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Using a knee arthrometer to evaluate tissue-specific contribution to knee flexion contracture in the rat --Manuscript Draft--

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Question	Response
If this article needs to be "in-press" by a certain date, please indicate the date below and explain in your cover letter.	

April 19, 2018

Dear Dr. DSouza,

We appreciate the helpful comments provided by yourself and the Reviewers for our invited manuscript entitled “Using a knee arthrometer to evaluate tissue-specific contribution to knee flexion contracture in the rat”.

Following our revisions, we believe that this article presents a novel, reproducible, and objective method for measuring knee range of motion in the rat and will be helpful for any laboratory that is interested in studying joint contractures. The appropriate ethics approval was obtained for the described methods, the submitted manuscript has been approved by all authors and we agree to bear any applicable publication charges. We trust that this study will be of interest to the many researchers who follow *JoVE*.

Sincerely,

A handwritten signature in black ink, reading "Mark Campbell". The signature is written in a cursive, flowing style. The first name "Mark" is written with a large, stylized 'M' and the last name "Campbell" follows in a similar cursive script. The signature is positioned above the printed name.

Mark Campbell MD MSc FRCPC

TITLE:

Using a Knee Arthrometer to Evaluate Tissue-Specific Contributions to Knee Flexion Contracture in the Rat

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KEYWORDS:

Joint contracture, immobility, arthrometer, mechanical testing, rat model, knee extension, range of motion, myogenic, arthrogenic

SUMMARY:

The goal of the protocol is to measure the extension range of motion of the rat knee. The effects of various diseases that increase the stiffness of the knee joint and the effectiveness of treatments can be quantified.

ABSTRACT:

Normal knee range of motion (ROM) is critical to well-being and allows one to perform basic activities such as walking, climbing stairs and sitting. Lost ROM is called a joint contracture and results in increased morbidity. Due to the difficulty of reversing established knee contractures, early detection is important, and hence, knowing risk factors for their development is essential. The rat represents a good model with which the effect of an intervention can be studied due to the similarity of rat knee anatomy to that of humans, the rat's ability to tolerate long durations of knee immobilization in flexion, and because mechanical data can be correlated with histologic and biochemical analysis of knee tissue.

Using an automated arthrometer, we demonstrate a validated, precise, reproducible, user-independent method of measuring the extension ROM of the rat knee joint at specific torques. This arthrometer can be used to determine the effects of interventions on knee joint ROM in the rat.

INTRODUCTION:

Having full range of motion (ROM) of the joints is critical for health and well-being¹. A loss in joint passive ROM is called a contracture². Joint contractures may arise from numerous conditions, including prolonged bedrest, paralysis, joint arthroplasty, burns, infection, and neurologic conditions^{1,3-5}. A contracture of the knee can be disabling as it accelerates joint degeneration, increases the risk of falls and detrimentally affects a person's ability to perform basic functional tasks including walking, sitting, and climbing stairs^{6,7}.

Once established, contractures of the knee are difficult to treat, and therefore determining which patients are at the highest risk of developing this condition is essential for prevention and avoidance of contracture-associated morbidity⁸. Experiments are designed to evaluate 1) the conditions causing or influencing knee joint contractures, 2) the severity of contractures, 3) their temporal progression, 4) the tissues involved in the contracture, 5) their reversibility as well as 6) the usefulness of various preventive and curative interventions on knee joint ROM. For all of these experiments, a valid, objective, precise and reproducible method for measuring the ROM is critical. Other ancillary measures (energy expenditure, histomorphometry, gene expression and protein content) are useful markers to understand the pathophysiology of joint contractures, but the mechanical limitation is what limits the patient and leads to disability. Some of the challenges in this area of research includes the heterogeneous methods by which knee ROM may be tested experimentally, as well as a lack of quantitative data⁹. The use of a variety of different experimental methods leads to results that are not comparable from laboratory to laboratory. This has led to controversy regarding the conditions (such as immobilization or joint arthroplasty) that cause joint contractures¹⁰. An automated method of experimentally measuring joint ROM following an intervention is therefore needed.

Here, we describe a user-independent, valid, precise and reproducible protocol for evaluating the rat knee ROM using a custom-built arthrometer linked to a digital camera to precisely measure the knee ROM in extension. We tested the effect of various periods of immobilization on knee ROM. We then describe the methods for measuring ROM at pre-specified torques on the resulting digital images using fixed bony landmarks. Overall, these methods reliably measure rat knee ROM and provide quantitative data.

PROTOCOL:

The rat knee immobilization model used has been approved by the University of Ottawa Animal Care and Veterinary Service and the local ethics committee.

1. Animal Preparation

1.1. At the end of the predetermined immobilization period, euthanize the rats by administration of carbon dioxide.

Note: Here we used 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.

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.

1.3. Using a scalpel, divide the skin to expose the plate and screws (see the note following step 1.1); insert the more proximal screw in the proximal femur and insert the more distal screw in the distal tibia. Palpate to locate the screws. 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.

1.4. Once the screws are removed, remove the plate manually or using forceps from a dissection kit.

1.5. Using scissors and forceps, deglove the lower extremity to remove skin from underlying fascia.

2. Animal Positioning on the Motor-Driven Arthrometer

Note: All testing should be performed at room temperature. Here the arthrometer is powered by a standard North American 120 V input. The adapter output is 12 V and 500 mA.

2.1. Position the animal to be tested on its side with the experimental leg (the leg to be tested) facing upwards (**Figure 2**).

2.1.1. Secure the femur in the grooved metal clamp that is integrated into the mounting stage of the arthrometer. Punch holes through the muscle using a precision screwdriver to place the clamp distal to the greater trochanter and secure the femur. Adjust the lateral femoral condyle over the center of rotation of the arthrometer (**Figures 1, 2**).

2.1.2. Position the movable arm with two upright posts behind the leg, just superior to the calcaneus, to push the knee into passive extension once the electric motor is activated.

2.1.3. Tighten the femur clamp at its base using a hex key until it is secured.

2.2. Ensure the camera is correctly mounted on the arthrometer using a screwdriver and is on **Manual Focus**. Focus the camera on the femoral condyle.

2.3. Select the direction setting on the arthrometer (clockwise or counterclockwise) depending on the direction of the knee ROM being tested and the position of the rat.

2.4. Activate the arthrometer motor by simultaneously pushing the **Power** and **Start** button.

Note: The necessity of pushing the power and start button simultaneously is a safety feature of the device, which prevents accidental activation.

2.4.1. Observe that the arthrometer motor will move at a speed of 6.6 RPM and then stop for 2.1 seconds upon reaching the first pre-set torque.

2.4.2. Note that when the first torque is reached, the corresponding LED will light up and the digital camera will take a picture of the knee automatically.

Note: Once the picture is taken, the arthrometer will continue to the next, higher preset torque. Once the four torques have been applied, the arthrometer will stop. Once the rat is positioned on the arthrometer and testing is initiated, the total time for testing one knee is approximately 18.8 seconds. Times may vary slightly depending on the condition of the joint contracture. The images taken are used to measure the extension at each torque.

3. Capturing the Angle of Knee Extension Using the Motor-Driven Arthrometer

Note: Once the motor has stopped at each applied torque, a digital camera is triggered to take a picture. The camera is positioned on the frame such that it is directly above the knee joint being tested and focused on the femoral condyle.

3.1. Continue testing with the same knee from the same animal but in a different situation, *e.g.*, after a myotomy of the posterior transarticular muscles is performed to isolate the arthrogenic (non-muscular) component of a contracture, or with a knee from another animal.

3.1.1. When completing the myotomy, dissect the muscle proximal enough to the knee joint to ensure that the capsule is not cut.

Note: It is easiest to complete the myotomy when the leg is in extension, following application of torque setting 4 (17.53 N-cm). Then, repeat steps 2.1 through 3.1.

3.2. Once both legs have been tested in all conditions (*e.g.*, before and after myotomy), dispose of the animal carcass and all biohazardous materials following institutional protocol, and clean the arthrometer.

4. Knee ROM Measurement Analysis

4.1. Analyze ROM using ImageJ.

Note: Here version 1.45s was used.

4.2. Open the file containing the digital image taken by the camera mounted on the rat arthrometer.

Note: The person performing the analysis should be blinded to the experimental grouping of the animal (*e.g.*, immobilized versus control).

4.3. Select the **Angle tool** from the main toolbar and trace the femorotibial angle by drawing a femoral line from the lateral condyle to the middle of the femur clamp (aligned with the femoral diaphysis, **Figure 2**), and a tibial line from the lateral femoral condyle to the lateral malleolus (**Figure 2**).

Note: The femoro-tibial angle corresponds to the maximal angle of knee extension reached at each preset torque.

4.4. Use the measuring tool by clicking **Analyze | Measure** to show the calculated angle produced by the 2 lines drawn above. Use the convention of 0° to mean full extension.

REPRESENTATIVE RESULTS:

The amount of knee extension determined for various periods of immobility are summarized for increasing durations of immobility and show that more severe contractures were produced following increasing lengths of immobilization. Representative results using ImageJ are shown in **Figure 3**.

The ability to measure maximum extension of rat knees in a valid, precise and reproducible, user-independent manner that 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 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. The results are presented in **Figure 3**. For the immobilized knee, the ability for maximum extension was reduced compared to the contralateral. Division of transarticular muscles eliminates the myogenic component of the flexion contracture. Following myotomy, the maximal extension capability for the experimental and contralateral knee increased; however, the experimental knee continued to demonstrate a flexion contracture (**Figure 3**).

FIGURE LEGENDS:

Figure 1. Rat knee arthrometer. A) Entire apparatus B) Representative image of animal within arthrometer C) Electronics display and clamps for arthrometer. D) Enlarged image of electronics display. Numbers indicate the various torques being applied: torque 1 = 2.53 N-cm, torque 2 = 7.53 N-cm, Torque 3 = 12.53 N-cm, torque 4 = 17.53 N-cm. E) Enlarged image of the femoral clamp and tibial pushing apparatus. The femoral clamp is fixed to the stage. The tibial moving arm has 2 upright posts that fix the distal lower extremity and move the knee into extension. Arrowhead and chevron indicate the femur clamp and the moveable arm, respectively.

Figure 2. Arthrometer and experimental rat for evaluation. A) Knee extension of the posterior limb is measured using the tibial line drawn from the lateral femoral condyle to the lateral malleolus and the femoral line drawn from the lateral condyle to the middle of the femur clamp. Arrowhead and chevron indicate the femur clamp and the moveable arm, respectively. B) High magnification images of the femoral condyle and C) High magnification of lateral malleolus.

Figure 3. Immobilized and contralateral rat knee range of motion following 16 weeks immobilization. For both knees, after testing knee extension with all articular structures intact (n=7), a myotomy of the trans-articular muscles was performed to determine the arthrogenic limitation on ROM. Data are presented as mean degrees from full extension (using the convention 0° = full extension) with error bars representing standard deviation. * represents $p < 0.01$ using the independent-samples T-test.

DISCUSSION:

The rat knee arthrometer was developed to reproducibly and reliably determine the maximum extension of the rat knee following an intervention. Advantages of this device include the consistent generation of torque across the knee joint with a constant arm length and extension force. Another advantage includes the ability to set the torque at a level that allows repetitive testing on the same joint to evaluate the influence of different articular structures on knee ROM, such as muscle, capsule, or ligament. For example, following testing of the fully intact joint, the posterior transarticular knee muscles could be divided and arthrometer testing repeated in order to determine the arthrogenic contribution to extension limitation¹¹.

Specific mechanical features of the arthrometer that optimize measurement accuracy and precision include the grooved clamp, which is designed to prevent rotation of the femur during testing (**Figure 1**). The distal two metal upright posts engage the leg posteriorly, pushing the knee into extension in a clockwise direction, and minimize the risk of posterior dislocation of the tibia on the femur during testing (**Figure 1**). The height of the posts and anterior overhang of the upper upright link ensure that the tibia does not slip off the posts. The ability of the upright posts to rotate and maintain their position on the tibia just proximal to the calcaneus ensures constant torque. 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.

Other methods for measuring joint ROM at specific torques have been described for both rat and other animal models¹²⁻¹⁶. Some of the advantages of our model over these other systems include a convenient size that allows benchtop placement of the device without the need for special facilities. Other models may also require disarticulation of the limb being studied while the model presented here does not. Mechanically, the arcing pathway of the post applies the extension force to the distal leg, following the angular progression of the knee, thus maintaining a consistent angle of force application. The stage of the arthrometer allows the placement of the entire rat on the measuring tool, allowing the testing of all *in situ* articular structures that may contribute to lost ROM without violating joint anatomy. While the ethics protocol for our lab excludes testing on live animals, this would theoretically be possible with appropriate analgesia and post-test sacrificing protocols.

The arthrometer does present some disadvantages. The device was sized to the adult rat knee to ensure that the moveable arm was at a length to ensure it would not slip off the leg and the leg would not slip off the arm. Younger rats, or smaller and larger species would benefit from appropriately-sized components. Additionally, a reprogramming of the optimal moment arm and force (torque) would be required. If, for example a larger or smaller animal was used, adjusting the length of the moment arm and/or amount of force applied to reach optimal torque may be necessary. Other parts of the arthrometer might also need to be resized, according to the size of the animal. While the movements of the arthrometer are user-independent, joint measurement using ImageJ may be subject to human error. We have found however that, using the methods presented here, there is high intra-rater and inter-rater reliability with intraclass correlation coefficients of 0.987 and 0.903, respectively. Because the highest torque often damages the articular structures during testing, a valid inter-rater reliability for the animal placement and activation of the arthrometer is difficult to determine. To avoid measurement error that might be associated with having more than one investigator perform this part of the protocol, we recommend having the same investigator secure the rats to the arthrometer for the duration of a study so that any bias is consistent between experimental and control knees. 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. Finally, results may vary from true *in vivo* ROM as the protocol was developed to test euthanized rather than live animals.

While we described the use of a device for evaluating the effects of immobility on the rat knee joint, other conditions affecting joint ROM could also be studied. There are numerous examples, some of which include effects of trauma, increased muscular tone secondary to central nervous system insult, or genetic modifications relating to neuromuscular disease. Respective interventions such as stem cell application to the knee joint, neuromuscular junction blockade, or gene therapy treatments that help discover new treatment options could also be assessed using the device.

ACKNOWLEDGMENTS:

The authors would like to thank Joao Tomas for his technical assistance with the device and Khaoula Louati for assistance in developing the image analysis methods.

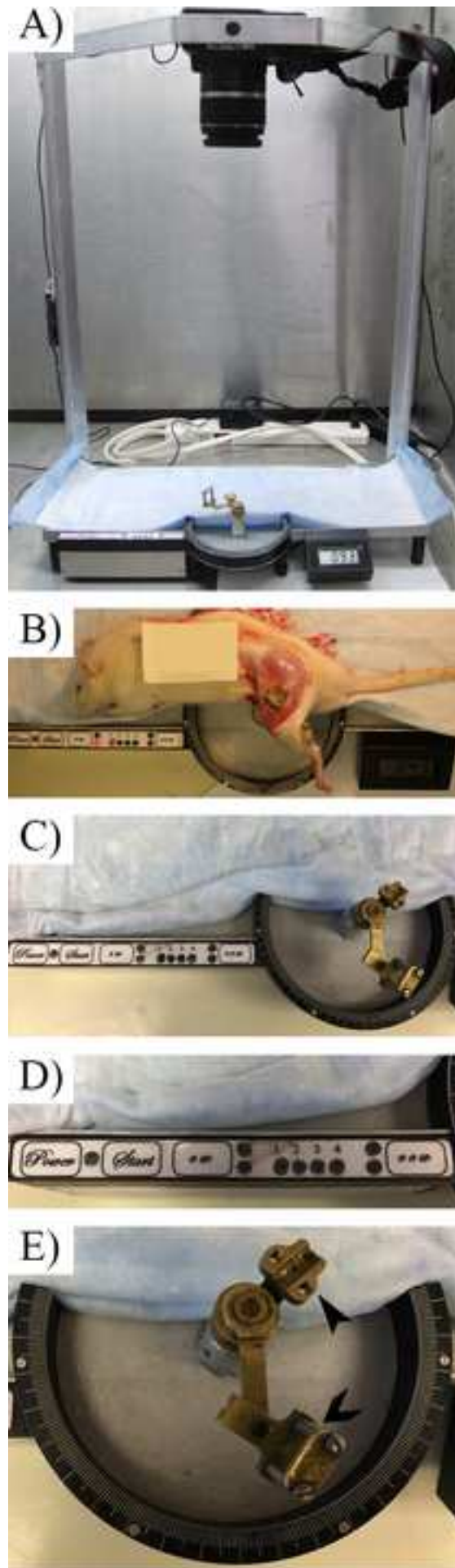
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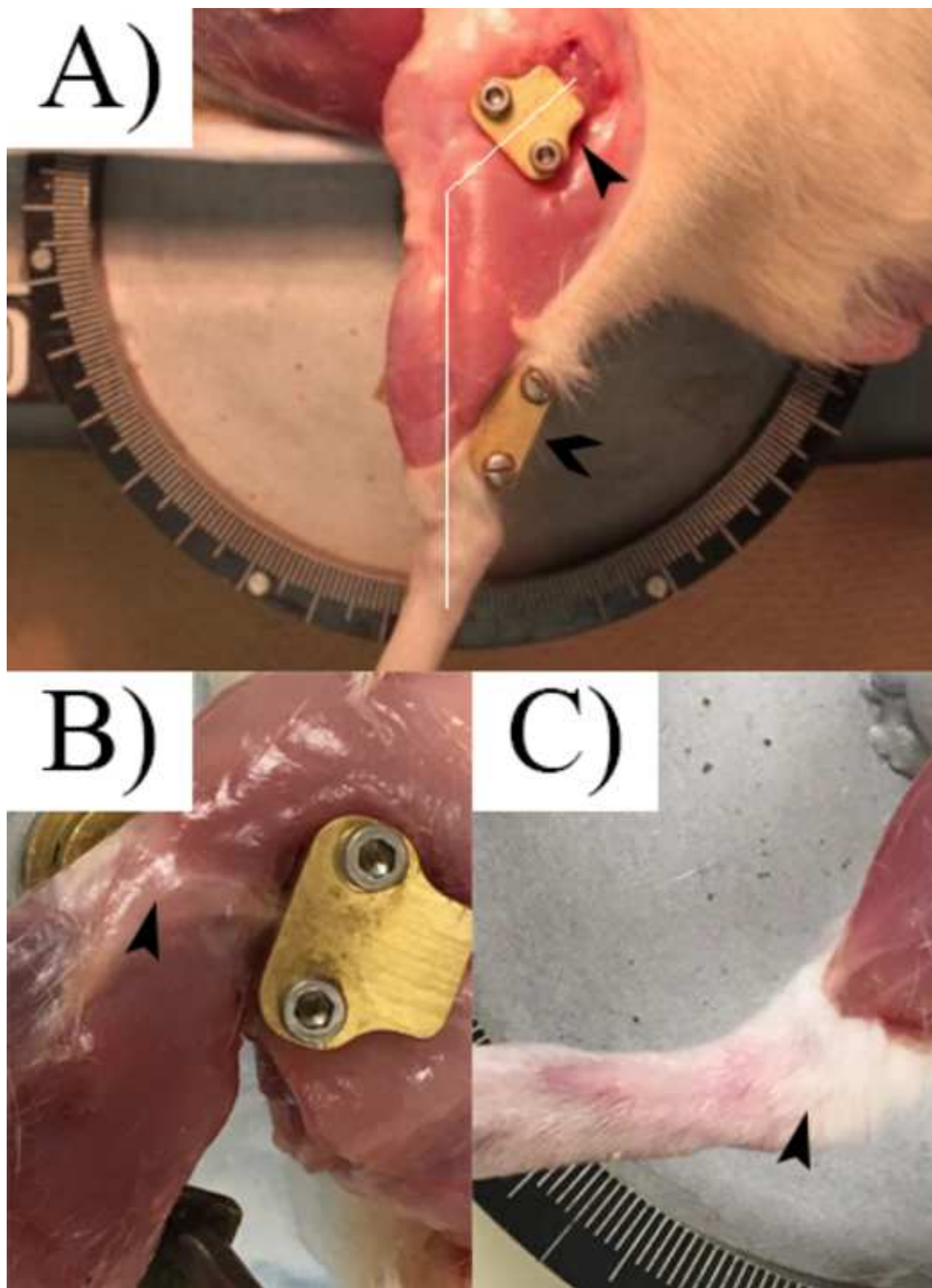
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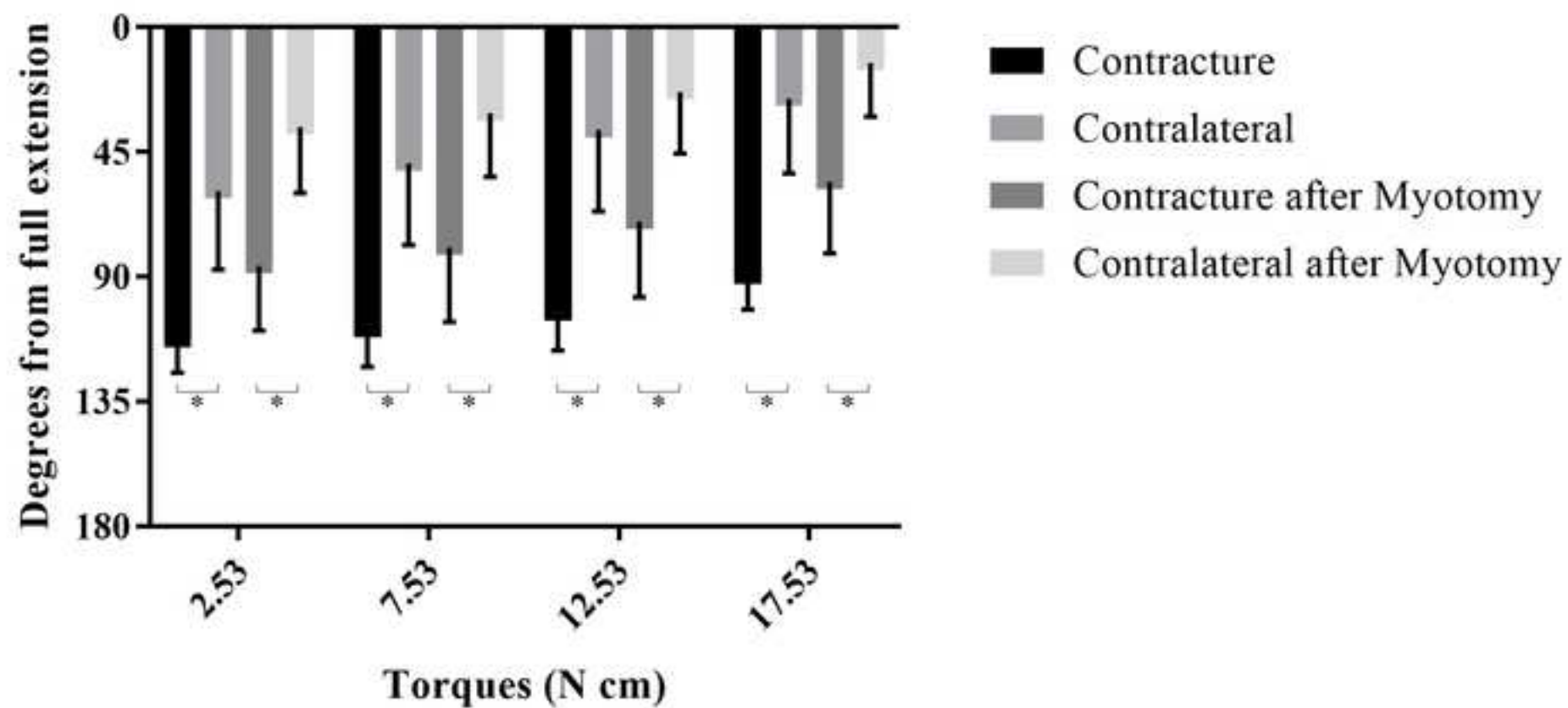
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Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Arthrometer	The Ottawa Hospital Rehabilitation Centre - Rehabilitation Engineering	N/A	
Camera	Canon	EOS-500D	Commonly known as EOS Rebel T1i
ImageJ	National Institutes of Health	Version 1.45s	
Absorbent Underpads	VWR	820202-845	
Dissection Kit	Fisher	08-855	Kit Includes: Forceps: medium points, nickel- plated Scissors: 1.5 in. (40mm) blades, stainless steel Dissecting knife handle: nickel-plated Knife blades: stainless steel, pack of 3 Dropping pipet: glass Bent dissecting needle: stainless steel with plastic handle Straight dissecting needle: stainless steel with plastic handle Vinylite Ruler 6 in. (15cm)
Precision Screw Driver	Mastercraft	057-3505-8	
Scalpel Blades - #10	Fine Science Tools	10010-00	
Screwdriver	Stanley	057-3558-2	
Hex Keys	Mastercraft	058-9684-2	

Universal AC to DC powder adapter	RCA	108004951	
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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)

1.3 “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.