Over the past 2 decades, many studies have shown that a number of factors contribute to the exercise intolerance of patients with chronic heart failure (CHF). Factors suggested include skeletal muscle underperfusion caused by central hemodynamic alterations such as diminished cardiac output, as well as left ventricular (LV) and right ventricular dysfunction, and abnormalities in peripheral mechanisms such as skeletal muscle vasodilation, skeletal muscle histology, metabolism, and muscle oxygen uptake. Blunt vasodilation during exercise is also reported in apparently healthy, coronary high-risk subjects. Among these factors, cardiac output and skeletal muscle vasodilation are the important determinants of skeletal muscle blood flow during exercise. Particularly in patients with CHF, impaired skeletal muscle vasodilation plays a key role in their exercise intolerance by reducing muscle blood flow. Therefore, the evaluation of muscle vasodilation during exercise is an important issue; however, until recently, no non-invasive method by which to assess muscle vasodilation during dynamic exercise had been available.

Near-infrared spectroscopy (NIRS) is a non-invasive method suitable for the measurement of tissue oxygenated and deoxygenated hemoglobin (Hb) and myoglobin (Mb) content. This technique utilizes the principal that the absorbance of light by oxygenated Hb and Mb (oxy-Hb + Mb) and deoxygenated Hb and Mb (deoxy-Hb + Mb) differs at different near-infrared wavelengths. By using NIRS, oxy-Hb + Mb, deoxy-Hb + Mb and total-Hb + Mb can be measured. As the amount of Mb does not change over a short period of time, the changes in total-Hb + Mb represent the changes in total Hb, that is, the blood volume, and because blood exists in blood vessels, we hypothesized that changes in working muscle total-Hb + Mb indicate muscle vasodilation, that is, the conductance of the vessel.

The present study was designed to investigate whether the changes in total-Hb + Mb from NIRS reflected muscle vasodilation during sub-maximal dynamic exercise in patients with and without cardiac dysfunction, indicating that NIRS provides a valuable method to assess the working muscle vasodilation.

**Methods**

**Subjects**

Ten male patients without LV systolic dysfunction (normal LV systolic function group) and 6 male patients with cardiac dysfunction (cardiac dysfunction group) participated in this study. The clinical characteristics of the participants are shown in Table 1.

The mean age of the normal LV systolic function group was 64±10 years. Clinical diagnoses were angina pectoris in 6 patients, arrhythmia (paroxysmal atrial fibrillation or
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premature ventricular contraction) in 3, and hypertension in 1. All patients with angina pectoris had received successful percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG), and no residual myocardial ischemia was documented. All patients with arrhythmia were under successful medical control, and no arrhythmia was detected during exercise. A case of hypertension was normotensive under medication. The LV systolic function in this group had been maintained satisfactory; all subjects had a LV ejection fraction (LVEF) of \( \geq 55\% \).

The mean age of the cardiac dysfunction group was 56±16 years. Four patients had previous myocardial infarction, and in 3 of them, coronary stenoses had been revasculized by either PCI or CABG. One patient had dilated cardiomyopathy, and the other had mitral stenosis. Their LVEF (assessed with left ventriculography) was 41±9% (range: 26–50%).

The present study was performed without discontinuance of the medication. The study was approved by the Ethical Committee of our institute (Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan), and written informed consent was obtained from all patients prior to participation in the study.

**Exercise Testing and Expired Gas Analysis**

All subjects underwent symptom-limited incremental exercise testing using an electromechanically braked upright bicycle ergometer (CORIVAL400; Lode, Holland). The exercise test began at a workload of 20 W, which increased by 10 W every minute. Just before the start of the exercise, we helped to turn the cycling pedals of the ergometer at a speed of 60 rotations per minute (rpm) using our hands before placing the patient’s feet on the pedals, so that patients were able to start pedaling without additional loading. Subjects were instructed to maintain a speed of 60 rpm. Exercise testing was terminated when the target heart rate (85% of maximal age-predicted heart rate: (220–age) beats/min) was achieved or symptoms such as severe exhaustion, leg fatigue or dyspnea developed. Before and during exercise, the heart rate was monitored by a 12-lead ECG, and brachial arterial blood pressure was measured every minute in the left arm by using a mercury sphygmomanometer.

Fig 1 The near-infrared spectroscopy (NIRS) probe of the OM-200. The probe has 2 detectors (A, B) and 1 light source (C). The distances between the light source and detectors were 25 mm (BC) and 40 mm (AC).

Inspired and expired gases were measured at rest and throughout exercise by using a hot wire flowmeter and a breath-by-breath measuring technique (Minato-280AE; Japan), and the following variables were measured; minute ventilation (VE) (L/min), oxygen uptake (\( \dot{V}O_2 \)) (L/min), carbon dioxide production (\( \dot{V}CO_2 \)) (L/min). These values were used to estimate the ventilatory threshold (VT) and peak \( \dot{V}O_2 \).

**Leg Blood Flow (LBF) and Vessel Conductance Measurements**

LBF was measured by using a thermodilution technique. In all subjects, 1 h before exercise testing, a 5-Fr thermodilution catheter was inserted via the right femoral vein. The distal thermister tip was positioned approximately 10 cm above the inguinal ligament in the right common iliac vein. The catheter was interfaced with a thermodilu-

### Table 1 Clinical Characteristics of the Subjects

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age/sex</th>
<th>Diagnosis</th>
<th>Medications</th>
<th>LVEF (%)</th>
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<tbody>
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<td>PVC</td>
<td>AA, CCA</td>
<td>70</td>
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<td>BAA, CCA, ISDN</td>
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<td>Cardiac dysfunction group</td>
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<td>ACEI, ISMN</td>
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</tbody>
</table>

LVEF: left ventricular ejection fraction; LV: left ventricular; PVC: premature ventricular contraction; AA: antiarrhythmic agent; CCA: calcium-channel antagonist; AP: angina pectoris; CABG: coronary artery bypass grafting; PCI: percutaneous coronary intervention; BAA: \( \beta \)-adrenergic antagonist; ISDN: isosorbide dinitrate; PAF: paroxysmal atrial fibrillation; HT: hypertension; ACEI: angiotensin-converting enzyme inhibitor; AAA: \( \alpha \)-adrenergic antagonist; MS: mitral stenosis; MI: previous myocardial infarction; ISMN: isosorbide mononitrate; DCM: dilated cardiomyopathy.
NIRS Device and Measurement

The NIRS device used was an OM-200 (Shimadzu Corp, Japan), which utilizes 4-wavelength (690, 780, 805, 830 nm) NIR lights from 4 laser diodes. The NIRS probe of the OM-200 has 2 detectors to 1 light source (Fig 1), allowing the absolute values of oxy-Hb + Mb, deoxy-Hb + Mb and total-Hb + Mb to be calculated by using the spatially resolved method based on diffusion theory. The distances between the light source and detectors were 25 and 40 mm, respectively. The NIRS probe was placed on the right thigh skin over the vastus lateralis muscle, 20 cm above the knee joint, and total-Hb + Mb was monitored before and during exercise at every second. To extract the value of total-Hb + Mb for the comparison with leg vessel conductance, we used the average value over the 10 s.

Absolute values of oxy-Hb + Mb, deoxy-Hb + Mb and total-Hb + Mb, using 780 nm, 805 nm, and 830 nm NIR lights are calculated as follows:24

$$\text{oxy-Hb} + \text{Mb} = a_{780} + 2.966a_{805} + 2.966a_{830} - (a) - 1$$

$$\text{deoxy-Hb} + \text{Mb} = a_{780} + 1.364a_{805} - 5.190a_{830} - (a) - 2$$

$$\text{total-Hb} + \text{Mb} = \text{oxy-Hb} + \text{Mb} + \text{deoxy-Hb} + \text{Mb} - (a) - 3$$

where $a$ is the absorption coefficient for each wavelength, and it can be calculated by using the spatially resolved method based on diffusion theory. In this method, the effective attenuation coefficient, $\alpha_{\text{eff}}$, is the most important parameter, and it can be calculated from the equation:

$$\alpha_{\text{eff}} = 1.54 \times (\text{abs}40 \text{mm} - \text{abs}25 \text{mm}) - 0.0627$$

As

$$\alpha_{\text{eff}} = (3\alpha_a \times \eta')^{1/2}$$

where $\eta'$ is the reduced scattering coefficient and is considered to be approximately constant. We can know $\alpha_a$ as follows:

$$\alpha_a = \alpha_{\text{eff}} / 3 \eta'$$

By using $\alpha_a$ for each wavelength, the absolute value of oxy-Hb + Mb, deoxy-Hb + Mb and total-Hb + Mb by using the equations (a)-1, -2, and -3 can be determined.

Reproducibility of the Measurements of NIRS and Leg Vessel Conductance

The reproducibility of the measurements of NIRS and leg vessel conductance was tested in 6 subjects (5 from the normal LV systolic function group, and 1 from the cardiac dysfunction group). These subjects performed repeated exercise tests after a 1.5–2.0-h rest period.

Statistical Analysis

The data from each subject is expressed as absolute values. The results are presented as mean ± SD. Comparisons between 2 groups were analyzed by using the non-paired Student’s t-test. Correlations between total-Hb + Mb from NIRS and skeletal vessel conductance by the thermodilution method during exercise were analyzed using linear regression. A p-value < 0.05 was considered to be statistically significant. Bland and Altman plots were also utilized to test the reproducibility of the measurements of NIRS and leg vessel conductance. Desirable agreement was defined as the mean difference between 2 measurements close to zero with 95% of individual differences being within 2 SD.
Normal LV systolic function group

Case 1

Case 2

Case 3

Case 4

Case 5

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Normal LV systolic function group

Case 6

Case 7

Case 8

Case 9

Case 10

\[ y = 203.69x + 46.70 \quad r = 0.83, p = 0.0008 \]

\[ y = 217.91x + 43.87 \quad r = 0.889, p < 0.0001 \]

\[ y = 534.58x + 63.56 \quad r = 0.820, p = 0.0001 \]

\[ y = 116.31x + 42.51 \quad r = 0.884, p = 0.00018 \]

\[ y = 248.17x + 57.79 \quad r = 0.792, p = 0.0012 \]
Cardiac dysfunction group

Case 1

Case 2

Case 3

Case 4
Results

Hemodynamic Responses to Dynamic Exercise
The peak exercise heart rate was 122.1±18.4 beats/min, and was slightly lower than their target heart rate (134.7±10.9 beats/min). The reasons for the termination of the exercise were achievement of target heart rate in 9 subjects, and leg fatigue in the other 7 subjects; the distribution of these reasons showed no significant difference between the 2 groups. Hemodynamic responses to the exercise testing are shown in Table 2 and Fig 2. There was no significant difference in heart rate and blood pressure responses between the 2 groups. The LBF and leg vessel conductance at the peak exercise period of the patients in the cardiac dysfunction group were significantly lower than those of the patients in the normal LV systolic function group. Total-Hb+Mb and Leg Vessel Conductance Baseline total-Hb+Mb and peak exercise total-Hb+Mb were significantly lower in the cardiac dysfunction group than in the normal LV systolic function group (Table 2).

The changes in total-Hb+Mb and leg vessel conductance during exercise in the 2 groups are shown in Fig 3. Immediately after starting exercise, the total-Hb+Mb showed an initial rapid decrease, which may reflect the initial increase in venous return caused by the pumping of muscles at the start of exercise. This initial rapid decrease of the total-Hb+Mb appeared within 15–20 s after the start of exercise in all subjects (see examples in case 1 in the...
normal LV systolic function group or case 1 in the cardiac dysfunction group in Fig 3). We set the total-Hb + Mb at the initial fall as the baseline level of total-Hb + Mb. In most cases, regardless of cardiac function, the changes in total-Hb + Mb and those of leg vessel conductance showed striking similarities. In addition, the levels of total-Hb + Mb and the levels of leg vessel conductance every 30–60 s during exercise in each case showed a strong positive correlation, except for 1 case (case 6 in the cardiac dysfunction group) (normal LV systolic function group: cases 1–10; r=0.792 to 0.974, p=0.0018 to <0.0001; cardiac dysfunction group: cases 1–5; r=0.805 to 0.963, p=0.0016 to <0.0001; case 6: r=0.509, p=0.1376). However, at the later phase of exercise, especially over 60–80% of peak oxygen uptake, discordance between total-Hb + Mb and vessel conductance was recognized in 7 cases (cases 1, 7, 9, and 10 in the normal LV systolic function group and cases 1, 2, 6 in the cardiac dysfunction group).

Interpersonal comparison revealed that the increases in total-Hb + Mb from baseline to peak exercise (delta total-Hb + Mb) also tended to correlate with peak VO2, and was significantly correlated with VT, which indicated the importance of working muscle vasodilation in determining the exercise capacity.

Reproducibility of the Measurements of NIRS and Leg Vessel Conductance

The changes in total-Hb + Mb and leg vessel conductance in the repeated exercise testings showed close similarity in each subject (changes in total-Hb + Mb and leg vessel conductance in the first and second testing are shown in Fig 6), and total-Hb + Mb every 30–60 s during exercise strongly correlated with those of leg vessel conductance in both testing periods (first testing: r=0.831 to 0.974, p=0.0018 to <0.0001; second testing: r=0.787 to 0.975, p=0.0091 to <0.0001). Furthermore, values of total-Hb + Mb, and leg vessel conductance acquired every minute during exercise showed a close correlation between the 2 testings (leg vessel conductance: r=0.893, p<0.0001; total-Hb + Mb: r=0.990, p<0.0001; Fig 7). Bland and Altman plots showed that the mean differences between the 2 testing periods were −0.003 (L·min⁻¹·mmHg⁻¹) for leg vessel conductance, and 0.016 (AU) for total-Hb + Mb, with 95% of individual differences within ±2 SD.

Discussion

Blood flow to skeletal muscle is usually 5–10 ml/100 ml limb volume/min at rest, and can increase up to 150–500 ml/
A (Normal LV systolic function group case 1)

B (Normal LV systolic function group case 3)

C (Normal LV systolic function group case 5)

D (Normal LV systolic function group case 6)
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100 ml limb volume/min at peak exercise.\(^{25,26}\) Thus, muscle blood flow can increase 50–100-fold above baseline levels. An increase in blood flow to active skeletal muscle during exercise is primarily achieved by: (1) elevated perfusion pressure (arterial minus venous pressure) with increased cardiac output through increased cardiac activity and venous return; and (2) a decrease in vascular resistance in working skeletal muscles and increase in vascular resistance in inactive organs/tissue such as viscera and skin.\(^{21,27}\) As the increase in arterial blood pressure is limited (Table 2, Fig 2; mean arterial blood pressure at peak exercise is at most 1.5 times as high as at rest), and the venous pressure is normally maintained within small limits, the increase in blood flow is accomplished in large part by the decrease in vascular resistance in working skeletal muscle,\(^{21,27,28}\) especially in CHF, because the increase in cardiac output in those cases is limited. Several previous studies have suggested that an inadequate increase in muscle blood flow resulting from impaired vasodilation contributes to exercise intolerance in patients with CHF.\(^{5–7}\) However, the mechanisms of impaired vasodilation and its clinical significance in exercise intolerance in CHF are not known. A simple and accurate method by which to evaluate active muscle vasodilation and blood perfusion during exercise would allow in depth analysis of the causes of exercise intolerance in patients with CHF.

Relationship Between Total-Hb + Mb and Vessel Conductance During Dynamic Exercise, and Clinical Significance for Use of NIRS Measurement

We found that total-Hb + Mb detected by NIRS very closely paralleled working muscle vessel conductance during dynamic exercise, and that the changes in total-Hb + Mb from the baseline level correlated well with the increases in muscle vessel conductance in most cases regardless of cardiac dysfunction. These observations indicate that NIRS allows evaluation of vasodilation non-invasively during large muscle dynamic exercise, which is impossible with other devices such as plethysmographies, the thermodilution method, and Doppler ultrasonography.\(^{29,30}\) Moreover, our data showed that working muscle vasodilation expressed by the increase in vessel conductance or by the increase in total-Hb + Mb is an important determinant of exercise tolerance, indicating that NIRS provides useful information on the causes of exercise intolerance in each subject.

A few studies documented that total-Hb + Mb detected by NIRS, in combination with the venous occlusion method, provides a relative value that significantly correlates with forearm blood flow assessed by using strain-gauge plethysmography at rest and after hand exercise.\(^{31,32}\) Our findings are important in that NIRS can evaluate muscle vasodilation during large muscle dynamic exercise without the requirement for a supplementary method such as venous occlusion.
Invalidity of Total-Hb + Mb Analysis on the Evaluation to Working Muscle Vasodilation

Total-Hb + Mb detected by NIRS reflects the amount of Hb or blood volume in small vessels, including arterioles, capillaries, and venules. The blood in the venules differs remarkably in volume according to the posture of the examinee at rest. However, during dynamic and rhythmic exercise, effective muscle contraction continuously pumps out venous blood to the central circulation, and thus the blood volume in the venules is thought to stay relatively constant and so the total-Hb + Mb during exercise is expected to reflect tissue vasodilation. Accordingly, in analyzing the total-Hb + Mb level, we set the baseline point as the nadir of the initial rapid decrease in total-Hb + Mb observed immediately after starting exercise, because this point might be due to increased venous return caused by muscle contraction. As was stated above, total-Hb + Mb correlated with vascular conductance very well (mean correlation coefficient: 0.865) in the present study. Moreover, the muscle content of total-Hb + Mb was significantly decreased in the cardiac dysfunction group compared to the normal LV systolic function group at baseline as well as at peak exercise level. This observation might reflect the decreased baseline and peak exercise blood flow and leg vessel conductance in these patients.

Validity of Vessel Conductance Measurement on the Evaluation to Working Muscle Vasodilation

Vascular conductance during exercise is determined primarily by the diameters of resistance arteries and/or arterioles, which are controlled by the contraction and/or relaxation of vascular smooth muscle through the myogenic vascular control, metabolic vascular control and endothelium-mediated vascular control; the opening of capillaries also is regulated. Thus, we used the vessel conductance as an index of the response of the vascular bed. In the present study, the levels of vessel conductance were 0.00110–0.0064 L·min⁻¹·mmHg⁻¹ at rest and 0.02042–0.07091 L·min⁻¹·mmHg⁻¹ at peak exercise in the normal LV systolic function group, and 0.00094–0.00385 L·min⁻¹·mmHg⁻¹ at rest and 0.01485–0.03182 L·min⁻¹·mmHg⁻¹ at peak exercise in the cardiac dysfunction group. These values correspond fairly with those reported by Lejemtel et al. and Sullivan et al. (the data of leg vessel resistance reported by Sullivan et al. were converted into vessel conductance in order to compare with our data), however, those data from the normal LV systolic function group were slightly lower than those reported in the normal control, and those in the cardiac dysfunction group were slightly higher than those reported in patients with heart failure. These differences may be attributed to the
normal LV systolic function group patients who might have had other cardiac insufficiencies such as diastolic dysfunction, and/or might have been less physically active, and to the fact that the cardiac dysfunction group patients had relatively mild cardiac impairment.

Limitations of NIRS Measurement and Clinical Implication

The results of the present study raise some questions, namely why (1) total-Hb+Mb did not parallel vessel conductance near peak exercise; and (2) total-Hb+Mb did not significantly correlate with those values in the inter-personal comparison. Generally, bicycle exercise is a rhythmic and isotonic exercise, however, an isometric exercise begins as the intensity increases. This may be the key mechanism explaining the discrepancy between the total-Hb+Mb and the leg vessel conductance in the later phase of exercise. In addition, previous studies have documented that slow red muscle fibers play a key role in increasing blood flow during continuous exercise. As the NIRS mechanism explaining the discrepancy between the total-CV and CV begins as the intensity increases. This may be the key mechanism explaining the discrepancy between the total-Hb+Mb and the leg vessel conductance in the later phase of exercise. In addition, previous studies have documented that slow red muscle fibers play a key role in increasing blood flow during continuous exercise.

Assessing Muscle Vasodilation

NIRS is a non-invasive and valuable method with which to assess the working muscle vasodilation during sub-maximal dynamic exercise in patients with and without LV systolic dysfunction, and might be particularly useful in individual follow-up, such as assessing the effects of cardiac rehabilitation and/or medications.

Conclusions

NIRS is a non-invasive and valuable method with which to assess the working muscle vasodilation during sub-maximal dynamic exercise in patients with and without LV systolic dysfunction, and might be particularly useful in individual follow-up, such as assessing the effects of cardiac rehabilitation and/or medications.

Acknowledgments

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