Evaluation of energy metabolism in skeletal muscle of patients with heart failure with gated phosphorus-31 nuclear magnetic resonance

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ABSTRACT Exertional fatigue is a major limiting symptom in patients with heart failure. To investigate the metabolic basis of this fatigue, we used gated nuclear magnetic resonance spectroscopy to compare inorganic phosphate (P_i), phosphocreatine (PCr) and pH levels, and fatigue (1 to 4+) during mild forearm exercise in eight normal men and nine men with heart failure. Wrist flexion every 5 sec for 7 min was performed at 1, 2, and 3 J (average power output = 0.2, 0.4, and 0.6 W). In both groups linear relationships were noted between power output and P_i/PCr; the slope of this relationship was used to compare PCr depletion patterns. At rest both groups had similar P_i/PCr ratios (normal subjects 0.12 ± 0.06, those with heart failure 0.15 ± 0.03) and pH (normal subjects 7.04 ± 0.13, those with heart failure 7.10 ± 0.11). In normal subjects exercise resulted in a progressive increase in P_i/PCr (slope = 1.17 ± 0.20 P_i/PCr units/W), a reduction in pH only at 0.6 W (0.2 W: 7.03 ± 0.10, 0.4 W: 7.01 ± 0.10, 0.6 W: 6.88 ± 0.16) and moderate fatigue (0.2 W: 0 ± 0, 0.4 W: 1.3 ± 0.5, 0.6 W: 1.9 ± 0.6). In patients with heart failure exercise resulted in significantly greater fatigue at all workloads (0.2 W: 1.0 ± 0.5, 0.4 W: 1.9 ± 0.6, 0.6 W: 2.9 ± 0.5). This fatigue was associated with twice as rapid an increase in P_i/PCr (slope = 2.63 ± 2.14 P_i/PCr units/W, p < .04 vs normal) and greater acidosis (0.2 W: 6.89 ± 0.11, 0.4 W: 6.78 ± 0.22, 0.6 W: 6.75 ± 0.23; p < .02 at 0.2 and 0.4 W vs normal). These data suggest that exertional fatigue in patients with heart failure may result from greater than normal PCr depletion and/or acidosis in the working muscle.


EXERTIONAL FATIGUE is a major limiting symptom in patients with chronic heart failure both during maximal exercise and during normal daily activities.1–3 Therefore, identification of therapeutic modalities that would improve this fatigue is an important clinical objective. To achieve this objective, however, it is imperative that the mechanism responsible for exertional fatigue in patients with heart failure be identified.

Recently we have demonstrated that the exertional fatigue that characteristically limits patients with heart failure during maximal bicycle exercise is associated with reduced leg blood flow and achievement of what appears to be a critical level of marked leg oxygen extraction and lactate release.3 We therefore have postulated that this fatigue is due to lactic acidosis in working muscle.4,5 In normal subjects and experimental animals, muscular fatigue during maximal exercise also appears to result from intracellular acidosis.4,6

Patients with heart failure do not, however, usually perform maximal exercise during normal daily activities. Therefore, it remains unclear whether the exertional fatigue experienced by such patients during less strenuous exercise is also associated with metabolic abnormalities in working muscle. The coupling of forearm exercise with phosphorus-31 nuclear magnetic resonance (31P NMR) provides a new noninvasive approach to the continuous monitoring of skeletal muscle metabolism in normal subjects5–9 and patients with pathologic conditions.10,11 This approach allows dynamic evaluation of inorganic phosphate (P_i), phosphocreatine (PCr), and ATP levels in intact tissues. In addition, 31P NMR can be used to access intracellular pH since the chemical shift between P_i and PCr is dependent on pH.12

These phosphate compounds and pH are key varia-
bles in muscle metabolism. Therefore, fatigue during submaximal exercise in patients with heart failure may be due to altered phosphoenergetics and/or to the earlier than normal onset of intracellular acidosis. This study was undertaken to evaluate this hypothesis.

Methods

Patients. Nine male patients with chronic congestive heart failure and an average age of 60 ± 7 years (± SD; range 46 to 67 years) were studied. All patients were in New York Heart Association functional class II or III and were receiving digoxin and diuretics. None had peripheral edema, ascites, angina pectoris, intermittent claudication, or reduced pulses in their limbs at the time of study. All patients had diffuse left ventricular dysfunction (ejection fractions 24 ± 7%). All patients had been ambulatory for at least 4 weeks before the study. All vasodilator therapy was withheld for 24 hr before the study. For comparison, a group of eight aged-matched (55 ± 8 years, range 46 to 70) sedentary male control subjects were studied. These subjects had no history of heart disease and no cardiac abnormalities discovered on physical examination. Six of these subjects underwent maximal exercise testing. All were found to have normal maximal oxygen consumption (VO$_2$) (> 20 ml/min/kg).

The protocol was approved by the Committee on Studies Involving Human Subjects at the University of Pennsylvania. Written informed consent was obtained from all subjects.

Protocol. The muscle exercise protocol followed that employed in previous reports. In these prior studies, subjects performed forearm tensor/flexor exercise while their arms were within the magnet, thereby enabling metabolic changes within the forearm to be measured continuously during exercise.

In the present study forearm exercise was performed with the patient’s forearm placed into a 1.9 tesla. 26.7 cm bore superconducting magnet interfaced with a TMR 32 Oxford Research Systems spectrometer operating at 32.5 MHz for phosphorus. The forearm was positioned over a 4.5 cm diameter surface coil that allowed examination of approximately 25 ml of tissue within the flexor compartment. Data collection was accomplished with 24 radiofrequency pulses (90 degree pulse width = 55 msec) applied every 5 sec. An exponential multiplication equivalent to a line broadening of 13 Hz was used that yielded a width at half height for PCR of less than 1 ppm.

After positioning of the forearm within the magnet, a 5 min resting NMR spectrum was obtained. The patient then performed wrist flexion every 5 sec for 7 min at a load of 1 J (average power output = 0.2 W). Forearm flexion elevated a bar. Workload was varied by hanging different weights from the end of this bar. The workload was determined with a standard formula from the weight lifted and the distance moved by the weight.

As in previous protocols, the steady state of arm exercise is established by an initial period in which NMR spectra are not recorded. In this study, a shorter interval was elected (1 min) than previously. Information was then accumulated just before each contraction in three 2 min scans (total time = 6 min). After completion of exercise, the patient was allowed to rest for 10 min and his forearm was removed from the magnet. At the end of this rest period, a repeat 2 min resting scan was obtained to ensure full recovery to baseline. Full recovery was always noted by 10 min. The subject then repeated the exercise protocol at a level of 2 and then 3 J. After the last exercise period and a subsequent 10 min rest, a 1 min resting scan was obtained. The patient then performed 2 min of exercise with accumulation of 1 min spectra at a workload estimated to produce a P/PCr ratio of 1. After termination of exercise data were collected at 1 min intervals for 6 min to evaluate recovery of PCr. Ten minutes later, the maximal force development of the subject’s forearm was tested by connecting the bar that elevated the weights to a Cybex II ergometer. Maximal forearm flexion capacity was measured five times and the three peak outputs were averaged.

After each exercise bout the subject was asked to quantify the degree of forearm fatigue from 1+ (mild) to 4+ (severe).

Spectral analysis. Quantification of metabolic components was obtained from the NMR spectra by peak-height analysis with a computer program. P and PCr peak levels were measured and used to calculate the P/PCr ratio. Changes in levels of PCr and P$_i$ were expressed as a ratio since these two compounds are in a relatively fixed pool. Intracellular pH was measured as the chemical shift of P$_i$ from the resonance signal of PCr (pka = 4.6). Prior studies as well as observations during this study indicate that the P/PCr ratio at a given workload is stable after 3 min of exercise. To gain the most accurate estimate of this ratio during the course of exercise, the data accumulated over the last 4 min of exercise were used in its calculation. In contrast, the chemical shift decreased over time in some subjects. Therefore, pH was calculated from the spectrum accumulated over the last 2 min of exercise.

Maximal VO$_2$. Maximal VO$_2$ was determined by analysis of expired respiratory gases during maximal upright bicycle exercise. Exercise was initiated at 20 W with subsequent 20 W increments. It was required that the respiratory gas exchange ratio exceed 1 for peak VO$_2$ to be taken as maximal VO$_2$. Maximal VO$_2$ was not measured in two of the control subjects. Respiratory gas analysis was performed with a Beckman Horizion II system.

Statistical analysis. Data from patients with heart failure and from normal subjects at rest and during exercise were compared by nonpaired Student’s t test. Changes in each variable during exercise within each group was evaluated by paired Student’s t test. The relationships between variables were examined by linear regression analysis. A p value <.05 was considered indicative of a significant difference. Data are expressed as mean ± SD.

Results

Forearm energy metabolism in normal subjects. The characteristic energy spectrum of a near-resting normal muscle showed a PCR peak, a P peak, and the three peaks of ATP (α, β, λ) (figure 1). α and β peaks were clearly resolved while the λ peak was fused with that of PCR. At rest, P/PCr averaged 0.12 ± 0.06 and pH averaged 7.04 ± 0.13.

Exercise led to a progressive decrease in PCR and an increase in P$_i$, a new steady state being achieved in 1 to 3 min (figures 1 and 2). In all subjects linear relationships were noted between power output and average P/PCr measured during the last 4 min of exercise (r = .93 ± .03; slope = 1.17 ± 0.20 P/PCr units/W, intercept = 0.20 P/PCr) (figures 2 and 3).

Exercise resulted in a decrease in pH only at 0.6 W (0.2 W: 7.03 ± 0.10; 0.4 W: 7.01 ± 0.10; 0.6 W: 6.88 ± 0.16; p < .03 vs at rest) (figure 4). No subject experienced fatigue at 0.2 W. The higher work rates produced only moderate fatigue (0.4 W: 1.3 ± 0.5, 0.6 W: 1.9 ± 0.6) (figure 4).
Forearm energy metabolism in patients with heart failure. The near-resting spectra obtained from the patients with heart failure were comparable to those obtained in the normal subjects (figure 1). P/PCr averaged 0.15 ± 0.03 and pH 7.10 ± 0.11, not significantly different from the values noted in the normal subjects.

Exercise led to a progressive decrease in PCr and increase in P, a new steady state being achieved in 1 to 3 min (figures 1 and 2). As in the normal subjects, linear relationships were noted between power output and average P/PCr measured over the last 4 min of exercise (r = .92 ± .05). However, the mean slope of these relationships was significantly greater than in the normal subjects (slope = 2.63 ± 2.14 P/PCr units/W; p < .04 vs normal) (figures 2 and 3). Seven of the nine patients had slopes above the normal range, but the intercept of the relationships (0.21 ± 0.22) was not significantly different from values noted in the normal subjects (figure 3).

Muscle pH decreased below resting levels at all levels of power output (0.2 W: 6.89 ± 0.11, 0.4 W: 6.78 ± 0.22, 0.6 W: 6.75 ± 0.23) (figure 4). The pH achieved was significantly lower than in the normal subjects at 0.2 and 0.4 W (both p < .02), but not at 0.6 W because of the drop in pH noted at 0.6 W in the normal subjects. The patients reported greater forearm fatigue than the normal subjects at all power outputs (0.2 W: 1.0 ± 0.5, 0.4 W: 1.9 ± 0.6, 0.6 W: 2.8 ± 0.5; all p < .03 vs normal).

Recovery of PCr after exercise. In the normal subjects exercise was performed for 2 min at 0.84 ± 0.18 W (0.6 to 1.0 W). This level of exercise increased the P/PCr ratio to 1.06 ± 0.30. After termination of exercise P/PCr rapidly recovered. This recovery was linearly related to time over the first 2 min of recovery (slope = −0.44 ± 0.07 P/PCr units/min) (figure 2). Recovery occurred in 2 min or less in six subjects and in three min in one.

In the patients with heart failure exercise was performed at only 0.62 ± 0.22 W (0.4 to 1.0 W). Although this mean power output was lower than in the normal subjects, the P/PCr achieved during the last minute of exercise (1.78 ± 0.97) was significantly higher than in the normal subjects.

After termination of exercise P/PCr recovery correlated linearly with time over the first 2 min of recovery (figure 2). The slope of this relationship (−0.81 ± 0.36 P/PCr units/min) was significantly greater than in

FIGURE 1. Characteristic rest and exercise NMR spectra obtained at 1 of exercise in a normal subject (top) and a patient with heart failure (bottom).

FIGURE 2. Relationship between P/PCr and power output in a normal subject and a patient with heart failure. Also shown is the P/PCr recovery pattern in these two subjects.
subjects (5.4 ± 1.4 J) and that in the patients with heart failure (4.8 ± 0.9 J).

**Discussion**

In the present study we used $^3$P NMR to determine if the fatigue experienced by patients with heart failure during submaximal exercise is associated with altered skeletal muscle metabolism. Two variables central to muscle metabolism were evaluated: the P/PCr ratio and intracellular pH.

The normal subjects ($p < .02$). Recovery time varied from 1 to 4 min, higher exercise P/PCr ratios being associated with longer recovery times.

To further evaluate recovery characteristics, the P/PCr ratios measured at each point during recovery were correlated with the change in P/PCr noted over the following 1 min (figure 5). A significant positive correlation was noted ($r = .86$), with results in normal subjects and patients with heart failure being similar.

**Relationship of energy metabolism in muscle and clinical characteristics.** Maximal $\text{VO}_2$ averaged 23.5 ± 3.4 ml/min/kg in the normal subjects ($n = 6$) and 14.3 ± 2.7 ml/min/kg (range 11 to 17.2 ml/min/kg) in the patients with heart failure. There was no correlation noted between maximal $\text{VO}_2$ and the slope of the P/PCr–power output relationship ($r = .07$).

There were modest correlations noted between the degree of fatigue and both pH ($r = -.46$) and P/PCr ratio ($r = .47$). There was no significant difference between the maximal developed force in the control

**FIGURE 3.** Comparison of the slopes and intercepts of the power output-P/PCr relationships in the normal subjects and the patients with heart failure.

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**FIGURE 4.** Comparison of P/PCr ratios, pH, and subjective fatigue (1 to 4+) during forearm exercise in normal subjects and patients with heart failure (CHF).
ADP is released from the breakdown of ATP in amounts equal to those of P\textsubscript{i}, although the resulting fractional change in the level of ADP is much larger than that in the level of P\textsubscript{i} since their basal levels are in the micromolar and the millimolar regions, respectively. Mitochondrial respiration is closely related to the ADP level. ADP at higher levels also exerts a regulatory effect on glycolysis. Thus, changes in P\textsubscript{i} and ADP levels exert a continuation of influences on energy production ranging from mitochondrial to mitochondrial plus glycolytic energy production. The control of this continuum can be interpreted largely in terms of the P/Pi ratio. Values in the 0.10 range shut down both mitochondrial and glycolytic metabolism. Values above this, continuing to 1, activate mitochondrial respiration in the state 4 to 3 transition. Values above 1 cause increasing glycolysis, leading to a drop in intracellular pH as lactic acid accumulates in the cell. Intracellular acidosis in turn is closely related to, and may be responsible for, muscle fatigue.

The resting pH and P/Pi ratios that we noted in our normal subjects are consistent with those in prior reports. The linear relationship noted between power output and P/Pi during exercise is also consistent with that previously reported in studies by Chance et al. These investigators have demonstrated that, although the whole relationship is best fitted by a rectangular hyperbola, the initial course of this relationship appears linear. Therefore, calculation of an initial slope of this function seems justified and affords a simple way of comparing the energy metabolism of normal subjects and patients with heart failure. We also observed, as did Chance et al., that changes in intracellular pH were within the experimental error until the P/Pi ratio reached approximately 1. Some lactic acid may have been produced at lower ratios. However, such changes would probably be obscured by the alkalization that results from the dephosphorylation of PCr.

In comparison, the patients with heart failure had normal resting P/Pi ratios and pH. However, during exercise these patients experienced more severe subjective fatigue associated with distinctly abnormal metabolic changes than did the normal subjects. Specifically, the degree of PCr depletion at any given power output was increased, as reflected by an increase in the slope of the power output–P/Pi relationship. This PCr depletion was associated with a more pronounced decrease in intracellular pH than was noted in the normal subjects.

There are several possible mechanisms responsible for this abnormal metabolic response. At each power output, the steady-state P/Pi is determined by the coupling between the energy used in muscular contraction and resynthesis of ATP. Resynthesis of ATP is in turn determined by the mitochondrial population, the efficiency of oxidative phosphorylation, substrate availability, and oxygen delivery.

The energy used in muscular contraction was comparable in both the normal subjects and patients with heart failure since the power outputs during exercise were comparable. Therefore, the increased P/Pi ratio in the patients with heart failure was probably the result of impaired resynthesis of ATP.

It is not possible from the experimental results of this study to identify the precise abnormality responsible for this impaired resynthesis. However, the most likely abnormality, based on data from other studies, is inadequate oxygen delivery to muscle. We and others have demonstrated that limb blood flow during exercise is impaired in patients with heart failure. Prior studies have also demonstrated that inadequate oxygen delivery to working animal muscle and to exercising human muscle produces an increased P/Pi ratio.

However, the recovery dynamics of P/Pi noted in the patients with heart failure is somewhat inconsistent with impaired oxygen delivery. Altered oxygen delivery, or any other factors influencing ATP synthesis, should theoretically interfere with recovery of PCr after exercise. In the patients with heart failure, recovery of PCr was normal if not enhanced. It is possible, however, that the type of recovery analysis undertaken in the present study is not sensitive enough to detect altered recovery in patients with heart failure. Alternatively, impaired oxygen delivery during exercise may interfere with ATP synthesis when ATP utilization is
marked, as during exercise, but not during recovery, when the energy required to replete PCr is more modest.

A second mechanism that could account, at least partially, for impaired ATP resynthesis in heart failure is a reduction in mitochondrial population and/or the efficiency of oxidative phosphorylation resulting from inactivity. However, studies in patients with peripheral vascular disease suggest that chronic underperfusion of working skeletal muscle produces an increase in muscle oxidative capacity. Since patients with heart failure also have chronic underperfusion of muscle, one might expect a similar increase rather than a decrease in muscle oxidative capacity.

In any event, our observations indicate that increased subjective fatigue during submaximal exercise in patients with heart failure is associated with altered muscle P/PCr and pH. These metabolic abnormalities, particularly the change in pH, may be responsible for the increased fatigue. Both human and experimental animal studies have demonstrated a close correlation between intracellular pH, PCr, and muscle fatigue. However, further studies are needed to establish the precise relationship between subjective fatigue and metabolic changes in patients with heart failure. Moreover, it is important to emphasize that no objective measurement of fatigue was made, such as an inability to sustain a given power output. Performance of exercise within a magnet requires that the arm be kept in an inconvenient position. Therefore, it is not always possible to differentiate between total arm fatigue and forearm fatigue. We are currently undertaking additional studies to examine the relationship between pH, P/PCr, and objective fatigue, as measured by an inability to sustain a given power output.

Whatever the direct relationship between altered muscle metabolism and fatigue in patients with heart failure, the ability to demonstrate altered metabolism noninvasively with use of 31P NMR has major clinical implications. If the observed abnormal metabolic responses are responsible for fatigue in patients with heart failure and/or are the result of impaired oxygen delivery, serial measurements with 31P NMR may provide a unique new tool for evaluation of fatigue and/or oxygen delivery in these patients. This tool would be particularly useful in the assessment of the value of therapeutic approaches designed to improve exercise tolerance.

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