Skeletal Muscle Metabolism During Exercise Under Ischemic Conditions in Congestive Heart Failure
Evidence for Abnormalities Unrelated to Blood Flow

Barry M. Massie, MD, Michael Conway, MB, MRCPI, Bheeshma Rajagopalan, BM, DPhil, Richard Yonge, DPhil, Simon Frostick, BM, FRCS, John Ledingham, DM, FRCP, Peter Sleight, DM, FRCP, and George Radda, DPhil, FRS

Previous studies with \(^{31}\)P nuclear magnetic resonance have demonstrated that patients with chronic congestive heart failure often exhibit increased glycolytic metabolism and impaired oxidative phosphorylation in exercising skeletal muscle, but the mechanism for these changes remains unresolved. This study was conducted to determine whether these abnormalities result from impaired blood flow or oxygen delivery. Nine patients with mild-to-moderate congestive heart failure and nine age- and size-matched, healthy control volunteers were studied during repetitive submaximal finger flexion exercise under aerobic and ischemic conditions. Skeletal muscle metabolism was assessed by \(^{31}\)P nuclear magnetic resonance of the flexor digitorum superficialis muscle. During steady-state aerobic exercise at 33% of each subject’s predetermined maximum workload, the patients with congestive heart failure exhibited significantly lower pH values (6.65 ± 0.22 vs. 6.97 ± 0.09, p < 0.002) and phosphocreatine concentrations, expressed as [phosphocreatine]/([phosphocreatine] + [inorganic phosphate]) (0.59 ± 0.14 vs. 0.79 ± 0.08, p < 0.002). Similar differences were also present throughout ischemic exercise at the same workload. Based upon these measurements, calculated lactate production and adenosine 5’-triphosphate consumption rates were significantly higher in the patients with congestive heart failure. These results indicate that in many patients with congestive heart failure exercising muscle exhibits increased glycolytic metabolism and appears to be metabolically less efficient in relation to external work performed. These changes cannot be explained by impaired blood flow or oxygen delivery alone. (Circulation 1988;78:320–326)

Exercise intolerance is the cardinal symptom of chronic congestive heart failure (CHF), yet its severity correlates poorly with hemodynamic indexes of cardiac function.\(^1\)\(^2\) A growing number of studies with \(^{31}\)P nuclear magnetic resonance (NMR) by us\(^3\)\(^4\) and others\(^5\)\(^6\) has demonstrated that metabolic abnormalities in skeletal muscle are common in patients with CHF, and our findings in a small number of patients suggest that these abnormalities may play a role in determining the degree of exercise limitation in these patients.\(^4\)

However, the characteristic \(^{31}\)P NMR findings in patients with CHF, that is, excessive depletion of phosphocreatine (PCr) and greater intracellular acidosis in relation to the level of exercise performed, are nonspecific. Similar changes have been noted during exercise with reduced muscle blood flow in normal subjects\(^7\) and in patients with peripheral vascular disease.\(^8\) Because reduced peripheral blood flow has been a common finding in patients with CHF,\(^9\)\(^10\) it has been uncertain whether these \(^{31}\)P NMR abnormalities reflect reduced nutrient blood flow to exercising muscle or primary alterations in muscle metabolism. Therefore, this study was undertaken to determine whether skeletal muscle metabolism in patients with CHF and in healthy subjects...
differs during exercise under ischemic conditions because such differences could not be explained by abnormalities of blood flow.

**Subjects and Methods**

**Subject Population**

The subject population consisted of nine male patients with chronic, stable CHF and nine age- and size-matched male volunteers serving as controls, who were studied after giving written consent to a protocol approved by the Ethics Committee of the Oxfordshire Health Authority.

The patients ranged in age from 42 to 61 years (54 ± 6 years, mean ± SD) and had CHF for at least 3 months. The cause of CHF was primary congestive cardiomyopathy in five patients and coronary heart disease in four patients. Six patients had symptoms consistent with New York Heart Association Class II, and the remaining three patients had symptoms consistent with Class III. Their mean ejection fraction by radionuclide angiography or two-dimensional echocardiography was 20 ± 7% (range, 13–33%), and their average exercise tolerance by upright bicycle ergometry was 90 ± 20 W (range, 50–125 W). At the time of study, all patients were receiving diuretics, four were taking digoxin, three were taking angiotensin-converting enzyme inhibitors, and two were taking other vasodilators. None of the patients had diabetes mellitus or clinical evidence of peripheral vascular disease or myopathy.

The controls were nonconditioned, healthy volunteers with a mean age of 51 ± 10 years (range, 30–65 years).

**Exercise Protocol**

The exercise routine consisted of repetitive finger flexion pulling a lever with the two distal phalanges at a rate of 40 pulls/min. The lever was attached by a two-pulley system to a fixed weight that was lifted a distance of 5 cm with each repetition. All subjects had performed forearm exercise previously with the apparatus used in this study in conjunction with 31P NMR. In addition to familiarizing the subjects with the procedure and experimental setting, the earlier studies had established a maximum workload for each individual during a protocol that gradually incremented the weight until exercise was limited by fatigue.

In this study, after a 256-scan resting 31P NMR spectrum of the flexor digitorum superficialis muscle was obtained, each subject lifted a weight equal to 33% of his own maximum workload for a minimum of 5 minutes or until four consecutive, 32-second 31P NMR spectra showed no change. The spectra from the final 2 minutes were summed and used to characterize steady-state exercise under aerobic conditions at this workload.

After a 30-minute recovery period and after obtaining a second resting spectrum that confirmed a return to baseline, a cuff sphygmomanometer was inflated to 250 mm Hg. Exercise was then performed at the same 33% of maximum workload until the subject could not continue because of discomfort. Serial, 32-second 31P NMR spectra were obtained during exercise under ischemic conditions. The recovery period after cuff release was monitored by six 32-second spectra, followed by seven 64-second spectra.

**Nuclear Magnetic Resonance Techniques**

NMR spectroscopy was performed with a 1.89 Tesla, 20-cm bore superconducting magnet (Oxford Instruments, Oxford, UK) interfaced with a Fourier transform spectrometer (TMR 32, Oxford Research System, Oxford, UK), operating at frequencies of 32.5 MHz and 80.285 MHz for 31P and 1H, respectively. The spectroscopy procedures have been described previously. In brief, subjects sat beside the magnet so that the flexor digitorum superficialis muscle of the dominant arm rested on a surface coil that was 2.5 cm in diameter. The magnetic field was adjusted for homogeneity during repetitive pulsing at the 1H NMR frequency so that the line width at half maximum for water protons was below 40 Hz. 31P NMR spectra were obtained by a 70° excitation pulse with a 1-second repetition rate.

**Spectral Analysis**

The time-averaged free-induction decays were baseline corrected, apodized, subjected to an exponential multiplication yielding a line broadening of 6 Hz, and subjected to Fourier transformation. Quantitative analysis was conducted by previously described methods. The relative concentrations of intracellular PCr, inorganic phosphate (Pi), and the β-phosphate of adenosine 5'-triphosphate (ATP) were determined by triangulation of their respective peaks, with correction for differential saturation due to the relatively short interpulse delay. The effects of partial saturation were examined by comparing long repetition rate (15 seconds) to 1-second spectra in four patients and four controls, and the effects were found to be comparable in the two groups.

Changes in [PCr] were followed as a normalized PCr ratio, [PCr]/([PCr] + [Pi]), in order to minimize the effect of fluctuations in total signal intensity due to arm movement. During recovery, there was often some loss in total signal compared with the spectra before exercise so that an assumption that PCr recovered fully after 12 minutes was used to back correct the [PCr] during early recovery. This assumption was supported by a return to the baseline PCr:ATP ratio at the end of the study. Intracellular pH was calculated from the chemical shift of Pi.

**Calculations**

With the data derived from 31P NMR and a number of assumptions, the amount of PCr used (ΔPCr), the amount of lactate produced (Δlactate), and the amount of ATP consumed (ΔATP) were calculated on a minute-by-minute basis. The
amount of PCr consumed can be approximated from the value of 42.5 mmol/l intracellular H2O for the sum of intracellular [PCr] + [Pi], which was previously derived from 31P NMR in muscle studies.11,13 Assuming that [PCr] + [Pi] does not change during exercise,13 ΔPCr per unit of time can be estimated from

\[ ΔPCr = \Delta \left( \frac{[PCr]}{[PCr] + [Pi]} \right) \times 42.5 \text{ mmol/l intracellular H2O} \quad (1) \]

The amount of lactate produced can be estimated assuming that each molecule of lactate produced by glycolysis liberates one proton, that the buffering capacity of skeletal muscle is approximately 30 mmol/l intracellular H2O/pH unit,11,12,14,15 that the hydrolysis of each PCr molecule by the creatine kinase reaction consumes one proton (Equation 2), and that protons are produced or consumed because of the partial ionization of Pi, the amount depending on the pH and the pKa of Equation 4 (6.75) as indicated in Equation 5:

\[ [PCr] + [ADP] + [H^+] \rightarrow [ATP] + [Cr] \quad (2) \]
\[ [Pi] = [Pi^{i-}] + [Pi^{2-}] \quad (3) \]
\[ [Pi^{i-}] \rightarrow [Pi^{2-}] + [H^+] \quad (4) \]
\[ 6.75 - pH = \log \left( \frac{[Pi^{i-}]}{[Pi^{2-}]} \right) \quad (5) \]

The amount of ATP consumed was calculated by

\[ \text{ATP used} = ΔATP + 1.5Δlactate - ΔPCr \quad (6) \]

Thus, because [PCr], [Pi], and [H+] can be determined from 31P NMR spectra, the amount of lactate produced per unit of time can be calculated by

\[ Δ \text{lactate} = -ΔpH \times 30 - Δ[PCr] - Δ[Pi^{2-}] \]

in mmol/l intracellular H2O. \quad (7)

Because oxidative metabolism may have continued during the 1st minute of exercise under ischemic conditions because of residual tissue oxygen stores, the values for PCr broken down, lactate produced, and ATP consumed were determined during the 2nd and 3rd minutes of exercise and were averaged.

Statistics
The significance of differences between the patients and controls was determined by the Student’s t test. Where serial measurements of the same metabolite were performed during exercise, Bonferroni’s correction was applied. All values are expressed as mean ± SD.

Results
Exercise Load
Because maximum workload of the patients was considerably lower than that of the controls, the weight (33% of maximum) assigned for these workloads was significantly less in the patients (761 ± 120 vs. 1,100 ± 270 g, p<0.01).

Resting Spectra
The initial resting values of the patients and the controls for intracellular pH (7.01 ± 0.02 vs. 7.02 ± 0.02, respectively) and the normalized PCr ratio, [PCr]/([PCr] + [Pi]) (0.88 ± 0.02 vs. 0.87 ± 0.02, respectively), were almost identical. Values for the resting measurements between exercise under aerobic and ischemic conditions returned to baseline.

Steady-State Exercise Under Aerobic Conditions
Metabolic steady state, as reflected by unchanged 31P NMR spectra during a 2-minute period, occurred within 5 minutes of exercise in all controls and in seven of nine patients. Steady state was achieved within 6 minutes in the remaining patients. The pH and PCr ratios at steady state are illustrated in Figure 1. The pH was significantly lower in the CHF group than in the control group (6.65 ± 0.22 vs. 6.97 ± 0.09, p<0.002), as was the PCr ratio (0.59 ± 0.13 vs. 0.79 ± 0.08, p<0.002).

Exercise Under Ischemic Conditions
The serial measurements of pH and the PCr ratio during exercise under ischemic conditions are illustrated in Figure 2. Only the results from the initial 3 minutes are shown because many subjects did not exercise beyond that point. During the 1st minute of exercise, when tissue oxygen stores were being depleted, the intergroup differences in pH were not significant. However, at each subsequent 32-second interval, the patients had significantly lower intracellular pH values. During this period, the rate of fall in pH, as determined by the slope of the pH and time plot, was greater in the patients than in the controls.

Beginning with the first exercise spectrum, the PCr ratio declined to significantly lower values in the patients than in the controls, and the intergroup differences remained significant throughout the exercise period. However, after the 1st minute of exert-
exercise under ischemic conditions, the rate of fall of the PCr ratio was similar in the two groups. Figure 3 illustrates the calculated values during the 2nd and 3rd minutes of exercise under ischemic conditions for the amount of PCr used, the amount of lactate produced, and the amount of ATP consumed in the two groups. Although the patients tended to use more PCr, the difference was of borderline statistical significance, and the values from the two groups overlapped considerably. However, the patients produced markedly more lactate and, hence, consumed more ATP for each minute of exercise than did the controls.

Recovery After Exercise Under Ischemic Conditions

The rate of oxidative recovery after release of the cuff sphygmomanometer was assessed by the time required to achieve 50% recovery of PCr. This occurred after 66 ± 36 seconds in the patients and 58 ± 23 seconds in the controls, a nonsignificant difference. Although pH recovery to 6.90 or above took longer in the patients (4.9 ± 2.4 minutes vs. 2.9 ± 1.2 minutes, $p<0.05$), this reflected the lower pH values at the end of exercise. The rate of pH recovery was similar in the two groups.

Discussion

Background

The most frequent complaint of patients with mild-to-moderate CHF is exercise intolerance. Although dyspnea during exertion is often a prominent symptom, the commonest results during exercise testing are muscle fatigue and exhaustion.16 Although, intuitively, one might have expected that the degree of impairment of exercise capacity would reflect the severity of the underlying heart disease, the numerous groups investigating this relation have consistently reported poor correlations between most indexes of cardiac function and objective measurements of exercise capacity.1,2

This discordance has shifted interest from central hemodynamics to the periphery. Based upon widened arteriovenous oxygen differences and increased venous lactate concentrations, it has long been suspected that inadequate blood flow to skeletal muscle is responsible for exercise limitation in CHF.17,18 In a series of studies, Zelis and coworkers9 and Zelis and Flaim19 demonstrated that forearm blood flow measured by venous occlusion plethysmography was reduced during handgrip exercise. Similar results from thermodilution techniques have been reported during lower-extremity exercise.10,20 These studies and others have also demonstrated reduced vasodilator reserve in the periphery, suggesting that the
dissociation between central hemodynamic and exercise capacity may be due to mechanical, neurogenic, or circulating vasoconstrictor-induced limitation to blood flow.\textsuperscript{9,10,19,20}

More recently, exercise testing has become the standard method for assessing the therapeutic response to new pharmacological agents in CHF. An intriguing, but nearly universal, finding has been that exercise tolerance improves only gradually over a period of weeks to months after the initiation of effective agents such as the angiotensin-converting enzyme inhibitors even though the same medications produce immediate hemodynamic improvement.\textsuperscript{21,22}

Although this discrepancy could be explained by gradual changes in the peripheral circulation, a more likely mechanism would be alteration in skeletal muscle metabolism or performance itself, which in a broad sense is a kind of "conditioning" process.

The advent of \textsuperscript{31}P NMR has made possible the nondestructive characterization of skeletal muscle metabolism and energetics during exercise.\textsuperscript{11} Studies performed in patients with CHF have demonstrated many metabolic abnormalities.\textsuperscript{3-6} The most prominent finding has been an increase in glycolytic metabolism at submaximal workloads as evidenced by excessive intracellular acidosis, but impaired oxidative phosphorylation has also been frequently observed. Although the data are limited, the severity of these metabolic abnormalities in many patients appears to correspond with the degree of exercise limitation.\textsuperscript{4} However, similar metabolic changes can be induced by limiting blood flow to the exercising limb,\textsuperscript{7} and patients with impaired circulation due to peripheral vascular disease exhibit the same changes.\textsuperscript{8} Thus, the question of whether the abnormalities revealed by \textsuperscript{31}P NMR are secondary to reduced skeletal muscle flow or are a direct result of altered muscle metabolism was not settled by these studies. Some support for the latter mechanism was provided by parallel experiments in the same subjects with CHF who showed no abnormality of forearm blood flow by plethysmography,\textsuperscript{5,6} but this technique is not specific for nutrient flow to exercising muscle, and hence, impairment of the skeletal muscle metabolism could not be excluded.

\textbf{Major Findings}

The present study was designed to characterize skeletal muscle metabolism by \textsuperscript{31}P NMR in patients with CHF and controls during submaximal exercise under aerobic conditions and during exercise under ischemic conditions, which would eliminate intergroup differences in blood flow as a factor. The findings during steady-state exercise under aerobic conditions at a workload of 33% of each individual's maximum workload are consistent with previous studies of patients with CHF.\textsuperscript{3-6} Compared with age- and size-matched controls, the patients had lower intracellular pH values and PCr values. The principal finding, however, was that during ischemic exercise pH fell faster and further in the patients. This indicates a higher rate of glycolytic metabolism in the patients with CHF. PCr also was lower in the patients, but this difference primarily reflected a greater fall during the 1st minute of exercise when fully anaerobic conditions might not have prevailed.

Because there was no blood flow or oxygen delivery to the exercising muscle during the anaerobic phase, the rates of the metabolic changes can be related to the external work. This analysis involves a number of assumptions and possible sources of error that are discussed below, but the findings are striking. Although the rate of PCr utilization is not significantly different between the patients with CHF and controls, the rate of lactate production is markedly greater in the patients with CHF. This translates into a nearly 50% greater rate of ATP consumption (23.1 vs. 15.6 mmol/l intracellular H\textsubscript{2}O/min exercise). Assuming that the workloads were appropriately matched, this suggests reduced "efficiency," or work output, in relation to energy consumption, in the patients with CHF.

\textbf{Potential Mechanisms for Observed Differences}

The reasons for these differences are unclear and beyond the scope of this investigation. However, several potential mechanisms warrant consideration. Fast glycolytic muscle fibers (Type IIb) are more susceptible to acidosis and fatigue than are slow oxidative fibers (Type I). At the same stimulation frequency, Type IIb fibers also exhibit greater depletion of PCr and increases in Pi than do Type I fibers.\textsuperscript{23,24} Many, though not all, studies have shown that in patients and experimental models with peripheral vascular disease, there is an increase in glyco- genolytic and glycolytic enzymes, whereas oxidative capacity is normal or reduced.\textsuperscript{25-27} This may reflect selective atrophy of Type I fibers or increased recruitment of Type IIb fibers during submaximal exercise. In addition, the unusual occurrence in patients with CHF of "split" or "double" Pi peaks during some exercise protocols suggests a mixed population of active fibers with one population becoming severely acidotic.\textsuperscript{3} Of note, previous \textsuperscript{31}P NMR studies in peripheral vascular disease show abnormalities similar to those observed in this study.\textsuperscript{8} Hence, the CHF results could be explained by excessive dependence on glycolytic fibers. Another potential mechanism relates to increased adrenergic stimulation in patients with CHF. This could enhance glycogenolysis in skeletal muscle, exacerbating the intracellular acidosis.

The higher rate of calculated ATP consumption in patients with CHF, assuming that the workloads were properly matched (see next section), suggests a loss of efficiency as defined by the rate of ATP consumption relative to power output. This finding could explain the increase in glycolytic metabolism, but it is also possible that an increase in glycolysis is responsible for decreased efficiency. Fast twitch fibers are known to use more energy
than slow fibers.\textsuperscript{23,24} In addition, lactate accumulation and acidosis impair muscle performance.\textsuperscript{23,28} Other causes for altered efficiency would be changes in contractile protein regulation, which could be induced by altered isomyosin composition or changes in myosin phosphorylation.\textsuperscript{29}

Limitations

There are a number of potential sources of error in the calculations and interpretation of these results. The primary source is the assignment of workloads. If patients with CHF have a smaller mass of muscle performing the same exercise task, the amount of work performed for each gram of muscle would be greater, and the observed metabolic changes would be more marked. Muscle atrophy, particularly of the lower extremities, is not uncommon in patients with advanced CHF.\textsuperscript{30} Although our patients only had mild-to-moderate symptoms and although forearm muscles rather than weight-bearing muscles were studied, some loss of forearm flexor mass might have been present. Indeed, the cross-sectional area corrected for skin-fold thickness of the forearm at the level of the flexor digitorum superficialis muscle where the coil was positioned was slightly smaller in the patients than in the controls (49.9 ± 1.9 cm\textsuperscript{2} vs. 51.7 ± 2.8 cm\textsuperscript{2}). Unfortunately, techniques were not available to quantify the mass of the appropriate muscle. Therefore, an attempt was made to match the workload to each individual’s exercise capacity with a value of 33\% of maximum workload, based upon the feasibility of continuing exercise under ischemic conditions for at least 3 minutes at this load. The workloads of the patients averaged 69\% of the workloads of the controls.

In fact, this probably provides a conservative estimate of the relative muscle mass in the two groups because factors such as deconditioning, decreased blood flow, and tissue acidosis all tend to decrease exercise capacity on the initial exercise protocol and thus decrease the load for this study. Another artifactual explanation for the metabolic differences between these two groups could be differences in the manner in which they performed the exercise procedure. Intergroup differences in energy expended in isometric work or in the duration of flexion and relaxation could have been present. Additional muscles may have been recruited to accomplish the task. To avoid these differences between groups as much as possible, all subjects performed at least one (and often 2–4) previous exercise runs with the apparatus.

Although the serial measurements of pH and PCR establish the presence of metabolic differences between the patients and controls during exercise under ischemic conditions, the additional calculations provide some insight into the potential mechanisms causing differences. As noted above, these involve several assumptions. Some of these calculations, such as the sum of [PCR] + [Pi], its stability during exercise, the stoichiometry of proton production and consumption, and the ratio of lactate to ATP production, are supported by previous \textsuperscript{31}P NMR studies and by recognized biochemical concepts. However, the assumed value for muscle buffering capacity, 30 mM/l intracellular H\textsubscript{2}O/pH unit, and its constancy are less well documented. Higher and lower values are reported in the literature.\textsuperscript{14,15} This value was determined empirically during experiments in patients with McArdle’s syndrome, in whom glycolysis does not occur to counterbalance the consumption of protons during PCR hydrolysis in early exercise, thus permitting the calculation of buffering capacity.\textsuperscript{11,12} However, use of a different value would introduce only quantitative rather than qualitative changes in our results.

Summary and Clinical Implications

The results of this study confirm our earlier work\textsuperscript{3,4} and that of workers at the University of Pennsylvania,\textsuperscript{5,6} which demonstrated increased glycolytic metabolism and decreased oxidative phosphorylation in exercising skeletal muscle in many patients with CHF. In addition, these new results extend earlier observations by indicating that these metabolic changes are not due to reduced blood flow or impaired oxygen delivery alone. Thus, this study suggests that there are intrinsic changes in skeletal muscle energetics that could reflect alterations in fiber composition, recruitment patterns, contractile efficiency, and metabolism.

These results have important clinical implications. Individual differences in the severity of skeletal muscle changes could explain much of the dissociation between symptoms of fatigue and exercise limitation and measurements of cardiac performance in patients with CHF. A failure of these changes to reverse or a delay in improvement could explain why treatments cause immediate hemodynamic improvement but produce a variable or only a gradual benefit in exercise tolerance. Future studies of muscle metabolism in larger numbers of patients will be of great interest.

Acknowledgment

We would like to thank Dr. Peter Styles for his helpful comments and for his assistance in making the calculations based upon work included in his DPhl thesis (University of Oxford, Oxford, UK, 1984).

References

30. Lipkin DP, Jones DA, Round JM, Poole-Wilson PA: Maximal force, fibre type, and enzymatic activity in quadriceps of patients with severe heart failure: A mechanism for reduced exercise capacity (abstract). Br Heart J 1985;54:622

KEY WORDS: • skeletal muscle • congestive heart failure • exercise • metabolism • nuclear magnetic resonance
Skeletal muscle metabolism during exercise under ischemic conditions in congestive heart failure. Evidence for abnormalities unrelated to blood flow.
B M Massie, M Conway, B Rajagopalan, R Yonge, S Frostick, J Ledingham, P Sleight and G Radda

Circulation. 1988;78:320-326
doi: 10.1161/01.CIR.78.2.320

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/78/2/320