Changes in Blood Circulation of the Tendons and Heart Rate Variability During and After Acupuncture

Keitaro Kubo, PhD,¹ Yojiro Iizuka, MS,¹ Hiroyoshi Yajima, PhD,²
Miho Takayama, PhD,² and Nobuari Takakura, PhD²

ABSTRACT

Objective: The purpose of this study was to investigate the changes in blood circulation of tendons and heart rate variability (HRV) during and after acupuncture in order to elucidate the mechanisms of acupuncture-induced changes in tendon blood circulation.

Materials and Methods: During 10 minutes of acupuncture treatment and a recovery period of 40 minutes, blood volume (THb) and oxygen saturation (StO₂) of treated and nontreated tendons were measured using red laser lights. In addition, HRV was also measured during the experimental period.

Results: THb and StO₂ of the treated tendons significantly increased during acupuncture, and then remained high during the recovery period. THb and StO₂ of the nontreated tendons did not change during acupuncture but gradually increased during the recovery period. In addition, the increase in THb of the nontreated tendons were significantly correlated with that of the treated tendons during the latter half of the recovery period. Heart rate (HR) continued to decrease during acupuncture and suddenly returned to the initial level after removal of the needle. The low-frequency (LF)/high-frequency (HF) ratio tended to increase during the recovery period. During the latter half of the recovery period, the changes in THb for both the treated and nontreated tendons were negatively correlated with the change in HR but not with changes in the LF/HF ratio.

Conclusions: These results suggest that changes in blood circulation of tendons after acupuncture treatment were controlled by the autonomic nervous system, which could not be detected by HRV analysis.

Keywords: needle, blood volume, autonomic nervous system, recovery

INTRODUCTION

TENDON INJURIES CAUSED BY OVERUSE (e.g., tendinopathies) are common among recreational and competitive athletes. In athletic and medical fields, acupuncture has been used to heal connective-tissue (tendon and ligament) injuries.^{1,2} Healing of injuries was considered to be related to improved blood flow in each tissue.^{3,4} Indeed, in 2 earlier studies the current authors found that blood volume and oxygen saturation (StO₂) of the Achilles tendon increased during acupuncture.^{5,6} In addition, the blood volume and StO₂ of the nontreated (contralateral) tendon increased gradually after removal of the needle, whereas these values

did not change during acupuncture treatment.⁶ However, the mechanisms leading to the changes in blood circulation of tendons during and after acupuncture have not yet been elucidated.

Previous studies demonstrated a transient decline in heart rate (HR) during acupuncture.^{7–9} According to the findings of pharmacologic studies,^{7,10} reduction of the HR associated with acupuncture stimulation was due to inhibition of the sympathetic nervous system and activation of the parasympathetic nervous system. One of current authors' previous studies showed that the amount of increase in the blood volume of the non-acupuncture-treated (contralateral) tendon was highly correlated with that of the acupuncture-treated

¹Department of Life Science (Sports Sciences), The University of Tokyo, Tokyo, Japan.

²Department of Acupuncture and Moxibustion, Tokyo Ariake University of Medical and Health Science, Tokyo, Japan.

tendon during the last phase of the recovery period.⁶ This suggests that changes in blood circulation for both treated and nontreated tendons would be caused by the central nervous system. Yet, HR variability (HRV) analysis has been used to evaluate a condition of the autonomic nervous system (ANS) noninvasively.¹¹

According to the findings of several studies,^{9,12–14} parasympathetic activity (i.e., the high-frequency [HF] component of HRV) increased significantly and the sympathetic activity (i.e., low-frequency [LF] component and ratio of LF to HF components of HRV) decreased as a result of acupuncture stimulation. Using this technique, it might be possible to clarify the relationship between changes in tendon blood circulation and HRV (i.e., condition of the ANS) during and after acupuncture treatment.

The present study was conducted to investigate changes in blood circulation of tendons and HRV during and after acupuncture in order to elucidate the mechanisms of acupuncture-induced changes in tendon blood circulation. The hypothesis was that the parasympathetic activity (i.e., HF component of HRV) increased after removal of the needle and that this change would be related to changes in blood circulation of treated and nontreated (contralateral) tendons.

MATERIALS AND METHODS

Participants

Twenty-one healthy male volunteers (mean $\pm$ standard deviation [SD]: age: 28.5 ± 8.6 years; height: 171.6 ± 4.5 cm; body mass index [BMI]: 70.6 ± 11.1 kg) participated in this study. The participants were either sedentary or mildly to moderately active men. They were fully informed of the procedures to be utilized as well as the purpose of this study. Written informed consent was obtained from all participants. This study was approved by the office of the Department of Sports Sciences, of The University of Tokyo, in Tokyo, Japan, and complied with this institution's requirements for human experimentation.

Acupuncture

The procedure of acupuncture treatment was similar to that used in the current authors' previous study.⁶ Each subject lay in a comfortable prone position on a test bench to get used to the laboratory conditions for 20 minutes before

FIG. 1. The changes in (A) oxyhemoglobin (Oxy), (B) deoxyhemoglobin (Deoxy), (C) total hemoglobin (THb), and (D) oxygen saturation (StO₂) of treated (clear squares) and nontreated (black circles) tendons during acupuncture treatment and recovery periods. *Significantly different from the resting level (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

the experiment. At its start, the subject lay in a prone position for a 15-minute rest period (baseline). After that, a needle was inserted into the subject's right Achilles tendon by an experienced licensed acupuncturist (who is one of the authors). A stainless steel needle (0.16 mm in diameter and 40 mm in length) was inserted vertically into the skin at 40 mm proximal to the calcaneus in the right foot. After insertion to a targeted depth (3 mm), the needle was left in place for 5 minutes without any manipulations (Acu-1). Then, the tip of the needle was moved up and down (up-and-down manipulation) at 1-mm amplitude and 2 Hz for 3 minutes (Acu-2). After that, the needle was left for 2 minutes without any manipulations (Acu-3). After the needle was removed, the subject remained relaxed in the same position for 40 minutes.

For the control condition, each subject laid in a comfortable prone position for 50 minutes after a 15-minute rest period. Acupuncture and control conditions were performed for each subject on 2 separate days, with at least 2 weeks between sessions, but no longer than 3 weeks were allowed between the two conditions. The order of the performance of the two conditions was randomized for each subject.

Blood Circulation of the Achilles Tendon

During the experimental period, the blood circulation—oxyhemoglobin (Oxy), deoxyhemoglobin (Deoxy), total hemoglobin (THb), and StO_2 —of the Achilles tendon of each subject was measured on both the treated and nontreated sides using a previously described procedure.^{5,6} To measure the blood circulation of the tendon using red laser lights (BOM-L1TRSF, Omegawave, Tokyo, Japan), a probe was positioned at 30 mm proximal to the calcaneus. With three red laser lights (635, 650, and 690 nm), this instrument can be used to calculate the relative tissue levels of Oxy, Deoxy, and THb (corresponding to the blood volume) according to the Beer–Lambert law.¹⁵ Oxy, Deoxy, THb, and StO_2 at a specific depth (a measurement depth of 3–5 mm) of the tissue can be measured by changing the location of two point detectors.¹⁵ The details of this technique and principle of this instrument (red laser lights) were described in 2 more of the current authors' previous studies.^{16,17} In addition, the precision and validity of this procedure were presented and discussed in these previous studies.^{16,17}

In the present study, the units of Oxy, Deoxy, and THb were expressed as $\mu\text{mol/L}$, although this did not represent the actual physical volume. StO_2 was calculated from Oxy and THb values using the following formula:

$$\text{StO}_2(\%) = 100 * \text{Oxy}/\text{THb}.$$

FIG. 2. Relationship between the increase in total hemoglobin (THb) of treated and nontreated tendons during (A) 0–10 minutes, (B) 11–20 minutes, (C) 21–30 minutes, and (D) 31–40 minutes of the recovery period.

The resulting data were input into a personal computer at a sampling frequency of 10 Hz via an A/D transducer (Power Lab, ADInstruments, New South Wales, Australia). The mean values over a given duration (5 minutes for Acu-1, 3 minutes for Acu-2, 2 minutes for Acu-3, and every 10 minutes during the recovery period) were calculated using analytical software (Chart, version 7.1, ADInstruments). Oxy, Deoxy, THb, and StO₂ data were presented as the amount of increase from the resting level. The repeatability of the blood circulation (Oxy, Deoxy, THb, and StO₂) measurements for the tendon during resting had been investigated in these previous studies.^{16,17}

HEART RATE VARIABILITY

During the experimental period, HRV was measured using Biocom's HRV LIVE, version 1.0 (Biocom, Inc., Seattle, WA), which is equipped with software that incorporates algorithms of short-term HRV analysis. A Biocom HRM-02 Pulse Wave Sensor (the photoplethysmography monitor used in the present study) was clipped to each subject's right earlobe. This instrument for measuring HRV was recommended by The European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Spectral transformation of R-R interval variability generated HF power (> 0.15 Hz) and LF power (0.04–0.15 Hz). The HF and LF values represented the activities of the parasympathetic nervous system (PNS) and sympathetic nervous system (SNS), respectively.^{18,19} The LF/HF ratio was considered to reflect sympathovagal balance or sympathetic modulations.^{20,21} These data were presented as the amount of change from the resting level, and the mean values over a given duration (Acu-1, Acu-2, Acu-3, and every 10 minutes during the recovery period) were calculated.

Statistics

Descriptive data included means $\pm$ SD. Regarding the blood circulation of tendons, a two-way (side $\times$ time) analysis of variance (ANOVA) with repeated measures was used to detect significant differences in the measured variables from the resting level. For HRV, 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 an ANOVA was significant, differences from the resting level were assessed by the Bonferroni *post-hoc* test. Linear regression analysis was performed of the relationship between the measured variables. The level of significance was set at $P < 0.05$.

RESULTS

The changes in the measured variables of tendon blood circulation during and after acupuncture treatment are

shown in Figure 1. For Oxy, the effect of time ($P < 0.001$) was significant, although the effect of side ($P = 0.083$) and the interaction between side and time ($P = 0.081$) were not significant. For Deoxy and StO₂, the effect of time ($P = 0.011$ for Deoxy; $P < 0.001$ for StO₂) was significant,

FIG. 3. Changes in (A) heart rate, (B) low-frequency (LF), (C) high frequency (HF), and (D) LF/HF ratio during acupuncture treatment and recovery periods. min, minutes. *Significantly different from the resting level (* $P < 0.05$).

although the effect of side ($P=0.652$ for Deoxy; $P=0.271$ for StO₂) and the interaction between side and time ($P=0.648$ for Deoxy; $P=0.332$ for StO₂) were not significant. For THb, the effect of time ($P<0.001$) and the interaction between side and time ($P=0.045$) were significant, although the effect of side was not significant ($P=0.053$). During the 21–30 and 31–40 minutes of the recovery period, the increase in THb of the nontreated tendons was significantly correlated with that of the treated tendons (Fig. 2).

The changes in HR, LF, HF, and LF/HF ratio are shown in Fig. 3. The HR continued to decrease during acupuncture treatment regardless of the maneuvers (Acu-1, Acu-2, and Acu-3), and suddenly increased to the resting level after removal of the needle ($P=0.024$). LF ($P=0.457$) and HF ($P=0.807$) did not change during the experimental period. The LF/HF ratio did not change during acupuncture treatment, and then tended to increase during the recovery period. Although the effect of time on the change in the LF/HF ratio was significant ($P=0.013$), *post-hoc* analysis did not identify significant differences at any timepoint. During

Acu-2, the changes in THb for both the treated and nontreated tendons were not significantly correlated with those in the HR or LF/HF ratio (Fig. 4). During the latter half of the recovery period (21–40 minutes), the changes in THb for both the treated and nontreated tendons were significantly correlated with those in the HR, although they were not correlated with those in the LF/HF ratio (Fig. 5).

Under the control condition, none of the measured variables changed during the experimental period (Fig. 6).

DISCUSSION

The main findings of this study were:

- (1) HR continued to decrease during acupuncture treatment regardless of the maneuver and suddenly returned to the resting level after removal of the needle
- (2) LF, HF, and LF/HF did not change during the experimental period

FIG. 4. Relationship between changes in the heart rate (A and B) and low-frequency/high frequency ratio (C and D) and those in total hemoglobin (THb) of the treated (A and C) and nontreated (B and D) tendons during acupuncture with up-and-down manipulation (Acu-2).

FIG. 5. Relationship between changes in the heart rate (A and B) and low-frequency/high-frequency ratio (C and D) and those in total hemoglobin (THb) of the treated (A and C) and nontreated (B and D) tendons during the latter half of the recovery period.

- (3) Changes in THb for both the treated and nontreated tendons were negatively correlated with the change in HR during the latter half of the recovery period.

In the present study, the same needling point was used on the Achilles tendon as in 2 of the current authors' previous studies^{5,6} because there is neither a meridian nor an acupoint on this tendon to insert a needle directly into this tendon with ease. Increase in blood circulation of the skin and the muscles with acupuncture was observed via axon reflex or autonomic reflex by activation of somatic afferent nerves of group III and IV fibers.^{22,23} To increase blood circulation in the tendon via both mechanisms, the skin on the tendon could be the best place to insert needles. This applies especially to the axon reflex, a robust and essential response to peripheral tissue microinjury, for inducing vasodilation to increase local blood flow, which could facilitate healing.^{24,25} Considering the lack of acupoint specificity in the local musculoskeletal action of acupuncture and the clinical aspects of this phenomenon, the point on the center line of

the dorsal side of the Achilles tendon was used. This is a good site to insert a needle into this tendon without difficulty a subject who is face down.^{5,6}

As in 2 of the current authors' previous studies,^{5,6} THb and StO₂ increased significantly during Acu-2 in the acupunctured tendons, and these two high values remained during the recovery period. In the nontreated tendons, THb and StO₂ did not change during acupuncture treatment, but these values increased gradually during the recovery period. In addition, the increase in THb of the nontreated tendons was significantly correlated with that of the treated tendons during the latter half of the recovery period. These results agreed with the findings of 2 previous studies.^{5,6} These phenomena suggested that an axon reflex occurred in the acupunctured tendons, as reported in the skin, and that the muscles somatovisceral reflex via the CNS was slightly involved to increase blood circulation.^{5,6}

HR continued to decrease during acupuncture treatment regardless of the maneuver (Acu-1, Acu-2, and Acu-3) and began to recover to the resting value after removal of the

FIG. 6. Changes in (A) total hemoglobin (THb); and (B) oxygen saturation (StO_2) of treated (clear circles) and nontreated (black squares) tendons. (C) heart rate and (D) low-frequency/high frequency ratio during no treatment and recovery periods (control condition). min, minutes.

needle. Several studies showed that HR decreased during and after acupuncture.^{7-9,12,14} For example, Uchida et al.⁹ reported that HR during 2 minutes of acupuncture stimulation (needle moved vertically at a frequency of 1 Hz) was significantly lower than before and after acupuncture. Yet, other researchers showed that HR did not change during acupuncture stimulation and then significantly decreased after stimulation.^{12,14} According to the findings of Sugiyama et al.,⁸ when the needle was rotated intermittently for 30 seconds at 5-minute intervals, HR suddenly decreased during rotation of the needle (at 30 seconds) and returned to the resting value during the recovery period (5 minutes). Although the reasons for the discrepancy between the previous and present findings are unknown, differences in the stimulated sites might be involved. In the present study, a needle was inserted into the Achilles tendon, whereas a needle was inserted into a very specific acupuncture point in the previous studies.^{7-9,12,14}

Given the decrease of HR, in any case, involvement of autonomic reflexes is not totally deniable for THb changes in the acupunctured tendons. However, THb for the treated tendons increased during acupuncture with up-and-down manipulation (Acu-2), whereas THb for the nontreated (contralateral) tendons did not increase (Fig. 1C). Thus, the change in blood circulation of the acupunctured tendons (Acu-2) could be induced by the axon reflex as suggested by Kashiba and Ueda.²⁶

Several studies showed that the LF/HF ratio decreased significantly during and after acupuncture stimulation, which indicates that acupuncture inhibits activity in the SNS and has an opposite effect on the PNS.^{9,27,28} Furthermore, pharmacologic studies showed that HR reduction induced by acupuncture stimulation led to inhibition of the SNS function and enhancement of the PNS function as mentioned earlier.^{7,10} In the present study, however, such a decrease in the LF/HF ratio was not noted during acupuncture and the recovery period. Yet, the possibility of ANS involvement cannot be ruled out because the THb of the nontreated and the acupunctured tendons increased in parallel (Fig. 2) and correlated negatively with HR change (Fig. 5AB). The discrepancy between the present and previous studies could be explained by the differences in the stimulated sites (not an acupoint in the present study and an acupuncture point in the previous studies) and duration of acupuncture with up-and-down manipulation (3 minutes in the present study; 15–60 seconds in most previous studies).

With respect needling site in particular, acupoint specificity was shown by functional magnetic resonance imaging studies to elicit responses in specific areas of the brain by stimulation at an acupoint. This is a distinct phenomenon from stimulation of other points^{29,30} or neurophysiologic studies designed to elicit a cardiovascular response exclusively with stimulation at acupoints.³¹

When considering these studies, it is necessary to explore how ANS action participates in changes of blood circulation

in the Achilles tendon, using HRV analysis with respect to acupoint specificity. Furthermore, as Uchida et al.⁹ stated, a limitation of HRV analysis has to be pointed out with regard to using this kind of analysis to determine the state of the ANS. It is, thus, necessary to investigate the effect of the ANS on the change in tendon blood circulation caused by acupuncture stimulation with other techniques (e.g., a pharmacologic method).

There were several limitations of this current study. First, the time of day when the testing was performed was not uniform among the subjects. Measurement of each subject should be scheduled at the same time of day because HRV data are influenced by circadian fluctuations.³² Therefore, one cannot deny that these considerations could affect the main results of this study. Second, previous exposure to acupuncture could affect the changes in blood circulation of tendons and HRV. Boutouyrie et al.³³ reported that the arterial diameter in subjects previously exposed to acupuncture increased significantly during acupuncture treatment, whereas in naive subjects, this did not occur. Almost all of the subjects in the present study had previous acupuncture experiences. In a future study, it is necessary to clarify the effects of previous exposure to acupuncture on the measured variables.

CONCLUSIONS

The increase in THb of the treated tendons was not related to that in the HR or LF/HF ratio during acupuncture treatment. Furthermore, the subjects with lower HRs showed a higher THb in the treated and nontreated tendons after removal of the needle. These results suggest that the changes in blood circulation of tendons after acupuncture treatment might be controlled by the upper CNA, which the current authors were not able to detect by HRV analysis.

AUTHOR DISCLOSURE STATEMENT

No financial conflicts of interest exist..

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Address correspondence to:

Keitaro Kubo, PhD

Department of Life Science (Sports Sciences)

The University of Tokyo

Komaba 3-8-1

Meguro-ku, Tokyo 153-8902

Japan

E-mail: kubo@idaten.c.u-tokyo.ac.jp