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Title: Standardized Protocol for Monitoring Muscle Fatigue and Biomechanics in Amateur Cyclists Using Infrared Thermography

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

✓ Correct

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

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? Yes, all done

4. Proposed filming date: 06/27/2026

Introduction

NOTE: Please refer to the file JoVE69926_Shotlist for filenames and shot details

INTRODUCTION:

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

- 1.1. **Salvador Santos:** We study muscle fatigue and biomechanics in amateur cyclists using infrared thermography.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Georgina Valtierra:** This protocol can be applied in sports science, cycling research, and clinical studies of muscle fatigue and thermoregulation.
 - 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. **Salvador Santos:** This protocol allows researchers to measure muscle fatigue, thermal changes, and biomechanics during cycling.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Ma. Cristina Hernández:** This protocol can be combined with other analyses, such as muscle activation, fatigue, and thermal asymmetry assessment.
 - 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. **Blanca Ramírez:** Future studies can apply this protocol to different athletes, injuries, and exercise conditions to better understand fatigue and performance.

- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Ethics Title Card

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. All participants provided written informed consent prior to participation.

Protocol

2. Subject Preparation and Pre-Experimental Procedures

Demonstrators: Salvador Santos, Blanca Ramírez, Georgina Valtierra, Ma. Cristina Hernández

- 2.1. To begin, line up the subjects who have consented to be a part of the study [1]. Record the age and sex of each subject [2]. Then record the height and mass of the subject [3].
 - 2.1.1. WIDE: Show the participants lined up for the study and the talent ready to note down the measurements.
 - 2.1.2. Talent recording the subject's age and sex on a data sheet.
 - 2.1.3. Talent measuring and recording subject's height and mass.
- 2.2. Describe the Borg Rating of Perceived Exertion scale from 6 to 20 to the subjects [1]. Instruct the subject to point to the number that represents their exertion level before and after the test [2].
 - 2.2.1. Talent presenting the Borg scale to the subject
 - 2.2.2. Talent instructing the subject to point to the number that represents their exertion level. Also, please zoom in to show the chart where the values will be entered.
- 2.3. Prohibit the subject from consuming alcohol and nicotine for 24 hours prior to measurement [1]. Instruct the subject to avoid caffeine intake and intense exercise for 3 hours before the test [2].
 - 2.3.1. Talent indicating restriction instructions on a checklist.
 - 2.3.2. Show the talent displaying the checklist and then zoom in on the caffeine intake and intense exercise items on the checklist.
- 2.4. Instruct the subject not to apply creams, cosmetics, or ointments to the lower extremities during the 12 hours prior to the test [1]. After confirming the hydration status, record it on the pre-test data sheet [2].
 - 2.4.1. Talent explaining skin preparation restrictions to the subject.
 - 2.4.2. Talent recording hydration status on the pre-test data sheet.
- 2.5. Use a clinical scale to record the body mass before the test [1]. Ask the subject to consume 300 milliliters of water 30 minutes before the session [2].
 - 2.5.1. Talent measuring body mass using a scale.

- 2.5.2. Talent handing water to the subject for consumption.
- 2.6. Instruct the subject to fully undress the lower extremities [1] and change into standardized black Lycra cycling shorts with no visible seams [2].
 - 2.6.1. Talent instructing the subject to remove lower garments.
 - 2.6.2. Shot of the subject wearing standardized black lycra cycling shorts.
- 2.7. Place a digital thermo-hygrometer 1 meter from the subject at a height of 1 meter [1-TXT]. Record these values every 5 minutes in a spreadsheet [2].
 - 2.7.1. Talent positioning the thermo-hygrometer near the subject. **TXT: Ambient Temp 18–25 °C; Relative humidity 40–55%**
 - 2.7.2. SCREEN: 2.7.2.mp4: 00:14-00:18, 00:30-00:32.
- 2.8. Ensure that there are no direct drafts, sunlight exposure, or heat or cold sources affecting thermoregulation [1]. Perform a 15-minute seated rest period for acclimatization [2]. Post rest, position the subject on the stationary bicycle in the required posture [3].
 - 2.8.1. Talent checking the environment for external influences.
 - 2.8.2. Talent seated at rest.
 - 2.8.3. Talent positioning the subject correctly on the bicycle.

3. Thermal Imaging Setup, Calibration, and Data Acquisition

- 3.1. Open the thermal camera internal settings menu and navigate to **Object Parameters** to enter an emissivity value of 0.98 [1]. Set the **Reflected Apparent Temperature** to match the previously measured ambient temperature [2].
 - 3.1.1. Talent navigating to **Object Parameters** and entering emissivity value.
 - 3.1.2. Talent Adjusting reflected temperature setting to match ambient value.
- 3.2. Use the infrared thermograph thermal camera with a minimum resolution of 320 by 240 pixels [1]. Set emissivity to 0.98 for human skin [2].
 - 3.2.1. Talent holding and positioning the thermal camera.
 - 3.2.2. Talent sets the emissivity to 0.98
- 3.3. Use a laser distance meter or a measuring tape to position the tripod exactly 100 centimeters from the lateral plane of the bicycle crankset [1]. Mark the tripod leg positions with adhesive tape [2].
 - 3.3.1. Talent measuring 100 centimeters from the lateral plane of the bicycle crankset
 - 3.3.2. Talent marking tripod positions on the floor using adhesive tape.
- 3.4. Enter the relative humidity and atmospheric temperature values into the instrument settings [1].

- 3.4.1. Inputting humidity and temperature into device settings.
- 3.5. Place the thermograph on a tripod and set the camera height to 30 centimeters above the floor [1]. Position the lens at a perpendicular angle to the lower extremity of the subject [2].
 - 3.5.1. Talent adjusting tripod height and mounting the camera.
 - 3.5.2. Talent aligning the camera perpendicular to the subject's leg.
- 3.6. In the thermal analysis software, select the **Polygon/Rectangle ROI** (*Polygon or Rectangle R-O-I*) tool [1]. Click and drag to delineate a 10 by 5 centimeter box over the Rectus Femoris [2]. Click Calculate to extract the mean temperature value [3].
 - 3.6.1. Selecting **Polygon/Rectangle ROI**.
 - 3.6.2. Show 10 x 5 cm box being drawn over the Rectus Femoris.
 - 3.6.3. Clicking **Calculate** to display mean temperature.
Video editor: Please highlight the mean value displayed on the screen.
- 3.7. For setting the measurement point protocols, capture a thermographic image immediately after the 15-minute rest period [1]. Before the end of the exercise period, stand behind the thermographic camera and pre-set the focus [2]. At the signal to stop pedaling, extend the dominant leg to the 6 o'clock position [3]. Press the **Capture/Freeze** (*Capture or Freeze*) button within 5 seconds [4]. Capture a third thermographic image 10 minutes after exercise completion [5].
 - 3.7.1. Talent capturing an image with the camera immediately after rest.
 - 3.7.2. Talent standing behind the thermographic camera and pre-setting the focus
 - 3.7.3. Talent extending the leg to the 6 o'clock position
 - 3.7.4. Talent pressing the **Capture/Freeze** button within 5 seconds
 - 3.7.5. Talent capturing post-exercise recovery image.
- 3.8. Set the stationary bicycle to ergometer mode with a fixed workload of 150 watts [1]. Use a metronome or console to maintain a cadence of 80 rpm [2]. Instruct the subject to maintain this pace within a 5 RPM margin [3]. Verify that the thermal camera is in standard recording mode, with a temperature range of 20 to 45 degrees Celsius and an emissivity of 0.98 [4].
 - 3.8.1. Talent adjusting bicycle workload settings.
 - 3.8.2. Talent monitoring cadence on console or metronome.
 - 3.8.3. Talent instructing the subject to maintain this pace within a ± 5 RPM margin
 - 3.8.4. SCREEN: 3.8.4.mp4
- 3.9. Use the formulae presented to calculate thermal shift [1]. Preserve the original thermographic image while retaining full resolution and metadata for analysis [2].

3.9.1. TEXT ON A PLAIN BACKGROUND:

- Calculate thermal shift: $\Delta T = T_{post} - T_{pre}$
- Perform this for dominant and non-dominant limbs
- Calculate thermal asymmetry: $\Delta T_{asymmetry} = |T_{right} - T_{left}|$

3.9.2. SCREEN: 3.9.2.mp4: 00:00-00:10

Results

4. Results

Video editor: In all the images mentioned below, show only the blue-outlined rectangles in the centre panels of A, B, and C. When referring to Fig. 3, show only the centre panels (blue rectangles) of 3A, 3B, and 3C.

4.1. When compared with the resting phase [1], the Immediate Post-Exercise moment showed redistribution of blood flow [2], with a drop in surface temperature due to peripheral vasoconstriction [3], and a rise in temperature in areas of pain such as the knees [4].

4.1.1. LAB MEDIA: Figure 3. *Video editor: Emphasize panel A and label it "Resting Phase"*

4.1.2. LAB MEDIA: Figure 3. *Video editor: Emphasize panel B and label it "Start of exercise"*

4.1.3. LAB MEDIA: Figure 3b. *Video editor: Highlight the darker blue areas around the yellow parts*

4.1.4. LAB MEDIA: Figure 3b. *Video editor: Highlight the bright yellow knee area and the value 32.8 °C*

4.2. The recovery confirmed peripheral cooling [1], despite active heat retention in the central muscle bellies [2].

4.2.1. LAB MEDIA: Figure 3. *Video editor: Emphasize panel C and label it "End of exercise". Simultaneously highlight the blue area around the yellow central portion in Figure 3c*

4.2.2. LAB MEDIA: Figure 3c. *Video editor: Highlight the yellow central areas of the legs compared with the cooler outer edges*

1. Borg Rating of Perceived Exertion
Pronunciation link: <https://www.howtopronounce.com/borg-scale>
IPA: /bɔrg 'reɪ.tɪŋ əv pə'si:v dɪg'zɜ:.ʃən/
Phonetic Spelling: borg ray·ting uhv per·seevd ig·zur·shuhn
2. Thermo-hygrometer
Pronunciation link: <https://www.howtopronounce.com/thermohygrometer>
IPA: /θɜ:.moʊ.haɪ'grɑ:.mə.tə/
Phonetic Spelling: thur·moh·hy·grom·uh·ter
3. Thermoregulation
Pronunciation link: <https://www.merriam-webster.com/dictionary/thermoregulation>
IPA: /θɜ:.moʊ.reg.jə'leɪ.ʃən/
Phonetic Spelling: thur·moh·reg·yuh·lay·shuhn
4. Acclimatization
Pronunciation link: <https://www.merriam-webster.com/dictionary/acclimatization>
IPA: /ə.klaɪ.mə.tə'zeɪ.ʃən/
Phonetic Spelling: uh·kly·muh·tuh·zay·shuhn
5. Emissivity
Pronunciation link: <https://www.merriam-webster.com/dictionary/emissivity>
IPA: /i:.mə.sɪ'vɪ.ti/
Phonetic Spelling: ee·muh·siv·ih·tee
6. Infrared
Pronunciation link: <https://www.merriam-webster.com/dictionary/infrared>
IPA: /ɪn.frə'red/
Phonetic Spelling: in·fruh·red
7. Thermograph
Pronunciation link: <https://www.merriam-webster.com/dictionary/thermograph>
IPA: /θɜ:.mə'græf/
Phonetic Spelling: thur·muh·graf
8. Thermographic
Pronunciation link: <https://www.merriam-webster.com/dictionary/thermographic>
IPA: /θɜ:.mə'græf.ɪk/
Phonetic Spelling: thur·muh·graf·ik
9. Atmospheric
Pronunciation link: <https://www.merriam-webster.com/dictionary/atmospheric>
IPA: /æt.mə'sfer.ɪk/
Phonetic Spelling: at·muh·sfer·ik
10. Polygon
Pronunciation link: <https://www.merriam-webster.com/dictionary/polygon>
IPA: /'pɑ:.lɪ.gən/
Phonetic Spelling: pol·ee·gon
11. Delineate
Pronunciation link: <https://www.merriam-webster.com/dictionary/delineate>
IPA: /dɪ'liːn.i.eɪt/
Phonetic Spelling: dih·lin·ee·ayt

12. Rectus Femoris
 Pronunciation link: <https://www.merriam-webster.com/medical/rectus%20femoris>
 IPA: /'rek.təs fə'mər.ɪs/
 Phonetic Spelling: rek·tuhs fuh·mor·iss
13. Ergometer
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ergometer>
 IPA: /ɜː'gɑː.mə.t̬ər/
 Phonetic Spelling: er·gom·uh·ter
14. Cadence
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cadence>
 IPA: /'keɪ.dəns/
 Phonetic Spelling: kay·duhns
15. Metadata
 Pronunciation link: <https://www.merriam-webster.com/dictionary/metadata>
 IPA: /'met̬.ə,deɪ.t̬ə/ ; /'met̬.ə,dæ.t̬ə/
 Phonetic Spelling: met·uh·day·tuh ; met·uh·da·tuh
16. Redistribution
 Pronunciation link: <https://www.merriam-webster.com/dictionary/redistribution>
 IPA: /,riː.dɪs.trə'bjuː.ʃən/
 Phonetic Spelling: ree·dis·truh·byoo·shuhn
17. Vasoconstriction
 Pronunciation link: <https://www.merriam-webster.com/dictionary/vasoconstriction>
 IPA: /,veɪ.zoʊ.kən'strɪk.ʃən/
 Phonetic Spelling: vay·zoh·kun·strik·shuhn
18. Peripheral
 Pronunciation link: <https://www.merriam-webster.com/dictionary/peripheral>
 IPA: /pə'rɪf.ə.əl/
 Phonetic Spelling: puh·rif·uh·ruhl
19. Thermal Asymmetry
 Pronunciation link: <https://www.merriam-webster.com/dictionary/asymmetry>
 IPA: /θɜː.məl ,eɪ'sɪm.ə.tri/
 Phonetic Spelling: thur·muhl ay·sim·uh·tree
20. Thermographic Analysis
 Pronunciation link: <https://www.merriam-webster.com/dictionary/thermographic>
 IPA: /θɜː.mə'græf.ɪk ə'næl.ə.sɪs/
 Phonetic Spelling: thur·muh·graf·ɪk uh·nal·uh·sis