Role of Skeletal Muscle Metabolism in Exercise Capacity of Patients With Myocardial Infarction Studied by Phosphorus-31 Nuclear Magnetic Resonance

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Patients with congestive heart failure reportedly show a poor correlation between cardiac function and exercise tolerance. Recent studies have demonstrated that skeletal muscle is the main factor that limits exercise tolerance. However, the relationship between high-energy phosphate metabolism in skeletal muscle and exercise tolerance has not been well defined. Exercise capacity was assessed in 35 subjects with myocardial infarction in terms of peak oxygen consumption (peak VO_2) during treadmill exercise with an analysis of expired gases. On the same day, changes in high-energy phosphates in finger flexor muscle during handgrip exercise were measured by magnetic resonance spectrometry. Phosphocreatine (PCr) utilization and the decrease in pH during handgrip exercise were significantly greater in patients with a poor exercise capacity and their time constant of recovery of PCr was prolonged. The ratios of PCr/Pi (Pi: inorganic phosphate) and PCr/HMPA (HMPA: hexamethylphosphoramide) during exercise and the time constant of recovery of PCr were significantly correlated with peak VO_2 . These results suggest that skeletal muscle metabolism governs exercise tolerance. (*Jpn Circ J* 1995; **59**: 315–322)

PERIPHERAL factors are important in determining the exercise capacity of patients with congestive heart failure. Patients with heart failure exhibit muscle atrophy, depressed oxidative capacity of muscle, and altered metabolic responses to exercise, as assessed by ^{31}P -nuclear magnetic resonance (NMR) spectrometry;^{1–7} all of which may contribute to exertional fatigue. However, there has been no examination of the relationship between the extent of metabolic changes in skeletal muscle and exercise capacity in normal subjects and in patients

with or without heart failure.

In this study, we investigated the relationship between changes in high-energy phosphate metabolism in skeletal muscle and exercise capacity in patients with old myocardial infarction. In addition, we sought to determine whether these metabolic changes could serve as indicators of exercise tolerance for use in evaluating therapeutic interventions, such as cardiac rehabilitation.

METHODS

Subjects

We studied 35 Japanese patients with old myocardial infarction more than 1 month after onset. The 32 males and 3 females

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Fig. 1. Relationship between peak oxygen consumption and left ventricular ejection fraction (A; $n=35$, $r=0.45$, $p=0.015$) or cardiac index at rest (B; $n=35$, $r=0.22$, $p=0.250$).

ranged in age from 39 to 76 (mean $\pm$ SD, 56 ± 8). All of the patients were categorized as New York Heart Association functional class I or II and participated in a non-supervised home exercise program after their discharge from the hospital. None had angina pectoris, peripheral edema, paroxysmal nocturnal dyspnea, intermittent claudication, pressure difference among extremities, or exercise disability at the time of the study. Thirty-two patients were receiving aspirin, 25 were receiving oral nitrates, and 20 were receiving calcium antagonists; 6 patients were treated with angiotensin converting enzyme inhibitor and 3 with diuretics. All cardiovascular medications were withheld for at least 24 h before exercise testing. Of the 35 patients, 16 had anterior myocardial infarction (MI), 12 had inferior MI and 7 had posterolateral MI. All of the subjects had undergone coronary arteriography and left ventriculography (LVG) 1–3 months prior to the study. Ejection fraction, measured by left ventriculography, ranged from 18 to 78% ($54 \pm 17\%$). All of the subjects gave their informed consent to participate in the study.

Study Protocol

Exercise tolerance was determined by a treadmill exercise test with an analysis of expired gases. After their exercise tolerance was determined, all of the subjects were

transferred to the NMR spectrometry laboratory, where they performed forearm exercise for 10 min, and the ^{31}P -NMR spectrum (MRS) of the flexor digitorum superficialis muscle was obtained.

Measurement of Oxygen Consumption

All of the subjects had undergone at least one previous symptom-limited graded exercise test, and were thus familiar with exercise testing procedures. They each performed an exercise test on a motorized treadmill, using the Weber and Janicki protocol⁸ with continuous 12-lead electrocardiogram and expired gas analysis. Blood pressure was measured with an automated cuff at 1-min intervals.

Peak oxygen consumption (pVO_2) was defined as the highest 15-sec average oxygen consumption measured by the breath-by-breath method with an MMC-4400 (Sensor Medics Co., CA, USA) during the graded treadmill exercise test.

^{31}P -MRS Measurements

Forearm exercise was performed with the subject's forearm placed in the field of a 2.0 T, 25-cm bore superconducting magnet interfaced with a BEM-250/80 spectrometer (Otsuka Electronics, Shiga, Japan). This spectrometer was operated at 34.56 MHz for phosphorus. The forearm was positioned over a 4.0-cm diameter surface coil. A test

Fig. 2. Phosphorus-31 nuclear magnetic resonance spectra obtained during handgrip exercise in a subject with good exercise capacity (peak $\text{VO}_2=36.8$, A) and a subject with poor exercise capacity (peak $\text{VO}_2=16.8$, B). ATP=adenosine triphosphate; Pi=inorganic phosphate; PCr=phosphocreatine; VO_2 =oxygen consumption; HMPA=hexamethylphosphoramide.

tube containing 200 mmol of hexamethylphosphoramide (HMPA) was positioned on the surface coil (on the side opposite the forearm) as a control for phosphorus chemical shift. Data collection was accomplished with 30 radiofrequency pulses applied every 2 sec.

After the forearm had been positioned in the magnet, a 2-min resting MRS was obtained. The subject then performed wrist flexion every 2 sec for 4 min, at a load equal to 10% of maximal grasping force, by elevating a corresponding weight. After 4 min of exercise, the subject was allowed to rest for 4 min. Data were collected throughout the rest, exercise and recovery periods.

Spectral Analysis

Quantification of the high-energy phosphate components in forearm muscle was achieved by peak height analysis with a computer program, FIT (version 1.2, Otsuka Electronics), using data from the MRS. The Fourier-transformed data obtained from the NMR spectrometer were transferred to a personal computer (PC-9801, NEC Co., Tokyo, Japan). Peaks of the phosphocreatine (PCr), inorganic phosphate (Pi), and HMPA signals were measured with the FIT program and the PCr/HMPA and PCr/Pi ratios were obtained. Intracellular pH was measured as the chemical shift of Pi from the resonance signal of PCr. To nullify the variance of data acquisition, HMPA was

TABLE 1 HEMODYNAMIC AND METABOLIC PARAMETERS IN FOUR GROUPS CLASSIFIED BY PEAK OXYGEN CONSUMPTION (VO_2)

	Group 1	Group 2	Group 3	Group 4
peak VO_2 (ml/kg/min)	<15	15–20	20–30	>30
n	8	11	12	4
age	64.6±11.9	54.0±4.1*	56.0±6.5*	50.0±7.7*
sex (male : female)	7 : 1	9 : 2	12 : 0	4 : 0
<i>Hemodynamic parameters</i>				
LVEF (%)	46.0±26.2	55.3±12.0	54.1±10.2	71.8±5.9*
LVEDVI (ml/m ²)	95.0±42.9	79.1±22.8	67.4±24.5	61.0±6.1
CI (l/min/m ²)	2.72±0.45	3.07±0.64	3.34±0.69*	3.17±0.26
<i>MRS parameters</i>				
PCr/HMPA (Ex 1 min)	0.59±0.22	0.63±0.14	0.77±0.26	0.73±0.21
time constant (sec)	124.9±38.1	117.0±33.1	100.7±33.1	94.0±10.5
PCr-Pi shift (ppm)	3.5±0.3	3.6±0.6	4.0±0.3*§	4.2±0.3*§
pH	5.9±0.3	6.1±0.5	6.4±0.3*§	6.7±0.3*§

*: $p \leq 0.05$ vs Group 1§: $p \leq 0.05$ vs Group 2

always positioned at the opposite site of the surface coil and used to calibrate the spectrum.

To assess the recovery of PCr after exercise, the time constant of recovery was calculated by fitting PCr areas to a single exponential curve according to Mancini et al.⁶ This curve can be described by the equation $\text{PCr} = C_1 + C_2 (1 - e^{-kt})$, (PCr=concentration of PCr; C_1 =initial concentration of PCr; C_2 =difference between the final and initial PCr; t =time; k =rate constant). The time constant of recovery was derived from the reciprocal of k and was expressed in seconds.

Measurement of Cardiac Functions

Right-sided cardiac catheterization with a Swan-Ganz catheter and femoral artery cannulation were performed. Thermodilution cardiac index (CI) was measured in triplicate at rest. The coronary angiogram and left ventriculogram were performed via brachial or femoral approaches. Left ventricular ejection fraction and end-diastolic volume index were calculated with the area-length method.

Statistical Analysis

Data are expressed as the mean±SD. Differences were evaluated by using ANOVA or unpaired Student's t-tests. The relationships between variables were ex-

amined by linear regression analysis. A p value <0.05 was considered to indicate a statistically significant difference.

RESULTS

Exercise Capacity and Left Ventricular Function

Peak oxygen consumption (peak VO_2) ranged from 10.6 to 36.8 (mean, 20.6 ± 7.2) ml/kg/min and left ventricular ejection fraction (LVEF) and CI were $54.9 \pm 16.8\%$ and 3.06 ± 0.63 liters/min/m², respectively. Correlations between hemodynamic variables and peak VO_2 are shown in Fig. 1. LVEF and left ventricular end-diastolic volume index (LVEDVI) correlated roughly with peak VO_2 (LVEDVI data not shown), but CI was not correlated with peak VO_2 .

MR Spectroscopy

Fig. 2A shows the MRS of a subject with good exercise capacity before, during, and after handgrip exercise. During exercise, PCr slowly decreased and Pi slowly increased. In contrast, a patient with poor exercise capacity showed an early decrease in PCr, a marked reduction of the PCr-Pi chemical shift, and a slower recovery of PCr after exercise (Fig. 2B).

To clarify the differences in MRS changes among patients with various exercise capacities, subjects were classified into four groups

Fig.3. Mean PCr/HMPA changes in four different exercise-capacity groups during handgrip exercise and recovery. Each point represents a mean value for each group (Group 1, $n=8$, $\bullet$; Group 2, $n=11$, $\blacktriangle$; Group 3, $n=12$, $\triangle$; Group 4, $n=4$, $\circ$). PCr=phosphocreatine; HMPA=hexamethylphosphoramide.

Fig.5. Relationship between the time constant of PCr recovery and peak oxygen consumption ($n=35$, $r=0.37$, $p=0.030$). PCr=phosphocreatine; VO_2 =oxygen consumption.

Fig.4. Relationship between PCr/HMPA level after 1 min of exercise and peak oxygen consumption ($n=35$, $r=0.35$, $p=0.041$). PCr=phosphocreatine; HMPA=hexamethylphosphoramide; VO_2 =oxygen consumption.

Fig.6. Relationship between PCr-Pi shift (pH) and peak oxygen consumption ($n=35$, $r=0.54$, $p=0.001$). PCr=phosphocreatine; Pi=inorganic phosphate.

based on their peak VO_2 : Group 1: 8 subjects with a peak VO_2 of less than 15 ml/kg/min; Group 2: 11 subjects, between 15 and 20 ml/kg/min; Group 3: 12 subjects, between 20 and 30 ml/kg/min, and Group 4: 4 subjects, more than 30 ml/kg/min. Their hemodynamic and MRS parameters are summarized in Table I. LVEF in Group 4 was significantly greater than that in Group 1. LVEDVI did not differ among the four groups. CI in Group 3 was significantly

greater than that in Group 1.

Fig. 3 shows the mean changes in PCr/HMPA in each group during and after exercise. The subjects in Group 1 had the most rapid decrease and the slowest recovery of PCr/HMPA. There were no differences in PCr, ATP, or Pi levels or in PCr-Pi shift (pH) at rest among the four groups.

The PCr level decreased more rapidly during handgrip exercise in subjects with a severe impairment of exercise capacity

(Group 1). The PCr/HMPA level after 1 min of handgrip exercise was used as an index of high-energy phosphate reserve. There were no significant differences in PCr/HMPA among the four groups, but there was a significant correlation between PCr/HMPA level and peak VO_2 (Fig. 4).

In the recovery phase after exercise, recovery of PCr was more rapid in subjects with good exercise tolerance. There were no significant differences in the time constant among the four groups. The time constant of PCr recovery was significantly correlated with peak VO_2 (Fig. 5).

The minimal PCr-Pi shift, which is indicative of intracellular pH, showed the closest correlation with exercise tolerance ($r=0.54$, $p=0.0008$, Fig. 6). Groups 3 and 4 had significantly large PCr-Pi shifts, compared to Groups 1 and 2. Subjects with poor exercise tolerance showed signs of severe acidosis in skeletal muscle.

DISCUSSION

We found that subjects with poor exercise capacity showed a rapid decrease in PCr during handgrip exercise and a slow recovery from that exercise. Subjects with poor exercise capacity showed signs of severe acidosis in skeletal muscle during exercise. The speed of turnover of high-energy phosphate during exercise correlated well with exercise tolerance. Due to the small number of subjects in each group, it was not possible to demonstrate any significant differences in PCr/HMPA or in time constant of PCr recovery between any of the groups. These results indicate that these parameters of high-energy phosphate metabolism can be used as an index of exercise tolerance.

For the possible clinical use of PCr/HMPA or the time constant of PCr recovery, it is important to know whether skeletal muscle metabolism is an independent limiting factor of exercise. Many reports have suggested that skeletal muscle energetics is the primary limiting factor in exercise capacity. Massie et al suggested that skeletal muscle abnormalities, due to either muscle atrophy or impaired blood flow, might explain the marked heterogeneity of symptom status and exercise capacity in patients with similar degrees of cardiac function.⁹ They assessed skeletal

muscle metabolism by ^{31}P -MRS and found that delayed PCr resynthesis and greater acidification of muscle were consistent with an impairment of substrate availability and altered biochemistry.¹⁰ Our results are consistent with the notion that skeletal muscle energy metabolism is the primary factor that limits exercise capacity.

In heart failure in rats, excessive depletion of PCr, increased glycolytic metabolism, depressed capacity of aerobic metabolism, and impaired activity of citrate synthetase in skeletal muscle have been demonstrated.¹¹ Those results coincide with the results of our present MRS study. Histologically, skeletal muscle in patients with congestive heart failure shows significant decreases in mitochondrial density and cytochrome oxidase activity in mitochondria, which result in a depressed oxidative capacity of working muscle.⁷

Shephard et al reported that muscle mass is a determinant of exercise capacity in normal subjects.¹² Mancini et al reported that skeletal muscle atrophy also limits exercise tolerance. Patients with heart failure exhibit skeletal muscle fiber atrophy that contributes to abnormalities in skeletal muscle metabolism.⁶

Another possible explanation for this depressed muscle metabolism is insufficient blood flow to skeletal muscle. Exertional fatigue limits exercise capacity in patients with heart failure. This fatigue is associated with abnormally elevated blood lactate levels. It has been speculated that reductions in blood flow and delivery of oxygen to skeletal muscle may be important in this elevation of lactate.¹³⁻¹⁵ In our study, skeletal muscle metabolism was correlated with left ventricular ejection fraction, but not with cardiac index. Leithe et al showed that resting forearm blood flow is directly related to the cardiac index at rest.¹⁶ Although we did not measure forearm blood flow during exercise, the lack of a relationship between muscle metabolism and the cardiac index and the findings of Leithe et al might indicate that abnormalities in muscle metabolism are not primarily due to muscle blood flow. Many studies have demonstrated that metabolic alterations in forearm muscle are not caused by a decrease in blood flow in the muscle.^{3,17}

Skeletal muscle metabolism is reportedly independent of exercise tolerance in patients with symptomatic mitral stenosis following percutaneous transvenous mitral commissurotomy (PTMC)^{18,19} The symptoms and hemodynamic conditions in patients with mitral stenosis improved rapidly after PTMC. In contrast, exercise capacity and leg muscle mass were dissociated over the course of recovery. Peak oxygen consumption increased gradually up until 6 mo after PTMC. These findings indicate that skeletal muscle metabolism governs exercise capacity.

The results shown in Table I suggest that high-energy phosphate metabolism may be influenced by the patient's age. However, due to the small number of subjects in each group, we could not conclude that skeletal muscle metabolism changes with age. Such a relationship has not been demonstrated in any other studies.

The present study has two important limitations. First, we examined and compared the high-energy phosphate metabolism in the forearm muscle during and after handgrip exercise with using oxygen consumption during treadmill exercise. Treadmill exercise primarily uses the muscles of the lower extremities. Since we used an MR spectrometer with a small bore size (25 cm), we could not measure directly high-energy phosphate metabolism in the working muscle that is actually used in treadmill exercise. It is unclear if the metabolic conditions in leg muscle are the same as those in forearm muscle. McCully et al reported metabolic differences between trained and untrained human muscle²⁰ based on comparisons of forearm muscle metabolism in highly trained rowers who had competed in the Olympics with that in untrained controls. However, their study did not indicate any difference between the trained forearm muscle and other untrained muscles in the same subject. No reports have shown a difference in metabolism between the muscles of the upper and lower extremities in the same subject. The second limitation of our study is related to the signal quality of the MR spectrometer. Subjects with poor exercise capacity showed a markedly reduced and atrophic forearm muscle mass. MR spectrometric evaluation involves positioning the forearm over a 4-cm

surface coil, which allows data collection within an area of approximately 12.6 cm² over the coil. If the subject's forearm is slender and atrophic, both the muscle mass and the total amount of PCr in the area of data collection are small and the MRS signals obtained are weak and noisy. During handgrip exercise, the amount of PCr is decreased and accurate positioning of the muscle over the surface coil becomes difficult. However, we are trying to improve the signal-to-noise ratio by modifying the spectrometer. This problem probably did not influence the overall results, since we always used the signal ratio in reference to a standard substance, HMPA.

In conclusion, wrist flexor muscle metabolism was significantly correlated with exercise capacity in cardiac patients, suggesting that muscle metabolism assessed by MRS is an independent limiting factor of exercise capacity, and that this oxidative capacity may be used as an index of exercise tolerance.

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