Video Article

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

T. Mark Campbell[1,2,3], Katherine Reilly[2,3], Louis Goudreau[4], Odette Laneuville[2,5], Hans Uhthoff[2,3], Guy Trudel[2,3,4]

[1]Elizabeth Bruyère Hospital

[2]The Bone and Joint Research Laboratory, University of Ottawa

[3]Department of Medicine, University of Ottawa

[4]The Ottawa Hospital Rehabilitation Centre

[5]Department of Biology, University of Ottawa

Correspondence to: T. Mark Campbell at tcampbell@bruyere.org

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.

Video Link

The video component of this article can be found at https://www.jove.com/video/58084/

Introduction

Having full range of motion (ROM) of the joints is critical for health and well-being[1]. A loss in joint passive ROM is called a contracture[2]. Joint contractures may arise from numerous conditions, including prolonged bedrest, paralysis, joint arthroplasty, burns, infection, and neurologic conditions[1,3,4,5]. 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 morbidity[8]. 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 data[9]. 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 contractures[10]. 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. 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 previously[6]. Over a period of time, this produces a knee flexion contracture[11].
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.
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.
4. Once the screws are removed, remove the plate manually or using forceps from a dissection kit.
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.

1. Position the animal to be tested on its side with the experimental leg (the leg to be tested) facing upwards (**Figure 2**).
   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 (**Figure 1**, **2**).
   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.
   3. Tighten the femur clamp at its base using a hex key until it is secured.
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.
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.
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.
   1. Observe that the arthrometer motor will move at a speed of 6.6 RPM and then stop for 2.1 s upon reaching the first pre-set torque.
   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 s. 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.

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.
   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.
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

1. Analyze ROM using ImageJ.
   NOTE: Here version 1.45s was used.
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).

3. Select the **Angle tool** from the main toolbar and trace the femorotibial angle by drawing a femoral line from the middle of the femur clamp to the lateral condyle (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. 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**).
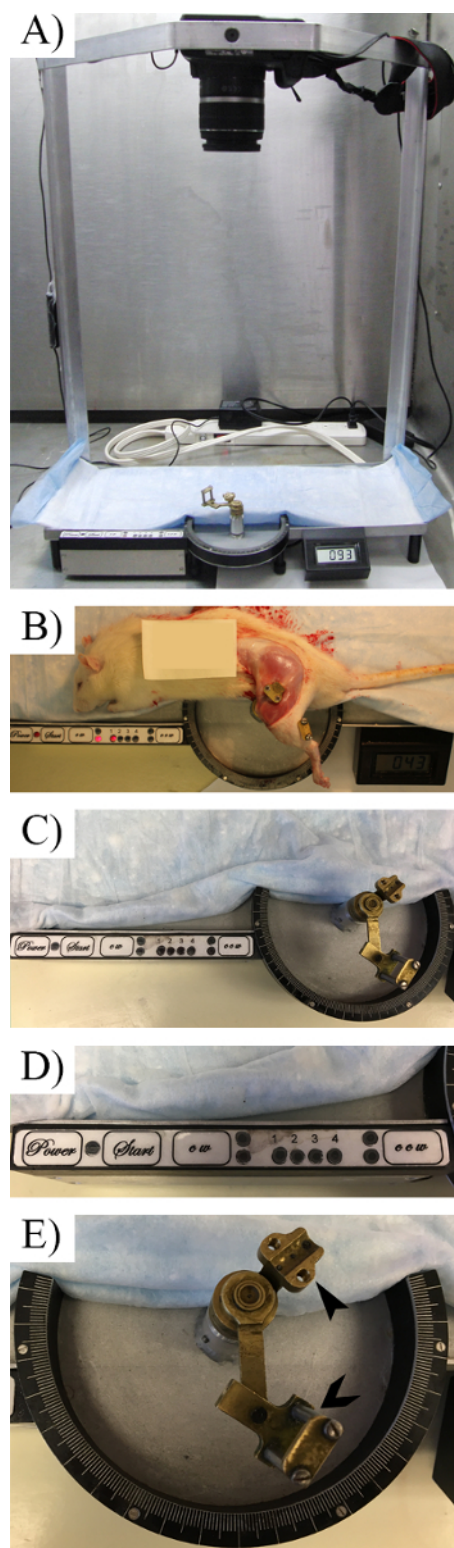
Figure 1: Rat knee arthrometer. (**A**) Entire apparatus (**B**) Representative image of animal within arthrometer (**C**) Electronics display and clamps for arthrometer. (**D**) Enlarged image of electronics display. Numbers indicate the various torques being applied: torque 1 = 2.53 N-cm, torque 2 = 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. Please click here to view a larger version of this figure.
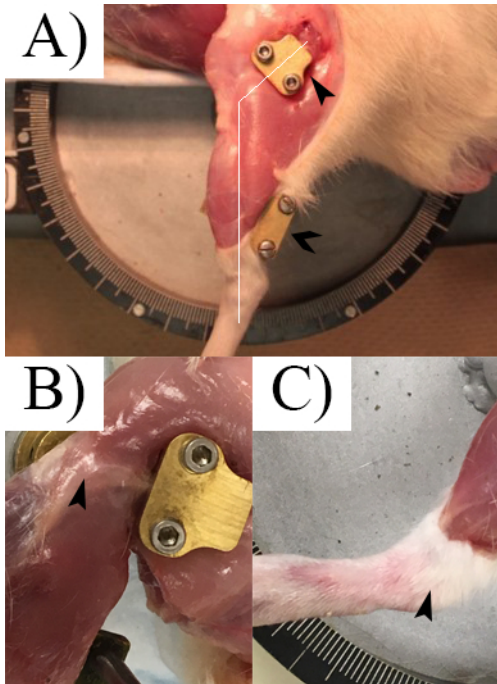
Figure 2: Arthrometer and experimental rat for evaluation. (**A**) Knee extension of the posterior limb is measured using the tibial line drawn from the lateral femoral condyle to the lateral malleolus and the femoral line drawn from the lateral condyle to the middle of the femur clamp. Arrowhead and 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.
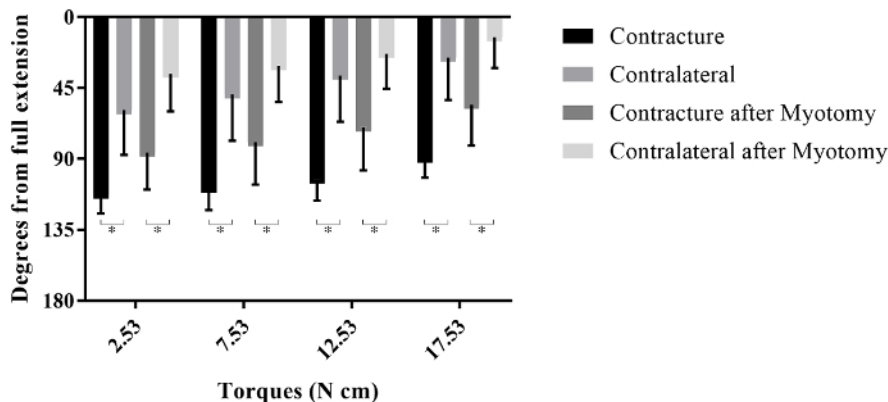

Figure 3: Immobilized and contralateral rat knee range of motion following 16 weeks immobilization. For both knees, after testing knee extension with all articular structures intact (n=7), a myotomy of the trans-articular muscles was performed to determine the arthrogenic limitation on ROM. Data are 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. Please click here to view a larger version of this figure.

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 limitation[11].

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 models[12,13,14,15,16]. 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.

Disclosures

The authors have no disclosures or conflicts of interest to declare.

Acknowledgements

References

1. Clavet, H., Hébert, P. C., Fergusson, D., Doucette, S., & Trudel, G. Joint contracture following prolonged stay in the intensive care unit. *Canadian Medical Association Journal.* **178** (6), 691-697, (2008).
2. Campbell, T. M., Dudek, N., & Trudel, G. in *Essentials of Physical Medicine and Rehabilitation: musculoskeletal disorders, pain, and rehabilitation.* (ed Silver JK Frontera WR, Rizzo TD.) Ch. 126, Saunders, (2015).
3. Dehail, P. *et al.* Acquired deforming hypertonia and contractures in elderly subjects: definition and prevalence in geriatric institutions (ADH survey). *Annals of Physical and Rehabilitation Medicine.* **57** (3), 11-23, (2014).
4. Korp, K., Richard, R., & Hawkins, D. Refining the idiom "functional range of motion" related to burn recovery. *Journal of Burn Care and Research.* **36** (3), 136-145, (2015).
5. Elliott, L., & Walker, L. Rehabilitation interventions for vegetative and minimally conscious patients. *Neuropsychological Rehabilitation.* **15** (3-4), 480-493, (2005).
6. Campbell, T. M., Reilly, K., Laneuville, O., Uhthoff, H., & Trudel, G. Bone replaces articular cartilage in the rat knee joint after prolonged immobilization. *Bone.* **106** 42-51, (2017).
7. Trudel, G. *et al.* Mechanical alterations of rabbit Achilles' tendon after immobilization correlate with bone mineral density but not with magnetic resonance or ultrasound imaging. *Archives of Physical Medicine and Rehabilitation.* **88** (12), 1720-1726, (2007).
8. Harvey, L. A. *et al.* Stretch for the treatment and prevention of contractures. *Cochrane Database of Systematic Reviews.* **1** Cd007455, (2017).
9. Trudel, G., Himori, K., & Uhthoff, H. K. Contrasting alterations of apposed and unapposed articular cartilage during joint contracture formation. *Archives of Physical Medicine Rehabilitation.* **86** (1), 90-97, (2005).
10. Trudel, G., Uhthoff, H. K., Goudreau, L., & Laneuville, O. Quantitative analysis of the reversibility of knee flexion contractures with time: an experimental study using the rat model. *BMC Musculoskeletal Disorders.* **15** 338, (2014).
11. Trudel, G., & Uhthoff, H. K. Contractures secondary to immobility: is the restriction articular or muscular? An experimental longitudinal study in the rat knee. *Archives of Physical Medicine and Rehabilitation.* **81** (1), 6-13, (2000).
12. Chimoto, E., Hagiwara, Y., Ando, A., & Itoi, E. Progression of an arthrogenic motion restriction after immobilization in a rat experimental knee model. *Upsala Journal of Medical Sciences.* **112** (3), 347-355, (2007).

13. Ando, A. *et al.* Remobilization does not restore immobilization-induced adhesion of capsule and restricted joint motion in rat knee joints. *Tohoku Journal of Experimental Medicine.* **227** (1), 13-22, (2012).

14. Abdel, M. P. *et al.* Effects of joint contracture on the contralateral unoperated limb in a rabbit knee contracture model: a biomechanical and genetic study. *Journal of Orthopaedic Research.* **30** (10), 1581-1585, (2012).

15. Hildebrand, K. A., Sutherland, C., & Zhang, M. Rabbit knee model of post-traumatic joint contractures: the long-term natural history of motion loss and myofibroblasts. *Journal of Orthopaedic Research.* **22** (2), 313-320, (2004).

16. Klein, L., Player, J. S., Heiple, K. G., Bahniuk, E., & Goldberg, V. M. Isotopic evidence for resorption of soft tissues and bone in immobilized dogs. *Journal of Bone and Joint Surgery. American Volume.* **64** (2), 225-230, (1982).