The Functional Characteristics of Tendon Blood Circulation in the Rabbit Hindlimbs

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Abstract Neurohormonal, mechanical, and muscle exercise effects on tendon blood flow were studied in thirty-five rabbits. After anesthesia by urethane, experiments were performed on in situ preparation of the hindlimb under stable state in systemic blood pressure. Tendon and muscle blood flow were measured simultaneously by the hydrogen gas clearance method, and their temperature and $P_{O_2}$ were continuously observed by thermocouple and oxygen sensor, respectively. The resting blood flow in the denervated tendon tibialis anterior, gastrocnemius, and soleus (ml/(100 g·min)) was 39.0 ± 4.0, 34.5 ± 8.2, and 30.2 ± 4.3, respectively, whereas in their muscles it was 17.8 ± 1.5, 17.1 ± 2.1, and 12.6 ± 1.1, respectively. The tendon tissue temperature and $P_{O_2}$ increased gradually until 15–20 min after cutting the sciatic nerve, and the increasing rate depended upon the initial control level before denervation. Intravenous injection of noradrenaline in the dose of 1–9.9 µg/kg produced a marked decrease in the tendon tissue temperature of the tibialis anterior, but a mild one in the muscle. The longitudinal tension force produced a decrease in the tendon tissue temperature of the tibialis anterior, but no change in the muscle. During muscle exercise, tendon blood flow and temperature tended to decrease, whereas the muscle blood flow and temperature increased markedly from the beginning of muscle exercise. There was no evidence to demonstrate the existence of exercise hyperemia in the tendon tissue. These data suggest that tendon blood circulation can be modified by many factors, and that mechanical and exercise effects may play a role in regulation of tendon blood flow channels and fluid transfer for the lubrication of tendon fiber movements.

Key words: hydrogen gas clearance, tissue temperature, noradrenaline, tension force, muscle exercise.

Although a detailed structural study of tendon blood circulation in both animal and human preparations has been made (Arai, 1907; Mayer, 1916; Edwards, 1946; Brockis, 1953; Peacock, 1959; Nisbet, 1960; Smith, 1965), the
functional observation of tendon blood flow is less known (Håstad, et al., 1958/1959; Barlow et al., 1961; Schatzker and Bränemark, 1969; Strömberg, 1971). Barlow et al. (1961) have shown a slower clearance of $^{24}$Na in the resting tendon, as compared with the muscle. Strömberg (1971) has described an accelerated disappearance rate of $^{133}$Xe in in situ tendon fibers by exercise, and an inhibited one by adrenaline. Takemiya et al. (1982) have shown a significantly higher level of resting blood flow in the tendon of the rabbit hindlimbs, as compared with that in the adjacent muscle. However, more information about the functional characteristics of tendon blood circulation will be necessary to confirm this fact.

This paper deals firstly with the observation of tendon tissue blood flow by the hydrogen gas clearance method, and secondly with time-dependent changes of tendon tissue temperature and partly tissue $P_0_2$ in the conditions of intact, denervated, noradrenaline infused, and longitudinal tension developed, as well as during muscle exercise. Finally, this study has been designed to examine whether or not exercise hyperemia in the tendon exists.

**MATERIALS AND METHODS**

*Animals and preparations.* Thirty-five adult rabbits of both sexes weighing 2.5–3.5 kg were used in the experiment. The animals were weighed and anesthetized with an intraperitoneal injection of urethane (1 g/kg body weight). After a tracheal tube had been inserted, other surgical procedures were performed: the femoral artery and brachial vein were exposed for cannulation, the skin in the right lower limb was partially opened to insert electrodes, and the right sciatic nerve was exposed for the purpose of denervation and for the stimulation experiments. Before insertion of polyethylene catheters, sodium heparin (400–1,000 U) was infused intravenously. The rectal temperature (38°C) was monitored with a thermistor probe connected to a thermometer. Room temperature was constantly maintained at approximately 26°C using an air conditioner.

The mean arterial pressure was measured from the left femoral cannula with a pressure transducer at the level of the heart and recorded on a polygraph (Nihon Kohden, Co., Japan). The cannula tube was filled with Ringer solution containing heparin (1 U/ml). The heart rate and respiratory movement were monitored continuously throughout the experiments.

*Measurements of local blood flow, tissue temperature and $P_0_2$.* Tissue blood flow was measured in accordance with the hydrogen gas clearance method devised by Aukland (1964). A wire-type electrode of black Pt-Pt sensitive to hydrogen gas was used (Unique Medical Co., Ltd., Japan). The diameter was approximately 80 μm. Hydrogen gas within about 5% of the tidal volume was introduced through the tracheal cannula. The inhalation of hydrogen gas was stopped after it had been saturated in the tissue, and the subsequent clearance curve was subjected to calculation based on Kety's analytical method (Kety, 1960). Most of the data showed bi-exponential curve, and therefore their total blood flow (ml/(100 g·min))
was determined by the application of two-compartment analysis. The indifferent electrode (Ag-AgCl₂) was embedded in the distal subcutaneous tissue. The hydrogen gas clearance curve was recorded until the current returned to the control line.

Although the hydrogen gas clearance method is useful for the analysis of the mean tissue blood flow, it is inappropriate for the observation of time-dependent changes in the tissue blood flow. Therefore, as shown by RANDALL et al. (1953), we made observations of the tissue temperature instead of measuring the time-dependent changes in the tissue blood flow. Measurements of tissue temperature in the tendon and muscle were performed simultaneously by thermocouples. A sensor was made by processing the copper-constantan thermocouple into a needle-type electrode. The current from the sensor was designed to compensate linearly with the operational amplifier (Unique Medical Co., Ltd., Japan).

In vivo recording of oxygen tension was carried out according to the polarographic method, using a pair of wire oxygen sensors (Pt, 80 μm in diameter) covered by polymer membrane and an indifferent silver electrode. During a series of experiments, simultaneous measurements of systemic blood pressure, heart rate, and respiratory movement were made.

Experimental protocol. Neural control of the resting blood flow and time-dependent changes in tissue temperature and \( P_{O_2} \) in the tendon and muscle were observed and compared with each other by cutting the sciatic nerve. The effect of intravenous injection of noradrenaline on the tibialis anterior tendon tissue temperature was found effective in the doses of 0.5 to 9.9 μg/(kg·min). Mechanical effect of tension force on the tibialis anterior tendon tissue temperature was recorded continuously. The tendon bundle near the bone was hooked with a wire and pulled longitudinally toward the foot via a force transducer within the range of 20 to 200 g. Muscle exercise was produced by applying square-wave electrical stimuli with 1 ms duration and a voltage of 2–6 V, sufficient to elicit a maximal response from the peripheral end of the severed sciatic nerve. Two types of muscle exercise were performed with the frequency of 1 and 5 Hz for the periods of 1 min. Total blood flow was calculated from the fast and slow components of the clearance curve, and time-dependent changes in tissue temperature in the tendon and muscle were observed during exercise.

Compilation of determined values. The total blood flow in the tendon (and muscle) was calculated in ml/(100 g·min), and the tissue temperature was expressed in degrees Celsius (°C). Statistical comparison of the results was carried out by unpaired Student’s t-test.

RESULTS

Resting blood flow in tendon vs. muscle

Under the conditions of stable systemic blood pressure, resting blood flow in the denervated tendon and muscle tissue was determined in both fast and slow
components (Fig. 1). Average resting blood flow in the tendon of tibialis anterior, gastrocnemius, and soleus (mean ± S.E.(n)) was 36.1 ± 3.0(17), 33.5 ± 4.6(11), and 31.6 ± 2.7(28) ml/(100 g min), respectively, whereas in the muscles it was 17.9 ± 1.1(35), 17.3 ± 1.5(15), and 12.5 ± 1.2(37) ml/(100 g min), respectively. Significant differences were observed between tendon and muscle (*p<0.05, **p<0.01).

Effect of sciatic nerve cutting on tendon blood flow and $P_{O_2}$

The time-dependent change of local blood flow in tendon and muscle was observed by recording the local tissue temperature. A typical example of time-dependent increase in tissue temperature of the tendon and muscle tibialis anterior after cutting the sciatic nerve is shown in Fig. 2. Tissue temperature increased gradually until 15–20 min after denervation. Rate of increasing tissue temperature in both tendon and muscle depended upon the initial level before cutting the sciatic nerve, as seen in Fig. 2. In this case, the room temperature was 19°C. The rectal temperature was 38°C.

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Figure 3 shows a typical example of rapid increase in Achilles tendon tissue $P_O_2$ as compared with M. gastrocnemius after sectioning the sciatic nerve. The resting $P_O_2$ in average in the tendon fiber (mean ± S.D.($n$)) was 31.0 ± 14.7 (10) mmHg, and the value after denervation reached 39.2 ± 16.2 (10) mmHg. Reactive hyperemia-like $P_O_2$ increment after release of femoral arterial occlusion was not observed in the tendon tissue, although vasomotor-like oscillation of $P_O_2$ and reactive hyperemia-like $P_O_2$ changes were observed in the muscle. During muscle exercise, the adjacent tendon tissue $P_O_2$ tended to decrease.

**Effect of noradrenaline on tendon tissue temperature**

Tendon tissue temperature decreased markedly by intravenous injection of noradrenaline after the sciatic nerve had been cut, whereas the decrease was mild in the muscle. No remarkable effects on systemic blood pressure and respiratory rhythm were observed by noradrenaline infusion in the range of 3 μg/kg. However, when the amount of infusion exceeded 4 μg/kg, systemic blood pressure increased markedly. Figure 4 shows the average decrement of tendon tissue temperature against the increasing concentrations of noradrenaline. The resting tissue temperature (mean ± S.E.($n$)) before infusion ranged from 34.60 ± 0.39 (6) to 34.93 ± 0.22 (6)°C. The maximal decrement in tissue temperature ($\Delta$) was −0.60°C with 9.9 μg/kg noradrenaline.

**Effect of applying longitudinal tension force on tendon tissue temperature**

Figure 5 shows a typical change in tissue temperature in the denervated tendon
of the tibialis anterior. Temperature decreased gradually during a step-wise elevation in tension force, and returned completely to the control level after tension release. Figure 6 shows the average decrement of tendon tissue temperature as the function of the increasing tension force. The maximal depression of 0.95°C was obtained by the tension force of 167 g. The tension force within the range of 200 g did not have any influence on the change of muscle tissue temperature.

Effect of muscle exercise on tendon blood flow and tissue temperature

Muscle exercise evoked by stimulation of the sciatic nerve terminal produced a decrease in the total blood flow in the tendon which was calculated by the fast and slow components of the hydrogen gas clearance curve. As shown in Fig. 1, the average blood flow in the tendons tended to decrease by the contraction of their adjacent muscles, although there was no significant difference between resting and exercising tendon blood flow. Figure 7 represents a case of simultaneous measurement of time-dependent tissue temperature in the soleus tendon and the muscle. During exercise with 1 Hz stimulation for 2 min, tendon tissue temperature de-
Fig. 4. The average decrement of tibialis anterior tendon tissue temperature as a function of increase in noradrenaline concentration.

Fig. 5. A typical pattern of time-dependent depression of tibialis anterior tendon tissue temperature against the step-wise elevation of 150 g tension force. This pattern was obtained repeatedly in the range of level of tension applied.
Fig. 6. Decrement of average tissue temperature in tibialis anterior tendon against increase in external tension forces. The same external tension forces showed no effect on the muscle tissue temperature.

Fig. 7. A typical pattern of time-dependent change of soleus tendon tissue temperature during exercise of hindlimb induced by stimulation of the terminal of the cut sciatic nerve. Tendon tissue temperature in the soleus decreased gradually after start of exercise for 2 min and returned to the control without any over-shooting response, while simultaneous measurement of tissue temperature in muscle showed a typical pattern of both exercise hyperemia and post-exercise hyperemia.
creased gradually by about 0.8°C, and returned to the control without any sign of functional hyperemia. On the other hand, muscle tissue temperature showed an obvious feature of exercise hyperemia.

As shown in Fig. 1, average tendon blood flows (mean ± S.E. (n)) in muscle exercise (ml/(100 g • min)) were 29.3 ± 5.7 (6) at 1 Hz and 30.7 ± 3.6 (6) at 5 Hz in the tibialis anterior, 31.7 ± 5.6 (5) at 1 Hz and 37.3 ± 8.3 (5) at 5 Hz in the gastrocnemius, and 30.1 ± 5.6 (8) at 1 Hz and 23.8 ± 3.4 at 5 Hz in the soleus, respectively, whereas muscle blood flows were 34.6 ± 4.1 (17) at 1 Hz and 53.2 ± 7.1 (10) at 5 Hz in the tibialis anterior (p <0.05), 16.6 ± 2.3 (12) at 1 Hz and 21.9 ± 3.8 (8) at 5 Hz in the gastrocnemius, and 13.6 ± 3.0 (15) at 1 Hz and 15.5 ± 1.7 (16) at 5 Hz in the soleus, respectively.

DISCUSSION

The present data provide new evidence indicating significant biomechanical effect on functional tendon blood circulation. The major findings are summarized hereafter. 1) The resting blood flow in the tendon was larger than that in the muscle. 2) The resting tendon tissue temperature and tissue $P_{O_2}$ increased gradually after cutting the sciatic nerve. 3) Noradrenaline injection caused a significant decrease in tendon tissue temperature. 4) During application of mechanical tension force toward the osteotendinous terminal, the tendon tissue temperature decreased as a function of increasing tension load, but the muscle showed no change. 5) During local muscle exercise, the tendon tissue temperature decreased markedly, despite the profound increase in its adjacent muscle.

The resting tendon blood flow. Before applying the hydrogen gas clearance method to measure tendon tissue blood flow, we compared the values obtained by hydrogen gas clearance and drop count methods in the muscle, where the average muscle tissue blood flow and total venous outflow in the rabbit hindlimbs were 16.1 and 13.0 ml/(100 g • min), respectively (TAKEMIYA et al., 1981, 1982). These data are closely in accord with the report of MISHRA and HAINING (1980) who obtained the average value of 12.7 ml/(100 g • min) in the muscles of rabbit hindlimb by hydrogen gas clearance method. Therefore, we decided to extend our experiments to the adjacent tendon tissue.

In the previous report, TAKEMIYA et al. (1982) indicated the evidence of an increased resting blood flow in the tendon as compared with the adjacent muscle. The data also showed no linear relationship between the resting blood flow in the tendon, and the arterial blood pressure. The present data confirm the increase of resting tendon blood flow in the denervated tissues. Moreover, the regional and time-dependent characteristics of tendon blood flow were observed along a longitudinal tendon tissue. Most of the present data were obtained as the total blood flow calculated from the fast and slow components.

In 1958, Håstad et al. first tried to observe functional tendon tissue blood flow in man by the radioisotope clearance method. They showed only the mean
clearance constant for Na\(^+\) injected locally into the Achilles tendon to be significantly higher in the younger age groups than in the higher age groups (HASTAD et al., 1958/1959). In an animal experiment, BARLOW et al. (1961) observed slower clearance of \(^{24}\text{Na}\) in the resting intramuscular septa and tendon than in the muscle. It is worthy of note that the experiment was performed on the intramuscular tendon, and their semi-isolated biceps preparations were made by a tight ligature of the tendon near its origin. Furthermore, it can be assumed that segmental blood supply was isolated from the neighboring tissues. In the superficial flexor tendon of a horse, STRÖMBERG (1971) showed that the disappearance curve could be resolved into 2 or 3 components from the registered curve. He also observed that there was no difference in the disappearance rate between the three sites of injection at rest. Although the paper presented a mean tendon blood flow of 2.91 (ml/(100 g • min)) using the partition coefficient for \(^{133}\text{Xe}\) between the tendon and blood, simultaneous measurement of adjacent muscle blood flow was not shown in his results.

Careful consideration must be given to the fact that in his experimental condition the resting value of the disappearance rate of \(^{133}\text{Xe}\) was determined in the tendon of standing horses and was thus presumably affected by the influence of the nervous and mechanical control.

In contrast to the data obtained by the method of counting the radioisotopes, the present data measured by a wire-electrode sensor has shown the regional and time-dependent characteristics of tendon tissue blood flow along a longitudinal portion of tendon fibers. These functional values seem to coincide with the structural evidence presented by ARAI (1907), MAYER (1916), and EDWARDS (1946). In particular, the existence of longitudinal vessels with numerous transverse branches appears to be essential for the explanation of our higher level of tendon blood flow and \(P_{\text{O}_2}\) at rest compared with the muscle. Judging from the investigations on paratenon and mesotenon (MAYER, 1916; EDWARDS, 1946; NISBET, 1960; SMITH, 1965) and the functional and the anatomical basis of non-nutritive circulation in the tendon (BARLOW et al., 1961; GRANT and WRIGHT, 1970), the regional differences and time-dependent changes in tendon blood flow in the present experiments seem to be fully or partially dependent on the activity of non-nutritive channels and/or segmental blood vessels, and its regulation by other factors (see below). Since it is known that the tendon is a predominantly extracellular tissue with low metabolic requirements (ELLIOTT, 1965), the blood flow is considered to play another important role.

**Neurohormonal effect.** The present experiments show that the resting tendon blood flow with the denervated sciatic nerve is larger than that with the innervated. Furthermore, a gradual increase in tendon tissue temperature and \(P_{\text{O}_2}\) was observed just after the denervation, apparently indicating the increased tendon blood flow. It is well known that vascular resistance in the resting skeletal muscle is usually controlled by the sympathetic vasoconstrictor nerve, and that the surgical procedure of sympathectomy has been performed to effect improvement of muscle blood flow (BARCROFT, 1963; FOLKOW and NELL 1971; POWIS, 1974). Although no
information is available for the control of the sympathetic vasoconstrictor on tendon blood circulation, it is considered that the resting tendon blood flow may depend upon this nerve activity. Since the sympathetic vasoconstrictor runs down largely within the sciatic nerve to the muscle and partly along the vessel wall, the increase in muscle and tendon blood flow after nerve section appears to indicate the presence of activity of the sympathetic vasoconstrictor nerve.

Noradrenaline infusion in the doses of 1–4 μg/kg body weight markedly decreased tendon tissue temperature, but only slightly depressed muscle temperature, without causing any change of systemic blood pressure. After the maximal suppression by a single injection of noradrenaline, the tendon tissue temperature returned rapidly to the control level. Generally, noradrenaline and a little more amount of adrenaline have a constrictive effect on the vessels of the skeletal muscle and tendon in cats (Barlow and Walder, 1965) and horses (Strömberg, 1971), and of the bone marrow in dogs, cats, and baboons (Gross et al., 1979). Particularly, Strömberg (1971) observed a prompt decrease in the disappearance rate of 133Xe after administration of adrenaline. An intravenous administration of adrenaline appears to have induced a strong vasoconstriction in the superficial flexor tendon of race horses. Thus, it is considered that the resting tendon blood flow is regulated by neurohormonal factors.

Biomechanical-exercise effects. During local muscle exercise, venous outflow from the muscle increased in proportion with the intensity of exercise, under the condition of stable systemic blood pressure (Barcroft, 1963; Hudlická, 1973; Takemiya et al., 1981). The exercise hyperemia in the rabbit hindlimb was also confirmed by our hydrogen gas clearance method (Takemiya et al., 1982), but tendon tissue blood flow showed a tendency to decrease on increasing the stimulation frequency from 1 to 10 Hz. This tendency was marked at the early stage of muscle exercise. However, as observed in our other data, during long-lasting exercise, tendon tissue temperature increased slightly, presumably due to the secondary effect of blood flow increase from the neighboring tissues, including musculotendinous, osteotendinous, and mesotenon channels (Edwards, 1946; Smith, 1965). Strömberg (1971) reported only a slight effect of muscle exercise on intratendinous blood flow, in which he showed a post-exercise increase in the 133Xe disappearance rate. The data on horses were obtained after 10-min exercise on a treadmill, with a distinct rise in post-exercise venous hematocrit and heart rate. This is in agreement with the present observations mentioned above.

The mechanism of decreasing tendon blood flow during muscle exercise was investigated by the application of biomechanical tension force. As presented in Fig. 6, the longitudinal tension force toward the osteotendinous terminal obviously decreased the tendon tissue temperature against the increase in tension forces. Since Mayer (1916) has already sketched the disappearance of blood vessels on the surface of the tendon fibers during muscle contraction, the functional change in tendon blood flow seems to be influenced by neuromechanical factors. Thus, the author’s interest was focused on the in vivo characteristics of the tendon vascular
structure and the related function. As shown by Edwards (1946), the tendon microvascular system consists of longitudinal arteriolar vessels with transverse channels and arcades of decreasing size, and of venolymphatic channels. It is reasonable to postulate that the mechanical factor may at first influence the venous channel to restrict blood flow, then the capillaries, and finally the other vessels, including the arterioles (Schatzker and Bränemark, 1969). Furthermore, microvascular dimensional configuration of the segmental blood supply system appears to have a decreasing effect on intratendinous blood circulation during forced to-and-fro movements of the tendon (Edwards, 1946; Smith, 1965; Colville et al., 1969). However, as shown in time-dependent experiments, the tendon vascular capacity seems to be large enough to respond to the enhanced systemic circulation in daily work or active exercise.

The author was further interested in the postulation that the exercise-induced mechanical compression and configuration of the tendon vasculature are closely associated with the plasma fluid filtration and drainage (Lundborg and Rank, 1978; Knight and Levick, 1982). According to the observation of lymphatics in the tendon tissue (Edwards, 1946), lymph vessels are seen to be parallel with blood vessels, and, furthermore, each artery is accompanied by two veins and four lymphatics. Therefore, it is reasonable to assume that the mechanical movement of the tendon, in addition to the neurohormonal controls, induces a proper pressure difference between arterioles and venules to regulate tendon blood flow in the fast capillary channel accompanying fluid transfer in the slow capillary channel.

In summary, the present study confirms that the resting blood flow in the tendon is larger than that in the muscle. Effects of sciatic nerve section and/or noradrenaline infusion demonstrate the existence of neurohormonal control on tendon blood circulation. Furthermore, there is no evidence to demonstrate the existence of exercise hyperemia in the tendon tissue. It seems reasonable to assume that longitudinal tension force and muscle exercise play a role in mechanical regulation of tendon blood flow channels and fluid transfer so as to lubricate tendon fiber movements.

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