Effects of percutaneous acupuncture stimulation on the viscoelastic properties of tendon during isometric contraction

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Abstract

This study aimed to investigate the effects of percutaneous acupuncture stimulation on the viscoelasticity of human tendon structures during isometric contraction. Nine healthy men participated. The experimental order was pre-test, acupuncture stimulation, and post-test. Real and sham acupuncture applications were used at the stimulus site of the medial gastrocnemius muscle (MG), and a crossover trial was performed on the same subjects at a later date. Before and after acupuncture stimulation, tendon elongation and MG aponeurosis were directly measured by ultrasonography while the subjects performed isometric plantar flexions up to the maximum voluntary contraction (MVC) followed by relaxation. The relationship between the estimated MG muscle force (Fm) and tendon elongation (L) during the ascending phase was fitted to a linear regression, the slope of which was defined as the stiffness of the tendon structures. Additionally, the ratio (%) of the area within the Fm-L loop to the area under the curve during contraction and relaxation was calculated and defined as hysteresis. Stiffness rate of change (RC) in real and sham acupuncture was 137.5 ± 116.5% and 55.0 ± 10.4%, respectively (p < 0.05). Thus, real acupuncture demonstrated significantly higher values than sham acupuncture. The hysteresis measurement results in real acupuncture indicated a downward tendency (pre-treatment: 25.6 ± 5.1%, post-treatment: 16.1 ± 13.0%), while sham acupuncture indicated an upward tendency (pre-treatment: 26.5 ± 10.9%, post-treatment: 28.4 ± 6.9%). These results indicated that percutaneous acupuncture stimulation reduces hysteresis, enhances stiffness, and improves the viscoelasticity of tendon structures.

Keywords: Acupuncture, elasticity, tendon, ultrasonography, viscosity
1. Introduction

Acupuncture has been used in various fields with an expectation of efficacy. The primary purpose of using acupuncture in the sports field is conditioning, such as the prevention of disorders, relieving muscle tension and fatigue, relaxation, and health management. Improving sports performance is such an important issue for all athletes that many of them would employ every available means to realize this purpose, including acupuncture, even if the scientific validation of its effect is not yet fully established.

The U.S. National Institutes of Health (NIH) 1997 consensus statement pointed out that it is necessary to for the effects of acupuncture to be researched and evaluated based on scientific evidence, rather than by ambiguous experiential evaluation [1]. Some of the physiological mechanisms and scientific explanations of acupuncture have gradually become clear, such as the sensory receptors that become activated in response to acupuncture stimulation [2], the reflection and communication mechanisms [3], and its impact on endocrine regulation and the autonomic nervous system [4]. However, very little research can be found that has verified how acupuncture stimulation acts upon exertions during actual physical exercises [5].

Every movement the human body performs, including during sports, is comprised of joint motions and achieved through skeletal muscle contractions [6]. The skeletal muscles are attached to bones by tendon structures, and move joints by exerting contractile forces. This function is finely controlled by the nervous system, and allows the performance of physical movements. Skeletal muscles comprise the muscle-tendon complex (MTC), which includes muscle and tendon structures with contractile and elastic forces, respectively [7]. The MTC enables us to perform various physical movements.

Therapies such as tack needles are used in percutaneous acupuncture for sports conditioning, and are expected to attain sustained stimulation of the low-invasive plaster type. The mechanism by which acupuncture acts upon MTC activities and regulates the smoothness
of physical movements has yet to be fully elucidated.

Recently, the use of ultrasonography has enabled direct observations of muscle-tendon activities in vivo. It has also made it possible to determine MTC functions with clues to the dynamics of tendon structures during muscle contraction. Using ultrasonography, previous research has observed the dynamic states of tendon structures during isometric contraction and investigated the dynamic properties of the MTC [8-10]. By utilizing the same method, we aimed to observe biological tendon and muscle responses to acupuncture stimulation and to clarify its effects on the viscoelasticity of human tendon structures.

Acupuncture has been experientially applied for releasing stiffness. If the mechanism by which acupuncture influences MTCs could be clarified, it could be used more appropriately for sports conditioning and could contribute to sports performance improvements. Therefore, by using tack needles that provide sustained stimulation, even during physical movements, we attempted to elucidate the effects of acupuncture on the viscoelastic properties of tendon structures that significantly contribute to MTC exertion.

2. Material and Methods

2.1. Subjects

Nine healthy volunteer men with no history of serious injuries to the lower limbs (age: 31.1 ± 8.7 years, height: 171.3 ± 5.6 cm, body mass: 66.8 ± 8.0 kg, mean ± SD) were enrolled. Prior to the experiment, the purpose, details, and potential risks of this study were explained to the subjects, and written, informed consent was obtained.

2.2. Experimental procedure
The experiment proceeded in the order of pre-measurement (pre-test), acupuncture stimulation (with tack needles), and post-measurement (post-test). Before and after acupuncture stimulation, the torque produced during isometric plantar flexion and elongation in the medial gastrocnemius muscle (MG) tendon structures were determined by a dynamometer (Cybex 770-NORM, Cybex International, Medway, MA, USA) and ultrasonography, respectively.

2.3. Acupuncture stimulation

During a 15-min break after the pre-task, real and sham acupuncture stimulation was applied by attaching tack needles (Pyonex) to the right leg. In order to avoid placebo effects, both real and sham acupuncture needles were prepared with identical shapes; the needles were randomly chosen without informing the subjects, and applied to the stimulus site (the middle of the gastrocnemius muscle belly) (Fig. 1). Furthermore, as a crossover experiment, a second trial was performed on each subject by replacing the needle with the needle type not used during the first trial, i.e., real to sham, or vice versa. To ensure the effects from the first trial would not interfere, a sufficient interval was arranged between the trials.

To ensure the validity of the blinded test, subjects were asked about sensations from acupuncture stimulation during each trial. The results of the inquiries were as follows. After each trial, all were asked if they felt the sensation of a needle being inserted. During real acupuncture, five replied “yes” and four replied “no;” during sham acupuncture, six replied “yes” and three replied “no” (kappa coefficient $[\kappa] = -0.11$). Thus, it was concluded that the blinded test was valid.
2.4. Measurement of the Viscoelastic Properties of Tendon Structures

2.4-1. Measurement of torque

Each subject lay prone on a bed and his right ankle joint was fixed to the dynamometer pedal. The pelvis and thighs were both fixed to the bed of the Cybex with suitable belts. The knee of the tested leg (the right) was fully extended, and the ankle joint was placed in a neutral anatomical position on a footplate and firmly fixed with a strap (Fig. 1).

Firstly, to measure maximum voluntary contraction (MVC), the subjects were instructed to exert plantar flexion MVC torque and hold it for 3 s. Measurements of each task were performed at least twice. To detect accidental errors of greater than 5%, one more trial was added, and the maximum value was determined as the MVC torque. Secondly, after being provided ample time to rest after the MVC task, each subject was instructed to develop a gradually increasing force from relaxation to MVC for 3 s, hold it for 1 s, and release back to a relaxed state for 3 s. Measurements of each task were performed at least three times. To detect accidental errors of greater than 5%, one more trial was added, and the maximum torque value was used for the analysis. At least a 3-min interval was provided between trials. The torque signals were converted from analog to digital at a sampling rate of 1 kHz (Contac ADA16-32/2(CB)F) and analyzed by a personal computer. Then, the measurements, along with the post-task, were performed in the same manner as the pre-task.

2.4-2. Electromyogram (EMG) Measurements

The electromagnetic activities of the tibialis anterior (TA), MG, lateral gastrocnemius (LG), and soleus (SOL) muscles were recorded during isometric plantar flexion. Then, Ag/AgCl electrodes with a built-in preamplifier (DE-2.3 10, Delsys, Boston, MA, USA) were placed over the bellies of the respective muscles. The obtained EMG signals were amplified by an
EMG amplifier (Bagnoli Desktop EMG systems, Delsys, Boston, MA, USA; filtering frequency: 50 Hz), analog-to-digital converted, and transmitted to a computer at a sampling rate of 1 kHz. EMG signals were amplified with band-pass filtering from 15.92 Hz to 600 Hz. The maximal and mean EMG signal values were computed after full-wave rectification. The mean values used for the analysis were those of maximum torque for the MVC.

2.4-3. Measurement of tendon structure elongation

On the basis of the procedure described by Kawakami et al. [11], longitudinal ultrasonographic images of the MG muscle were obtained using a real-time ultrasonography apparatus (M-Turbo, SonoSite, Bothell, WA, USA) and recorded at 30 Hz. The probe used had a frequency of 7.5 MHz and was longitudinally attached to the dermal surface of the subject’s calf with double-faced adhesive tape and was held manually to prevent it from sliding. The site chosen for the probe attachment was approximately 30% distal from the popliteal crease to the center of the lateral malleolus.

At this point, longitudinal ultrasonographic images were obtained, recorded at 30 Hz, transmitted to a computer, and analyzed by image analysis software (ImageJ, NIH, Bethesda, MD, USA). On the basis of the procedure described by Muramatsu et al. [12], the displacement distance of the cross-point of the two echoes from the MG fascicles and deep aponeurosis was calculated as the subject performed a gradually increasing (ramp) isometric plantar flexion up to the MVC. The intersection of a clearly visible fascicle with the aponeurosis was identified for the MG and digitized at every 10% of the MVC pre-test torque by using software (ImageJ, NIH, Bethesda, MD, USA). The displacement of each intersection was defined as the tendon elongation during the ramp isometric contractions. In this calculation, the muscle contraction speed was not identified as a distinguishing condition.
2.5. Calculations of stiffness and hysteresis

The obtained torque values were converted to MG muscle force according to the following equation [13]:

\[
F_m = k \times TQ \times MA^{-1}
\]

where \( F_m \) (N) is the muscle force produced by the MG resulting in the load on the Achilles’ tendon during plantar flexion, \( k \) is the relative physiological cross-sectional area (PCSA) of the MG to the total PCSA of the plantar flexors of the ankle, as reported by Fukunaga et al. [14], and \( TQ \) (Nm) is the measured torque. \( MA \) is the moment arm length of the triceps surae muscles at the ankle joint at 90°, which was estimated from the leg length of each subject. The \( MA \) value was evaluated according to the procedure reported by Rugg et al. [15], which Kubo et al. [16] also applied.

The torque value during the ramp isometric contraction was synchronized to the tendon force output evaluated in the above-mentioned procedure. Since the performed ramp isometric contraction plantar flexion up to the MVC both in pre- and post-tests was 80% of the MVC for all subjects, 50–80% of the MVC was analyzed with a linear regression equation by using minimum multiplication, the slope of which was adopted as the stiffness of the MG muscle/tendon [17] (Fig. 2).

The ratio (%) of the area within the \( F_m-L \) loop to the area under the curve during the ramp isometric contraction and relaxation was calculated in the following manner [18]. Firstly, elastic energy 1 (Ee1) was calculated from the area of the \( F_m-L \) relationship in the ascending phase. Secondly, in the same procedure, elastic energy 2 (Ee2) was calculated from the area of the \( F_m-L \) relationship in the descending phase (Fig. 3). Thus, the following equation is formulated:
$H = \frac{(Ee1 - Ee2)}{Ee1} \times 100\%$

2.6. Statistics

For all descriptive data, the values are presented as the mean ± SD. The values are presented to the first decimal point after rounding off the second decimal point. Values of each item were compared using a paired Student’s t-test between the groups. F ratios were considered significant at $p < 0.05$.

3. Results

3.1. MVC and EMG

EMG changes in the lower leg muscles (TA, MG, LG, and SOL) were measured to evaluate changes in their forces during MVC exertion and their contributions during isometric plantar flexion before and after percutaneous acupuncture stimulation. Table 1 shows the relative changes in MVC torque and integrated electromyogram (iEMG) values of the TA, MG, LG, and SOL during ramp isometric plantar flexion before and after acupuncture stimulation. No significant difference was found between pre- and post-acupuncture stimulation values.

3.2. Stiffness

Stiffness changes between the pre-test and post-test were measured for both real and sham acupuncture and compared as rates of change. Fig. 4 shows relative changes in stiffness between pre- and post-acupuncture stimulation. In real acupuncture, no significant difference was observed (pre: $31.4 \pm 17.9$ N[mm]$^{-1}$, post: $32.5 \pm 18.0$ N[mm]$^{-1}$). In sham acupuncture, stiffness changed significantly after stimulation (pre: $31.7 \pm 14.4$ N[mm]$^{-1}$, post: $16.8 \pm 6.7$ N[mm]$^{-1}$, $p < 0.05$). The rate changes (RCs) in real and sham acupuncture were $137.5 \pm$
116.5% and 55.0 ± 10.4%, respectively. Thus, there was a significant difference between real and sham acupuncture (p < 0.05).

3.3. Hysteresis

Hysteresis percentages (%) in the pre-test and post-test were compared for both real and sham acupuncture. Fig. 5 shows the relative change in hysteresis between pre- and post-acupuncture stimulation. In real acupuncture, no significant difference between pre- and post-acupuncture was observed, but a downward tendency was indicated (pre: 25.6 ± 5.1%, post: 16.1 ± 13.0%, RC: 63.0 ± 46.4%). In sham acupuncture, no significant difference between pre- and post-acupuncture was observed, but an upward tendency was indicated (pre: 26.5 ± 10.9%, post: 28.4 ± 6.9%, RC: 107.2 ± 98.3%).

4. Discussion

In some previous studies, the mechanical functions of the MTC have been discussed in terms of the dynamics of active contractile components and elastic components that elongate passively [19-20].

The entire length of an MTC is calculated by adding up the length of the tendon and muscle. In the case of isometric contractions that involve no joint movement, the length remains the same. That is, while the length of the MTC remains the same during an isometric contraction, the tendon structure is elongated to the same extent that the muscle contracts.

The relationships between tendon length and force are represented as three regions (toe, linear, and yield) [21]. Among them, the linear region is identified as the region in which the length/force relationship is represented linearly [22-23]. The slope obtained from investigating the force deformation curve in this region is said to be equivalent to the elastic coefficient, and thus defined as stiffness and used as an index for evaluating the elastic properties of
tendon structures [12, 24]. In the present study, we investigated stiffness before and after percutaneous acupuncture stimulation. The results after stimulation demonstrated that stiffness seemed to rise during real acupuncture, while a significant decrease was observed during sham acupuncture. This seems to suggest that acupuncture stimulation enhanced the elastic properties of the muscle during isometric contraction.

Since tendon structures have viscosity as well as elasticity, a clockwise hysteresis loop can be produced (vertical axis: force [load]; horizontal axis: tendon length [displacement]) during the stimulation and relaxation of muscles [25]. This phenomenon is called hysteresis, and is quantified as the ratio (%) between the area within the loop and the area under the unloaded curve [26]. It represents the part of the energy stored in tendons that stretching dissipates during contraction because of viscosity. In other words, a decrease in hysteresis implies a decrease of viscosity, i.e., a reduction of energy dissipation during muscle contractile movements. The results observed in the present experiment were a downward tendency during real acupuncture and an upward tendency during sham acupuncture. Thus, these findings may suggest that percutaneous acupuncture stimulation increases elastic properties and decreases viscoelastic properties of tendon structures. If the viscosity property (hysteresis) of the tendon structure decreases, the resistance to tendon elongation accompanied by muscle contraction of the MTC would also decrease. This implies the transmission efficiency of force has improved because of the reduction in resistance produced by tendon elongation and muscle contraction. That is, the same amount of muscle contraction could produce a greater amount of muscle force.

In the present study, no significant change in MVC or EMG was observed in ankle joint plantar flexion before or after percutaneous acupuncture stimulation (real or sham). This result suggests that the degree of muscle activation did not change from percutaneous
acupuncture stimulation. In other words, the change in stiffness observed after acupuncture stimulation does not seem to have been produced by a difference in muscle contraction output. From these findings, it is estimated that the increase in elastic tendon properties was induced by a decrease in its viscosity property.

While the present study does not intend to elucidate the whole mechanism of acupuncture, the following study by Kawakita et al. [27] provides us with clues to some effects of acupuncture stimulation. They observed impulses of human vasoconstrictor activities by using microneurography, showing that subtle, non-invasive acupuncture stimuli provoked responses. This fact implies that even a painless tack needle, as used in the present experiment, would have sufficient effects to induce biological responses. In regard to the effect of cutaneous stimulation, it has been shown that gentle mechanical cutaneous stimulation (touch) using a brush applied to the skin induced a morphine-like inhibitory effect on the somatocardiac sympathetic reflexes [28-29].

This report suggests that mechanical cutaneous stimulation stimulated low-threshold mechanoreceptors, released opioids in the spinal cord by activating low-frequency neural activities, and induced an analgesic effect by inhibiting the activity of unmyelinated C fibers and C-reflex potentials.

The sham acupuncture used in the present study did not include a needle body, but instead was an adhesive plaster. On the other hand, the real acupuncture included a needle 0.6 mm in length that is intended to reach the dermal layer. Thus, in real acupuncture, somatosensory activation provoked by dermal stimulation seems to have induced different reflexive responses in addition to those caused by cutaneous stimulation in sham acupuncture.

Although blood flow measurements were not included in a report by Watanabe et al. [29], they concluded that gentle mechanical cutaneous stimulation could inhibit sympathetic reflexes.
Thus, it is estimated that the percutaneous stimulation induced by the non-invasive tack needles used in the present study could inhibit sympathetic reflexes and vasomotor nerve activity, cause vasodilation in the muscle, and as a result, increase blood flow. Furthermore, the blood flow increases during isometric contractions in this experiment indicate a state of temporal congestion in the muscle, which would have increased the stiffness of the relevant muscle.

On the other hand, it is reported that muscle/tendon viscosity decreases with a rise in temperature accompanied by increased blood flow to the muscles [30]. Thus, in the present study, the blood flow increase induced by percutaneous acupuncture stimulation seems to have depressed the viscosity (i.e., hysteresis) in the muscle/tendon structures.

There were some limitations to the present study. The data acquisition status was limited, and further investigations acquiring additional data under various circumstances and during other forms of exercise are required. Additionally, the sample size was small, and it will be necessary for future studies to evaluate the effects in a greater number of individuals to validate the results of the present study.

5. Conclusions

In this study, persistent acupuncture application using tack needles demonstrated some improvements in the viscoelastic properties of tendon structures. This result indicates the potency of acupuncture. That is, if the acupuncture stimulation site is accurately selected and the application is performed appropriately in accordance with the targeted physical activity, it would be possible to adjust the viscoelasticity of tendon structures, affect the elongation of the MTC, and produce higher physical performance. If that is proven the case, acupuncture could become a powerful method for sports conditioning.
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Conflicts of Interest

The authors declare that there are no conflicts of interest.
References


**Figure legends**

Fig. 1. A schematic representation of the experimental setup. A dynamometer was used to fix the ankle joint angle at 0° plantar flexion from the anatomical position and to measure torque. The right foot was firmly attached to the footplate of the dynamometer with a strap. An ultrasonography apparatus with an electronic linear-array probe was used to obtain sectional images of the medial gastrocnemius muscle (MG). Sonograms, torque, and EMG data were synchronized by a timer in the PC.

Fig. 2. Sonograms of the MG musculotendinous intersection made by the fascicles and deep aponeurosis during isometric contraction. Typical ultrasonic images at 0, 50, 60, 70, and 80% of the maximal MVC are shown. The arrows indicate intersections made by the fascicles and deep aponeurosis.

Fig. 3. A typical example of the estimated muscle force (Fm)–tendon elongation (L) relationship in the MG during a ramp isometric contraction. The Fm-L curves during the ascending and descending phases of force development produced a loop. The areas of each of the curves under both the ascending phases (Ee1) and descending phases (Ee2) were calculated. Then, the ratio of the area (H) within the Fm-L loop to the area beneath the curve during the ascending phase was calculated as an index of hysteresis.
Fig. 4. The relative changes in muscle stiffness before and after acupuncture stimulation. The values are the mean ± SD (n = 9). *There was a significant difference between acupuncture and sham acupuncture, p < 0.05.

Fig. 5. The relative changes in hysteresis (%) pre- and post-acupuncture stimulation. A significant change was not observed, but downward and upward tendencies were indicated for acupuncture and sham acupuncture, respectively.
Table 1: Measured variables pre- and post-acupuncture stimulation

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<td>MVC (Nm)</td>
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<td>iEMG (mVs)</td>
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<td>TA</td>
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<td>MG</td>
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<td>SOL</td>
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<td>1.13 ± 0.84</td>
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The relative changes in MVC torque (Nm) and iEMG values (mVs) during isometric contraction. The values are the mean ± SD (n = 9). There were no significant differences observed.
Fig. 4

Relative change in stiffness (%)

* p < 0.05
Fig. 5

Relative change of Hysteresis (%)

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