Near-infrared Spectroscopy of Vastus Lateralis Muscle during Incremental Cycling Exercise in patients with Type 2 Diabetes

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ABSTRACT. Purpose: It is clinically important to elucidate the precise mechanism of exercise intolerance in patients with type 2 diabetes (T2DM). The aim of this study was to examine whether there is a difference in the time course change of the oxygenation in the vastus lateralis (VL) muscle during submaximal incremental cycling exercise between patients with T2DM and age-matched healthy subjects.

Methods: Nine elderly men with T2DM and 10 age-matched healthy men (CON) participated in this study. All participants performed an incremental cycling exercise. Total, deoxygenated and oxygenated hemoglobin/myoglobin in the VL muscle were assessed using near-infrared spectroscopy, and cardiorespiratory response was also evaluated during the exercise.

Results: There were no significant differences in the time course changes of deoxygenated hemoglobin/myoglobin between groups (p > 0.05). However, the oxygenated hemoglobin/myoglobin in T2DM was significantly higher than that in CON at an intensity above ventilatory threshold during the incremental cycling exercise (p< 0.05).

Conclusion: This study suggests that patients with T2DM had early limitation of oxygen extraction and lower capacity of oxygenated myoglobin dissociation in the VL muscle. The fact that patients with T2DM showed different oxygen kinetics in a peripheral tissue from healthy subjects may partly explain the potential mechanisms of exercise intolerance in T2DM.

Key words: Exercise, Type 2 diabetes, Near-infrared spectroscopy, Skeletal muscle oxygenation, exercise tolerance

Type 2 diabetes (T2DM) is a significant cause of premature mortality and morbidity related to cardiovascular disease, blindness, kidney and nerve disease, and amputation1-2. Physical activity is highly recommended as a cornerstone of treatment for patients with T2DM along with diet and medication3. However, there are many patients with T2DM who cannot perform the recommended exercise since exercise intolerance is common in these patients4-9. Further, their exercise intolerance is associated with an increased risk of mortality10. Therefore, it is clinically important to improve exercise intolerance in patients with T2DM.

However, the determinants of exercise intolerance in T2DM remain incompletely understood. Generally, the physiological factors that limit exercise tolerance include: 1) the pulmonary diffusing capacity, 2) maximal cardiac output, 3) oxygen carrying capacity of the blood, and 4) skeletal muscle characteristics11. The first three factors can be classified as central factors and the fourth as a peripheral factor. Previous studies examining cardiac output response to exercise showed that maximum cardiac output was reduced in a combined group of patients with type 112 and 2 diabetes13. According to the previous result that the maximum heart rates were not different, the reduced cardiac output during exercise suggested that maximum stroke volume was less in these patients compared with healthy subjects14.
In contrast, another study reported that the stroke volume response to exercise in T2DM was similar to healthy subjects. Taken together, whether cardiac output limits exercise tolerance in T2DM is still controversial.

It is fact that central factors such as cardiac dysfunction cannot fully explain exercise intolerance in T2DM and peripheral factor also contributes to it. Impaired oxygen utilization in skeletal muscles has been considered as one of contributors to exercise intolerance in patients with T2DM, and it depends on oxygen delivery and uptake to working muscles. Regarding the oxygen delivery, previous studies have reported that skeletal muscle hypoxia at the onset of exercise in rodents with diabetes and that patients with T2DM showed slowed microvascular blood flow at the onset of moderate ergometry exercise. These findings suggest that the imbalance between oxygen demand and oxygen supply to the working muscles occurs at the onset of exercise. Although several possible contributors such as reduced capillary density, loss of capillary perfusion, and heterogeneous distribution of blood flow have been noted, it remains unclear which specific factors may limit oxygen delivery. As for oxygen uptake to the muscles, there are much less studies examining whether the capacity of oxygen extraction to the muscles is impaired in patients with T2DM. Mason et al. demonstrated that whether patients with T2DM have impaired skeletal muscle oxygenation during ergometry exercise with constant load of 85 % lactate threshold and found that it is impaired in T2DM independent of blood flow or local recruitment of blood volume. However, this finding was obtained during exercise with constant load just below lactate threshold, and, hence, it is unclear whether skeletal muscle oxygenation is impaired at the other intensities in patients with T2DM. Additionally, despite the incremental exercise test is clinically common to evaluate cardiorespiratory fitness in T2DM, there are no data, to our knowledge, examining the time course change of skeletal muscle oxygenation in a working muscle during incremental exercise.

Therefore, the aim of this study was to examine whether there is a different time course change in skeletal muscle oxygenation during submaximal incremental cycling exercise between patients with T2DM and age-matched healthy subjects. Since patients with T2DM show impaired oxidative metabolism in the muscles such as decreased mitochondrial density, reduced oxidative enzymes and low percentage slow-twitch fibers, it is hypothesized that patients with T2DM have a lower oxygen utilization during the incremental exercise compared with healthy subjects. Additionally, since muscle strength and endurance in thigh muscles are strongly associated with mobility limitation and mortality, examining the oxygen utilization in thigh muscles is crucial to elucidate peripheral factors contributing to exercise intolerance in T2DM.

Methods

Participants

Outpatients with T2DM were recruited from a hospital, and community-dwelling healthy people were asked to participate in this study. Nine elderly men with T2DM and ten age-matched healthy men (Control; CON) participated in this study (Table 1). Age, body mass and body mass index were matched between the groups. All patients with T2DM had been diagnosed with T2DM according to the World Health Organization for classification of diabetes criteria. Patients with T2DM had been treated by diet and/or any oral hypoglycemic agents (sulfonylureas, glinides, thiazolidinediones, biguanides, alpha-glucosidase inhibitors, glucagon-like peptide 1 receptor agonists and/or dipeptidyl peptidase-4 inhibitors). In addition, all patients had normal cardiovascular, retinal, renal, hepatic, gastrointestinal, and neurological functions assessed by clinical

| Table 1. Differences in characteristics between groups |
|-----------------------------------------------|
|                  | T2DM (n=9) | CON (n=10) |
| Age (years)      | 68.8 ± 2.6 | 74.2 ± 1.2 |
| Height (cm)      | 166.0 ± 2.6 | 164.2 ± 1.2 |
| Weight (kg)      | 61.5 ± 2.7 | 61.4 ± 1.3 |
| Body Mass Index  | 22.3 ± 0.8 | 22.8 ± 0.6 |
| Duration of T2DM (years) | 18.2 ± 4.3 | - |
| Blood Glucose (mg/dl) | 120.2 ± 14.2 | 95.7 ± 7.9 |
| HbA1c (%)        | 7.3 ± 0.2 * | 5.4 ± 0.2 |
| Total Cholesterol (mg/dl) | 209.8 ± 16.6 | 179.5 ± 7.7 |
| LDL Cholesterol (mg/dl) | 122.4 ± 18.6 * | 64.4 ± 7.7 |
| Triglyceride (mg/dl) | 100.6 ± 13.1 | 108.7 ± 18.1 |
| Ankle Brachial Pressure Index | 1.1 ± 0.03 | - |
| Subcutaneous tissue thickness (cm) | 0.36 ± 0.06 | 0.41 ± 0.26 |

T2DM, Type 2 Diabetes Mellitus; CON, Control; LDL, Low-Density Lipoprotein; Values are means ± SE. *; p < 0.05 vs CON
because it can affect the NIRS values (Table 1). The NIRS taken to determine the thickness of the subcutaneous tissue sonographic image (SSD-900, ALOKA, Tokyo, Japan) was probe at the center of a prove location, a longitudinal ultra- and inferior lateral edge of the patella. Before placing the probe at midpoint of the line between the head of great trochanter dominant muscle during cyclin g exercise, and was located on the right vastus lateralis (skeletal muscle oxygenation. The NIRS probe was placed technique that offers functional insight into changes in NIRS (Heo-100, Omron, Kyoto, Japan), which has increas- 2) slope and ventilatory equivalencies for oxygen and car- diac output, and aging22,30. NIRS is a noninvasive technique that offers functional insight into changes in skeletal muscle oxygenation. The NIRS probe was placed on the right vastus lateralis (VL) muscle, which is the dominant muscle during cycling exercise, and was located at midpoint of the line between the head of great trochanter and inferior lateral edge of the patella. Before placing the probe at the center of a prove location, a longitudinal ultrasonographic image (SSD-900, ALOKA, Tokyo, Japan) was taken to determine the thickness of the subcutaneous tissue because it can affect the NIRS values (Table 1). The NIRS was then calibrated to obtain a zero value while subjects rested on the cycle ergometer with the right leg relaxed at the bottom of the pedal. The NIRS detects total, deoxygen- ated and oxygenated hemoglobin/myoglobin in blood and muscle at a given time and location, thereby providing an indirect level of oxygen utilization during exercise. A pair of two-wavelength light-emitting diodes, with wavelengths of 760 nm and 850 nm, was used as the light source as re- ported in a previous study31. The total, deoxygenated and oxygenated hemoglobin/myoglobin levels were respect- ively averaged over 30-s intervals for the duration of the testing protocol period.

Experimental Protocol
A nurse sampled overnight fasting blood to determine the concentration of glycated hemoglobin (HbA1c), total cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride levels before the testing protocol. All participants performed a submaximal incremental exercise test, using a cycle ergometer (Aerobike 75XL-II, Combi, Tokyo, Japan), 1 to 3 hours after a lunch. The experimental proto- col consisted of 2 min of rest, 4 min of 10 W loaded cy- cling (warming up period), followed by the incremental ramp exercise (10 W/min) as a previous study24. Once heart rate attained 70 % heart rate reserve (HRR), a 6 min recover- ery period at the intensity of 10 W started (cooling down period). The HRR was calculated as follows: HRR = predicted maximum heart rate (220 – age) – heart rate at rest30. Before the test, the seat was set so that range of motion of the participants’ knee was about 5 degree at the bottom of pedal, and handlebar height of the cycling ergometer was adjusted in order that they could perform the exercise with- out discomfort. The participants were asked to maintain a peda- frequency of 50 rpm with the aid of a visual pedal rate indicator during the test. During the experimental peri- od, near-infrared spectroscopy (NIRS), respiratory gas ex- change and electrocardiogram (ECG) were continuously monitored and recorded. The Ethical Committee for Kyoto Teishin Hospital (#22-4) approved all procedures.

Near-infrared Spectroscopy
Skeletal muscle oxygenation was evaluated using a NIRS (Heo-100, Omron, Kyoto, Japan), which has increas- ingly been used to evaluate the real-time levels of oxygenated hemoglobin/myoglobin in the small vessels and skele- tal muscle22. Studies have demonstrated NIRS to be highly sensitive to changes of skeletal muscle oxygenation due to exercise, hypoxemia, and aging22,30. NIRS is a noninvasive technique that offers functional insight into changes in skeletal muscle oxygenation. The NIRS probe was placed on the right vastus lateralis (VL) muscle, which is the dominant muscle during cycling exercise, and was located at midpoint of the line between the head of great trochanter and inferior lateral edge of the patella. Before placing the probe at the center of a prove location, a longitudinal ultrasonographic image (SSD-900, ALOKA, Tokyo, Japan) was taken to determine the thickness of the subcutaneous tissue because it can affect the NIRS values (Table 1). The NIRS...
HbA₁c was stabilized with EDTA-2K and NaF, and with NaF, respectively. After the blood collection, it was immediately centrifuged at 4 °C and the serum was frozen and stored at −20 °C until assay. Plasma glucose and HbA₁c were measured with an automatic analyzer (JCA-BM9030, Japan Electron Optics Laboratory, Tokyo, Japan), and serum cholesterol and triglyceride were measured with another automatic analyzer (AU 5400, Beckman Coulter, Brea, CA, USA). All procedures for blood analyses were performed by medical technologists.

**Statistical Analyses**

Percentage HRR (%HRR) was calculated as follows: \( \text{%HRR} = \frac{\text{HR} - \text{resting heart rate}}{\text{HRR}} \times 100 \). The NIRS-measured data were expressed at 20, 45 and 70 %HRR. Before the analysis, the normal distribution of the data was confirmed using Shapiro-Wilk test. The parametric analysis was used for normally distributed data and the non-parametric analysis was used for non-normally distributed data. Characteristic parameters, VT and \( \text{VO}_2 \text{peak} \) were compared between groups using independent Student’s t-test. Also, Friedman test was used for the analyses of total, deoxygenated and oxygenated hemoglobin/myoglobin. All data were provided as mean and standard error (SE). The level of statistical significance was set at \( p < 0.05 \). Statistical analyses were performed using SPSS (SPSS 25.0, IBM, Tokyo, Japan).

**Results**

Table 2 shows that percentage HRR, load and \( \text{VO}_2 \) at VT in patients with T2DM were significantly lower than those values in CON. However, there were no significant differences in the load and \( \text{VO}_2 \) at 70% HRR between T2DM and CON.

The time course changes of total, deoxygenated and oxygenated hemoglobin/myoglobin in each group are represented in Fig. 1. The total hemoglobin/myoglobin significantly increased in both groups as the exercise intensity increased (T2DM: 20% HRR −0.0053 ± 0.0073 vs 70% HRR 0.016 ± 0.011; \( p < 0.001 \), CON: 20% HRR −0.029 ± 0.0053 vs 70% HRR 0.016 ± 0.011; \( p = 0.029 \)). Although total hemoglobin/myoglobin in T2DM was significantly higher than that in CON at 20% HRR (T2DM vs CON: −
0.0053 ± 0.0073 vs −0.029 ± 0.0053; p = 0.018), there were no significant differences between groups at 45 and 70 % HRR (both p > 0.05). Whereas the deoxygenated hemoglobin/myoglobin at 70% HRR was significantly higher than that at 20% HRR in both groups (T2DM: 20% HRR −0.025 ± 0.013 vs 70% HRR 0.049 ± 0.011; p < 0.001, CON: 20% HRR −0.013 ± 0.013 vs 70% HRR 0.039 ± 0.019; p = 0.014), there was no significant difference in the deoxygenated hemoglobin/myoglobin at each time point between groups (all p > 0.05). The deoxygenated hemoglobin/myoglobin showed that there were no significant differences at 20 and 45% HRR between groups (20% HRR T2DM vs CON: 0.0080 ± 0.0073 vs −0.0032 ± 0.011; p = 0.416, 45% HRR T2DM vs CON: −0.014 ± 0.011 vs −0.036 ± 0.0088; p = 0.125). The deoxygenated hemoglobin/myoglobin in T2DM, however, was significantly higher than that in CON at 70% HRR (T2DM vs CON: −0.024 ± 0.010 vs −0.056 ± 0.0064; p = 0.014).

**Discussion**

NIRS provides real-time level of skeletal muscle oxygenation during exercise\(^6\). Generally, as work rate increases, muscle oxygenation initially either remains constant near resting levels or decreases, and, then, muscle oxygenation decreases more steeply near the work rate where lactic acidosis is detected in healthy subjects\(^6\). Our present result in the both groups had good agreement with this response reported in the previous study. However, there was a significant difference in total hemoglobin/myoglobin between T2DM and CON at 20 % HRR. Previous studies suggested that patients with T2DM showed slowed oxygen uptake kinetics during the initial period of submaximal exercise with constant load\(^6,15,21,35\). These studies concluded that mismatching between the amount of oxygen demand and oxygen supply was induced by slowed oxygen uptake kinetics during the initial period of submaximal exercise. These suggestions might explain the significant difference in total hemoglobin/myoglobin at the intensity of 20% HRR between the groups. On the other hand, our present result showed no significant difference in total hemoglobin/myoglobin between the groups at the intensity of 45 and 70% HRR. This finding implies that blood flow to the VL muscle was identical between T2DM and CON at moderate to high intensity. Our finding might be line with a previous result indicating that blood flow to the working muscle during moderate-intensity exercise was not impaired in patients with T2DM\(^20\). However, the decrease of muscle oxygenation level at the intensity of 70% HRR in T2DM was significantly smaller than that in CON. The deoxygenated hemoglobin/myoglobin detected by NIRS can be considered as a surrogate of microvascular oxygen extraction\(^30,38\), and our present study indicated that there was no significant difference in the deoxygenation level at 70% HRR between groups. Therefore, the fact that the decrease of muscle oxygenation level at the intensity of 70% HRR in T2DM was significantly smaller than that in CON suggests that oxygen supplied to peripheral tissues might not be extracted into the VL muscle in patients with T2DM. Thus, this finding might indicate that oxygen extraction in T2DM has nearly reached its limitation earlier than CON.

The measured oxygenation level using NIRS does not represent that of a single vessel, but includes that in arterial, capillary, and venous oxygenated hemoglobin and intracellular myoglobin\(^17\). Arterial deoxygenated hemoglobin does not normally change as work rate is increased\(^8\). In contrast, the major change in venous oxygenated hemoglobin occurs over a lower work rate period, decreasing from 50% to about 25% at VT and to 15% at V\(_{O2\text{peak}}\)\(^9\). A previous study showed that deoxygenated hemoglobin dissociation at intensities below VT was due to decreasing capillary partial pressure of oxygen (Po2), and that above VT, lactic acidosis allows end capillary oxygenated hemoglobin saturation to decrease further without decreasing Po2\(^9\). Therefore, Po2 gradient between capillary and working muscles widens and the dissociation of oxygenated myoglobin occurs above VT. To sum up, it has been considered that the major desaturation during incremental exercise is due to oxygen loss from hemoglobin at the intensities below VT and also to oxygenated myoglobin dissociation at the intensities above VT\(^34\). In our present study, there was no significant difference in oxygenated hemoglobin/myoglobin at the intensities below VT (averaged intensity of VT in T2DM and CON were 32.0 and 41.2% HRR, respectively). This result suggests that there was no significant difference in the capacity of oxygen extraction from capillary to working muscles between T2DM and CON at the intensities below VT. However, the decrease of oxygenated hemoglobin/myoglobin level in T2DM was significantly smaller than that in CON at the 70% HRR intensity. This result might indicate that patients with T2DM have low dissociating capacity of myoglobin at the intensities above VT besides early limitation of oxygen extraction. Previous studies found that patients with T2DM have a reduction in type I slow-twitch oxidative muscle fibers, which contain a larger amount of myoglobin when compared with type II muscle fibers\(^38,91\) and that a higher percentage of type II fast-twitch glycolytic muscle fibers was demonstrated in the VL muscle of patients with T2DM\(^20\). The fiber-type distribution peculiar to T2DM might partly explain the difference in the myoglobin dissociation between T2DM and CON.

Our study showed that %HRR and V\(_{O2\text{peak}}\) at VT in T2DM were significantly lower than that in CON. The VT and V\(_{O2\text{peak}}\) are parameters that have been widely considered as clinical markers of aerobic fitness\(^40,41\), and lower aerobic fitness in T2DM is widely recognized independent of cardiovascular disease or other comorbidities such as obesity\(^45\). Our result that VT in patients with T2DM was significantly
lower than that in CON is agree with these previous studies\(^{42,43}\). The VT depends on oxidative metabolism in the muscles (mitochondrial density, oxidative enzymes and percentage slow-twitch myosin heavy chain types) and oxygen supply to the mitochondria (cardiac output, capillary density, haematocrit, arterial-venous oxygen difference, and oxygen diffusion)\(^{44}\). Since it has been reported that these functions are impaired in the patients with T2DM\(^{17,18-22}\), our present result of low VT in T2DM might be a net result of these impaired functions peculiar to T2DM. Therefore, the early occurrence of VT might lead to early limitation of oxygen extraction in patients with T2DM.

There are some limitations to this study. First, since the sample size was small, our results need to be carefully interpreted and further studies with larger sample sizes are required to confirm our findings. Second, the average age in T2DM and healthy participants were 68.6 and 74.2 years old, respectively. Although each group included both middle-aged and elderly people, the majority of the participants were 65 and more years old. A previous study reported that elderly people showed NIRS-measured lower skeletal muscle oxygenation compared to young adults\(^{45}\). The effect of age on the vasculature, which parallel the influence of T2DM\(^{46}\), are themselves spatially heterogeneous in blood flow perfusion\(^{47}\), and aging degenerates skeletal muscle metabolism\(^{48}\). Therefore, in the cases of young or middle-aged patients with T2DM, our present results should be carefully interpreted. Third, although the difference of deoxygenated and oxygenated hemoglobin/myoglobin between T2DM and CON may be possibly noticeable at intensities above 70% HRR, the participants were prohibited from performing the incremental exercise at intensities above 70% HRR due to avoiding the onset of myocardial ischemia. Fourth, although NIRS probe can reflect the oxygenation in only a part of the VL muscle, it cannot represent tissue oxygenation in whole VL muscle. Therefore, for evaluating the spatially heterogeneous oxygenation in the tissue, it is required to use multi-channel NIRS equipment. Finally, there are some probable contributing factors to exercise intolerance in patients with T2DM, such as decreased cardiac function, increased blood flow in conduit vessels, increased precapillary vasoconstriction and abnormal endothelium-mediated vasodilation, and capillary recruitment\(^{49}\). Hence, only our study focusing on tissue oxygenation cannot fully elucidate the precise mechanism of exercise intolerance in T2DM. However, since impaired skeletal muscle oxygenation has been considered to be one of the causes of exercise intolerance in patients with T2DM\(^{50}\), our results would partly contribute to clarifying the peripheral mechanism of exercise intolerance in T2DM.

**Conclusion**

The patients with T2DM showed a higher oxygenated hemoglobin/myoglobin level at an intensity of 70 % HRR compared to healthy subjects. Our study suggested that patients with T2DM have early limitation of oxygen extraction and lower capacity of oxygenated myoglobin dissociation in the VL muscle. Therefore, this study may provide further insights on the relationship between peripheral factor and exercise intolerance in T2DM.

**Acknowledgments:** This research was supported by a Grant-in-Aid for physical therapy research from the Japanese Physical Therapy Association (No. B23) and the Japanese Council for Science, Technology and Innovation, SIP Project ID 14533567, “Technologies for creating next-generation agriculture, forestry and fisheries” (funding agency: Bio-oriented Technology Research Advancement Institution, NARO).

**Conflict of Interest:** The authors have no conflicts of interest relevant to this study.

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