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Title: Reliability of a Vibration-based Elastography Protocol for Assessing Achilles Tendon Stiffness Across Multiple Joint Angles in Elite Athletes

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

3. Filming location: Will the filming need to take place in multiple locations? **No**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 15

Number of Shots: 46

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Yixiong Cui:** We utilize a novel elastography protocol to evaluate Achilles tendon stiffness across multiple joint angles, aiming to characterize biomechanical adaptations in elite athletes from various sports.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.7.2*

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Weiqiang Xu:** Unlike existing methods, our vibration-based elastography reliably assesses continuous tendon stiffness changes across the full range of motion.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Yixiong Cui:** This protocol non-invasively measures the Achilles tendon's functional stiffness spectrum, capturing its non-linear mechanical behavior across dynamic angles.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.13.1.*

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Wenpu Yang:** The key challenge during dynamic acquisition is maintaining constant contact pressure and probe perpendicularity to avoid motion artifacts.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Manshuang Yang:** Future studies can map tendon behavior to monitor tendinopathy rehabilitation, assess training adaptations, and predict dynamic sports performance.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Research Ethics Committee of Beijing Sport University. Written informed consent was obtained from the participants.

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Functional Stiffness Spectrum Acquisition Protocol

Demonstrators: Wenpu Yang & Manshuang Yang

- 2.1. To begin, recruit participants from national-level sports teams, including professional male athletes aged 18 to 26 years across multiple sport disciplines [1]. Determine the dominant leg by asking the participant to kick a ball [2].
 - 2.1.1. WIDE: Shot of a single representative participant arriving at the laboratory and being briefed on the experimental procedure by the talent. ***Videographer:** Please obtain CU shot as well for this shot*
 - 2.1.2. Shot of the participant simulating a kick (or kicking a soft ball) in the lab space while the talent observes to select the dominant leg.
- 2.2. Prepare the equipment, M5 Soft Tissue Elastometer equipped with a 9L3 (Nine-L-Three) to 8.5 megahertz linear array probe and a mechanical excitation module [1]. Gather additional equipment, including an Achilles boot, ultrasound gel, and an examination bed [2].
 - 2.2.1. Shot of the talent with M5 Soft Tissue Elastometer with the linear array probe and mechanical excitation module.
 - 2.2.2. Talent placing the Achilles boot, ultrasound gel, and examination bed in the preparation area
- 2.3. Switch on the main power supply [1] and confirm that the system status indicator illuminates [2]. Then, power on the tablet interface [3], launch the ultrasound system software by selecting the designated application icon [4], and verify that the system enters the main ultrasound operating interface with real-time B-mode imaging displayed [5].
 - 2.3.1. Talent switching on the main power supply.
 - 2.3.2. Shot of the system status indicator illuminated.
 - 2.3.3. Talent powering on the tablet interface.
 - 2.3.4. Show the designated application icon being selected on the tablet interface.
 - 2.3.5. Show the main ultrasound operating interface with real-time B-mode imaging displayed.
- 2.4. Set the elastography mode parameters to a frequency of 6 megahertz [1], 4 acquisition

lines [2], a 5 millimeter depth range [3], and a 300-millisecond acquisition time [4]. Activate the excitation module [5] and position the excitation tip 3 to 6 millimeters in front of the probe protrusion side and perpendicular to the probe imaging plane [6].

2.4.1. Show the elastography mode settings panel with frequency set to 6 megahertz.

NOTE: 2.4.1 to 2.4.6 are filmed in a single shot

2.4.2. Show the acquisition lines parameter set to 4.

2.4.3. Show the depth range set to 5 millimeters.

2.4.4. Show the acquisition time set to 300 milliseconds.

2.4.5. Talent activating the excitation module.

2.4.6. Talent positioning the excitation tip 3 to 6 millimeters in front of the probe protrusion side and perpendicular to the probe imaging plane.

2.5. Next, instruct the participant to remove shoes and socks [1]. Position the participant prone on the examination couch with the ankles fully extended over the edge by approximately 5 centimeters [2].

2.5.1. WIDE: Talent instructing the participant to remove shoes and socks.

2.5.2. Talent positioning the participant prone on the examination couch with ankles extended over the edge.

2.6. Palpate to locate the superior apex of the calcaneal tuberosity [1]. Mark a point 5 centimeters proximal to this landmark using a skin marker to define the initial measurement site [2].

2.6.1. Talent palpating the heel to locate the superior apex of the calcaneal tuberosity.

2.6.2. Talent marking a point 5 centimeters proximal to the landmark using a skin marker.

2.7. Apply a uniform layer of prewarmed coupling gel to the transducer surface [1]. Lightly place the probe against the measurement site [2] and align the target point under the anterior side of the probe [3].

2.7.1. Talent applying a uniform layer of prewarmed coupling gel to the transducer surface.

2.7.2. Talent lightly placing the probe against the measurement site.

2.7.3. Talent aligning the target point under the anterior side of the probe.

2.8. Now, switch the ultrasound system to elastography mode [1]. Position the reference line so that the acquisition depth range begins just below the superficial tendon fascia [2]. Adjust the region of interest to cover the tendon thickness while avoiding the skin, subcutaneous tissue, and Kager fat pad [3].

2.8.1. Show the ultrasound system being switched to elastography mode.

- 2.8.2. Show the reference line positioned just below the superficial tendon fascia.
- 2.8.3. Show the region of interest adjusted to cover the tendon thickness while avoiding surrounding tissues.
- 2.9. Before acquisition, confirm imaging quality by ensuring that the transducer plane is nearly perpendicular to the skin surface at greater than 75 degrees [1]. Confirm that the transducer-to-skin distance is approximately 5 millimeters [2]. Verify that no visible air bubbles are present and that the fascia and tendon fibers are clearly visualized [3].
 - 2.9.1. Talent checking that the transducer plane is nearly perpendicular to the skin surface at greater than 75 degrees.
 - 2.9.2. Talent confirming an approximately 5-millimeter distance between the transducer and skin.
 - 2.9.3. Show the ultrasound image with no visible air bubbles and clearly visualized fascia and tendon fibers.

Data Acquisition and Quality Control

NOTE: Please insert a chapter title

- 2.10. Initiate continuous measurement by clicking the **On** button [1]. Allow the system to automatically calculate the shear modulus and display mean and standard deviation values of valid data [2]. Maintain constant participant and operator posture during acquisition to obtain at least 10 valid continuous data points [3]. Stop data acquisition by pressing the **Freeze** function once sufficient data points are collected [4]. Review the dataset for outliers and remove abnormal data points using the system editing function [5].
 - 2.10.1. Show the On button being clicked to initiate continuous measurement.
 - 2.10.2. Show the system displaying shear modulus values with mean and standard deviation.
 - 2.10.3. Talent maintaining steady probe position on the measurement site.
 - 2.10.4. Show the Freeze function being pressed to stop acquisition.
 - 2.10.5. Show the dataset with outliers being removed using the editing function.
- 2.11. Conduct measurements sequentially on both Achilles tendons under relaxed, 0 degrees neutral, 20 degrees plantarflexion, 40 degrees plantarflexion, 20 degrees dorsiflexion, and 40 degrees dorsiflexion conditions [1].
 - 2.11.1. LAB MEDIA: Figure 1
- 2.12. Repeat measurements at least three times at each ankle angle [1]. Validate each measurement only if the standard deviation of the continuous data points is less than 10 percent of the mean, as required by the device's internal validation criteria [2]. ~~Discard and repeat any measurement that does not meet this criterion [3].~~ Save B-mode images and mechanical imaging maps for documentation [4]. **NOTE: The VO is edited**

for the deleted shot

2.12.1. Talent repeating measurement acquisition at a set ankle angle.

2.12.2. Show the standard deviation value compared to the mean value within the system display.

~~2.12.3. Show an invalid measurement being discarded and acquisition restarted.~~ NOTE: This is not filmed

2.12.4. Show B-mode images and mechanical imaging maps being saved for documentation.

Boot Installation and Angle Setting NOTE: Please insert a chapter title

2.13. After completing the relaxed state measurement, place the participant's foot into the adjustable ankle testing boot, ensuring the heel rests completely flush against the posterior heel cup of the boot base [1]. Secure the forefoot, midfoot, and lower leg using the attached hook-and-loop straps to prevent heel lift or lateral shifting during testing [2]. Loosen the bilateral locking knobs on the boot hinge mechanism [3].

2.13.1. Talent placing the participant foot into the adjustable ankle testing boot with the heel flush against the posterior heel cup.

2.13.2. Talent fastening the hook-and-loop straps around the forefoot, midfoot, and lower leg.

2.13.3. Talent loosening the bilateral locking knobs on the boot hinge mechanism.

2.14. Manually guide the ankle to the target angle by aligning structural markers with the goniometric scale [1]. Tighten the locking knobs firmly to secure the ankle joint at the target angle [2]. Perform ultrasound measurement immediately after locking the angle to prevent viscoelastic tendon relaxation [3].

2.14.1. Talent aligning structural markers with the goniometric scale while guiding the ankle to the target angle.

2.14.2. Talent tightening the locking knobs to secure the ankle joint.

2.14.3. Talent performing ultrasound measurement with the probe on the tendon.

2.15. After that, instruct the participant to remove the ankle boot [1]. Clean and sanitize all instruments and ultrasound probes [2].

2.15.1. Talent assisting the participant in removing the ankle boot.

2.15.2. Talent cleaning and sanitizing the instruments and ultrasound probes.

Results

3. Results

- 3.1. A successful measurement was visually characterized by a high-quality B-mode image with a clear, continuous tendon fibrillar structure parallel to the skin surface [1] and a stable, homogeneous elastography color map within the predefined Region of Interest [2].
 - 3.1.1. LAB MEDIA: Figure 2.
 - 3.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the boxed measurement area on the right side of the image.*
- 3.2. Average coefficient of variation values ranged from 14.0 to 25.2 percent across different joint angles [1].
 - 3.2.1. LAB MEDIA: Table 2. *Video editor: Highlight the row labeled "Average CV" across all joint angle columns.*
- 3.3. Intraclass correlation coefficient values ranged from 0.871 to 0.974 across all assessed joint angles [1].
 - 3.3.1. LAB MEDIA: Table 2. *Video editor: Highlight the row labeled "ICC(2,1)" across all joint angle columns.*
- 3.4. The highest reliability was observed in the relaxed state [1], and the lowest reliability was observed at the neutral 0-degree position [2].
 - 3.4.1. LAB MEDIA: Table 2. *Video editor: Highlight the "Relax" column in the row labeled "ICC(2,1)".*
 - 3.4.2. LAB MEDIA: Table 2. *Video editor: Highlight the "0" column in the row labeled "ICC(2,1)".*
- 3.5. Achilles tendon stiffness increased non-linearly from plantarflexion to dorsiflexion in all participants [1].
 - 3.5.1. LAB MEDIA: Figure 3.
- 3.6. A linear mixed model revealed a significant main effect of joint angle [1], whereas side and sport type showed no main effects [2].
 - 3.6.1. LAB MEDIA: Table 3. *Video editor: Highlight the row labeled "Angle".*
 - 3.6.2. LAB MEDIA: Table 3. *Video editor: Highlight the rows labeled "Side" and "Sport".*
- 3.7. The Angle and Sport interaction was significant, indicating sport-specific stiffness differences at certain ankle angles [1].
 - 3.7.1. LAB MEDIA: Table 2. *Video editor: Highlight the row labeled "Angle × Sport".*

4. Elastometer
Pronunciation link: <https://www.howtopronounce.com/elastometer>
IPA: /ɪ,læ'stɑːmɪtər/
Phonetic Spelling: ih·las·taa·muh·tur
5. Megahertz
Pronunciation link: <https://www.merriam-webster.com/dictionary/megahertz>
IPA: /'megə,hɜːts/
Phonetic Spelling: meg·uh·hurts
6. Ultrasound
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultrasound>
IPA: /'ʌltrə,saʊnd/
Phonetic Spelling: ul·truh·sound
7. Elastography
Pronunciation link: <https://www.howtopronounce.com/elastography>
IPA: /ɪ,læ'stɑːgrəfi/
Phonetic Spelling: ih·las·taa·gruh·fee
8. Acquisition
Pronunciation link: <https://www.merriam-webster.com/dictionary/acquisition>
IPA: /,ækwə'zɪʃən/
Phonetic Spelling: ak·wuh·zi·shuhn
9. Millimeters
Pronunciation link: <https://www.merriam-webster.com/dictionary/millimeter>
IPA: /'mɪlə,mi:tər/
Phonetic Spelling: mil·uh·mee·tur
10. Mechanical
Pronunciation link: <https://www.merriam-webster.com/dictionary/mechanical>
IPA: /mə'kænɪkəl/
Phonetic Spelling: muh·kan·ih·kuhl
11. Perpendicular
Pronunciation link: <https://www.merriam-webster.com/dictionary/perpendicular>
IPA: /,pɜːpən'dɪkjələr/
Phonetic Spelling: pur·puhn·dik·yuh·lur
12. Palpate
Pronunciation link: <https://www.merriam-webster.com/dictionary/palpate>
IPA: /'pæl,peɪt/
Phonetic Spelling: pal·payt
13. Calcaneal
Pronunciation link: <https://www.merriam-webster.com/dictionary/calcaneal>
IPA: /kæl'kemiəl/
Phonetic Spelling: kal·kay·nee·uhl
14. Tuberosity
Pronunciation link: <https://www.merriam-webster.com/dictionary/tuberosity>
IPA: /,tu:bə'rɑːsəti/
Phonetic Spelling: too·buh·raa·suh·tee

- 15. Proximal**
Pronunciation link: <https://www.merriam-webster.com/dictionary/proximal>
IPA: /'prɑ:k səməl/
Phonetic Spelling: prok·suh·muhl
- 16. Transducer**
Pronunciation link: <https://www.merriam-webster.com/dictionary/transducer>
IPA: /trænz'du:sər/, /træns'du:sər/
Phonetic Spelling: tranz·doo·sur; trans·doo·sur
- 17. Tendon**
Pronunciation link: <https://www.merriam-webster.com/dictionary/tendon>
IPA: /'tendən/
Phonetic Spelling: ten·duhn
- 18. Subcutaneous**
Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneous>
IPA: /,sʌbkju:'teɪniəs/
Phonetic Spelling: sub·kyoo·tay·nee·uhs
- 19. Fascia**
Pronunciation link: <https://www.merriam-webster.com/dictionary/fascia>
IPA: /'fæʃə/, /'feɪʃiə/
Phonetic Spelling: fash·uh; fay·shee·uh
- 20. Dorsiflexion**
Pronunciation link: <https://www.merriam-webster.com/dictionary/dorsiflexion>
IPA: /,dɔ:rsi'flekʃən/
Phonetic Spelling: dor·see·flek·shuhn
- 21. Plantarflexion**
Pronunciation link: <https://www.howtopronounce.com/plantarflexion>
IPA: /,plæntə:r'flekʃən/
Phonetic Spelling: plan·tar·flek·shuhn
- 22. Shear Modulus**
Pronunciation link: <https://www.howtopronounce.com/shear-modulus>
IPA: /ʃɪr'mɑ:dʒələs/
Phonetic Spelling: sheer mod·juh·luhs
- 23. Standard Deviation**
Pronunciation link: <https://www.merriam-webster.com/dictionary/standard%20deviation>
IPA: /'stændərd'di:vi'eɪʃən/
Phonetic Spelling: stan·derd dee·vee·ay·shuhn
- 24. Outliers**
Pronunciation link: <https://www.merriam-webster.com/dictionary/outlier>
IPA: /'aʊt,laiər/
Phonetic Spelling: out·ly·ur
- 25. Viscoelastic**
Pronunciation link: <https://www.howtopronounce.com/viscoelastic>
IPA: /,vɪskoʊ'læstɪk/
Phonetic Spelling: vis·koh·ih·las·tik

- 26. Fibrillar**
Pronunciation link: <https://www.howtopronounce.com/fibrillar>
IPA: /faɪˈbrɪlər/
Phonetic Spelling: fy·bril·ur
- 27. Homogeneous**
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogeneous>
IPA: /ˌhoʊməˈdʒiːniəs/, /ˌhɑːməˈdʒiːnjəs/
Phonetic Spelling: hoh·muh·jee·nee·uhs; hah·muh·jee·nyuhs
- 28. Intraclass**
Pronunciation link: <https://www.howtopronounce.com/intraclass>
IPA: /ˌɪntrəˈklæs/
Phonetic Spelling: in·truh·klas
- 29. Correlation**
Pronunciation link: <https://www.merriam-webster.com/dictionary/correlation>
IPA: /ˌkɔːrəˈleɪʃən/
Phonetic Spelling: kor·uh·lay·shuhn
- 30. Non-linearly**
Pronunciation link: <https://www.merriam-webster.com/dictionary/nonlinear>
IPA: /ˌnɑːnˈlɪniər/
Phonetic Spelling: non·lin·ee·ur
- 31. Participant**
Pronunciation link: <https://www.merriam-webster.com/dictionary/participant>
IPA: /pɑːrˈtɪsəpənt/
Phonetic Spelling: par·ti·suh·puhnt
- 32. Goniometric**
Pronunciation link: <https://www.howtopronounce.com/goniometric>
IPA: /ˌɡoʊniəˈmetrɪk/
Phonetic Spelling: goh·nee·uh·me·trik
- 33. Achilles**
Pronunciation link: <https://www.merriam-webster.com/dictionary/Achilles>
IPA: /əˈkɪliːz/
Phonetic Spelling: uh·kil·eez