

Association between Muscle Oxygenation and Vascular Occlusion Determined by Near-infrared Spectroscopy in the Tibialis Anterior Muscle

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ABSTRACT

Many previous studies on near-infrared spectroscopy (NIRS) based evaluation of muscle oxygenation, have assessed the association of muscle oxygenation with muscle fatigue and exercise tolerance. To examine the changes in oxyhemoglobin (O₂Hb) and deoxyhemoglobin (HHb) levels, some studies performed occlusion of arterial blood flow in the upper arm and measured the biarticular forearm muscle oxygenation; however, these muscles are subject to contractions based on the position of the arm, which could have led to discrepancies in the findings. Therefore, this study aimed to assess the association between muscle oxygenation and vascular occlusion in the tibialis anterior muscle during isometric exercise using NIRS. Twenty-nine women of mean age of 20.00 ± 1.56 years were included. Maximum isometric contraction performed for successive 30 sec and 60 sec was assessed under occlusive and non-occlusive conditions of the unilateral femoral artery, with and without the use of a tourniquet, at the same time. The O₂Hb level was reduced in both conditions, with a significant decrease observed earlier during an initial quarter of the exercise ($p < 0.05$). This significant decrease in the O₂Hb level may be due to decreased oxygenation in the muscle associated with muscle contraction, primarily during aerobic exercise. Under the occlusive condition, the total rate of increase in the HHb levels was smaller than the total rate of decrease in the O₂Hb levels. Therefore, adequate oxygen supply to meet the increased demand for the O₂Hb cannot be achieved and the increase in the HHb level is suppressed during muscle contractions.

Key words: *near-infrared spectroscopy, tibialis anterior muscle, muscle oxygenation, vascular occlusion*

INTRODUCTION

In recent years, many methods of measuring muscle oxygenation have been developed and applied in research and clinical practices. One of these methods is near-infrared spectroscopy (NIRS), which has also been used in a variety of exercises and sports as a non-invasive method for the continuous measurement and assessment of muscle oxygenation during exercise. The difference in the near-infrared light absorption is characteristic of hemoglobin in a biological tissue based on its oxygenated or deoxygenated state. It facilitates the non-invasive measurement of blood oxygenation by NIRS. As reported in 1977 by Jobsis¹⁰⁾, blood oxygenation can be non-invasively measured by near-infrared light illuminating the target site. Although NIRS was originally used to visualize brain activity, it has also been used in the studies which measured differences in muscle oxygenation between athlete and non-athlete, as well as in the studies which measured the degree of recovery from fatigue after exercise^{2,13)}. Oka¹⁵⁾ concluded that post-exercise of recovery oxygen level time was a promising

indicator of muscle fatigue, while the minimum of oxygen level was a promising indicator during maximal contraction. Many other studies have also examined of quadriceps femoris muscle the contraction in incremental cycling exercise using a bicycle ergometer^{2,8)}. These results have suggested that the exchange rate and critical point of muscle oxygenation during exercise were reproducible and reflect exercise loads.

However, these studies, only suggested that comparisons of muscle oxygenation and blood oxygen levels can be used to assess muscle fatigue and exercise tolerance. Hamaoka et al.⁵⁾ indicated that the changes in muscle oxygenation during exercise are determined by the changes in the consumption of oxygen by the muscle tissue or the supply of oxygen from blood flow. It suggested the necessity to examine the changes in muscle oxygenation based on its oxygen consumption. Therefore, it is necessary to examine the changes in oxyhemoglobin (O₂Hb) and deoxyhemoglobin (HHb) observed during exercise to assess the muscle oxygenation. Previous studies have used arterial occlusion to assess the levels of muscle oxygen consumption based on the changes of O₂Hb and HHb between rest and during exercise^{3,5)}. In

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the present study, muscle oxygenation in the tibialis anterior muscle was continuously measured during the isometric exercise of ankle dorsiflexion. Since only the popliteal artery supplies blood to the lower leg without blood flow bypass, tourniquet use on the quadriceps femoris further ensures vascular occlusion. This study aimed to assess the relationship between muscle oxygenation and vascular occlusion during exercise by measuring the O_2Hb and HHb levels using quantitative NIRS during isometric contraction of the tibialis anterior with and without vascular occlusion.

MATERIALS AND METHODS

Subjects and muscle measured

The subjects were women aged 18–22 years of age. We choose them as subjects because, as reported by Maughan et al.¹³⁾ and Petrofsky et al.¹⁸⁾, women exhibit less individual difference in muscles and more muscle endurance than men. The isometric-contraction exercise of the ankle joint with maximum voluntary contraction dorsiflexion was measured continuously in 30-sec and 60-sec durations. Thirty-six women consented to participate in our study. After excluding women whose measurements were difficult to obtain, 29 women were finally included as subjects for analysis (mean age: 20.00 ± 1.56 years). The mean height and body weight were 158.34 ± 5.25 cm and 52.93 ± 7.97 kg, respectively. The right and left thigh circumference, 15 cm superior to the patella, was 45.09 ± 3.95 cm and 44.72 ± 4.47 cm, respectively. Maximum right and left calf circumference were 34.59 ± 2.65 cm and 34.53 ± 2.63 cm, respectively. Right and left calf length from the knee lateral joint space to the lateral malleolus was 38.31 cm and 38.31 ± 1.61 cm, respectively.

In this study the right tibialis anterior muscle oxygenation was measured under occlusive and non-occlusive conditions, since for most Japanese, regardless of age or sex, the right leg is the dominant leg²⁵⁾. O_2Hb and HHb were measured during isometric exercise of ankle dorsiflexion in the tibialis anterior muscle with quantitative NIRS in the occlusive and non-occlusive condition to analyze the relation between muscle oxygenation and vascular occlusion.

Experiment design

At the start of the experiment, each subject sat in a chair with their knee flexed at a 90° angle. To eliminate the effect of variations in the upper body, each subject's upper body was secured to the back of a chair. The fore-foot was placed on a stationary plate, while the bases of the metatarsals were held in place with a stabilizing belt (Figure 1). Prior to the start of exercise, vascular occlusion was induced using 300 mmHg of pressure; this pressure was maintained and monitored throughout the measurements. Next, after having the subject rest for 1 min, the measurement of the relaxed tibialis anterior muscle was started using the NIRS; the first minute of measurement was considered as the baseline. To minimize the effects of vascular occlusion induced by the



Figure 1 Illustration of each subject sat in a chair with their knee flexed at a 90° angle, while the bases of the metatarsals were held in place with a stabilizing belt.

pressure from the tourniquet (MT-870, Mizuho Medical Co., Ltd., Tokyo, Japan; tourniquet), the patients were required to perform isometric exercise of ankle dorsiflexion for 30-sec followed by 60-sec under the non-occlusive condition. Subsequently, the subjects were required to perform isometric exercise of ankle dorsiflexion for 30-sec and then for 60-sec in the occlusive condition. Figure 2 shows the protocol for measurement of muscle oxygenation and vascular occlusion. In this study, continuous changes in oxygenation and vascular occlusion of the tibialis anterior muscle at rest and during isometric exercise of ankle dorsiflexion were measured. Two conditions for exercise were established: occlusive and non-occlusive. In the occlusive condition, a digital tourniquet was used to apply approximately 300 mmHg of pressure to the thigh, 15 cm superior to the fossa, thereby occluding arterial blood flow. As a result of measuring the maximum exertion force in the dorsiflexion direction of the ankle joint, there was no significant difference between the occlusive and non-occlusive condition (data not shown).

The protocol in this study was performed using a tourniquet⁹⁾. In the occlusive condition, pressure was applied for 2-min using the tourniquet that had already been wrapped around the subject's right thigh, after which the subject exercised for 30-sec followed by for 60-sec. Two min after this exercise, the tourniquet was removed and the subjects were required to rest for 5-min. This concluded the measurements. In the non-occlusive condition, subjects performed maximum isometric exercise of ankle dorsiflexion for 30-sec and then for 60-sec without the application of pressure.

Measurement methods

Muscle oxygenation of the tibialis anterior muscle was quantified by measuring the concentrations of O₂Hb and HHb with a near-infrared time-resolved spectroscopy (tNIRS-1; Hamamatsu Photonic K.K., Hamamatsu, Japan). In this study, continuous changes in muscle oxygenation and vascular occlusion of the tibialis anterior muscle were measured using quantitative NIRS. The quantitative NIRS consists of the three wavelengths, but the qualitative NIRS consists of the two wavelengths with a probe and a non-invasive oximeter. The three wavelengths (755 nm, 816 nm, and 850 nm) emitted the infrared light from the probe's transmitter, which can analyze absolutely the temporal spread of short-pulse light. The reflected near-infrared light scattered by the muscle tissue was converted to optical densities with the NIRS at a sampling rate of 12 Hz. The infrared light is absorbed by O₂Hb in blood in the arterioles, veins, and capillary plexus, and by myoglobin in the muscle; the light then returns to the sensor at the receiver⁶⁾. Absolute values were difficult to measure with a qualitative NIRS. With the above three wavelengths, O₂Hb and HHb in tissue blood flow can be measured quantitatively¹⁹⁾.

The near-infrared light transmitter and receiver were 3 cm apart at all times. The probe, with its near-infrared ray transmitter and receiver, was attached to the center of the belly of the tibialis anterior muscle, perpendicular to the muscle fibers. Thus, the levels of O₂Hb and HHb were measured. Arithmetic means of measured values are shown in 5-sec intervals, with 0 sec defined as the start of the measurement. Initial NIRS measurements differed in both conditions and had to be compared. Therefore, with measurements at the start of exercise defined as a zero point correction.

The data obtained from measurements with quantitative NIRS is represented in terms of mean and standard deviation. The paired student-t test was used for the analysis. The measurements of the occlusive and non-occlusive conditions, and between 30-sec and 60-sec were compared. The significance level was set at 5% or 1%.

Ethical considerations

All subjects provided consent after receiving a sufficient explanation of the study both in written and oral forms. Personal information of the subjects were anonymized to protect their privacy. All study data was processed statistically and is presented in a manner that does not allow individual subjects to be identified. Subjects' information will not be used for any purposes unrelated to the objective of the study. Experiment data will be used only for the study, and confidentiality will be strictly maintained. This study was approved by the Prefectural University of Hiroshima (Approval no. 18MH005).

RESULTS

Comparison of changes in O₂Hb between 30-sec and 60-sec exercise in the occlusive and non-occlusive conditions

Figure 3 shows means and standard comparison of concentration changes in O₂Hb between 30-sec and 60-sec exercise in the occlusive and non-occlusive conditions. The change in O₂Hb concentration was measured from start to finish during the 30-sec and 60-sec exercise, and the mean value was calculated for 29 subjects. Comparisons between 30-sec and 60-sec exercise in O₂Hb showed that a longer duration of exercise resulted in a significant decrease levels by both the occlusive and non-occlusive conditions ($p < 0.05$, $p < 0.01$). Comparisons between the occlusive and non-occlusive conditions in O₂Hb did not reveal a significant decrease levels associated with the duration of the exercise.

Comparison of changes in HHb between 30-sec and 60-sec exercise in the occlusive and non-occlusive conditions

Figure 4 shows means and standard comparison of concentration changes in HHb between 30-sec and 60-sec exercise in the occlusive and non-occlusive conditions. The change in HHb concentration was measured from start to finish during the 30-sec and 60-sec exercise, and the mean value was calculated for 29 subjects. Comparisons between 30-sec and 60-sec exercise

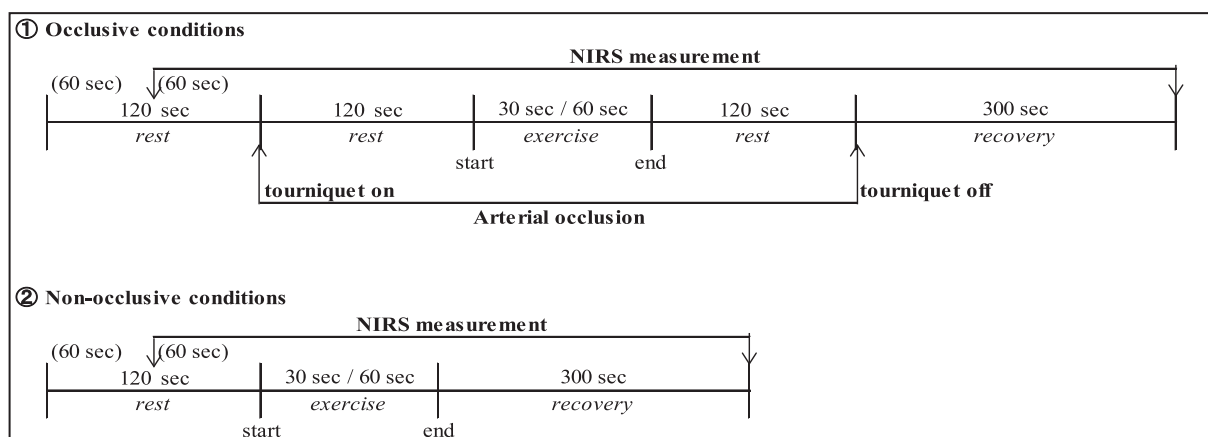


Figure 2 The protocol for measurement of muscle oxygenation and vascular occlusion. NIRS: near-infrared spectroscopy.

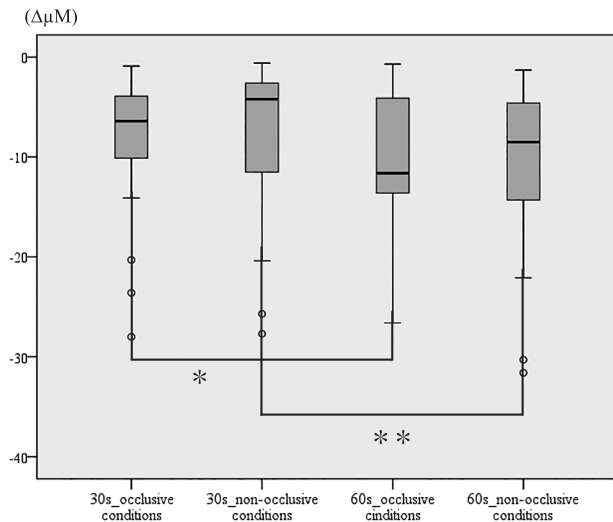


Figure 3 Comparisons of the changes in the O_2Hb level during 30-sec and 60-sec exercise under the occlusion and non-occlusion conditions. **: $p < 0.01$, *: $p < 0.05$. O_2Hb : oxyhemoglobin; ○: an extreme deviation from the mean.

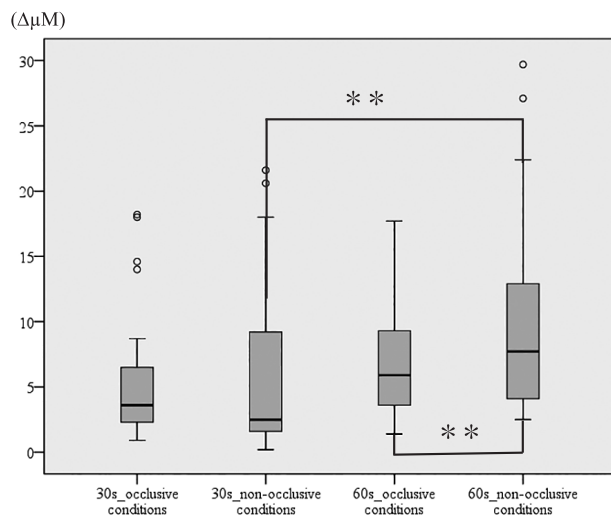


Figure 4 Comparisons of the changes in the HHb level during the 30-sec and 60-sec exercise under the occlusive and non-occlusive conditions. **: $p < 0.01$. HHb: deoxyhemoglobin; ○: an extreme deviation from the mean.

showed that a longer duration of exercise resulted in a significant increase in the HHb level in the non-occlusive condition ($p < 0.01$). However, in the occlusive condition, a significant difference was not observed. Comparisons between the occlusive and non-occlusive conditions revealed that the increase in the HHb level was significantly greater during the 60-sec exercise in the non-occlusive condition ($p < 0.01$). In the 30-sec exercise, the difference between both conditions was not significant.

Changes in HHb during 30-sec exercise by the occlusive and non-occlusive conditions

The change in HHb concentration was measured from start to finish during the 30-sec exercise under occlusive and non-occlusive conditions, and the mean value was calculated every 5 seconds for 29 subjects. Figure 5

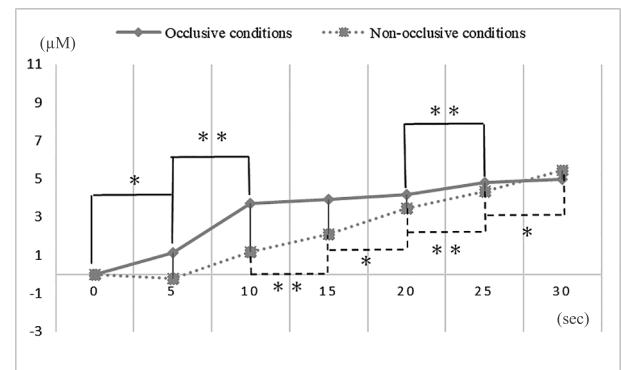


Figure 5 Changes in HHb level during 30-sec exercise under the occlusive and non-occlusive conditions. **: $p < 0.01$, *: $p < 0.05$. HHb: deoxyhemoglobin.

shows changes in the HHb during 30-sec maximum isometric exercise of ankle dorsiflexion under each condition. In the occlusive condition, the HHb level increased significantly in the first 10 sec of exercise ($p < 0.01$, $p < 0.05$). Another significant increase was observed from 20 sec to 25 sec ($p < 0.01$). From 25 sec to 30 sec, there was no significant difference in the HHb levels and the level had stabilized. In the non-occlusive condition, HHb was stable and showed no significant difference between 0 sec and 5 sec, but the levels increased significantly from 10 sec to 30 sec ($p < 0.01$, $p < 0.05$). Additionally, comparisons at each measurement point between the two conditions revealed that the level of HHb relative to baseline was significantly higher in the occlusion condition at 5 sec, 10 sec, and 15 sec ($p < 0.05$).

Changes in HHb during 60-sec exercise by the occlusive and non-occlusive conditions

The change in HHb concentration was measured from start to finish during the 60-sec exercise under occlusive and non-occlusive conditions, and the mean value was calculated every 5 seconds for 29 subjects. Figure 6 shows changes in HHb during the 60-sec maximum isometric exercise of ankle dorsiflexion for each condition. In the occlusive condition, the HHb level increased significantly from 5 sec after the start of exercise till 15 sec ($p < 0.05$). From 15 sec to 25 sec, the HHb level increased gradually but not significantly, while from 25 sec to 30 sec the HHb level decreased, but not significantly. After 30 sec, the HHb level increased continuously, with significant increases observed from 30 sec to 35 sec, 40 sec to 45 sec, and 50 sec to 55 sec ($p < 0.05$). In the non-occlusive condition, HHb level was stable from 0 to 5 sec and showed no significant difference. At 10 sec, the HHb level increased but not significantly. After that, the HHb level increased significantly until 45 sec after the start of exercise ($p < 0.01$). From 45 sec to 60 sec, the HHb level increased gradually but not significantly. Additionally, comparisons at each measurement point between the occlusive and non-occlusive conditions revealed that HHb levels relative to baseline were significantly lower in the occlusion condition at 30 sec ($p < 0.05$) and 40 sec ($p < 0.01$).

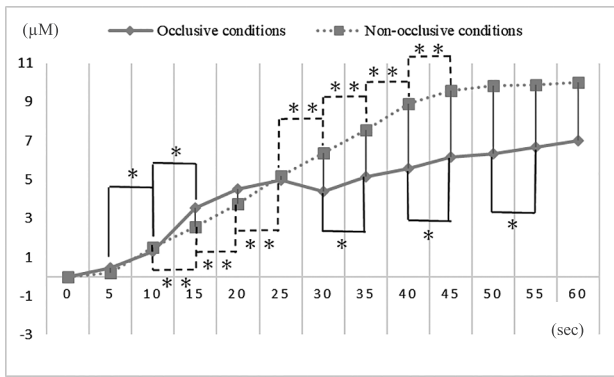


Figure 6 Changes in the HHb level during 60-sec exercise under the occlusive and non-occlusive conditions. **: $p < 0.01$, *: $p < 0.05$. HHb: deoxyhemoglobin.

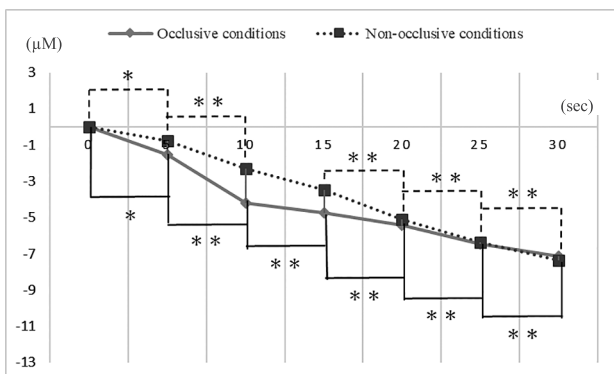


Figure 7 Changes in O_2Hb level during 30-sec exercise under the occlusive and non-occlusive conditions. **: $p < 0.01$, *: $p < 0.05$. O_2Hb : oxyhemoglobin.

Changes in O_2Hb during 30-sec exercise by the occlusive and non-occlusive conditions

The change in O_2Hb concentration was measured from start to finish during the 30-sec exercise under occlusive and non-occlusive conditions, and the mean value was calculated every 5 seconds for 29 subjects. Figure 7 shows changes in O_2Hb during 30-sec isometric exercise of ankle dorsiflexion by condition. In the occlusive condition, the O_2Hb level significantly decreased throughout the 30 sec from the start to the end of the exercise ($p < 0.01$, $p < 0.05$). Significant decreases in the O_2Hb level were also observed during 30 sec of exercise in the non-occlusive condition ($p < 0.01$, $p < 0.05$). In the non-occlusive condition, O_2Hb did not significantly differ from 10 sec to 15 sec. Additionally, comparisons at each measurement point between the occlusive and non-occlusive conditions revealed no significant differences in O_2Hb between the conditions relative to the baseline.

Changes in O_2Hb during 60-sec exercise by the occlusive and non-occlusive conditions

The change in O_2Hb concentration was measured from start to finish during the 60-sec exercise under occlusive and non-occlusive conditions, and the mean value was calculated every 5 seconds for 29 subjects. Figure 8 shows changes in O_2Hb during the 60-sec maximum isometric exercise of ankle dorsiflexion for each condition. In the occlusive condition, significant decreases were

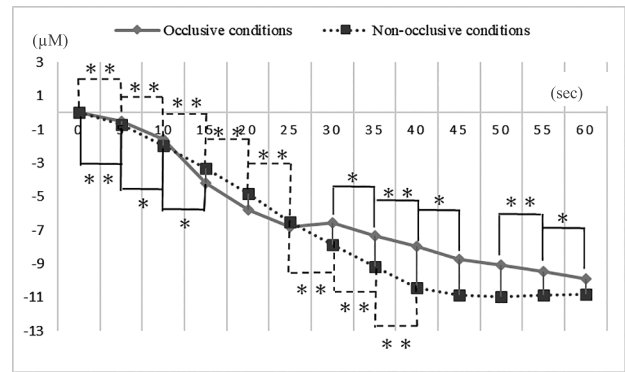


Figure 8 Changes in the O_2Hb level during 60-sec exercise under the occlusive and non-occlusive conditions. **: $p < 0.01$, *: $p < 0.05$. O_2Hb : oxyhemoglobin.

observed from the start of the exercise to 15 sec ($p < 0.01$, $p < 0.05$). After that, the O_2Hb level decreased gradually, with a slight increase from 25 sec to 30 sec; however, none of these differences were significant. After 30 sec, the O_2Hb level decreased significantly at all intervals except during 45 sec to 50 sec ($p < 0.01$, $p < 0.05$). In the non-occlusive condition, significant decreases were observed from the start of the exercise to 40 sec ($p < 0.01$). Additionally, comparisons at each measurement point between the occlusive and non-occlusive conditions revealed that in the occlusive condition, O_2Hb levels relative to baseline were significantly lower at 30 sec ($p < 0.05$) and 40 sec ($p < 0.01$), and 55 sec ($p < 0.01$).

DISCUSSION

The effects of HHb and vascular occlusion on muscle oxygenation in isometric contraction

According to De Blasi et al.⁴⁾, HHb is not affected by changes in blood flow during exercise and is thus more suitable than O_2Hb for assessing local muscle oxygenation. Additionally, Hoshi et al.⁹⁾ have reported that changes in HHb levels were decreased by venous return. During the 30-sec isometric exercise of ankle dorsiflexion in occlusive condition, the increase in the HHb level observed especially in the first 5 sec of exercise in the occlusive condition may have been due to a temporary increase in the venous return. This caused venous blood flow to be pushed out by increased intramuscular pressure associated with muscle contraction. During the 30-sec and the 60-sec isometric exercise of ankle dorsiflexion under the occlusive condition and the non-occlusion condition, the HHb levels of muscle oxygen consumption based on the changes showed similar responses up to 30 sec. In the 60-sec of exercise, a significant difference was observed in muscle oxygenation of HHb since 30 sec. It may have been the result of the oxygen necessary for aerobic metabolism to arteriolar vasodilation, which had little effect in circulation output associated with arterial occlusion.

In the non-occlusive condition, the O_2Hb level decreased for the first to 40 sec of exercise and then plateaued, whereas the HHb level increased for the first to 45 sec of exercise and then plateaued. Comparisons of

changes in the HHb level during 60-sec exercise in the occlusive and non-occlusive conditions revealed that the increase rate of HHb levels was significantly increasing in the non-occlusive condition. Thus, the occlusive condition in this study suggested that HHb, as an indicator of local muscle oxygenation, reflects the balance between local muscle oxygen supply and consumption with particularly strong reflection of oxygen consumption⁴⁾. The occlusive condition demonstrated not only the contrasting changes in O₂Hb and HHb, but also the rate of increase in HHb was smaller than the rate of decrease in the O₂Hb level. This may have occurred because vascular occlusion made it impossible to supply enough oxygen to meet the increased demand for oxygen in the muscle, thereby reducing the rate of increase in HHb levels.

The increase in HHb levels was higher in the occlusive condition than in the non-occlusive condition in the first 30 sec of exercise. There was no significant difference in the mean increase during the first 15 sec of the 60-sec exercise. In our previous study, comparisons between 30-sec and 60-sec exercise of ankle dorsiflexion showed that a longer duration of exercise resulted in a significant decrease in strength over time²³⁾. Therefore, the fact that the subjects tried to maintain the 60-sec contraction suggests that there may be no significant difference in the mean value of HHb during the initial 30 sec.

The effects of O₂Hb and vascular occlusion on muscle oxygenation in isometric contraction

For the exercise performed for 60 sec under the non-occlusive condition, the O₂Hb level decreased gradually from 40 sec onwards and then plateaued. Mori et al.¹⁴⁾ assessed the erector spinae muscles using NIRS and found, muscle oxygenation decreased from the beginning of the exercise, then gradually decreased from 40%MVC (Maximal voluntary contraction) after the start of exercise, and then plateaued. This plateauing, which was the result of increased cardiac output and metabolites causing arterioles to vasodilate, suggests that oxygen supply and consumption were balanced¹⁶⁾. In the non-occlusive condition, which involved only isometric muscle contraction without arterial occlusion, the O₂Hb level decreased sharply. This was conceivably because as isometric concentration time elapsed from 30 sec to 60 sec, compared to the occlusive condition, in which blood flow was markedly reduced by vascular occlusion. The non-occlusive condition, which involved only isometric muscle contraction, demonstrated the increasing blood flow in muscle tissue as well as greater aerobic energy metabolism, which exceeded the supply¹²⁾.

During the 60-sec exercise, the occlusive condition involved not only isometric muscle contraction but also arterial occlusion, which markedly reduced blood flow. This resulted in a prominent decrease in the O₂Hb level compared to the non-occlusive condition, which only involved isometric muscle contraction, from 30 sec to 50 sec after the start of the exercise ($p < 0.05$). This decrease in the O₂Hb levels was assumed to be due to arterial occlusion and reduced blood flow resulting from the mechanical compression associated with muscle con-

traction. Paterson et al.¹⁷⁾ reported that if the load is 15–20% of maximum voluntary contraction, the muscle receives enough blood flow; however, if the load is higher than 20%, blood flow begins to decrease. Similarly, in this study, the early increase in pressure in the tibialis anterior muscle prevented enough blood flow to the muscle. This explained why oxygen blood supply exceeded oxygen consumption in the muscle.

The decrease in O₂Hb levels was smaller in the occlusive condition than in the non-occlusion condition. This indicated that the vascular occlusion reduced the blood remaining in the muscle tissue, leading to a reduction in O₂Hb in the muscle. The temporary increase in the O₂Hb level from 25 sec to 30 sec may have been due to the oxygen necessary for aerobic metabolism of arteriolar vasodilation, which had little effect in the circulation output associated with arterial occlusion. This suggested that muscle oxygenation can be better measured with the occlusive condition than the non-occlusive condition.

Assessment of O₂Hb and HHb in muscle tissue with the occlusive condition and the non-occlusion condition

In this study, the compression in the muscle decreased O₂Hb and increased HHb with the occlusive condition and the non-occlusion condition. In the occlusive condition, the volume of blood in the muscle will be decreased without the influx of arterial blood, whose condition shall force the muscle to rely entirely on the stored oxygen in occlusive conditions. It may have been the result of the oxygen necessary for aerobic metabolism to arteriolar vasodilation, which had little effect in circulation output associated with arterial occlusion. It could take an accurate measurement of muscle oxygenation during exercise in occlusive conditions.

In this study, the quantitative NIRS is capable of quantitative measurement, in an attempt to assess muscle oxygenation in occlusive and non-occlusive conditions. A quantitative NIRS can measure absolute values on the tissue oxygenation index. A qualitative NIRS allows relative values for calculations in percentages based on the tissue oxygenation index. The former measures not only absolute changes due to the facility in determining optical path lengths, but also it can be applied in absolute fashion for measurements during exercise. Conventionally qualitative NIRS does not measure absolute levels of O₂Hb in muscles, but rather relative levels of O₂Hb in blood. Therefore, intramuscular O₂Hb is considered to analyze suitably with quantitative NIRS rather than qualitative NIRS. The above suggests that quantitative assessment is a suitable method for analyzing muscular oxygenation, and that future studies must examine exercise load and muscle strength.

In the NIRS data of this study, it is necessary to compare and verify the oxygen dynamics of 60-sec muscle contraction above 30 sec and the oxygen dynamics under 30-sec muscle contraction. In this study, the same condition of muscle contraction was repeated as much as possible. In addition, muscle oxygenation due to vascular occlusion was evaluated using a tourniquet. It may be

possible to measure the contraction dynamics of the muscle by adding data analysis of the relationship between the NIRS and the electromyogram.

CONCLUSIONS

The quantitative NIRS measures O₂Hb and HHb levels during the isometric exercise of ankle dorsiflexion in the tibialis anterior muscle. The relation between muscle oxygenation and vascular occlusion indicates that muscle compression during contraction results in vascular occlusion, thereby reducing blood flow. During the 30-sec exercise, the occlusive condition demonstrated a much greater reduction in O₂Hb than the non-occlusive condition at every measured time-point. From 30 sec to 60 sec, in the non-occlusive condition, the O₂Hb level decreased gradually from 40 sec onwards and then plateaued. This plateauing, which resulted from decreased circulation output and arteriole vasodilation caused by metabolites, suggests that oxygen supply and consumption reached homeostasis.

The decrease in O₂Hb levels was smaller in the occlusive condition than in the non-occlusive condition, which was due to vascular occlusion reducing the blood remaining in the muscle tissue, leading to a reduction in O₂Hb in the muscle. Comparisons of the HHb levels during 60-sec exercise in the occlusive and non-occlusive conditions revealed that the increase in the HHb level was significantly greater than in the non-occlusive condition. Thus, the occlusive condition in this study suggested that HHb, as an indicator of local muscle oxygenation, reflects the balance between local muscle oxygen supply and consumption and is a particularly strong indicator of the latter.

The influx of arterial blood was more efficiently occluded before exercise to stop the supply of oxygen in the occlusive than in the non-occlusive condition. The occlusive condition revealed the retardation of oxygen from the influx of arterial blood. This phenomenon suggests that the changes in the O₂Hb and HHb levels reflect the oxygenation content of muscle. The results of this study suggest that quantitative NIRS enables a detailed assessment of the difference in stored oxygen in the muscle between the occlusive and the non-occlusive condition.

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