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An *in vivo* rodent model of contraction-induced injury in the quadriceps muscle

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ABSTRACT

Most animal studies of muscle contractile function utilise the anterior or posterior crural muscle (dorsiflexors and plantarflexors, respectively). An advantage to using these muscles is that the common fibular and tibial nerves are readily accessible, while the small size of the crural muscles is a disadvantage. Working with small muscles not only makes some *in vivo* imaging and the muscle testing techniques more challenging, but also provides limited amounts of tissue to study. The purpose of this study was to describe a new animal muscle injury model in the quadriceps that results in a significant and reproducible loss of force. The thigh of Sprague Dawley rats ($N = 5$) and C57BL/10 mice ($N = 5$) was immobilised and the ankle was attached to a custom-made lever arm. The femoral nerve was stimulated using subcutaneous electrodes and injury was induced using 50 lengthening ("eccentric") contractions through a 70° arc of knee motion. This protocol produces a significant and reproducible injury, with comparable susceptibility to injury in the rats and mice. This novel model shows that the quadriceps muscle provides a means to study whole muscle contractility, injury, and recovery *in vivo*. In addition to the usual benefits of an *in vivo* model, the larger size of the quadriceps facilitates *in vivo* imaging and provides a significant increase in the amount of tissue available for histology and biochemistry studies. A controlled muscle injury in the quadriceps also allows one to study a muscle, with mixed fibre types, which is extremely relevant to gait in humans and quadruped models.

Introduction

Muscle strains are one of the most common complaints treated by physicians and account for the majority of all sports-related injuries.[1,2] It is difficult to study muscle injuries in humans, as the incidence is a random event that is difficult to predict, and the clinical presentation varies greatly. Much of the data regarding muscle injuries has therefore been ascertained from studies on animals, which provide control over many variables and the ability to study mechanisms of injury and recovery. An *in vivo* injury provides a method for assessing contractile function without dissecting the muscle, and thus without the need to euthanise the animal under study. In the laboratory setting, investigators have used toxins,[3,4] lacerations,[5,6] freeze damage, and contusions[7,8] to study muscle damage, but by far the majority of muscle injuries in humans are attributable to excessive strain of an activated muscle (e.g., forceful lengthening, or "eccentric", contractions). Submaximal lengthening contractions are used in everyday activities, but it is well known that high force lengthening contractions are associated with muscle damage and pain.[9]

One problem with many of the biological markers used to assess muscle injury, including those used in most animal studies, is that they usually do not correlate with loss of force. Since full contractile function can persist despite the presence of injury markers, loss of force may be the most valid measure of injury,[10] and probably the most relevant. Most animal studies of *in vivo* or *in situ* muscle contraction utilise the dorsiflexor or plantarflexor muscles (the crural muscles), which offer the advantage of accessibility to the nerves innervating them, and the ability to compare results to previous literature.[11–15] A disadvantage of using the crural muscles is their small size (especially in mice), which not only makes the contractility assay and injury more challenging, but also provides limited amounts of harvested tissue for examination. Variation from muscle to muscle with regards to architecture, fibre type, regeneration properties, etc., also underlies the importance of studying different muscle groups. Despite a common nerve supply and insertion, the quadriceps muscle is often described as four muscles (rectus femoris, vastus lateralis, vastus intermedius and vastus medialis) due to the different points of origin. The quadriceps femoris was chosen as an alternative to

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crural muscles due to its large mass and multiple fibre types. For example, approximately half the fibres in the vastus intermedius are slow (Type I), but the other "heads" of the quadriceps contain primarily fast (Type II) fibres.[16] An injury model using the quadriceps allows the study of a larger muscle that has a significant role in gait, even in the quadruped.

The purpose of this study was to describe a novel muscle injury model in the quadriceps that results in a significant and reproducible loss of force. Force production and force loss are compared in both mouse and rat quadriceps. To the best of our knowledge, this is the first study to utilise a controlled lengthening contraction-induced injury model in the quadriceps femoris.

Materials and methods

Injury

All protocols were approved by the University of Maryland Institutional Animal Care & Use Committee (IACUC). We have previously described a model to injure the tibialis anterior muscle (TA) of rats and mice, which results in a significant and reproducible injury.[17–20] Here we tested a modified version of this apparatus on the quadriceps muscles. We used five age-matched male mice (Jackson Laboratory, Bar Harbor, ME C57BL/10ScSn, $N = 5$, 28.5 ± 1.4 g) and five age-matched male rats (Charles River Laboratories, Wilmington, MA, Sprague-Dawley, $N = 5$, 242.3 ± 5.5 g). Animals were anaesthetised (~4–5% isoflurane for induction in an induction chamber, then ~2% isoflurane via a nosecone for maintenance) using a precision vapouriser (cat# 91103, Vet Equip, Inc., Pleasanton, CA) for all experiments. Sterile ophthalmic cream (Paralube Vet Ointment, PharmaDerm, Floham Park, NJ) was applied to each eye to protect the corneas from drying. During the procedure, the animal was kept warm by use of a heat lamp.

With the animal supine, the thigh was stabilised with a fine (27 gauge) needle through the femoral condyles and the ankle was secured onto a lever arm. The quadriceps lever arms (Fig. 1B and C) for the mice and rats were custom-made and machined from aluminum. The mouse lever arm has a total length of 32.16 mm and weighs 2.3 g. The protruding rod adjusts from 16 to 24 mm at increments of 0.84 mm and is adjusted until it lies anterior to the ankle joint. The rat lever arm has a total length of 49.71 mm and weighs 3.7 g. The rat ankle rod adjusts from 28 to 40 mm, also at increments of 0.84 mm. The mouse or rat lever arm connects to a stepper motor and rotates through a pre-determined motion (up to 360°, Fig. 1A). For experiments, the axis of the knee was aligned with the axis of the stepper motor (model T8904, NMB Technologies, Chatsworth, CA) and a torque sensor (QWFK-8M, Sensotec) to measure torque in Newton millimeters (N mm). The femoral nerve was stimulated via subcutaneous needle electrodes (J05 Needle Electrode Needles, 36BTP, Jari Electrode Supply, Gilroy, CA). Proper

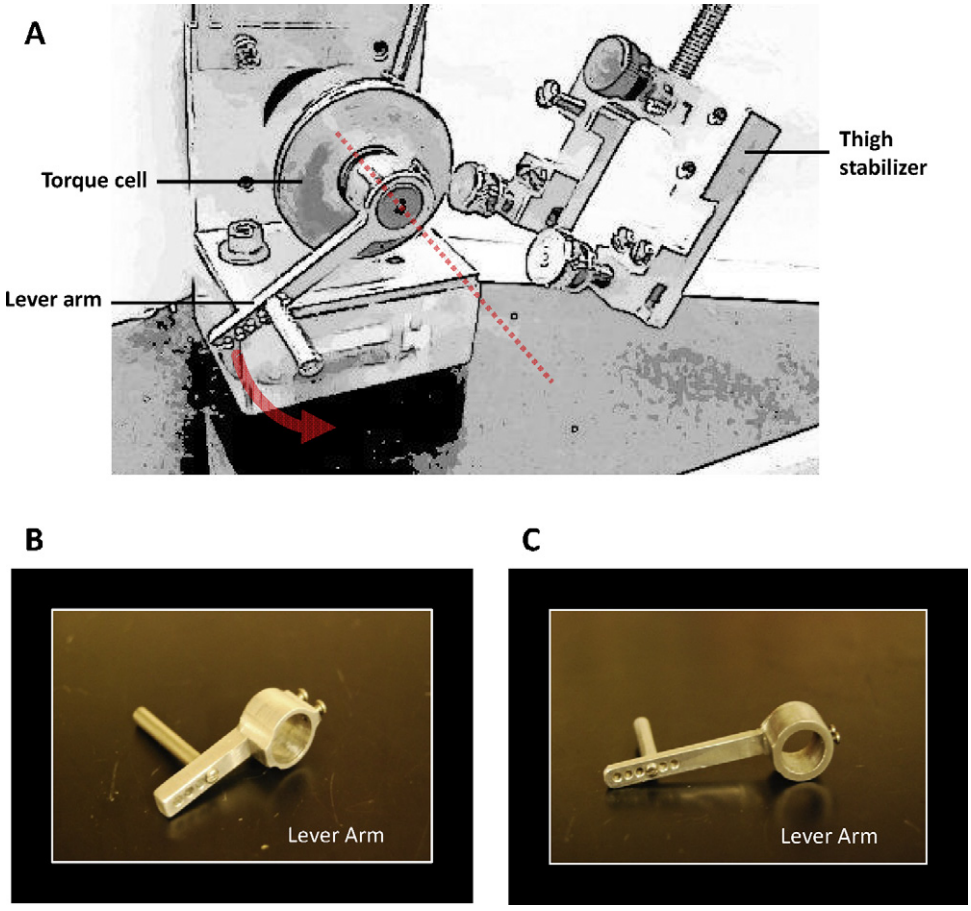

Fig. 1. Injury model and lever arm specifications. The cartoon describes the injury model for the quadriceps. (A) Animals are positioned supine on a raised bed (not shown) with a fine needle through the femoral condyles. The needle is secured into the thigh stabiliser, which can be adjusted in multiple planes and then stabilised. The needle/thigh stabiliser complex is aligned with the axis of the torque cell (dotted red line). The lever arm is adjusted so that the protruding arm is positioned over the anterior aspect of the ankle joint. Electrodes (not shown) are used to stimulate the quadriceps muscle. The isometric torque (towards extension) is recorded. For lengthening contractions, a stepper motor superimposes flexion (red arrow) onto a maximally contracting quadriceps muscle. (B) Mouse lever arm has a total length of 32.16 mm and weighs 2.3 g. Protruding ankle rod adjusts from 16 to 24 mm at increments of 0.84 mm. (C) Rat lever arm has a total length of 49.71 mm and weighs 3.7 g. The rat ankle rod adjusts from 28 to 40 mm also at increments of 0.84 mm. Both lever arms connect to the stepper motor and rotate through a pre-determined motion (up to 360°). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)