Combined Use of Near-infrared Spectroscopy with $^{31}$P-Magnetic Resonance Spectroscopy to Study Cardiovascular Pathophysiology

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Abstract: In order to better understand the pathophysiology of cardiovascular diseases, both peripheral muscle metabolism and blood flow should be examined. Conventionally, blood flow measurements were performed by venous occlusion plethysmography or catheter based procedures, which evaluate non-selective blood flow. Plethysmography measures the whole limb blood flow, including the flow in skin and non-exercising muscles. A catheter is usually inserted into the large vein and also provides the whole limb blood flow measurement. In contrast, near-infrared spectroscopy might provide information about the nutritive flow in localized exercising muscle. A combined use of NIRS with $^{31}$P-magnetic resonance spectroscopy, which can measure muscle metabolism, is a novel tool to elucidate the pathophysiology in cardiovascular disorders.

Key words: cardiovascular, chronic heart failure, near-infrared spectroscopy, $^{31}$P-magnetic resonance spectroscopy, exercise intolerance

Introduction

Chronic heart failure (CHF) is a common and debilitating pathophysiologic condition in cardiovascular diseases with high rates of mortality and morbidity. CHF is characterized as a clinical disorder with exercise intolerance. At first vision, it may be thought simply due to failure of perfusion of the exercising musculature and the consequent early onset of intramuscular acidosis in CHF. However, studies show that the degree of exercise intolerance in patients with CHF is not correlated with the extent of the central hemodynamic disturbance. Moreover, improved circulation by cardiotonic agents is not contributed to increase in exercise tolerance. The challenging studies have shown that reductions in skeletal muscle mass and aerobic enzyme activity, and an increased percentage of fast-twitch–type (IIb) fibers in skeletal muscle can induce early anaerobic metabolism during exercise and may limit exercise in patients with CHF. These findings have been confirmed by studies using $^{31}$P-magnetic resonance spectroscopy (MRS), which revealed intrinsic abnormalities of skeletal muscle metabolism manifested by a greater magnitude (and increased rate) of phosphocreatine (PCr) depletion and a decreased intramuscular pH in patients with CHF. Experimentally demonstrated mechanisms of skeletal muscle dysfunction and exercise intolerance in CHF were shown in Fig. 1. Thus, skeletal muscle dysfunction might be a principal factor for exercise intolerance in CHF. Yet, there is a possibility that nutritive blood flow is selectively impaired and contributes to exercise intolerance in CHF.

Interestingly, studies using near-infrared spectroscopy (NIRS) have shown that peripheral muscle oxygenation is impaired during exercise in patients with CHF. Although both muscle metabolism and muscle oxygen kinetics are important determinants of exercise capacity, these factors have only been separately evaluated in patients with CHF. Even in normal subjects, only a few studies have assessed both muscle metabolism and oxygen kinetics. McCully et al. measured both oxygenated hemoglobin (oxy-Hb) and PCr recovery after submaximal exercise in normal subjects using NIRS and $^{31}$P-MRS respectively, and found that the time constants of these indices were similar. In patients with CHF, local oxygen delivery as...
well as skeletal muscle energetic metabolism might be impaired and these abnormalities are potential contributors to exercise intolerance. In this review, I will discuss the relation between muscle metabolism and oxygen kinetics in CHF by presenting the date obtained from a useful combination of $^{31}$P-MRS and NIRS measurements.\(^{15}\)

**Experimental procedures**

Fifteen patients with CHF, mean (SD) age 58 (8) years, and 16 age matched normal subjects were studied. Patients were in New York Heart Association class II–III with ejection fraction of 29 (13)%. Heart failure was attributed to idiopathic dilated cardiomyopathy in all patients. Patients with peripheral vascular disease were excluded from the study.

Subjects performed supine plantar flexion of the right calf muscle. Before the study, muscle strength was measured by the one repetition maximum (1 RM) method, which measures the maximum weight that can be lifted only once. Using magnetic resonance imaging, the maximum calf flexor muscle cross sectional area (MCA) was determined. The workload was adjusted to 50% of 1 RM. Plantar flexion was performed once every 1.5 seconds for six minutes against a pedal. Measurements were obtained from the 1-minute rest period before exercise through the six-minute recovery period after exercise.

$^{31}$P-MRS was performed by an 80-mm surface coil in a 55-cm bore, 1.5-Tesla superconducting magnet (Magnetom H15, Siemens, Erlangen, Germany). Shimming was adjusted using a proton signal from water. Spectra were obtained with a pulse width of 500 ms, a transmitter voltage of 20 V, and a repetition time of 1000 ms. Four scans were performed and averaged for each spectrum. PCr is expressed as $\frac{[PCr]}{([PCr]+[Pi])}$, where $Pi$ is inorganic phosphate. The degree of PCr change was calculated as: $PCr$ depletion = $\frac{rest\ PCr - peak\ PCr}{rest\ PCr}$. The muscle pH was calculated from the changes in the chemical shifts of $Pi$ relative to PCr as previously described.\(^{16}\) As previous studies have shown the appropriateness of using monoexponential fitting to describe the rate of PCr recovery,\(^{17}\) we estimated it using time constants. PCr recovery after exercise was fitted to a single exponential curve obtained by least squares regression, and the time constant for PCr recovery ($\tau$) was calculated as follows: $[PCr] = C1 + C2(1 - e^{-kt})$, where $[PCr]$ is the PCr concentration, $C1$ is the initial $[PCr]$, $C2$ is the difference between the final and initial $[PCr]$, $t$ is time, and $k$ is the rate constant ($1/k = \tau$).

NIRS was performed with a dual wave spectrometer (HEO100, Omron, Tokyo, Japan), a tissue oximeter that uses a two wavelength light emitting diode (LED), with wavelengths of 760 and 840 nm, as a light source. The basic principles of NIRS and in vitro results obtained with this tissue oximeter have been described previously.\(^{18,\ 19}\) The NIRS probe, which has a photo-
A diode in the centre and a near-infrared LED on each side, was attached to the medial portion of the calf muscle and fixed with a rubber strap to prevent displacement during exercise. All studies were performed using the same auto gain settings on the spectrometer. Subjects rested during the 1 minute gain setting. After exercise begins, oxy-Hb saturation is depleted from the stable baseline and then reaches a plateau indicating the balance of oxygen demand and supply in muscle tissue. After exercise is completed, the oxy-Hb saturation increases until it reaches a plateau. Data were sampled every 0.5 seconds. As in previous studies discussing the recovery rate of oxy-Hb,14, 20) we evaluated the recovery kinetics by means of time constants.

The oxy-Hb recovery was fitted to a single exponential curve and the time constant for oxy-Hb recovery ($\tau_{\text{oxy-Hb}}$) was calculated as the $\tau_{\text{PCr}}$. Measurements by $^{31}$P-MRS and NIRS were obtained with the same protocol.

Peak oxygen uptake and the ventilatory anaerobic threshold were measured by a breath by breath apparatus with an upright bicycle ergometer.

**Muscle metabolism and tissue oxygenation in CHF**

Representative data of $^{31}$P-MRS and NIRS after exercise in a patient and a normal subject are shown in Fig. 2. The rate of PCr recovery, evaluated as the $\tau_{\text{PCr}}$, was significantly greater in patients with CHF than in normal subjects (Fig. 3), indicating that PCr recovery was impaired in patients with CHF. In Fig. 3, normal subjects showed a significantly smaller value of the $\tau_{\text{PCr}}$ and greater anaerobic threshold, while patients with CHF showed greater $\tau_{\text{PCr}}$ and lower anaerobic threshold (left panel). The $\tau_{\text{PCr}}$ was significantly correlated with anaerobic threshold when both groups were included ($R=0.54$, $p<0.01$). The rate of oxy-Hb recovery evaluated as the $\tau_{\text{oxy-Hb}}$ was significantly greater in patients with CHF than in normal subjects (Fig. 3). As well as the $\tau_{\text{PCr}}$, normal subjects showed significantly smaller $\tau_{\text{oxy-Hb}}$ and greater anaerobic threshold, while patients with CHF showed greater $\tau_{\text{oxy-Hb}}$ and lower anaerobic threshold (Fig. 3, right panel). The $\tau_{\text{oxy-Hb}}$ was correlated with the anaerobic threshold when both groups were included ($R=0.70$, $p<0.01$). Comparison between $^{31}$P-MRS and NIRS measurements are shown in Fig. 4. The $\tau_{\text{PCr}}$ and the $\tau_{\text{oxy-Hb}}$ were similar in normal subjects. In patients with CHF, however, the $\tau_{\text{PCr}}$ was significantly greater than the $\tau_{\text{oxy-Hb}}$. Moreover, the difference in the $\tau_{\text{PCr}}$ between the two groups was remarkably greater than the difference in the $\tau_{\text{oxy-Hb}}$. The $\tau_{\text{PCr}}$ in patients with CHF showed a wide variance.

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**Figure 2** Representative spectra showing recovery of phosphocreatine (PCr, filled symbols) and oxygenated hemoglobin (oxy-Hb, solid line) in normal subjects (A1) and patients with CHF (B1). Each dataset is fitted with a single exponential curve (A2 and B2). A dotted line indicates the fitting curve of PCr and a solid line shows that of oxy-Hb. The time constants are as follows: A (normal subject): $\tau_{\text{oxy-Hb}}=28$ s; $\tau_{\text{PCr}}=33$ s. B (patient with chronic heart failure): $\tau_{\text{oxy-Hb}}=53$ s; $\tau_{\text{PCr}}=110$ s. $\tau_{\text{oxy-Hb}}$, time constant for oxy-Hb resaturation; $\tau_{\text{PCr}}$, time constant for PCr resynthesis.
Both the $\tau$ PCr and the $\tau$ oxy-Hb after exercise were greater in patients with CHF than in normal subjects. These time constants correspond to PCr resynthesis and hemoglobin resaturation. Thus, our present findings indicate that PCr resynthesis and hemoglobin resaturation were impaired after exercise in patients with CHF. In normal subjects, the $\tau$ oxy-Hb was similar to the $\tau$ PCr, which was consistent with previous findings. It suggested that the rate of hemoglobin resaturation matches the rate of oxygen utilization after exercise to resynthesise PCr in skeletal muscle in normal subjects. However, the $\tau$ PCr was significantly greater than the $\tau$ oxy-Hb in patients with CHF in the present study, indicating that PCr resynthesis was significantly delayed compared with hemoglobin resaturation. Therefore the rate of hemoglobin resaturation does not appear to be a major determinant of muscle metabolic recovery, evaluated as PCr resynthesis in patients with CHF. A possible reason for the significant delay in PCr resynthesis in muscles is impaired oxygen utilization in muscle mitochondria or impaired oxygen diffusion to mitochondria from capillaries. In other words, muscle metabolic recovery may depend to a greater extent on the capacity of oxygen utilization rather than on hemoglobin resaturation or oxygen delivery in patients with CHF.

**Summary**

Impaired intramuscular energetics and oxygen delivery are two important factors that determine exercise capacity in patients with CHF. Estimating both variables with $^3$P-MRS and NIRS independently, we could acquire detailed information about the mechanism that predominantly determined the exercise capacity in each patient. We could assume that different kinds of treatment may have a differential effect on muscle metabolism or oxygen delivery, for example, exercise training, medication, heart transplantation, and so on. This may help us to plan better treatments to improve their exercise intolerance and enable us to evaluate their effects appropriately.
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組織内エネルギー代謝計測との併用による循環病態解説

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**Key words:** cardiovascular, chronic heart failure, near-infrared spectroscopy, $^{31}$P-magnetic resonance spectroscopy, exercise intolerance

**要 旨:** 循環障害を有する疾患の動的病態を解析するには、中心循環のみならず末梢組織の血流(酸素供給)および筋内代謝を調べる必要がある。後者は、磁気共鳴分光法により測定可能であるが、血流測定に用いられる血管内カテーテル、ブレチスモグラフィおよび超音波ドップライは、必ずしも局所筋血流を評価できるわけではない。われわれは、磁気共鳴分光法と近赤外線分光法を用いた循環器疾患の病態解析を行ってきたので、その有用性を概説する。

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