

Paraffin therapy induces a decrease in the passive stiffness of gastrocnemius muscle belly and Achilles tendon

A randomized controlled trial

Ya Peng Li, MD^a, Ya Nan Feng, BSc^a, Chun Long Liu, MPT^b, Zhi Jie Zhang, PhD^{a,*}

Abstract

Background: The purposes of this study were to examine the feasibility of using the MyotonPRO digital palpation device in measuring the passive stiffness of gastrocnemius muscle belly and Achilles tendon; to determine between-days test-retest reliability of MyotonPRO; and to evaluate the acute effect of paraffin therapy on stiffness measurements in healthy participants.

Methods: It is a randomized controlled trial. Twenty healthy participants (male, n = 10; female, n = 10; total, n = 20) were recruited to evaluate the passive stiffness of gastrocnemius muscle belly and Achilles tendon. Dominant and nondominant legs were randomly divided into an experimental side (20 cases) and a control side (20 cases). The experimental side received 20 minutes of paraffin therapy.

Results: The stiffness of muscle and tendon in the experimental side decreased significantly after paraffin therapy ($P < .01$), whereas no significant differences in stiffness measurements were found in the control side ($P > .05$). The passive stiffness of muscle and tendon was positively correlated with the ankle from 30° plantar flexion to 10° dorsiflexion for dominant legs. Between-days test-retest reliability in stiffness measurements was high or very high (ICCs were above 0.737).

Conclusion: Paraffin therapy induces a decrease in the passive stiffness of gastrocnemius muscle belly and Achilles tendon. Furthermore, the MyotonPRO can reliably determine stiffness measurements.

Abbreviations: AT = Achilles tendon, CONSORT = Consolidated Standards of Reporting Trials, CV = coefficient of variance, DF = dorsiflexion, ICC = intraclass correlation coefficient, LG = lateral gastrocnemius, MDC = minimal detectable difference, MG = medial gastrocnemius, MTU = muscle-tendon unit, NE = neutral position, PF = plantar flexion, PRE-paraffin therapy = before paraffin therapy, POST-paraffin therapy = after paraffin therapy, RCT = randomized controlled trial, ROM = range of motion, SD = standard deviation, SEM = standard error measurement.

Keywords: Achilles tendon, gastrocnemius muscle belly, paraffin therapy, stiffness

1. Introduction

An increase in the passive stiffness of muscle belly in the medial gastrocnemius (MG) and lateral gastrocnemius (LG) muscles as well as the Achilles tendon (AT) can result in various disorders

Editor: Anis Jellad.

This study was supported by Project of Science Research of Traditional Chinese Medicine of Henan Province of China (2017ZY1004 and 2019ZY1028).

The authors have no conflicts of interest to disclose.

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How to cite this article: Li Y, Feng Y, Liu C, Zhang Z. Paraffin therapy induces a decrease in the passive stiffness of gastrocnemius muscle belly and Achilles tendon: a randomized controlled trial. *Medicine* 2020;99:12(e19519).

Received: 23 July 2019 / Received in final form: 7 January 2020 / Accepted: 8 February 2020

<http://dx.doi.org/10.1097/MD.00000000000019519>

affecting the lower limb, including Achilles tendinitis^[1] and plantar fasciitis,^[2] and thus limit joint mobility.^[3] Normally the human gastrocnemius muscle-tendon unit (MTU), as a mechanical buffer, is mainly responsible for transmitting and absorbing energy during functional activities.^[4] Because of its viscoelastic properties, the MTU can be stretched, which is essential for the storing of elastic energy.^[5] These functions are more likely to be compromised if the MTU is too stiff and hence unable to absorb enough energy during functional activities such as walking, running, and jumping.^[6] Therefore, it is important to monitor such adaptations in clinical practice, employing practical, quantitative, and sensitive techniques for reducing the passive stiffness of gastrocnemius muscle belly and AT.

The behavior of gastrocnemius muscle belly and AT can be examined noninvasively by various methods such as simple palpation,^[7] supersonic shear wave imaging,^[8] and magnetic resonance elastography.^[9] However, the efficacy of palpation-based examinations is typically limited to the detection and qualitative assessment of muscle and tendon stiffness.^[7] Elastography ultrasound is feasible tool to quantify the elastic properties of soft tissues, but there are some limitations including the cost of equipment and operator dependent. Magnetic resonance elastography has inherent limitations due to its complex methodology and negative cost-effectiveness.^[10]

The portable MyotonPRO digital palpation device is a computerized electronic tissue compliance meter that is capable of the noninvasive, objective assessment of the mechanical properties of a muscle at rest and during contraction. Earlier study also had very high between-day reliability of testing the rectus femoris in older (ICC > 0.70) males.^[11] Our team found excellent intra- and intertester reliability in the measurement of upper trapezius stiffness using a handheld MyotonPRO device.^[12] In the study by Pożarowski et al,^[13] the MyotonPRO device was used to measure the stiffness of the AT in karate fighters. Therefore, it is vital to assess the reliability and precision of the MyotonPRO for accurately quantifying the stiffness of the passive stiffness of gastrocnemius muscle belly and AT by different testers for treatment.

Paraffin therapy, a form of heat therapy, has been in general use in hospitals as a physical modality of treating patients with musculoskeletal problems.^[14–16] Paraffin therapy provides superficial heat to the muscle and tendon tissue, which can relax the smooth muscle fibers in arterioles and improve local circulation.^[15] Previous studies found that paraffin therapy could relieve pain and improve the range of motion (ROM) of finger joints in patients with hand arthritis.^[16] However, to the best of our knowledge, few studies have examined the acute effects of paraffin therapy on the passive stiffness of gastrocnemius muscle belly and AT.

The purposes of this study were to examine the feasibility of using the MyotonPRO device in measuring the passive stiffness of gastrocnemius muscle belly and AT; to determine between-days test–retest reliability of MyotonPRO; and to investigate the acute effect of paraffin therapy on those stiffness values among healthy participants.

2. Methods

Participant recruitment was conducted in May 2019. The limited age range of study participants was from 18 to 28 years. The inclusion criteria were as follows: without neuromuscular disease; without a history of musculoskeletal injury in the lower limbs; without abnormal shape of feet (pes planus and hollow foot or people with internal and external valgus deformity); without obliquity of pelvis or scoliosis. The exclusion criteria were participants with a stretching or massage on the human gastrocnemius muscle belly before testing. Twenty-three participants were recruited in this study. Three participants did not meet the inclusion criteria. The convenience sample consisted of 10 males and 10 females.

The study protocol was approved by the ethics committee (KY2019-001-01) of Henan Provincial Luoyang Orthopedic Hospital. The trial was registered in International Clinical Trials Registry Platform (ChiCTR1900021304). The procedure of the study was fully explained to the participants and they provided informed consent before testing. The experimental procedures were conducted in accordance with the Declaration of Helsinki.

The MyotonPRO (Myoton AS, Estonia) was applied perpendicular to the surface of the gastrocnemius muscle belly and AT via a probe. A constant preload force (0.18 N) is applied before imposing an additional 15-millisecond impulse of 0.40 N of mechanical impulse.^[17] The muscle and tendon tissue respond as a damped natural oscillation to the imposed mechanical impulse, which is quantified by an acceleration sensor attached to the probe. Stiffness is represented by the ability of the muscle or tendon to resist its shape after the tap. The muscle or tendon stiffness is calculated from the acceleration signal measurements

and the double integral of the signal. The calculation formula for muscle or tendon stiffness was as follows:

$$S = \frac{a_{\max} \cdot m_{\text{probe}}}{\Delta l}$$

where m_{probe} is the mass (preload) of the probe of 0.18 N, a_{\max} is the maximum amplitude of the oscillation in the acceleration signal, and Δl is the maximum amplitude of the displacement signal following the end of the impulse time.^[13] S is representative of dynamic stiffness (N/m).

3. Experiment I

3.1. Procedure

This study was conducted at Rehabilitation Therapy Center, Luoyang Orthopedic Hospital of Henan Province, Orthopaedic Hospital of Henan Province, Luoyang, China. Each subject was instructed to lie prone with knee extension and the ankle in a resting position. The stiffness of MG and LG as well as AT was quantified using the MyotonPRO device by an experienced physical therapist (YPL). The gastrocnemius muscle was evaluated at 4 sites: at the junction of upper 1/3 and middle 1/3 of the lines joining the fibulae capitulum and AT insertion site (R1 and L1) and at the junction of upper 1/3 and middle 1/3 of the lines joining proximal medial condyle of tibia and AT insertion site (R2 and L2).^[18] AT were tested 5 cm above the tuber calcanei.^[13] For the measurement, the tip of the measurement device was vertically placed on the skin marker with the muscle relaxed. At each site, 3 sets of measurements were taken and the means were used for statistical analysis. Prior to the measurement participants performed a self-paced walking warm up for 5 minutes. All tests were performed in the afternoon in independent quiet spaces with an average indoor temperature of 23°C.

3.2. Passive stiffness of gastrocnemius muscle belly and Achilles tendon

Passive stiffness of gastrocnemius muscle belly and AT on the dominant side was examined. The dominant leg was determined as the preferred leg used to kick a ball.^[6] Localized passive muscle and tendon stiffness in 10 of the participants were assessed again at 30° plantar flexion (PF), anatomically neutral position (NE), and 10° dorsiflexion (DF) after the 1st measurement using the MyotonPRO device. An ankle brace was used to quantify the passive ROM. The same testing procedure as previously described was then performed.

3.3. Reliability tests

To determine test–retest reliability between sessions, 10 of the participants returned. The same testing procedure was repeated by the original operator (YPL). Two measurements were taken each day in 5-day intervals, and the average of these 2 measures was used for test–retest reliability calculations.

4. Experiment II

4.1. Paraffin therapy

Because there was no significant difference in muscle and tendon stiffness between the dominant and nondominant legs ($P > .05$) (Table 1), this study was designed as a prospective randomized

Table 1
Descriptive statistics (mean ± standard deviation) for the muscle and tendon stiffness (dominant vs nondominant) symmetries of the lower limbs.

	Mean ± standard deviation (N/m)		P
	Dominant	Nondominant	
MG	297.52 ± 44.45	292.25 ± 45.17	.519
LG	340.82 ± 69.25	331.48 ± 60.07	.419
AT	801.42 ± 72.90	821.77 ± 79.75	.254

AT = Achilles tendon, LG = gastrocnemius lateralis, MG = gastrocnemius medialis.

controlled trial (RCT). Dominant and nondominant legs were randomly divided into an experimental side (20 cases) and a control side (20 cases) in the ratio of 1:1.

Passive stiffness measurements of gastrocnemius muscle belly and AT were made before (PRE) and immediately after (POST) paraffin therapy intervention while participants lay prone on a bed. The detailed procedure was operated by an experienced paraffin therapist (YNF). We made sure that clothing was well out of the way of the treatment area in the experimental side. The prepared paraffin pieces were placed so as to cover the lower limb (from knee joint to calcaneal tubercle, and covered with plastic wrap and then lightweight towels to retain the heat longer. The paraffin pieces were removed after 20 minutes and the area then wiped with a soft towel to ensure that all the paraffin had been completely removed. Finally, the stiffness of muscle and tendon was immediately measured again. The paraffin pieces were 2 to 3 cm thick and their surface temperature was 58°C to 60°C, which was safe and medically acceptable (XYL-VIII, XIANGYU MEDICAL, An-yang, Henan, China).^[19]

4.2. Data analysis

Data processing and reliability analyses were performed using SPSS Version 15.0 software (SPSS Inc, Chicago, IL). Descriptive statistics were generated to summarize the measurements of all demographic data. A paired *t* test was used to compare gastrocnemius muscle belly and AT stiffness in both the dominant and nondominant legs and to examine change in stiffness values at 30° PF, NE, and 10° DF as well as PRE-paraffin therapy and immediately POST-paraffin therapy. Intraclass correlation coefficients (ICCs) with 95% confidence interval (CI) were used to determine the intraoperator reliability (1-way random model consistency). All reliability coefficients were interpreted as follows: negligible (0.00–0.30), low (0.30–0.50), moderate (0.50–0.70), high (0.70–0.90), or very high (0.90–1.00).^[20] The coefficient of variance (CV) and standard error measurement (SEM) was calculated (based on formula $CV = [\text{standard deviation}/\text{mean}] \times 100\%$ and $SEM = \text{standard deviation} \times \sqrt{1-ICC}$, respectively).

Table 2
Gastrocnemius muscle belly and Achilles tendon stiffness associated with the ankle in 30° plantar flexion (PF), neutral anatomical position (NE), and 10° dorsiflexion (DF).

Dominant legs	Mean ± standard deviation (N/m)			P	
	30° PF	NE	10° DF	30° PF to NE	NE to 10° DF
MG	323.87 ± 58.98	386.63 ± 45.21	437.37 ± 70.68	.003	.001
LG	343.60 ± 65.99	395.03 ± 72.42	415.83 ± 75.69	.000	.000
AT	696.97 ± 90.31	1143.60 ± 87.31	1329.70 ± 177.06	.000	.002

AT = Achilles tendon, DF = dorsiflexion, LG = lateral gastrocnemius muscle, MG = medial gastrocnemius muscle, NE = neutral anatomical position, PF = plantar flexion.

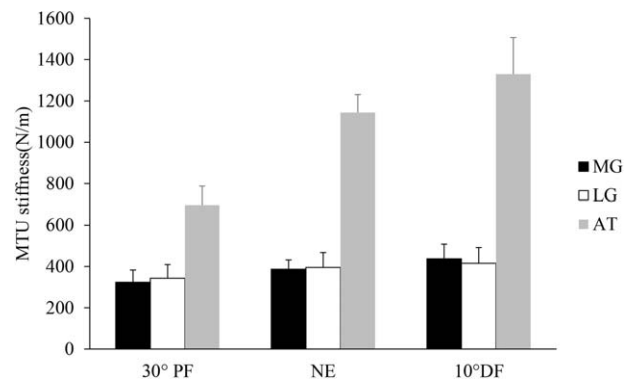


Figure 1. The passive stiffness of gastrocnemius muscle belly and Achilles tendon at 30° plantar flexion (PF), at anatomically neutral position (NE), and at 10° dorsiflexion (DF) on the dominant side. Relation of change in muscle and tendon stiffness at 30° PF, NE, and 10° DF. AT, Achilles tendon; LG, lateral gastrocnemius muscle; MG, medial gastrocnemius muscle; MTU = muscle-tendon unit.

Minimal detectable difference (MDC) was calculated (using the formula: $MDC = 1.96 \times SEM \times \sqrt{2}$). Reliability was further evaluated by using Bland–Altman plots, which measure bias between measurement methods as well as variability of scatter.^[12] Bonferroni correction for paired *t* test was used to correct the statistical analysis. Thus, an alpha of 0.02 was used to set the level of statistical significance. The data are presented as mean and standard deviation (SD) in the text.

5. Results

Demographic of 20 healthy participants in this study are as following: 10 males (age, 22.5 ± 3.7 years; body height, 173.7 ± 4.0 cm; body mass, 65.2 ± 8.1 kg) and 10 females (age, 20.8 ± 2.8 years; body height, 165.8 ± 4.5 cm; body mass, 61.1 ± 11.5 kg) (all presented as the mean ± SD).

There were no significant difference in the medial and lateral heads in gastrocnemius muscle and AT stiffness in the dominant and nondominant legs (*P* > .05) (Table 1). Our findings indicate symmetry was high between dominant and nondominant sides for gastrocnemius muscle belly and AT stiffness in healthy participants. However, averaged stiffness was slightly greater in the dominant legs than nondominant legs for muscle. This is the opposite for AT. Percentage mean differences between-sides for muscle and tendon values were 1.80% (MG), 2.82% (LG), and –2.48% (AT).

There was a significant difference in gastrocnemius muscle belly and AT stiffness at 30° PF, NE, and 10° DF (*P* < .02) (Table 2). Change in those stiffness values is presented in Figure 1. Muscle and tendon stiffness increased sharply with the ankle

Table 3
The between-days test-retest reliability of MyotonPRO in measurement of gastrocnemius muscle belly and Achilles tendon stiffness.

	Mean ± standard deviation (N/m)		SEM	ICC	95% CI
	Day 1	Day 2			
Dominant legs					
MG	302.53 ± 46.36	312.43 ± 54.33	14.66	0.836	0.379–0.959
LG	348.83 ± 84.67	339.80 ± 78.98	24.98	0.882	0.553–0.970
AT	815.61 ± 55.24	833.99 ± 41.80	13.22	0.737	0.008–0.934
Nondominant legs					
MG	293.93 ± 44.01	309.73 ± 51.94	13.92	0.873	0.521–0.968
LG	326.73 ± 64.55	333.27 ± 65.51	20.41	0.957	0.837–0.989
AT	802.11 ± 64.45	804.03 ± 42.25	13.36	0.832	0.364–0.958

AT = Achilles tendon, 95% CI = 95% confidence interval, ICC = intraclass correlation coefficient, LG = lateral gastrocnemius muscle, MG = medial gastrocnemius muscle, SEM = standard error mean.

from 30° PF to NE, with significant differences at MG (from 323.87 to 386.63 N/m, $P < .01$, an increase of 21.05%), LG (from 343.60 to 395.03 N/m, $P < .01$, an increase of 15.30%) and AT (from 696.97 to 1143.60 N/m, $P < .01$, an increase of 66.31%). Muscle and tendon stiffness increased slowly with the ankle from NE to 10° DF, with significant differences at MG (from 386.63 to 437.37 N/m, $P < .01$, an increase of 12.68%), LG (from 395.03 to 415.83 N/m, $P < .01$, an increase of 5.30%) and AT (from 1143.60 to 1329.70 N/m, $P < .01$, an increase of 16.17%). These results show that the MyotonPRO can be used to assess the changes in gastrocnemius muscle belly and AT stiffness from PF to DF.

In the evaluation of between-days test-retest reliability, 10 of the participants were retested 5 days later by the original operator. The gastrocnemius muscle belly and AT stiffness index is presented in Table 3. The calculated ICC reveals high test-retest reliability in MG (ICC = 0.836; 95% CI = 0.379–0.959; SEM = 14.66 N/m; MDC = 40.62 N/m), LG (ICC = 0.882; 95% CI = 0.553–0.970; SEM = 26.77 N/m; MDC = 69.24 N/m) and AT (ICC = 0.737; 95% CI = 0.008–0.934; SEM = 17.47 N/m; MDC = 36.64 N/m) in the dominant legs. The ICC values in the nondominant legs were high for MG (ICC = 0.873; 95% CI = 0.521–0.968; SEM = 13.92 N/m; MDC = 38.58 N/m) and AT (ICC = 0.832; 95% CI = 0.364–0.958; SEM = 20.38 N/m; MDC = 37.03 N/m) as well as very high for LG (ICC = 0.957; 95% CI = 0.837–0.989; SEM = 20.41 N/m; MDC = 56.57 N/m).

The Bland-Altman plot for muscle and tendon stiffness shows the bias line, or the mean difference in MG stiffness between 5 days was 9.9 N/m and the 95% limit of agreement was -65.6 to 85.4 N/m (Fig. 2A). The bias line, or mean difference in the LG stiffness between 5 days was -9.0 N/m and the 95% limit of agreement was -117.0 to 99.0 N/m (Fig. 2B). The bias line, or mean difference in AT stiffness between 5 days was 18.4 N/m and the 95% limit of agreement was -66.5 to 103.3 N/m (Fig. 2C).

There was no significant difference between the experimental and control sides PRE-paraffin therapy ($P > .05$). The effect of immediately after the intervention is shown in Table 4. In the experimental side immediately POST-paraffin therapy, MG stiffness significantly decreased by 5.67% (from 302.15 ± 47.62 to 285.03 ± 42.88 N/m; $P < .05$) (Fig. 3A). LG stiffness significantly decreased by 5.39% (from 346.63 ± 77.14 to 327.95 ± 64.26 N/m; $P < .05$) (Fig. 3B). A significant reduction of AT stiffness was found (from 814.88 ± 70.78 to 744.72 ± 63.99 N/m; $P < .01$) (Fig. 3C). There were no significant

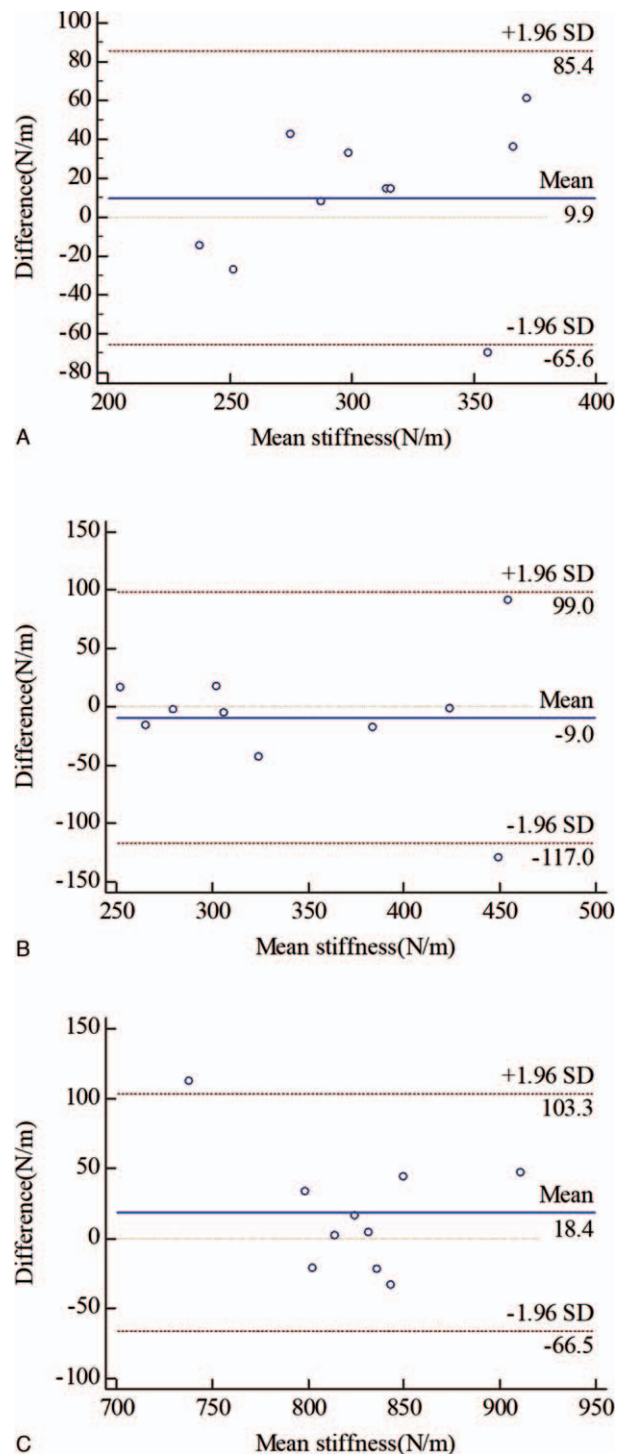


Figure 2. Bland-Altman plot showing mean differences in measurement of gastrocnemius muscle belly and Achilles tendon stiffness in dominant legs on different days by the same operator. (A) Stiffness of the medial gastrocnemius muscle. (B) Stiffness of the lateral gastrocnemius muscle. (C) Stiffness of the Achilles tendon. Broken lines indicate the 95% limits of agreement. SD = standard deviation.

differences in the control side ($P > .05$). There was a significant difference in the comparison of percentage change in gastrocnemius muscle belly and AT stiffness between the experimental and control sides PRE- and POST-paraffin therapy ($P < .01$) (Table 4).

Table 4

Changes in gastrocnemius muscle belly and Achilles tendon stiffness PRE and immediately POST-paraffin therapy.

	Mean \pm standard deviation (N/m)		
	PRE	POST	% Change
Experimental group			
MG	302.15 \pm 47.62	285.03 \pm 42.88*	-5.33 \pm 6.83 [†]
LG	346.63 \pm 77.14	327.95 \pm 64.26*	-4.71 \pm 5.62 [†]
AT	814.88 \pm 70.78	744.72 \pm 63.99* [‡]	-8.28 \pm 7.73 [†]
Control group			
MG	287.58 \pm 40.59	292.95 \pm 47.88	1.73 \pm 7.00
LG	325.67 \pm 47.62	327.73 \pm 45.22	0.91 \pm 5.43
AT	808.30 \pm 82.82	802.45 \pm 62.18	-0.26 \pm 6.74

AT=Achilles tendon, LG=lateral gastrocnemius muscle, MG=medial gastrocnemius muscle, POST=after paraffin therapy, PRE=before paraffin therapy.

*Significant change between PRE-paraffin therapy and immediately POST-paraffin therapy.

[†]Significant % change between experimental group and control group POST-paraffin therapy.

[‡]Significant difference between groups POST-paraffin therapy.

6. Discussion

A significant decrease in gastrocnemius muscle belly and AT stiffness POST-paraffin therapy was detected when compared with PRE- and POST-paraffin therapy in the experimental side. We found high or very high test-retest reliability for quantifying the stiffness of the gastrocnemius muscle belly and AT. The precision of the measurements is demonstrated by the relatively low SEM values. MDC is defined as the smallest amount of change that likely reflects true change rather than measurement error inherent in the value. Although there was a significant difference between the experimental and control sides immediately POST-paraffin therapy, the change of AT stiffness was greater than MDC. That was, the change in AT represented real and reliable change. Passive muscle and tendon stiffness increased with the ankle from PF to DF for dominant legs. Between dominant and nondominant sides symmetry was high for the gastrocnemius muscle belly and AT stiffness in healthy participants.

The gastrocnemius muscle belly and AT stiffness showed a significant decrement immediately POST-paraffin therapy in the experimental side. These results suggest that the acute effect of paraffin therapy on the stiffness of gastrocnemius muscle belly and AT was to a higher degree at tendon stiffness, not muscle stiffness. No similar study has been reported, so we cannot directly compare the results of other studies. A study published by Funk et al^[21] compared 30 seconds of static stretching with 20 minutes of heat application on hamstring flexibility. The results demonstrate that significant benefits to increase hamstring flexibility could be gained by using moist heat packs in comparison with static stretching. Robertson et al^[22] compared the effects of deep heating and superficial heating on tissue extensibility with 24 healthy participants. The extensibility of the calf muscles and associated soft tissues was measured by using an inclinometer in weight bearing. These results indicate that superficial heating is more effective than no heating and deep heating increases tissue extensibility more than superficial heating or no heating. Interestingly, Fujita et al^[23] compared the effects of thermal agents and physical activity on muscle flexibility, as well as their effectiveness when combined with static stretching. This study showed that although thermal agents and physical activity are effective in increasing knee-joint ROM ($P < .05$), there were no significant differences in MTU stiffness between pre- and postintervention measurements.

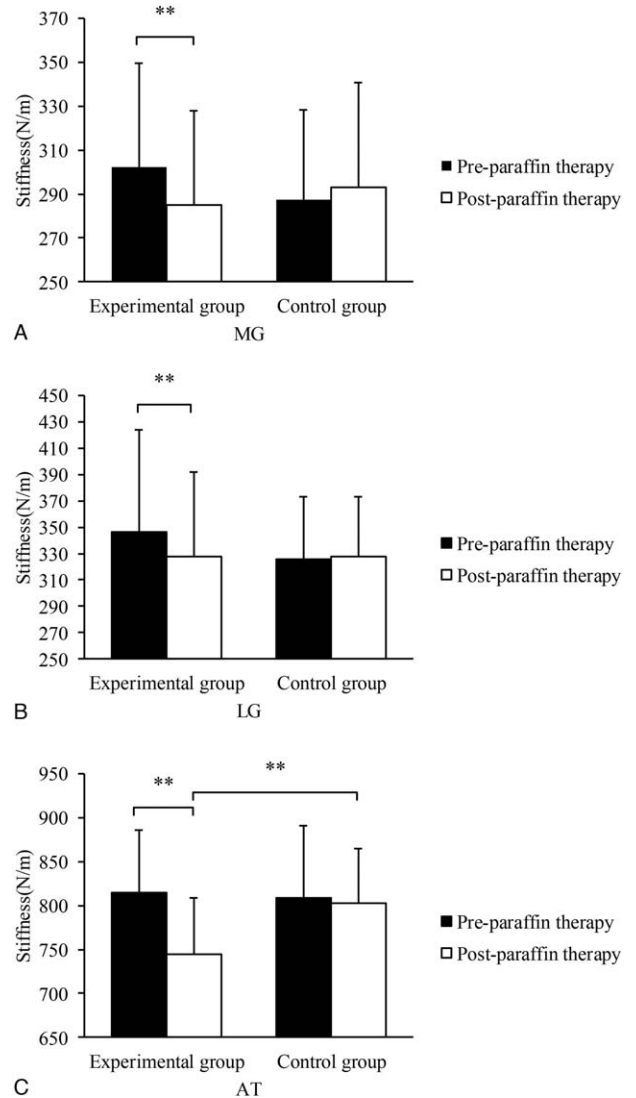


Figure 3. Changes in stiffness of gastrocnemius muscle belly and Achilles tendon (AT) PRE-paraffin therapy and immediately POST-paraffin therapy in the experimental and control sides. ** Represents significant change ($P < .01$). Values are mean \pm standard deviation. $N=20$. (A) Gastrocnemius medialis (MG). (B) Gastrocnemius lateralis (LG). (C) Achilles tendon (AT).

Skeletal muscle is comprised of series elastic elements and parallel elastic elements.^[3] The parallel elastic elements is involved in the intramuscular connective tissue (e.g., the endomysium, perimysium, and epimysium).^[24] The perimysium is a major extracellular membrane contributor to passive stiffness.^[2,5] Therefore, a reduction in stiffness of muscle after paraffin may be associated with modulation in the viscoelasticity of intramuscular connective tissue. No evidence for the effects of temperature on the tendon properties on humans was found in the literature. Since the force exerted by muscle fibers stretches AT before it is transmitted to the bone, the changes in AT stiffness may be accompanied by the alterations of the muscle fibers and the intramuscular connective tissue. Furthermore, the myotendinous junction displacement may have increased, likely leading to a decrease in tendon stiffness. Borrell et al^[26] reported that paraffin bath therapy causes temperature increases of 7.5°C in the joint capsule and 4.5°C in muscle. Paraffin therapy could induce

the relaxation of the muscle fibers due to vasodilatation of the peripheral blood vessels.^[16] Furthermore, paraffin therapy also could contribute to reducing pain and muscle spasm as well as increasing metabolism in the tissues. Its advantages may be attributed to improving transduction of tissue fluid and lymph flow.^[27]

The previously described results reflect the high to very high intraoperator reliability of MyotonPRO measurements, with strong agreement and insignificant measurement errors on repeated tests. Between-days reliability is important to establish. A recent study published by Lo et al^[28] assessed the within-session relative and absolute interrater reliability of the MyotonPRO. They reported that ICCs ranged between 0.63 and 0.97 in patients with within 1 month of the first occurrence of stroke. The SEM of all muscles ranged within 6.42 and 20.20 N/m for stiffness. Drenth et al^[29] found that ICC ranged from 0.54 to 0.71 for intrarater reliability in dementia patients with paratonia. The reliability of most of the biomechanical measures in the human patellar tendon and AT tests by Sohirad et al^[30] was adequately high (ICC=0.96). The results of the present study indicate that the MyotonPRO could potentially be useful in monitoring individual changes in the biomechanical properties of gastrocnemius muscle belly and AT.

In our study, changes in passive gastrocnemius muscle belly and AT stiffness during active passive ROM were quantified using the MyotonPRO device. We demonstrated that passive muscle and tendon stiffness was significantly increased with the ankle from 30° PF to DF for dominant legs. This finding is in keeping with multiple studies that tested muscle stiffness at a variety of angles and found increased stiffness with greater stretch/extension.^[31] Our team revealed a 14.2% increase in stiffness on shoulder flexion between 0° and 60° of flexion using the MyotonPRO device.^[12] In addition, there is a significant increase of the passive stiffness in the biceps brachii at full elbow extension when compared with 30° of flexion using shear wave elastography.^[32] These investigators also demonstrated significantly higher passive biceps brachii stiffness in the right arm for women at both elbow positions. In a previous study, Bell et al^[33] reported a negative correlation between estrogen levels and musculotendinous stiffness. In Chen et al,^[32] study, they also found that the stiffness of muscle was affected by the level of estrogen among women.

The present study was the first to investigate between-limb symmetry of mechanical properties in healthy young people. Our findings revealed that the mean stiffness of MG and LG were 297.52 ± 44.45 N/m and 340.82 ± 69.25 N/m in dominant legs as well as 292.25 ± 45.17 N/m and 331.48 ± 60.07 N/m in nondominant legs, respectively. Our findings were in line with the previous studies (LG = 33.5 ± 6.3 kPa; MG = 27.6 ± 7.3 kPa).^[18] This study showed that AT stiffness in both dominant and nondominant legs were 801.42 ± 72.90 N/m and 821.77 ± 79.75 N/m, slightly higher than that measured by Schneider et al.^[17] The result was in accordance with AT stiffness POST-paraffin therapy. In a study by Aird et al,^[11] MyotonPRO measurements of quadriceps in healthy older males were symmetrical between sides. Experiment I demonstrates the asymmetry of gastrocnemius muscle belly and AT stiffness between dominant and nondominant legs. This finding was useful in the design of Experiment II protocol, which assumed no difference in gastrocnemius muscle belly and AT stiffness between the dominant and nondominant legs.

We are aware of potential limitations of this study. First, the study included solely healthy younger participants; therefore its

results cannot be generalized to the whole population. Further studies will be needed to examine the changes of gastrocnemius muscle belly and AT stiffness in different populations (including people with Achilles tendinopathy or plantar fasciitis). Second, n=10 is a small sample size for between-days test-retest reliability. It is necessary to assess test-retest reliability with a larger sample population in further research. Third, our study examined only the acute effects of paraffin therapy on gastrocnemius muscle belly and AT stiffness. Further studies are needed to make these measurements changes with time POST-paraffin therapy. Finally, we assumed that right and left sides within the same participants was considered as separate cases in this RCT. However, there could be physiological cross-effects from heating the limb on one side of the body.

7. Conclusion

Paraffin therapy induces a decrease in the passive stiffness of gastrocnemius muscle belly and AT. The MyotonPRO can reliably determine the mechanical properties of gastrocnemius muscle belly and AT tissue. Furthermore, it is necessary to understand the effect of the paraffin therapy on the human MTU stiffness to prevent the occurrence of the sports injury and improve athletic performance.

Author contributions

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Funding acquisition: Zhi Jie Zhang.

Investigation: Ya Peng Li, Ya Nan Feng.

Methodology: Ya Peng Li.

Project administration: Zhi Jie Zhang.

Software: Chun Long Liu.

Supervision: Ya Nan Feng.

Writing – original draft: Ya Peng Li.

Writing – review & editing: Zhi Jie Zhang.

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