**JoVE58084**: Using a knee arthrometer to evaluate tissue-specific contribution to knee flexion contracture in the rat - *Response to Reviewer Comments*

**Editorial Comments**

Comment 1

Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Response

Done.

Comment 2

Please submit the figures as a as a vector image file to ensure high resolution throughout production: (.svg, .eps, .ai). If submitting as a .tif or .psd, please ensure that the image is 1920 pixels x 1080 pixels or 300dpi.

Response

All figured that were resubmitted were 300dpi

Comment 3

Figure 2: Is the yellow vertical line straight? It doesn’t look it.

Response

The line was redrawn to ensure it was straight

Comment 4

Figure 3: Please define the error bars: SD, SEM, etc.

Response

The figure legend for Figure 3 has been updated to indicate that the error bars represent standard deviation.

Comment 5

Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns.

Response

The table of materials has been updated to include a more comprehensive list of materials.

Comment 6

Please provide an email address for each author.

Response

Email addresses have been added to the ‘Authors and Affiliations’ section

Comment 7

JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

Response

Commercial language was removed from the body of the manuscript and left in the ‘Table of Materials and Reagents’

Comment 8

Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

Response

The protocol has been re-written in the imperative tense.

Comment 9

The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion.

Response

Done.

Comment 10

Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

Response

More details have been added throughout the protocol.

Comment 11

Please describe the immobilization model in more detail.

We have added details to the Discussion. Lines (starting on line 218).

“The rat knee immobilization model used in our lab has been approved by the University of Ottawa Animal Care and Veterinary Service and the local ethics committee. Our lab uses an immobilization model with a plate and 2 screws (one inserted in the proximal femur and the other in the distal tibia) which avoids violation of any knee joint structures, and maintains a knee-flexed position of 135° as described previously6. Over a period of time, this produces a knee flexion contracture11.”

Comment 12

1.2: How are the skin and the screws divided? How are the plate and the screws removed?

Response

More detail has been provided (section 1.3)

* 1. “Using a scalpel, divide the skin to expose the plate and screws. The more proximal screw is inserted in the proximal femur and the more distal screw in the distal tibia. The screws should be easily located by palpation. Once the screw heads are accessible, remove the screw using a screwdriver.

NOTE: during the period of immobilization, the heads of the screws may become covered by soft tissue. If this occurs, use the scalpel to gently remove the tissue and uncover the screw heads.”

Comment 13

Please specify all surgical tools and the surgery as well.

Response

A dissection kit with specific components was included in the ‘Table of Materials’.

Comment 14

2.1: What is an appropriate area?

Response

This has been clarified (section 1.2)

“1.2. Cover the area both on and around the surface that the arthrometer will be placed upon with absorbent, water-proof protection pads. Wear gloves, lab coat, and eye protection, while completing the experiment.”

Comment 15

Please provide all experimental parameters.

Response

These have been added under Section 2.

“NOTE: all testing should be performed at room temperature. Our arthrometer is powered by a standard North American 120V input. The adapter output is 12V and 500mA.”

Comment 16

Please do not abbreviate journal titles.

Response:

The referenced journals are now cited by their full names.

**Reviewer 1**

Comment 1

The authors should describe in the discussion section the disadvantages that present this technique.

Response:

The limitations in the Discussion have been expanded, starting at Line 251.

Comment 2

One of the major limitations is the death of the animal according to point 1.2. (line 89). The authors should improve this method to allow testing live animals. Please, discuss.

Response:

This has been added to the Discussion (line 247).

“While the ethics protocol for our lab does not include testing on live animals, this would theoretically be possible with appropriate analgesia and post-test sacrificing protocols.”

Comment 3

The authors should add an image of the whole animal in the arthrometer.

Response

An image was added to Figure 1 which showing the whole animal on the arthrometer.

Comment 4

Please, explain better the aim of step 2.5.2. (line 120).

Response

Details regarding how we determined the 4 torques have been added to the Discussion (starting line 232).

“Four torques are tested in sequence: 2.53 N-cm, 7.53 N-cm, 12.53 N-cm, and 17.53 N-cm. The highest torque level was determined to be the amount of force that led to capsular failure in normal (unoperated) rat knee joints (i.e., extension surpassing 0°) after division of all transarticular muscle. The lowest torque was the point of resistance to angular motion just above measurable amounts on normal rat knee joints. The middle two torques were set to be approximately midway between the highest and lowest torques.”

**Reviewer 2**

Comment 1

Line 104 - 107 - how do the upright posts engage the leg? Is it posterior compression? Or does the tibia pass through the uprights? If the tibia does not pass through the uprights, do the uprights have to be repositioned anterior to the leg in order to push the tibia posterior to flex the knee?

Response

The metal posts engage the tibia posteriorly, as suggested. The action of the upright posts has been clarified in the Discussion (paragraph starting on line 225).

“The distal two metal upright post engage the leg posteriorly, pushing the knee into extension, and are designed to minimize the risk of posterior dislocation of the tibia on the femur during mechanical testing (Figure 1).”

Comment 2

Line 155 - 159 - the lateral femoral condyle and lateral malleolus are not easily defined on the pictures. Is there any work on the intra and inter observer repeatability of the measures?

Response

We have included higher magnification images to show these landmarks, as they are more easily identified on high-resolution, high-magnification images. Intra-observer intraclass correlation coefficient is 0.987. Inter-observer intraclass correlation is 0.903. These have been added to the manuscript (line 261).

Comment 3

Line 172 - 175 - are any of the mean angle measures statistically different? How many animals were used to generate the data? Was the immobilized knee randomized, or was it always the same side?

Response

Statistical indicators have been added to Figure 3. Seven rats were used to generate this representative data. The laterality of the immobilized knee alternated between left and right (e.g. rat 1 left immobilized, rat 2, right immobilized). Note that the person measuring the angles was blinded to which side was immobilized during our measurements.

This has been more fully explained in the last paragraph of the Results:

“The ability to measure maximum extension of rat knees in a valid, precise and reproducible, user-independent manner reduces bias in the data. In the example provided, we evaluated the maximum knee extension following 16 weeks of immobilization for 7 rats, comparing the immobilized (experimental) limb to the non-immobilized contralateral limb. The limb chosen for immobilization was alternated from one rat to the next (e.g. rat 1 had the right knee immobilized, rat 2 the left). The investigator measuring the angles was blinded to which side was immobilized during measurements.”

Comment 4

Discussion - it is not clear about the tibia clamp, if it wraps around the bone or not like the femur clamp does, has it happened that the upright posts slip off the leg, or at least the leg slips on the clamp such that the application of the torque changes because the tibia is not rigidly clamped?

Response

This has been clarified in the Discussion. See Reviewer 2, Comment 1. In our experience with the arthrometer, the tibial posts maintain their position just superior to the calcaneus during testing.

Comment 5

Line 166 - 167 - clinical convention is that full extension is 0°.

Response

Agreed. The Y-axis in Figure 3 has been rescaled to conform to this convention.

Comment 6

Line 120 - 129 - why 4 torques? How were the torque values selected?

Response

See Reviewer 1, Comment 4.

Comment 7

Labelling Figure 1 to indicate the femur clamp, the tibia clamp, and the upright posts would be helpful.

Response

The femur clamp and moveable arm, or ‘upright posts’, have been labelled with an arrowhead and chevron respectfully. The corresponding Figure legend has also been updated.

**Reviewer 3**

Comment 1

The posterior transarticular muscles, for example hamstrings begin from ischial tuberosity. It means that the angle of pelvic influence of the knee extension ROM. How does the investigator control the pelvic position during measurement?

Response

We thank Reviewer 3 for this excellent comment and we have discussed it at length. We agree that our arthrometer does not control the movement of the pelvis during testing. It is possible that at lower torques (torques 1 and 2), the relative change in knee extension going from one torque to the next would be greater than if the pelvis were fixed (ie. as the pelvis moves into retroversion due to the pull on the hamstrings). Note however that this would be true of both experimental and control knees and so a difference due to experimental conditions (e.g. immobility) should still be detectable (though the percentage change between torques might be different). At the higher torques (torques 3 and 4), this is unlikely to affect the knee ROM measurements and the pelvis would be in maximum retroversion by torque 2 (no movement of the pelvis is visible on the arthrometer above torque 2) and ROM measurements are taken using fixed bony landmarks below the pelvis. Of course, once myotomy is performed, this issue would be resolved.

We have updated the Limitations paragraph (line 267):

“Finally, because the hamstrings cross both the knee and hip joints, retroversion of the pelvis may occur during testing at torques 1 and 2 prior to myotomy. This may contribute to increases in knee extension for both experimental and control knees at these torques.”

Comment 2  
The authors mention that joint angle measurement is investigator-independent. On the other hand, there are some possibility that there are difference of the results caused by between investigator skill. Inter-examiner reliability should be confirmed about this apparatus.

Response  
A valid inter-rater reliability for the use of the arthrometer is very difficult to calculate due to the fact that the highest torque often damages the articular structures, which would alter (increase) the maximum extension that could be achieved if a second person attempted to re-test the same rat. We generally avoid try to avoid changing the person securing the rats to the arthrometer during a study so that any investigator-dependent bias is consistent between experimental and control knees. This has been added as a limitation to the Discussion (lines).

We have added intra and inter-rater reliability for ImageJ analysis to the Discussion (see Reviewer 2, Comment 2).

Comment 3  
This protocol is to measure the range of motion in extension of the rat knee joint. Reviewer recommends author mention this point clearly in abstract and too; Line 43 from the ROM to the extension ROM.

Response

Though the arthrometer could be used to measure flexion, we agree that the protocol describes extension only. The abstract has been updated to clarify (line 50)

Comment 4

Line 301: There are no arrowhead and chevron in Figure 2.

Response

Agree that this was an omission. Arrowheads and chevron has been added to Figure 2.

**We would like to thank the Editor and Reviewers 1, 2, and 3 for their insightful comments that have helped strengthen our manuscript.**