PCr/Pi after exercise. Furthermore, alterations found in patients with CHF are compatible with the effects of exercise deconditioning [4, 5, 19, 20], suggesting that abnormality of skeletal muscle metabolism might be due to physical detraining [3, 4, 18]. Unfortunately, we could not examine the alteration in skeletal muscle histology and biochemistry, and there were no reports concerning altered skeletal muscle in patients with CLD.

However, the patients with CLD and CHF studied in this study were under more physical detraining compared to control subjects, and skeletal muscle metabolism in patients with CLD during exercise, as assessed by 31P-NMR, was similar to that in patients with CHF. Therefore, we assume altered skeletal muscle metabolism in patients with CLD, as well as in patients with CHF, may be partially due to physical detraining and probably a shift in fibre distribution and a decrease in oxidative capacity.

Malnutrition may also contribute to the abnormalities observed in our patients with CLD and CHF [6]. Skeletal muscle biopsies of severely malnourished patients have demonstrated extensive necrosis of muscle fibres, neurogen-like grouping of atrophic type II fibres and predominant atrophy of type II fibres. We measured anthropometric parameters and laboratory tests to evaluate the nutritional status of our patients. However, there were no significant differences in nutritional status among the three groups.

We investigated the relationship between the PCr/Pi ratio at 4 min after exercise and several variables. The PCr/Pi ratio at 4 min after exercise did not correlate with parameters of cardiac function, Pao2, and Pvo2. These data suggested that delayed recovery of skeletal muscle after exercise is not due to changes in these parameters. These results might, in part, explain the lack of correlation between parameters of cardiac function and exercise tolerance in these patients.

In conclusion, our data demonstrated metabolic abnormalities in skeletal muscle of patients with CLD and CHF which were not due to undernutrition. These may result from reduced Cao2 and AOO, and be partially due to physical detraining.

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References


