Measurement of blood flow in the human Achilles tendon in vivo

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Abstract  The purpose of this study was to apply a high-power laser blood flowmeter and laser oxygenation monitor to investigate 1) changes during a single isometric contraction and 2) changes after repeated contractions in blood flow (BF), blood volume (THb), and oxygen saturation (StO2) in the human Achilles tendon in vivo. Subjects (n = 17) performed isometric contractions at 10%, 30%, and 50% of the maximum voluntary contraction (MVC) for 1 min. Subjects (n = 10) also performed five sets of 50 repetitions at 70% of MVC for 1 second (s), and remained relaxed for 20 min. During isometric contractions at all torque levels, BF decreased significantly from resting level regardless of torque level, whereas THb during contractions tended to decrease with increases in torque level. After repeated contractions, BF, THb, and StO2 were higher during the recovery period than resting level. These results show that 1) BF in the tendon was already interrupted during 10% of MVC to the same degree as that during 50% of MVC, whereas THb during contractions slightly decreased with increases in the torque level and 2) blood circulation (BF, THb, and StO2) within the tendon was facilitated further after repeated contractions.

Keywords : blood volume, oxygen saturation, plantar flexion, laser blood flowmeter

Introduction

Blood circulation within tendons has been suggested to contribute to the repair of cumulative microtrauma in the tendons after numerous physical activities1). Therefore, a clearer understanding of blood circulation in tendons is important for preventing injuries. Previous in vitro and in vivo studies reported relationships between tendon blood circulation and degeneration (e.g., tendinopathy)2,3). According to previous findings obtained using human cadavers4,4), vascularization in the midsection was the poorest in the entire Achilles tendon, and, thus, tendon rupture frequently occurred in this region5,6). To date, blood circulation in human tendons has been measured in vivo using radioisotope scanning7,8) and laser Doppler flowmetry9). However, the radioisotope scanning technique exposes subjects to radiation, and laser Doppler flowmetry requires the invasive insertion of a probe. On the other hand, previous studies using ultrasound-based technology (e.g., Power Doppler sonography) demonstrated increases in blood flow and perfusion with chronic tendinopathy10,11). However, few quantitative techniques have been established to non-invasively assess the amount of blood flow in human tendons in vivo12).

Previous studies demonstrated that the blood flow in the muscles decreased towards or below the resting level at higher intensities, since intramuscular pressure is positively related to contraction intensity13,14). We recently reported that blood volume and oxygen saturation in human tendons could be determined using a laser oxygenation monitor15). With this technique, we found that blood volume in the tendons decreased with increases in the torque level16). This result implied that blood vessels within the tendon became thinner because the tendon was stretched by exerted muscle force. Strictly speaking, however, “blood volume” in the tendons is different from “blood flow”. Considering this point, it is necessary to grasp blood flow as well as blood volume in the tendons for correctly understanding changes in blood circulation in the tendons during contractions.

Furthermore, we found that changes in blood volume and oxygen saturation in the tendons differed among contraction modes17-19). We demonstrated that blood volume and oxygen saturation in tendons increased significantly after repeated ballistic contractions, but remained unchanged after repeated isometric contractions with a long duration18). However, we cannot deny the possibility that increases in blood volume in tendons after repeated contractions reflect a mere hyperemia after exercise as a physiological response5). On the other hand, Langberg et al.8) showed that blood flow in the Achilles tendon increased 3-4 times during intermittent isometric exercises and returned immediately to the resting level after the end of exercises. In any case, it is likely that blood circulation within the tendons changes with various exercises. Therefore, a clearer understanding of blood flow in the tendons as well as blood volume and oxygen saturation is considered essential for elucidating the mechanisms responsible

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A laser blood flowmeter has been widely used to non-invasively measure blood flow in surface tissues (i.e., skin)\(^20\). The probe of a laser blood flowmeter has generally been composed of one emitting and one detecting optical fiber. As described above, we succeeded in measuring blood volume and oxygen saturation in human tendons using the probe of a laser oxygenation monitor with one emitting and two detecting optical fibers\(^16\). With this instrument, we measured variables in blood circulation at different depths by changing the distance between the emitting and detecting optical fibers. In the present study, we attempted to measure blood flow in human tendons using the probe of a laser blood flowmeter with different distances between the emitting and detecting optical fibers. Using a laser blood flowmeter and laser oxygenation monitor, we investigated changes in blood flow, blood volume, and oxygen saturation in the Achilles tendon during a single isometric contraction and after repeated contractions.

**Materials and Methods**

**Experimental design and subjects.** Two tasks were performed by each subject on two separate days with at least 3 days between sessions: Experiment 1) three different torque levels (10, 30, and 50% of the maximum voluntary contraction [MVC]) of isometric contractions as a single task, Experiment 2) 250 isometric contractions at 70% of MVC as a repeated task. Seventeen healthy males (age: 31.9 ± 7.5 years, height: 172.4 ± 5.4 cm, body mass: 72.2 ± 11.7 kg, mean ± SD) participated in experiment 1 in the present study. Of these, ten subjects also participated in experiment 2. Subjects were physically active, but had not participated in any organized program of regular exercise for at least 1 year before testing. None of the subjects had any known metabolic, neuromuscular, or peripheral vessel disorders. They were fully informed of the procedures to be utilized, as well as the purpose of the study. Written informed consent was obtained from all subjects. This study was approved by the Ethics Committee for Human Experiments, Department of Life Science (Sports Sciences), The University of Tokyo (Issue Number: 504).

**Blood flow in the Achilles tendon.** In order to measure blood flow (BF) using a laser blood flowmeter (FLO-N1, Omega Wave, Tokyo, Japan), a probe (DM, Omega Wave, Tokyo, Japan) was positioned 25 mm proximal to the calcaneus. Although the probe of a laser blood flowmeter is generally composed of one emitting and one receiving optical fiber, the probe used in the present study was composed of one emitting and two detecting optical fibers. The diameter of the optical fiber was 100 μm. In the present study, the distances between one emitting and two detecting optical fibers were 1 and 2 mm, respectively.

Concerning the laser blood flowmeter used in the present study, the depth sensitivity of the plastic sheet model by a simulation of human skin was established by Kashima (personal communication) using a similar procedure of his previous study\(^21\). As a result, the measurement depth was estimated to be 2.2 – 4.2 mm when the distances between the emitting and two detecting optical fibers were 1 mm and 2 mm. Two-point detection and the differential calculation method were used to measure BF in the deep region of the tissue only (measurement depth of 2.2-4.2 mm). The longer the distance between the emitting and detecting optical fibers, the weaker the intensity of light received. Therefore, we herein used a high-power laser blood flowmeter (FLO-N1, Omega Wave, Tokyo, Japan).

In order to verify the precision and validity of FLO-N1 (laser blood flowmeter), we used a probe with a distance between the emitting and detecting optical fibers of 0.5 mm (measurement depth of 0-1 mm) in experiment 2 (n=8) (see Results and Discussion).

The repeatability of BF in the tendon was investigated on 2 separate days in a preliminary study with 9 young males. No significant differences were observed between the test and retest values of BF in the tendon. The test-retest correlation coefficient (r) and coefficient of variance were 0.925 and 15.8 %.

**Blood volume and oxygen saturation in the Achilles tendon.** In order to measure blood volume (total hemoglobin; THb) and oxygen saturation (oxyhemoglobin saturation; StO2) using a laser oxygenation monitor (BOM-L1TRSF, Omega Wave), a probe (SF-DS, Omega Wave, Tokyo, Japan) was positioned 35 mm proximal to the calcaneus. This instrument uses three red laser lights (635, 650, and 690 nm), and calculates the relative tissue levels of oxyhemoglobin (OxyHb), deoxyhemoglobin (DeoxyHb), and THb. The distance between the light source and photodetector was 5 mm. According to the findings of Kashima\(^22\), the measurement depth was estimated to be 3-5 mm when the distance between the emitting and detecting optical fibers was 5 mm. The details of this technique and principles of this instrument have been described elsewhere\(^16,22\). Briefly, two-point detection and the differential calculation method were used to measure blood volume and oxygen saturation in the deep region of the tissue only (measurement depth of 3-5 mm; Fig. 1 of Kubo et al.\(^16\)). THb and StO2 at specific depths in the tissue were measured by changing the location of the two detectors. The offset value for blood volume was reduced, and highly sensitive measurements were achieved using the two-point detection method.

In the present study, the units of OxyHb, DeoxyHb, and THb were expressed as μmol / l, although this does not represent the actual physical volume. Tissue StO2 was calculated from OxyHb and THb values using the following formula:

\[
\text{StO2} (\%) = 100 \times \frac{\text{OxyHb}}{\text{THb}}
\]
These data were input into a personal computer at a sampling frequency of 10 Hz via an A/D transducer (Power Lab, AD Instruments, Australia). The mean values over a given duration (5 min during baseline measurements, every 4 min over a 20-min recovery period) were calculated using analytical software (Chart ver. 7.1, AD Instruments, Australia). These data were presented as the amount of changes from the resting level. The repeatability of measurements in tendon THb and StO2 was confirmed in our previous studies16-18).

A single isometric contraction at different torque levels (experiment 1). The subject lay prone on a test bench and the waist and shoulders were secured using adjustable lap belts and held in position. The right ankle joint was set at 90 degrees with the knee joint at full extension, and the foot was securely strapped to a footplate connected to the lever arm of a specially designed dynamometer. After a standardized warm-up, the subjects performed two or three MVC for isometric plantar flexion. The peak torque was recorded in every trial, and the highest MVC value was used to establish the target torque during the single contraction (experiment 1) and repeated contraction (experiment 2, see below) tests. Following 10 min of baseline measurements, subjects performed isometric contractions at 10%, 30%, and 50% of MVC for 1 min. During contractions, the subjects were encouraged to maintain the target force displayed on an oscilloscope. The order of the measured value of each torque level was randomized in order to avoid any systematic effects. At least a 5-min rest period was permitted between two consecutive attempts in order to avoid the effects of fatigue. The measured variables were presented as the amount of changes (per 10 seconds [s]) from the resting level.

In a preliminary study, we confirmed that the measured variables returned to the resting level immediately after the end of contraction. In this experiment (experiment 1), therefore, we obtained the measured variables only during contraction. However, since obvious noises were observed at the beginning and end of contractions (approximately 1-2 s), these noises were removed to analyze the measured variables.

Repeated isometric contractions (experiment 2). The posture of the subjects and set-up were the same as those in experiment 1. Following 10 min of baseline measurements, the subjects performed five sets of 50 repetitions at 70% of MVC for 1 s, with a 2-s gap between repetitions and a 1-min rest between each set. According to our previous study18), THb and StO2 of the tendon increased significantly after this task. During contractions, the subjects were encouraged to maintain the target force displayed on an oscilloscope. All subjects fully performed 250 repetitions of the isometric plantar flexion task at 70% of MVC. After completion of the task, subjects remained relaxed for 20-min in the same position. In the present study, the measured variables during the exercises were not presented because they (particularly BF) contained more noise. The measured variables were presented as the amount of changes (per 4 min) from the resting level over the 20-min recovery period. Furthermore, we measured BF in skin (see above) using the same protocol to prove the validity of FLO-N1 (laser blood flowmeter) (n = 8).

Distance from the skin to the Achilles tendon. The posture of the subject was the same as that used in experiment 1 and 2. The distances from the skin to the upper and lower ends of the Achilles tendon were measured at 30 mm proximal to the calcaneus using B-mode ultrasonography (SSD-6500, Aloka, Japan).

Statistical analysis. Descriptive data included means ± SD. In experiment 1, a two-way (%MVC x time) ANOVA with repeated measures was used to detect significant differences in the measured variables from the resting level. In the event of significant values of F in ANOVA, Bonferroni’s post hoc test of the critical difference was used to identify significant changes from the resting level. In experiment 2, a one-way ANOVA with repeated measures was used to detect significant differences in the measured variables from the resting level. If the F statistic in ANOVA was significant, differences from the resting level were assessed by Bonferroni’s post hoc test. Significance was set at p < 0.05.

Results

The depth of the Achilles tendon from the surface skin was 1.9 ± 0.4 mm at its superficial side and 6.8 ± 0.8 mm at its deep side.

Regarding BF in the tendon, the effect of time (p < 0.001) was significant, whereas those of the torque level (p = 0.133) and interaction between time and the torque level (p = 0.103) were not (Fig. 1A). During isometric contractions at all torque levels, BF in the tendon decreased significantly from the resting level regardless of the torque level. Regarding THb in the tendon, the effects of time (p < 0.001) and the torque level (p = 0.015) as well as interaction between time and the torque level (p = 0.014) were significant (Fig. 1B). THb in the tendon during contractions tended to decrease with increases in the torque level. Regarding StO2 in the tendon, the effect of time (p = 0.003) was significant, whereas those of torque level (p = 0.511) and the interaction between time and torque level (p = 0.114) were not (Fig. 1C). Decreases in StO2 in the tendon during contractions were small regardless of the torque level.

In experiment 2, all subjects completed the repeated tasks with their individual torque production levels. BF in the tendon during the recovery period was significantly higher than the resting level, except for 16-min after exercise (p = 0.052) (Fig. 2A). THb in the tendon during the
recovery period was significantly higher than the resting level (all p < 0.01) (Fig. 2B). StO2 in the tendon during the recovery period was significantly higher than the resting level, except for 4-min after exercise (p = 0.123) (Fig. 2C). On the other hand, BF in the skin (n = 8) returned rapidly to the resting level after the end of the exercise (p = 0.224, Fig. 3).

Discussion

During a single isometric contraction, BF in the tendon decreased significantly from the resting level regardless of the torque level, whereas THb in the tendon slightly decreased with increases in the torque level (Fig. 1AB). We previously reported similar findings for THb in the tendon16). Considering the results obtained on THb, it is likely that blood vessels within the Achilles tendon are thin, and, thus, blood volume in the Achilles tendon decreases, when it is stretched by the muscle force exerted. On the other hand, the results on BF in the tendon implied that blood flow within the Achilles tendon was already interrupted during 10% of MVC to the same degree as that during 50% of MVC. These changes in blood flow in tendons markedly differ from those in muscles23). Previous studies using ultrasonography showed that the tendon force and elongation relationship was curvilinear consisting of an initial region (toe region) characterized by a large increase in tendon elongation with increasing force18,19). Therefore, one of the possible reasons is that BF within the tendons may be already stopped during contractions at even lower force level, because the tendon would be elongated considerably. In the future, further investigations are needed to clarify this point.

On the other hand, previous studies showed that the blood flow of muscles decreased during contractions at higher force levels, whereas that increased at lower force levels15,23). Therefore, it is likely that there are differences in blood flow during contractions between muscles and tendons. In particular, during contractions at lower force levels, blood flow in the muscles may increase and that in the tendons decrease. Considering this point, differences in training-induced changes in tendon properties among the exercise protocols used19,24) may be related to differences in the responses of blood flow in tendons.
We previously demonstrated that THb and StO2 in the Achilles tendon increased after the same protocol as that used in experiment 2 in the present study(18). The present results for THb and StO2 are consistent with our previous findings. Furthermore, BF in the tendon increased significantly after repeated contractions, and was maintained at a higher level until the end of the recovery period. However, other studies reported that blood flow in tendons suddenly returned to the resting level after repeated contractions(8,25). Possible reasons for these differences are the methods used to measure blood flow in tendons (xenon-133 washout in Langberg et al.(8,25), laser blood flowmeter in the present study), the intensity of exercise (workload equivalent to individual body mass in Langberg et al.(8,25), 70% of MVC in the present study). In the beginning of this study, we had concerns that increases in THb and StO2 after repeated contractions may reflect hyperemia after exercise as a physiological response(3). However, our results revealed that BF as well as THb and StO2 in the tendon remained high during the recovery period after repeated contractions. Therefore, the exercise protocol employed in the present study appears to have induced increased vascularity and the dilation of blood vessels in the tendon, but not hyperemia.

In the present study, an important methodological consideration is whether BF in the tendon, measured by laser blood flowmeter, truly reflects blood circulation in the tendon. In the present study, we confirmed using ultrasonography that the depth of the Achilles tendon from the skin was consistent with the penetration depth of the laser blood flowmeter. Knobloch et al.(13) attempted to compare blood flow in tendons at different depths (measurement depths of 0-2 mm and 0-8 mm) between tendinopathy patients and healthy subjects using the oxygen-to-See (O2C) system. However, we cannot deny the possibility that their findings on blood flow in tendons includes that in skin. In experiment 2, BF in skin returned rapidly to the resting level after the end of the exercise (Fig. 3), whereas BF in the tendon increased significantly after repeated contractions and remained high until the end of the recovery period (Fig. 2A). Therefore, we identified clear differences in BF changes between the tendon (measurement depth of 2.2-4.2 mm) and skin (measurement depth of 0-1 mm). Based on these points, the present results on BF in the tendon reflected changes in blood flow in the tendon, but not in the skin.

In conclusion, the present study showed that 1) BF in the tendon was already interrupted during contraction at lower force levels, whereas THb during contractions slightly decreased with increases in the torque level and 2) the increased BF in the tendon as well as THb and StO2 after repeated contractions were maintained until the end of the recovery period. These changes in blood circula-
tion in the tendons appeared to be related to the training-induced changes in the morphological and mechanical properties of tendons.

**Conflict of Interests**

There is no conflict of interests for any of the authors.

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