In Vivo Magnetic Resonance Spectroscopy Measurement of Deoxymyoglobin During Exercise in Patients With Heart Failure

Demonstration of Abnormal Muscle Metabolism Despite Adequate Oxygenation

Donna M. Mancini, MD; John R. Wilson, MD; Lizann Bolinger, PhD; Hao Li, MS; Keith Kendrick, BS; Britton Chance, PhD; John S. Leigh, PhD

Background Skeletal muscle metabolic abnormalities have been described in patients with heart failure that are independent of total limb perfusion, histochemical changes, and muscle mass. However, these skeletal muscle metabolic abnormalities may result from tissue hypoxia caused by redistribution of flow. Myoglobin is an O₂ binding protein that can indirectly assess tissue hypoxia.

Methods and Results In vivo measurement of deoxymyoglobin was performed by use of proton (¹H) magnetic resonance spectroscopy in 16 heart failure (HF) (left ventricular ejection fraction=20±6%; VO₂=14.5±5.1 mL/kg per minute) and 7 healthy (NI) subjects. Simultaneous phosphorus (³¹P) magnetic resonance spectroscopy and near-infrared spectroscopy also were obtained to examine muscle metabolism and oxygenation. Supine calf plantarflexion was performed every 4 seconds. Incremental steady-state work was performed. A second exercise protocol studied rapid incremental (RAMP) exercise with plantarflexion every 2 seconds. Arterial occlusion at end exercise provided physiological calibration for myoglobin and hemoglobin signals.

With steady-state exercise, the work slope, ie, inorganic phosphorus to phosphocreatine ratios versus work, was significantly greater in patients with heart failure (NI: 0.18±0.08; HF: 0.40±0.32 W⁻¹; P<.05). Intracellular pH was reduced significantly at end exercise in patients but not healthy subjects. Despite these metabolic abnormalities, muscle oxygenation derived from 760- to 850-nm absorption was comparable in both groups throughout exercise. The relation of inorganic phosphorus/phosphocreatine (P/Pc) ratio and muscle oxygenation was shifted upward in patients with heart failure such that at the same muscle oxygenation, P/Pc ratio in these patients was increased. No deoxymyoglobin signals were observed at rest. At maximal exercise, 4 of the healthy subjects and 3 of the patients exhibited deoxymyoglobin (P=NS).

With RAMP exercise, the work slope was again significantly greater in patients with heart failure (NI: 0.21±0.10; HF: 0.57±0.32 W⁻¹; P<.05). Intracellular pH again was significantly decreased at end exercise in patients but not healthy subjects. Five of the healthy subjects and 3 of the heart failure patients had deoxymyoglobin signal (P=NS). With arterial occlusion, deoxymyoglobin was seen in all subjects.

Conclusion Abnormal skeletal muscle metabolism in patients with heart failure usually occurs in the absence of myoglobin deoxygenation, suggesting that the abnormalities are not a result of cellular hypoxia during exercise with minimal cardiovascular stress. (Circulation. 1994;90:500-508.)

Key Words • myoglobin • muscle metabolism • muscle oxygenation

The limitation of exercise at the cellular level traditionally is thought to result from a limit on aerobic ATP production from inadequate O₂ delivery to mitochondria and thus cell hypoxia. However, aerobic metabolism also may be limited by saturation of the hydrogen ion shuttle mechanisms that transport reducing equivalents into the mitochondria and/or the rate of metabolic flux in the citric acid cycle. Reduced muscle mitochondrial content such as occurs with deconditioning also may produce a pattern of reduced aerobic metabolism.1-3

Simultaneous application of phosphorus (³¹P), proton (¹H), and near-infrared (NIR) spectroscopy provides a unique opportunity to examine muscle metabolism and oxygenation in humans. ³¹P magnetic resonance spectroscopy (MRS) allows noninvasive assessment of muscle oxidative and glycolytic metabolism by monitoring high-energy phosphate compounds.4-6

¹H MRS allows in vivo detection of deoxynegated myoglobin. Myoglobin, an oxygen binding protein located exclusively in the skeletal muscle, functions as an extra intracellular source of oxygen and facilitates the transfer of O₂ to mitochondria. Significant deoxygenation of myoglobin does not occur until the partial pressure of tissue oxygen falls below 2.5 mm Hg.7,11 Proton resonances from oxgenated myoglobin are usually concealed under the water peak. However, with deoxygenation, the electron spin of the ferrous ion changes from a low to a high spin state. A paramagnetic shift occurs making protons from deoxynegated myoglobin visible at approximately 70 ppm from the water peak.12 Thus, in vivo monitoring of deoxymyoglobin provides a noninvasive probe by which to assess severe tissue hypoxia.

NIR spectroscopy is another noninvasive technique that relies primarily on the optical properties of hemo-
globin to assess skeletal muscle oxygenation. Both oxygenated and deoxygenated forms absorb light at 850 nm, whereas at 760 nm absorption is primarily from deoxygenated forms.\textsuperscript{13,14} The correlation of 760- to 850-nm absorption changes with venous hemoglobin oxygen saturation has been demonstrated in exercising animal and human muscle.\textsuperscript{15,16}

Abnormal skeletal muscle metabolism, ie, reduced oxidative metabolism with earlier shift to glycolytic metabolism, has been demonstrated in patients with heart failure using \textsuperscript{31}P MRS.\textsuperscript{17-23} These abnormalities are independent of total limb perfusion,\textsuperscript{18,22,23} histochernical changes,\textsuperscript{20} and muscle mass.\textsuperscript{21} Whether these metabolic changes result from severe tissue hypoxia caused by a maldistribution of blood flow or inability to use available \(\text{O}_2\) stores during exercise remains unclear.

In this study, we investigated whether severe tissue hypoxia occurs during small-muscle mass exercise in normal subjects and patients with heart failure through the coupling of \textsuperscript{31}P, \(^1\text{H}\), and NIR spectroscopy. We hypothesized that a deoxymyoglobin signal during exercise in patients with heart failure but not healthy subjects would suggest inadequate \(\text{O}_2\) delivery and severe tissue hypoxia as the mechanism for the previously described metabolic abnormalities. Moreover, an elevated \(\text{P} / \text{PCr}\) ratio with reduced muscle oxygenation during exercise in patients with heart failure would further support reduced \(\text{O}_2\) delivery. Alternatively, absence of deoxymyoglobin signal with higher \(\text{P} / \text{PCr}\) ratios at similar muscle oxygenation during exercise would imply inability to use available cellular \(\text{O}_2\) stores from decreased mitochondrial content and/or function.

**Methods**

**Subjects**

Of the 16 patients participating in the MRS study, 15 were men and 1 was a woman. The average ±SD age was 57 ± 9 years. New York Heart Association classifications for the patients designated 19% in Class I, 38% in Class II, 38% in Class III, and 5% in Class IV for heart failure symptomatology. Cause of heart failure was coronary artery disease in 38% of patients and dilated cardiomyopathy in the remaining 62% of patients. Left ventricular ejection fraction (LVEF) averaged 20 ± 6%. Peak exercise oxygen consumption (\(\text{VO}_2\)) during treadmill exercise averaged 14.5 ± 5.1 mL/kg per minute. All patients were receiving treatment with digoxin, diuretics, and vasodilators. Before participation in the study, all patients were screened for peripheral vascular disease by observation of physical signs (eg, hair loss, dependent rubor), palpation of peripheral pulses, and determination of rest ankle and brachial blood pressures. All participants had preserved peripheral pulses and an ankle-to-brachial pressure ratio of one or more. Seven healthy men also were studied. The age of the subjects averaged 52 ± 10 years and was comparable to heart failure subjects (\(P=\text{NS}\)). In a sub-study, 16 different patients with heart failure (LVEF = 20 ± 7%; peak \(\text{VO}_2\) = 15.4 ± 4.7 mL/kg/min) and 9 sedentary normal subjects (peak \(\text{VO}_2\) = 28.0 ± 1.4 mL/kg per minute) underwent percutaneous calf muscle biopsies to measure muscle myoglobin content. The subjects were age-matched (healthy [NI]: 60 ± 9; heart failure [HF]: 57 ± 14 years; \(P=\text{NS}\)).

Both protocols were approved by the Committee On Studies Involving Human Beings at the University of Pennsylvania. All subjects gave written informed consent.

**Skeletal Muscle Biopsy**

To investigate whether skeletal muscle myoglobin content of heart failure and healthy subjects was comparable, percutaneous muscle biopsies were obtained from the gastrocnemius muscle with the biopsy technique described by Bergstrom.\textsuperscript{24} Gross blood and connective tissue were removed from the samples. One biopsy (approximately 20 to 40 mg) was frozen immediately in liquid nitrogen and stored at −80°C. Muscle samples were coded and then analyzed so that the technician was unaware of the subject’s group. A muscle sample of 10 to 20 mg was homogenized in 50 vol 50% glycerol containing 20 mmol/L NaH\(2\text{PO}_4\), buffer, pH 7.4, 5 mmol/L mercaptoethanol, 0.5 mmol/L ethylenediaminetetraacetic acid (EDTA), and 0.09% bovine serum albumin. All experiments were performed on the same day at 25°C. Muscle homogenates were assayed for myoglobin concentration by the method of Möller and Sylven.\textsuperscript{25}

\(^1\text{H}\) and \textsuperscript{31}P MRS and NIR Spectroscopy Measurements

One-minute \(^1\text{H}\) spectra were obtained using a 1.96-T 1-m bore superconducting magnet (Oxford Research Systems) and 9-cm surface coil. Water suppression was completed with a Super Weft (rapid pulsing) 0.1-second pulse sequence and with a pulse width of 0.08 millisecond. Selective excitation of the deoxymyoglobin signal was accomplished with a Gaussian pulse and a band-pass filter. Quantitation of myoglobin deoxygenation was provided by measuring peak heights. The maximal peak height noted during cuff ischemia was assumed to represent maximal myoglobin deoxygenation. Peak heights were normalized by this value and expressed as percent deoxygenation. This was then converted into percent oxygenation.\textsuperscript{12} Signal-to-noise ratio, calculated as the peak height divided by peak-to-peak noise times 2.5, averaged 19 ± 8.

Simultaneous \textsuperscript{31}P MRS spectra were obtained with the same 9-cm surface coil (Q factor = 150). This single coil was double-tuned to \(^1\text{H}\) and \textsuperscript{31}P with a 180° flip angle for small muscle samples placed at the center of the coil. Therefore, the sample volumes of the \(^1\text{H}\) and \textsuperscript{31}P MRS were from similar but not identical regions. Data acquisition was accomplished with the application of radiofrequency pulses (pulse width = 0.08 millisecond) every 4 seconds. After optimization of field homogeneity, 1-minute scans were recorded during rest and with exercise. Quantification of \(\text{P}\), to \(\text{PCr}\) ratio and ATP was obtained from the Fourier-transformed MRS spectra. An exponential multiplication to a line broadening of 15 Hz was used, yielding a width at half height for PCr of less than 1 ppm. Areas of the \(\text{P}\), and \(\text{PCr}\) signal were measured with computer-generated best-fit analysis as previously described.\textsuperscript{4,5,21} \(\text{pH}\) was calculated from the chemical shift difference of \(\text{P}\), from \(\text{PCr}\).

To assess an individual’s metabolic response to exercise, the \(\text{P}/\text{PCr}\) ratio was correlated with power output. The \(\text{P}/\text{PCr}\) ratio provides an estimate of ADP concentration. ADP level is closely linked to mitochondrial respiration. As described in the transfer function of Chance, the relation during low-level exercise between power output and the \(\text{P}/\text{PCr}\) ratio is linear. Therefore, the calculation of the slope of this relation affords a simple way of comparing oxidative metabolism between subjects. The work slope was calculated for all subjects.\textsuperscript{21}

NIR spectroscopic measurements were made by a dual-wave spectrometer (Runman, NIM, Inc), which filtered light at 760 and 850 nm. Specially designed nonmagnetic fiber-optic light guides were constructed. Light was transmitted to the tissue via one fiber-optic light guide. Reflected light was delivered via a second fiber-optic light guide to a photomultiplier. Changes in muscle oxygenation were expressed relative to the overall change in the signal noted during the reactive hyperemic phase of the protocol, ie, the physiological range. The signal noted at the end of arterial occlusion was presumed to represent near-maximal muscle deoxygenation and therefore was assigned a value of 0% oxygenation. The signal noted after release of the cuff was considered to represent near-maximal oxygenation and therefore was assigned a value of 100% oxygenation. Percent muscle deoxygenation was therefore
derived from the measured deflection of the baseline from the maximal oxygenation divided by the physiological range. This was then converted into percent oxygenation. 

Exercise Protocol

The experimental design used in MRS study is illustrated in Fig 1. Exercise consisted of supine plantarflexion of the foot. The subject's foot was placed on a pedal attached to a pneumatic device that was interfaced with a pressure transducer. The subject was immobilized by a system of Velcro straps used at the knee, ankle, waist, and shoulders. The nonmagnetic fiber-optic light guides were attached with Velcro straps to the medial aspect of the calf at the site of the MRS coil. Therefore the NIR, 31P, and 1H MRS sampled overlapping regions. A rapidly inflating pneumatic cuff was placed on the thigh.

Work was quantified with a computerized system. The pedal was fully depressed against varying pneumatic workloads to a maximum of 30 pounds per square inch (psi). This provided calibration from which force could be derived. The duration of contraction and tension development with each depression of the foot pedal was recorded by a MacLab system. These data were then downloaded to an IBM Interactive Data Language (IDL) system (Research Systems). Actual work performed was quantified by the IBM-IDL system, which integrated the area under each deflection to the point of maximal tension and summed these over 1-minute intervals.

Two exercise protocols were performed. The first protocol consisted of incremental steady-state exercise and was performed in all subjects. The initial workload was extension against a pneumatic load of 5 psi every 4 seconds performed for 4 minutes. Workloads were increased by 5 psi until exhaustion. The subject then rested 15 minutes. Resting data were collected for 3 minutes followed by repetition of the previously determined maximal workload for 3 minutes. At end exercise, pneumatic cuff inflation to a suprasystolic blood pressure (>200 mm Hg) was performed for 3 to 5 minutes depending on the subject's cooperation.

The subject then rested for 30 minutes. Because exercise performed every 4 seconds may enable recovery of deoxygenated myoglobin, a second exercise protocol was performed with plantarflexion every 2 seconds in a subgroup of 7 heart failure and 6 healthy subjects. Exercise workloads were of 1-minute duration and were begun at 4 psi and increased by 4 psi until exhaustion.

Statistical Analysis

Data were compared using unpaired or paired Student's t tests as appropriate. The relation between variables was examined by linear regression analysis. A value of P<.05 was considered statistically significant. All data are expressed as mean±SD.

Results

Skeletal Muscle Biopsies

The myoglobin content of the calf muscle of age-matched sedentary normal and heart failure subjects was comparable (NI: 4.0±0.9; HF: 4.4±1.5 mg/g; NS).

Steady-State Exercise

The maximal workload achieved in healthy and heart failure subjects during exercise was similar (NI: 3.9±1.4; HF: 4.1±2.4 W, NS). The work slope, ie, correlation of P;/PCr ratio and workload, was significantly greater in patients with heart failure (NI: 0.18±0.08; HF: 0.40±0.32 W−1; P<.05) (Fig 2, top). Muscle oxygenation as assessed from 760- to 850-nm absorption was similar at rest and throughout exercise (Fig 2, bottom). A statistically significant shift in the relation of P;/PCr versus muscle oxygenation was demonstrated in patients with heart failure, such that at similar muscle oxygenation P;/PCr was increased (Fig 3).
The results of $^1$H, $^{31}$P MRS, and NIR spectroscopy at rest and at the maximal workload are shown in Table 1. $P_i$/PCr ratio and pH at rest and at end exercise were similar between the two groups. pH decreased significantly with exercise in patients with heart failure but not healthy subjects. Muscle percent oxygenation at end exercise was not different between the two groups. Deoxymyoglobin signal was present in 57% of healthy subjects and 19% of HF subjects at end exercise ($P=NS$). Deoxymyoglobin signal was detected in all subjects at the end of cuff inflation. Of the 3 patients who had a deoxymyoglobin signal during exercise, 2 patients had New York Heart Association Class II congestive heart failure, and 1 had New York Heart Association Class IV. There was no significant correlation between work performed and percent of deoxymyoglobin present. Workload performed by subjects with and without myoglobin signal was similar (With Myo: 3.7±1.7; without Myo: 4.2±2.3 W; NS). Representative $^{31}$P, NIR and deoxymyoglobin spectra in healthy and heart failure subjects are shown in Figs 4 through 6.

In 16 subjects, no deoxymyoglobin signal during exercise could be detected despite marked skeletal muscle deoxygenation noted with the NIR spectrometer. The deoxygenation observed in these subjects was, therefore, almost exclusively caused by deoxygenated hemoglobin. With cuff ischemia, additional NIR deoxygenation occurred, which may represent a small contribution from myoglobin.

**Rapid Incremental Exercise**

The maximal workload attained by healthy subjects was significantly greater than heart failure patients (NI: 6.5±1.8; HF: 3.7±1 W; $P<.01$). The results of $^1$H, $^{31}$P, and NIR spectroscopy at rest and maximal rapid incremental (RAMP) exercise are shown in Table 2. The work slope again was significantly greater in patients with heart failure (NI: 0.21±0.10; HF: 0.57±0.32 W$^{-1}$; $P<.05$). $P_i$/PCr ratios at rest and at end exercise were comparable between the two groups. pH$\text{I}$ significantly decreased in heart failure subjects but tended only to decrease in healthy subjects. Muscle percent oxygenation derived from NIR spectroscopy was comparable in both groups at end exercise and was similar to that achieved during constant-rate exercise.

Deoxymyoglobin signal during exercise was detected in 29% of heart failure subjects versus 83% of the healthy subjects ($P=NS$). Both patients with deoxymyoglobin signal had New York Heart Association Class II CHF. There was no significant correlation between work performed and presence of deoxymyoglobin signal.

In those subjects who exhibited deoxymyoglobin, hemoglobin and myoglobin oxygenation during RAMP exercise was compared. Hemoglobin deoxygenation preceded myoglobin deoxygenation in all subjects. No deoxymyoglobin signal was detected until hemoglobin was at least 70% deoxygenated. Examples of the differential rates of deoxygenation for these proteins are shown in Fig 7 in a healthy and heart failure subject.

**Discussion**

Skeletal muscle metabolic abnormalities during exercise have been described in patients with heart failure. These metabolic abnormalities include a more rapid depletion of PCr with slowed recovery and an earlier shift to glycolytic metabolism.17-23 The results of this study are concordant with previous reports as the work slope, ie, the correlation of the $P_i$/PCr ratio with power output, was increased in patients with heart failure during both steady-state and RAMP exercise. pH$\text{I}$ was also decreased significantly during exercise.

In this study we coupled $^{31}$P, $^1$H and NIR spectroscopy to examine tissue oxygenation and metabolism. Prior studies have examined metabolism or oxygenation separately. Simultaneous application of NIR and $^1$H spectroscopy also enabled us to investigate the contribution of myoglobin to NIR absorption as well as to examine the differential deoxygenation of myoglobin and hemoglobin during exercise in humans.
This combined approach enabled us to demonstrate that during exercise with minimal cardiovascular stress, the metabolic abnormalities observed in patients with heart failure occur despite what appears to be adequate muscle oxygenation. The absence of deoxymyoglobin signal during the small-muscle mass exercise and the normal muscle oxygenation throughout exercise indicate that the $^{31}$P metabolic abnormalities in these patients do not result from inadequate $O_2$ availability. Prior studies demonstrated similar tissue perfusion by venous plethysmography between healthy and heart failure subjects. However, whether maldistribution of blood flow occurred resulting in ischemia to exercising muscle remained unclear. This study excludes this hypothesis and demonstrates that the metabolic changes are not from severe tissue hypoxia during small-muscle mass exercise.

$^1$H spectroscopy was used to monitor the development of deoxymyoglobin in humans during maximal calf exercise. Myoglobin is an oxygen-binding protein. Similar to hemoglobin, the myoglobin dissociation curve follows a hyperbolic function of oxygen pressure. However, myoglobin binds oxygen at low pressures much more readily than hemoglobin. The 50% desaturation (P50) of myoglobin does not occur until approximately 2.5 mm Hg at 37°C. In rapidly metabolizing muscle such as occurs during exercise, a steep oxygen gradient develops between myoglobin deoxygenation and cytochrome AA3, the rate-limiting step of mitochondrial respiration. A coherent relationship occurs between deoxygenated myoglobin and AA3 during high metabolic activity, enabling myoglobin to serve as a noninvasive indicator of tissue hypoxia.

Previous studies from this laboratory have demonstrated the detection of deoxymyoglobin in the resting ischemic forearm muscle, as well as detection of signal from standard deoxymyoglobin solutions that approximate normal human tissue levels. The sensitivity of this method is 5% to 10%. This is the first study to use this technique to examine deoxymyoglobin formation during calf exercise in a large series of healthy and heart failure subjects.

As prior studies have reported reduced muscle enzymatic content in patients with heart failure, including reduced citrate synthase and $\beta$-hydroxyacyl CoA reductase, percutaneous gastrocnemius muscle biopsies were performed to determine if muscle myoglobin content was comparable in age-matched healthy and heart failure subjects. Unlike the findings in animal experiments, intramuscular myoglobin concentration is similar in trained and sedentary human subjects.

Myoglobin levels measured in different individual human muscle fiber types indicate that Type II fibers contain on average two thirds as much myoglobin as Type I fibers. Our study demonstrates similar muscle myoglobin content in the heart failure and healthy subjects at concentration levels consistent with previous reports. Therefore, the results of $^1$H MRS should be comparable between healthy and heart failure subjects. Deoxymyoglobin signal was observed in only 3 of the 16 patients during maximal constant-rate exercise. Even

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**TABLE 1. Metabolic Measurements During Maximal Constant Rate Exercise**

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<thead>
<tr>
<th></th>
<th>Healthy (n=7)</th>
<th>Heart Failure (n=16)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
</tr>
<tr>
<td>$P_i/PCr$</td>
<td>0.08±0.02</td>
<td>0.77±0.48*</td>
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<tr>
<td>pH</td>
<td>7.29±0.19</td>
<td>7.19±0.18</td>
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<td>NIR muscle oxygenation, %</td>
<td>...</td>
<td>23±11</td>
</tr>
<tr>
<td>Subjects with deoxymyoglobin signal, n</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Myoglobin oxygenation, %</td>
<td>73±30</td>
<td>88±25</td>
</tr>
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</table>

$P_i/PCr$ indicates the ratio of inorganic phosphorus to phosphocreatine and NIR, near-infrared spectroscopy.

*P<.05 rest vs exercise; P<.05 healthy vs heart failure.

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**Fig 4.** Representative $^{31}$P magnetic resonance spectroscopy (MRS) spectra in a healthy (normal) subject and a patient with heart failure at rest, with maximal exercise, and cuff inflation. $P_i$ indicates inorganic phosphorus; $PCr$, phosphocreatine; ATP, adenosine triphosphate; ppm, parts per million; and PDE, phosphodiester.
with exercise at a more rapid rate, deoxymyoglobin signal was evident in only 2 of 7 heart failure subjects. These results strongly suggest that maximal small-muscle mass exercise in these patients is generally not limited by tissue hypoxia. Prior studies that measured mean myoglobin oxygen tension during maximal exercise also suggested that intracellular O₂ availability was not limiting exercise. Hydrogen ion shuttle mechanisms that transport reducing equivalents into the mitochondria, the rate of metabolic flux in the citric acid cycle, or simply reduced mitochondrial content are alternate sites of limitation of aerobic metabolism. Other factors such as muscle mass/strength and/or supratentorial input also may serve to limit the small-muscle mass exercise performance in our patients.

Deoxymyoglobin signal during both steady-state and RAMP exercise was more prevalent in the age-matched healthy subjects. Our finding of similar myoglobin content in the skeletal muscle biopsies between the healthy and heart failure subjects suggests that the greater prevalence of the deoxymyoglobin signal in healthy subjects is not from differences in muscle myoglobin concentration. There is no prior evidence to suggest any abnormalities in the O₂ binding characteristics of myoglobin between the groups. Myoglobin O₂ dissociation curve unlike hemoglobin does not exhibit a Bohr effect when exposed to varying pH, temperature, or 2,3 diphosphoglycerate concentration. Therefore, differences in pH cannot explain this finding. Alternatively, there may be a problem with transfer of O₂ from myoglobin to mitochondria in patients with heart failure. These patients have been shown to have reduced mitochondrial content with disorganized cristae and reduced mitochondrial enzymes, which may or may not be due to inactivity. Differences in the velocity of blood flow, blood viscosity, and/or muscle capillary density between the two groups may be additional factors affecting the desaturation of myoglobin. Work performed by the healthy subjects was significantly higher during RAMP exercise. Attainment of higher workloads, with increased O₂ demands, may explain the trend for increased myoglobin deoxygenation in the healthy subjects.

pH was significantly decreased during exercise in patients with heart failure. Therefore, a reduction in pH occurred in the presence of significant O₂ cellular reserve. Reduction in pH with lactate formation is often viewed as an indicator of tissue hypoxia. The absence of significant deoxymyoglobin signal in the majority of subjects suggests that muscles produce and release lactate under aerobic conditions. This would occur if mitochondrial FAD failed to reoxidize the proton shuttle of the mitochondrial membrane at a rate sufficient to keep cytosolic [NADH+H⁺]/[NAD] normal. The redox state of the cytosol would become reduced. NADH accumulation in the cytosol would
result in pyruvate oxidation of NADH to NAD with the subsequent accumulation of lactic acid. These results are consistent with Connett et al, who demonstrated formation of lactate without myoglobin desaturation in a canine gracilis muscle preparation.

In early work, it was thought that myoglobin as well as hemoglobin contributed significantly to muscle NIR light absorption. However, animal studies from this laboratory and by Seiyama et al suggest that almost all the absorption is from hemoglobin. In our study we were able to demonstrate a high degree of hemoglobin deoxygenation without any concomitant myoglobin deoxygenation during exercise in the majority of subjects studied. Therefore, the major signal from NIR spectroscopy was derived from hemoglobin. In some subjects, a small amount of deoxygenated myoglobin is evidenced during exercise. Nevertheless, the majority of the NIR signal from these subjects is derived from hemoglobin. This study also illustrates that hemoglobin deoxygenation precedes myoglobin deoxygenation. The transitional point for rapid deoxygenation of myoglobin appears similar in both heart failure and healthy subjects. However, with the small number of subjects studied, this conclusion is circumspect.

**Limitations of the Study**

A potential criticism of this study is that 1H NMR may be an insensitive method to detect small quantities of deoxymyoglobin. Also, 1H, 31P NMR, and the NIR spectroscopic measurements are from similar but not necessarily identical skeletal muscle regions. The spectra for deoxymyoglobin are summed signals over 1 minute. Local oxygen gradients can exist in muscle. It is therefore possible that local PO2 below 2.5 mm Hg can exist without influencing the average deoxymyoglobin signal, which is derived from a larger volume with a low signal-to-noise ratio. Moreover, with muscle fatigue, less work may be performed with a lessening of NIR deoxygenation. This may be sufficient to reoxygenate myoglobin. The effect of inorganic phosphate, which is higher in heart failure, on myoglobin saturation is unknown though being nonbond it is unlikely to significantly alter the saturation curve.

NIR spectroscopy is limited in that it provides qualitative data. The path length of NIR light in muscle is unknown for continuous light, although work by Chance et al suggests that the average path length in human skeletal muscle is ~2.6 cm. Consequently, the light absorption levels monitored in this study do not yield absolute levels of hemoglobin deoxygenation. However, a relative calibration can be provided in limb musculature by use of a physiological range.

This study also is limited in that only small-muscle mass exercise, which does not stress the cardiovascular system, was studied. If maximal exercise had been examined, we may have found a greater degree of deoxymyoglobin signal in the heart failure patients. We previously examined vastus lateralis muscle deoxygenation using NIR spectroscopy during upright bicycle exercise. With this cardiovascular limiting exercise, skeletal muscle deoxygenation was significantly greater in patients with heart failure. Reduced limb perfusion during bicycle exercise has been previously demonstrated in patients with heart failure by a variety of techniques. Our present findings of similar muscle oxygenation during small-muscle mass exercise does not conflict with our prior results. With single-limb plantarflexion, cardiovascular stress is minimal and limb perfusion is similar between normal and heart failure.

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**Table 2. Metabolic Measurements During RAMP Exercise**

<table>
<thead>
<tr>
<th></th>
<th>Healthy (n=6)</th>
<th>Heart Failure (n=7)</th>
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<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
</tr>
<tr>
<td>P1/PCr</td>
<td>0.08±0.02</td>
<td>2.41±1.19*</td>
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<tr>
<td>pH</td>
<td>7.32±0.12</td>
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<td>NIR muscle oxygenation, %</td>
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<td>26±7</td>
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<td>Subjects with deoxymyoglobin signal, n</td>
<td>...</td>
<td>5</td>
</tr>
<tr>
<td>Myoglobin oxygenation, %</td>
<td>53±27</td>
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</table>

P/PCr indicates the ratio of inorganic phosphorous to phosphocreatine and NIR, near-infrared spectroscopy. *P<.05 rest vs exercise; P<.05 healthy vs heart failure.
subjects \cite{18,22}; therefore, similar muscle deoxygenation would be expected.

Conclusions

In summary, the results of this study suggest that small-muscle mass exercise in patients with heart failure is not limited by severe tissue hypoxia. The metabolic abnormalities previously noted in these patients do not result from inadequate O\(_2\) delivery, but from inadequate O\(_2\) utilization by mitochondria. The mechanism by which this occurs remains unclear. Reduced mitochondrial content from deconditioning will decrease the rate of aerobic metabolism. Recently, it has been demonstrated that skeletal muscle metabolic abnormalities in patients with heart failure can be improved with training, though whether these changes can be reversed fully remains unclear.\cite{47} The majority of patients who had deoxymyoglobin signal had Class II CHF. Moreover, there was a tendency for healthy subjects to have an increased prevalence of deoxymyoglobin signal. Increased mitochondrial content or more normal mitochondrial function in these subjects may permit more optimal utilization of available O\(_2\) stores. Decreased mitochondrial content also could explain the tendency to decrease pH\(_i\) in CHF patients, as lactate will accumulate from mass action effect. Alternatively, there may be mitochondrial dysfunction. Drexler et al.\cite{38} have demonstrated mitochondrial abnormalities with decreased volume and surface density of cristae and inner boundary membranes. This may result in inability to transfer O\(_2\) across the mitochondrial membrane and/or abnormalities in the metabolic flux in the citric acid cycle. Future studies that investigate in vitro mitochondrial function in these patients are needed.

In this study we also demonstrate that NIR spectroscopy provides a useful, noninvasive method of monitoring skeletal muscle oxygenation. It monitors primarily muscle hemoglobin and not myoglobin deoxygenation. As NIR spectroscopy can be performed repeatedly without discomfort or risk, it can be used to serially assess the effect of therapeutic interventions in patients with heart failure.

Acknowledgment

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References

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