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METHODS AND TECHNIQUES

Contribution of individual quadriceps muscles to knee joint mechanics

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ABSTRACT

Many attempts have been made to determine the contribution of individual muscles in an agonistic group to the mechanics of joints. However, previous approaches had the limitations that muscles often could not be controlled in a precise manner, that individual muscles in an agonistic group could not be activated individually, and that individual muscle contributions could not be measured in an actively contracting agonistic group. Here, we introduce a surgical approach that allows for controlled activation of individual muscles of an agonistic group. The approach is illustrated for the vastus lateralis (VL), vastus medialis (VM) and rectus femoris (RF) of the rabbit quadriceps femoris group. We provide exemplar results for potential applications of the approach, such as measuring the pressure distribution in the patellofemoral joint, and the torque–angle relationship of VL, VM and RF when activated individually and when the three muscles are activated simultaneously.

KEY WORDS: Joint biomechanics, Distribution problem, Individual muscle activation, Nerve stimulation

INTRODUCTION

Understanding the contribution of individual agonistic muscles to the mechanics of joints has been a fundamental problem in biomechanics for decades (Crowninshield and Brand, 1981; Hodgson, 1983; Penrod et al., 1974). Solving this problem experimentally, or theoretically, has been associated with a series of non-trivial assumptions. For example, it has been assumed that the forces exerted by individual muscles are proportional to the muscles' physiological cross-sectional area (e.g. Ichinose et al., 1997; Kubo et al., 2001), independent of fibre-type distribution, and independent of force–length or force–velocity properties (e.g. Crowninshield and Brand, 1981), assumptions that seem to belie experimental findings from direct force-sharing measurements in animal models (Abraham and Loeb, 1985; Herzog, 1996; Herzog et al., 1993; Walmsley et al., 1978). Furthermore, it remains unclear how the mechanical properties of muscles within an agonistic group affect the resultant joint moments, or how individual muscle forces affect the joint contact mechanics. Also, with few exceptions (Maas and Sandercock, 2010; Sandercock and Maas, 2009; Tijs et al., 2014), it has been presumed implicitly that the forces produced by individual muscles, and their mechanical properties measured in isolated muscle experiments, remain the same when the same muscles are activated within their agonistic group (Herzog, 1996). However, these assumptions have not been tested critically, and their validity remains uncertain.

Various experimental approaches have been used to determine the contributions of selected individual muscles to joint biomechanics. For example, intramuscular activation of individual muscles, using indwelling fine wire electrodes, combined with tendon force measurements have been used to study muscle properties (Caldwell and Reswick, 1975; Crago et al., 1980). However, intramuscular activation does not guarantee complete and exclusive activation of single muscles, as it is virtually impossible to fully activate a given muscle without co-activating neighbouring muscles. Nerve stimulation of entire agonistic muscle groups with simultaneous measurement of the individual muscle forces has been used to determine the mechanical properties of individual muscles (Bernabei et al., 2015; Herzog et al., 1992). However, this approach is limited in that all muscles are always activated to the same degree, and muscle weakness, delayed activation of agonistic muscles or force imbalance among muscles are hard to simulate *in vivo*. In order to quantify muscle contributions to joint biomechanics, muscles have also been eliminated through nerve sectioning (e.g. Egloff et al., 2014; Tijs et al., 2014), surgical elimination of a muscle (e.g. Sawatsky et al., 2012), tendon transection (e.g. Maas and Sandercock, 2008) or muscle inhibition via chemical nerve ablation (e.g. Longino et al., 2005; Misiaszek and Pearson, 2002). However, the main limitation of these studies is that the interventions were irreversible, and thus only a single condition could be tested. Selective muscle stimulation has also been attempted using a nerve sleeve with a multi-electrode array (McNeal and Bowman, 1985; Rozman et al., 2000). However, this approach did not allow for isolated muscle activation, and was found to be unreliable, as small movements of the sleeve electrode relative to the nerve produced great changes in activation (McNeal and Bowman, 1985). In order to determine the contribution of individual, agonistic muscles to the biomechanics of joints, it is necessary to be able to activate the target muscles individually and simultaneously for all conceivable combinations, reliably and without cross-talk, and with complete control over the amount and the timing of activation of each muscle.

Therefore, the purpose of this technical note is to describe a surgical approach to activate individual muscles of an agonistic muscle group in a controlled manner, allowing individual and simultaneous stimulation of these muscles, and thereby permitting many mechanical scenarios of interest to be simulated *in vivo*. We describe the approach for muscles of the rabbit quadriceps group, providing exemplar results of pressure distributions in the patellofemoral joint and torque–angle relationships for activation of the vastus lateralis (VL), vastus medialis (VM) and rectus femoris (RF). The proposed method can be used, in principle, for agonistic groups other than the rabbit quadriceps muscles, and can be applied to study other basic properties of agonistic muscle function, such as

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how the forces and mechanical properties, e.g. the force–length and force–velocity relationships, change for muscles when they are activated in isolation compared with when they are activated as part of the entire agonistic muscle group.

MATERIALS AND METHODS

Animal preparation

Experiments were performed on skeletally mature New Zealand white rabbits (1 year old). Seven rabbits were used to study the anatomy of the neurovascular system around the femoral nerve and femoral artery in the inner thigh, to develop the surgical approaches, and to optimize the surgical procedures for implantation of nerve cuff stimulating electrodes for VL, VM and RF. Two additional rabbits were used for pilot studies, and exemplar results of these experiments are shown here. The area of the inguinal ligament and femoral nerve was carefully prepared for surgery (Horisberger et al., 2012; Longino et al., 2005). All experimental procedures were approved by the Animal Ethics Review Committee of the University of Calgary.

Surgical procedures

The purpose of the surgery was to implant a nerve cuff stimulating electrode on each of the individual nerves supplying the VL, VM and RF. A rabbit was positioned supine and was anaesthetized using a 2.5% isoflurane (Benson Medical) to oxygen mixture. An incision (6–7 cm) was made just above the location of the femoral nerve in the inguinal triangle (Fig. 1A). Connective tissues and fat were removed through blunt dissection to reach the inguinal ligament (Fig. 1B). The pectineus muscle was carefully removed to allow access to the femoral nerve (Fig. 1C). The adductor nerve was then removed to prevent co-contraction (Fig. 1D). The small vein running underneath the pectineus (Fig. 1D) was separated from its connective tissue, ligated and cut (Fig. 1E) to gain access to the femoral nerve branch. Removal of the vein typically results in exposure of the individual nerve branches to the VL, VM and RF. The nerve branches were separated carefully from associated connective tissue (Fig. 1F), and three custom-built nerve cuff electrodes, similar to but much smaller (length 1.2 mm, internal diameter 0.9 mm) than those used in previous experiments (Sawatsky et al., 2012), were implanted on the nerve branches supplying the VL, VM and RF (Fig. 1G). Each nerve cuff was connected to an individual stimulation channel (stimulator: Grass S8800, Astro/Med Inc., Longueil, QC, Canada), providing controlled and independent electrical current to each of the nerve branches. Intra-operative testing was performed to verify stimulation of the correct muscle, and lack of cross-stimulation of non-target muscles. A silicon Kwik-Cast (World Precision Instruments, Sarasota FL, USA) was applied around each nerve cuff to prevent cross-talk between muscles and to maintain proper placement of the electrodes (Fig. 1H). Once proper stimulation had been established and lack of cross-talk secured, the individual muscles could be stimulated at any level of activation, in any combination and at various knee joint angles.

Experimental setup

After the completion of surgery, rabbits were fixed in a custom-built stereotaxic frame with both pelvis and knee joint pinned. A servo

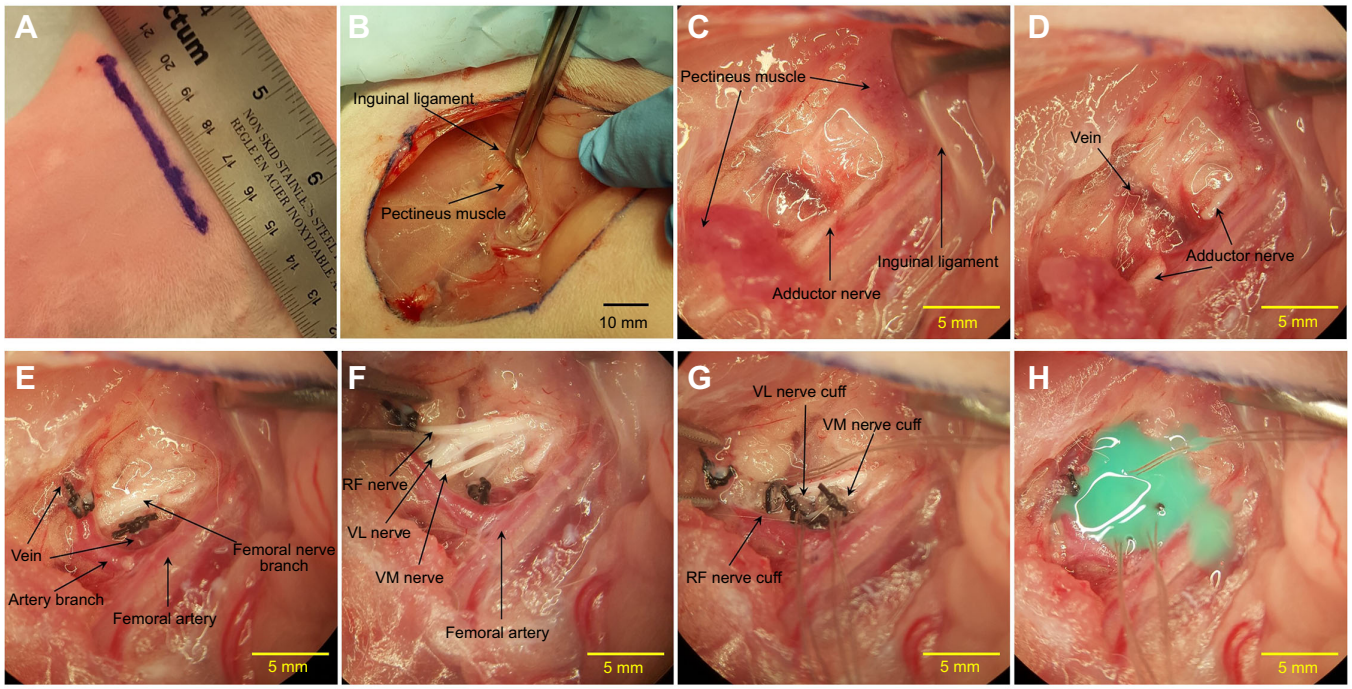


Fig. 1. Surgical procedures. (A) Rabbit in a supine position. A skin marker shows where the femoral nerve and the inguinal triangle are located. An incision was made along the marked line. (B) After blunt dissection, the inguinal ligament can be seen (pinched by forceps). The pectineus muscle is directly attached to the ligament. (C) The pectineus muscle was removed. Fibres of the pectineus muscle can be seen below the inguinal ligament. The adductor nerve can be seen in the middle of the photo. (D) The adductor nerve was surgically cut. The femoral nerve branches run underneath the vein in the middle of the photo. (E) The vein shown in D has been ligated by four knots, and cut. Following removal of the vein, the femoral nerve branch can be seen. (F) Connective tissues around the nerve branches were eliminated, which allows for separation of the individual nerve branches arising from the femoral nerve. VL, vastus lateralis; VM, vastus medialis; and RF, rectus femoris. (G) Three cuff-type nerve electrodes were implanted. Each cuff has two wires that conduct the electrical stimulation signals from a stimulator. (H) Silicone was injected around the nerve branches and each nerve cuff electrode; 8–10 min after injection, the silicon is fixed in place and cross-talk is prevented. Furthermore, the silicon keeps the nerve cuff electrodes firmly in place and ensures a constant threshold activation level.

Journal of Experimental Biology (2019) 222, jeb188292. doi:10.1242/jeb.188292

motor was attached to the end of the tibia in order to hold the knee joint at the target angle and measure knee extension torque (Egloff et al., 2014; Leumann et al., 2015).

The experimental setup used in this study represents a statically constrained configuration that can be used to measure isometric properties of the muscles. However, this approach can also be used for dynamic configurations, such as patellar tracking experiments and force–velocity and history-dependent properties of muscle activated in isolation or in the entire agonistic group.

Measurements

First, to test the reliability of this surgical technique, individual muscles were stimulated three times at the 90 deg knee joint angle at a frequency of 100 Hz, and the corresponding knee torques were recorded and compared. Also, in order to verify that a single target muscle was activated without recruiting non-target muscles, torque–voltage recruitment curves of individual quadriceps muscles were obtained in all cases. For a single experiment (not shown here), the torque–voltage recruitment curves were not stopped at the voltage where the target muscle reached its maximal torque, but voltage increments were continued to observe the point where additional non-target muscles were recruited. The recruitment of additional muscles was not only detected by a sudden increase in knee extension torque but also observed by eye and felt by muscle palpation. For the exemplar results illustrated here, pressure distributions in the patellofemoral joint were measured at 90 deg of knee flexion. Both medial and lateral retinacular incisions were made to insert a strip of pressure-sensitive film into the patellofemoral joint (Ronsky et al., 1995; Sawatsky et al., 2012). To achieve pressure distributions at various levels of muscle activation, quadriceps muscles were activated through a range of frequencies from 30 to 100 Hz. In addition, to obtain the corresponding torque–angle relationships, knee extension torques generated individually by VL, VM and RF were measured at various knee joint angles, ranging from 30 to 110 deg (0 deg is defined as full extension).

Calculation of cross-sectional area of individual quadriceps muscles

In order to compare the expected torque combination of each muscle with the experimentally measured torque, we estimated the physiological cross-sectional area of each muscle based on direct measurement of muscle mass, angle of pennation and fibre length (Koh and Herzog, 1998; Lieber and Blevins, 1989). To estimate the relative contribution of the VL, VM and RF to the knee extensor torques, the physiological cross-sectional area (PCSA, cm^2) was estimated according to Eqn 1 (Lieber and Blevins, 1989):

$$\mathrm{PCSA} = \frac{m \cdot \cos\theta}{l \cdot 1.054}, \quad (1)$$

where m is muscle mass, l is fibre length, θ is the average pennation angle and 1.054 g cm^{-3} is the density of skeletal muscle tissue.

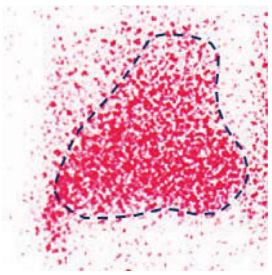
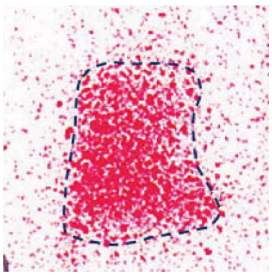
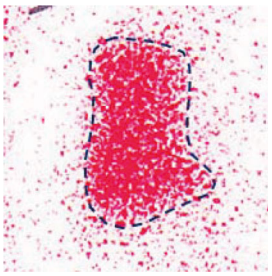
RESULTS AND DISCUSSION

Knee extensor torques were highly repeatable with a correlation coefficient of 0.99. The mean (±1 s.d.) torque for all three muscles was 3.0±0.1 N m, and the torques for VL, VM and RF individually were 2.0±0.09, 0.53±0.02 and 0.8±0.03 N m, respectively.

Through torque–voltage recruitment curves for each individual quadriceps muscle, we found a ‘safety margin’ where the knee extension torque reached its maximum value and did not increase despite increasing the stimulation voltage. The safety margins for VL, VM and RF in an exemplar experiment were 4.2–5.2 V, 2.8–4.4 V and 4.2–5.4 V, respectively. Once the safety margin was exceeded (i.e. at 5.4 V for VL, 4.6 V for VM and 5.6 V for RF), non-target quadriceps muscles were recruited. The end of the safety margin was identifiable by a sudden increase in knee extensor torque, and was also clearly detectable by visual and tactile inspection of the quadriceps muscles. Therefore, by carefully measuring the stimulation voltage versus torque recruitment curves, single muscle activation is possible. However, as the threshold for the safety margin differs for different muscles and animals, these recruitment curves need to be established for each muscle and each animal separately.

As an example for potential applications of the approach described above, we show the joint contact areas in the patellofemoral joint obtained for simultaneous activation of the VL, VM and RF, for simultaneous activation of the VL and RF only, and for isolated activation of the VL only, while matching the resultant knee extension torque within about 2% of the mean (Table 1). Note how the patellofemoral contact pressure distribution changes for the different conditions, and how the patellofemoral contact area decreases with decreasing numbers of muscles

Table 1. Pilot results of patellofemoral joint contact pressure distribution when muscles in the quadriceps group were stimulated

	3 muscles	2 muscles	1 muscle
Muscle(s) stimulated	VL+VM+RF	VL+RF	VL
Raw pressure stain			
Joint contact force (N)	45.1	43.0	44.1
Contact area (mm^2)	10.4	10.2	9.4

Vastus lateralis (VL), vastus medialis (VM) and rectus femoris (RF) were stimulated simultaneously (VL+VM+RF or VL+RF) or alone (VL). Comparison of joint contact area measured from super low pressure-sensitive film, which has a pressure range of approximately 0–2.6 MPa. Top and bottom of each pressure image represent proximal and distal, while left and right sides indicate medial and lateral parts in the patellofemoral joint, respectively. The dashed line on each image shows the joint contact area (region of interest).

Journal of Experimental Biology (2019) 222, jeb188292. doi:10.1242/jeb.188292

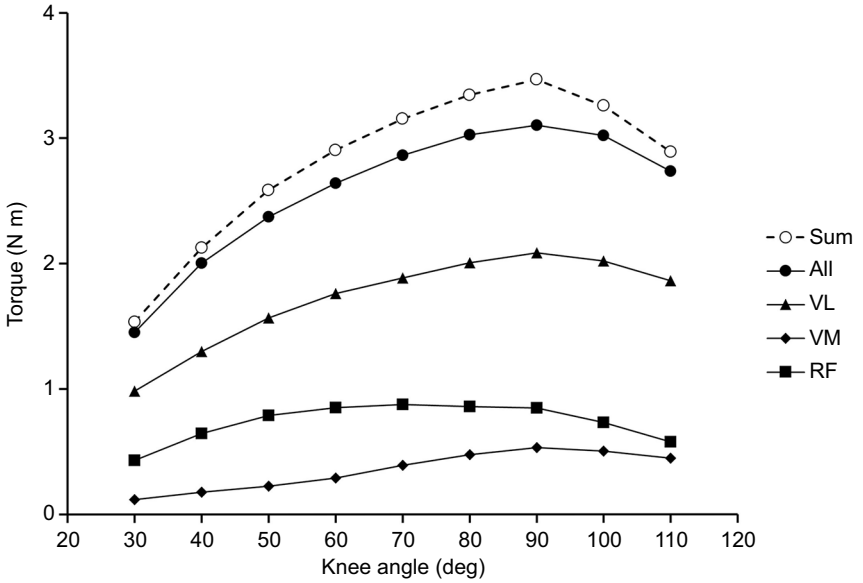


Fig. 2. Experimental torque–angle relationship of individual quadriceps muscles in one animal. A 0 deg knee angle indicates full extension. Sum, sum of VL, VM and RF torques; All, when VL, VM and RF were stimulated simultaneously; VL, vastus lateralis; VM, vastus medialis; RF, rectus femoris.

contributing to the same knee extensor torque. This result illustrates the dependence of joint contact pressure distribution on the muscles contributing to the joint moment.

Another application is the determination of the torque–angle relationships of individual muscles and combinations of synergistic muscle activation. In Fig. 2, we show the torque–angle relationship for the VL, VM and RF individually and simultaneously. The relative torque contributions of the VL, VM and RF across all joint angles were 61.4±1.7%, 12.0±3.1% and 26.6±3.4%, respectively. However, the relative physiological cross-sectional area of the VL, VM and RF was, respectively, 41.8±1.5%, 19.1±2.1% and 39.1 ±1.6% on average, indicating that the torque contribution measured for the VL was greater, and that for the VM and RF was smaller than expected based on estimates of their physiological cross-sectional area. Furthermore, adding the torques measured for activating the VL, VM and RF in isolation (Sum in Fig. 2) exceeded the potential to generate torque when all three muscles were activated simultaneously (All in Fig. 2) by 8.5±2.2% when averaged across all joint angles. These results indicate that estimating individual agonistic muscle contributions to the joint torque based on the physiological cross-sectional area of muscle might not be accurate. It also indicates that the force potential of the VL, VM and RF might not be same when they are activated simultaneously, compared with when they are activated in isolation.

Using the experimental approach described here, a variety of fundamental problems in muscle mechanics can be approached. For example, muscle weakness, muscle imbalance, or mal-coordination of muscles and its effects on joint contact mechanics can be studied. Furthermore, it has been shown that muscles activated within an agonistic group produce substantial inter-muscular pressures that may affect the deformation of muscles and their ability to produce forces (Siebert et al., 2014). Also, it has been argued that muscles within a group transmit forces to each other via connective tissues that are known to surround and connect individual muscles. These inter-muscular force transfers might affect the mechanical properties of the group of muscles to a substantial degree (Huijing, 2003). Finally, we have shown that when muscles are activated individually, and the sum of these individual muscle forces is calculated, the resulting force can be much greater (by 20% on average for the rabbit quadriceps) than the corresponding forces obtained when these same muscles are activated simultaneously (de Brito Fontana et al., 2018). Therefore, using the current approach enables the mechanical properties of individual muscles activated in isolation and activated in concert with their agonists to be studied, providing novel information on the function of muscles within their agonistic group, which has been rarely considered in muscle mechanics and biomechanical research.

Despite many advantages over previously used techniques, there remain limitations to the current approach. First, the technique described here (for the rabbit quadriceps muscles) is surgically difficult and requires substantial training. Second, the anatomy of the neurovascular system differs between animals, and may require individual surgical approaches. Finally, the quadriceps muscle group might not be an ideal system to investigate properties of individual agonistic muscles because of the merging of all muscles into a single tendon. Using an agonistic group where the individual muscles have separate tendons would allow direct quantification of changes in muscle properties between isolated muscle activation and agonistic muscle activation. In conclusion, using controlled stimulation of individual muscles of an agonistic group allows joint function and agonistic muscle properties to be studied in a novel way, providing insights into basic questions of musculoskeletal biomechanics.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: W.H.; Methodology: S.-W.H., A.S.; Validation: S.-W.H., A.S.; Formal analysis: S.-W.H.; Investigation: S.-W.H.; Resources: W.H.; Data curation: S.-W.H., A.S., H.d.B.F.; Writing - original draft: S.-W.H., W.H.; Writing - review & editing: A.S., H.d.B.F., W.H.; Visualization: S.-W.H., A.S., H.d.B.F.; Supervision: W.H.; Project administration: W.H.; Funding acquisition: S.-W.H., W.H.

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