Long-Term Measurement of Muscle Function in the Dog Hindlimb Using a New Apparatus


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Summary: The objective of this study was to develop an apparatus for reliable, reproducible, and minimally invasive measurements of long-term, myoneural function. Twenty conditioned dogs were anesthetized and placed supine with one hindlimb secured in a boot apparatus. The hindpaw was attached to a force transducer that was connected to a recorder for continuous monitoring of torque. Muscles within the anterolateral compartment were stimulated by percutaneous electrodes over the peroneal nerve near the fibular head. This elicited isometric dorsiflexion of the hindpaw. Twitch and tetanic torques correlated positively with dog weight whereas other skeletal-muscle function parameters (time to peak tension, one-half relaxation time, and endurance) were independent of dog weight. Muscle function results were consistent with an overall compartmental composition of 30% Type I and 70% Type I1 fibers. Repetitive testing of twitch and tetanic torques in the dog legs yielded coefficients of variance of 3–4% (intraday) and 7% (interday). Thus, about one-half of the interday variability may be accounted for by diet, exercise, and other physiological conditions that change daily. The apparatus was also used to detect myoneural degeneration following tourniquet ischemia. The results indicate that this procedure for evaluating muscle function yields reliable and quantitative results noninvasively, and thus allows long-term testing of muscle function in normal and diseased hindlimbs of dogs. Key Words: Skeletal muscle and nerve tests—Muscle contraction—Twitch and tetanic torques—Minimally invasive equipment—Joint position—Pneumatic tourniquet effects.

Maintenance and recovery of normal skeletal muscle function is of prime importance in health and disease. Exercise affects muscle function (2,11,17) and is implemented daily in rehabilitation programs to optimize recovery after musculoskeletal injuries, to increase athletic performance, and to prevent reinjury. Operative procedures such as fasciotomy, tendon lengthening, and muscle transfers may also affect skeletal muscle function.

In order to understand more thoroughly how various disease states affect muscle function, a quantitative, reproducible technique is needed for long-term studies. Edgerton and colleagues (7) developed an isolated distal tendon preparation for acute evaluation of muscle function. Garfin and coworkers (10) used this apparatus to study muscle function immediately following fasciotomy in canine hindlimbs. Patterson and collaborators (18) also used an isolated tendon preparation to study muscle function after tourniquet application on cynomolgus monkeys. Our previous muscle-function apparatus (13) for dogs allowed long-term testing, but the use of 1 mm Kirschner wires through the distal tibia prevented daily assessment of my-
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MATERIALS AND METHODS

Twenty conditioned dogs, unselected as to sex and weighing between 18 and 30 kg, were used in this study. The parameters measured were isometric twitch torque, time to peak tension, one-half relaxation time, isometric tetanic torque, and endurance tests at 15 and 10 Hz. Initially 34 hindlimbs of 17 dogs were studied at semiweekly intervals over a period ranging from 2 to 5 weeks for a total of 160 tests. To determine variability on a given test day, four hindlimbs of two dogs were studied three times in 1 day. The animals were removed completely from the apparatus between tests.

Long-term reliability of the technique was studied using two dogs of approximately equal weight (26 and 27 kg), studied at weekly intervals over a period of 2 months (seven tests per dog). Functional changes were studied following placement of a pneumatic tourniquet (inflated to 350 mm Hg for 6 h) on the experimental hindlimb of one dog just proximal to the stifle joint as previously described (14). Muscle function was determined 2 days after tourniquet application, and then at weekly intervals for 4 weeks.

Animal Preparation

Prior to studies of muscle function, dogs were anesthetized with intramuscular ketamine (30 mg/kg body weight) after a preanesthetic, intramuscular injection of 20 mg of xylazine. Supplemental doses of ketamine were administered thereafter as needed. The dogs were placed supine in the testing apparatus. The dog undergoing tourniquet application was preanesthetized with intramuscular morphine (30 mg), anesthetized with intravenous pentobarbital sodium (25 mg/kg body weight), and intubated. Supplemental doses of pentobarbital sodium (100 mg every 2 h) were administered thereafter as required. One day prior to tourniquet application, the dog was denied food but had free access to water. During tourniquet application, brachial artery pressure was continuously recorded and sterile normal saline (2 ml/min) was continuously infused into the brachial vein to maintain fluid homeostasis.

Muscle Function Apparatus

The leg under study was placed in the muscle function apparatus (Fig. 1). The thigh was stabilized by the foam support and the ankle was secured by tightening the noninvasive aluminum clamps (2.0 cm diameter) over the malleoli (Fig. 2). Under these conditions, tissue fluid pressure as measured by Wick Catheters (Intermedics Orthopedics, Inc., Dublin, CA) in subcutaneous regions below the clamps ranged between 40 and 200 mm Hg during the 30–40 min testing period. However, none of the 40 hindlimbs undergoing frequent, multiple testing in this study exhibited evidence of skin trauma or soft tissue injury, which would have prevented subsequent testing. The hindpaw was attached to a load cell (Statham 20 lb model, Gould-Statham Instruments, Inc., Oxnard, CA) and force transducer (Statham, model UC3), which was connected to a strip-chart recorder (model 7754A, Hewlett Packard, San Diego, CA) for continuous monitoring of muscle tension. Maintaining the tibiotalar angle at 175°, a 100 g preload tension was applied to the hindpaw in order to standardize initial conditions. Tibiotalar angle was optimized by measuring the muscle function parameters at varying angles within the dog’s full range of motion (tibiotalar angles of 145–195°) and recording the angle that maximized the reproducibility of measured forces. In humans the full range of ankle motion in conventional terms is about 20° dorsiflexion to 30° plantarflexion (tibiotalar angles of 70–120°). Each tibiotalar angle was measured using a goniometer. In two dogs these angles were also verified directly by lateral X-ray films. To stimulate the peroneal nerve, percutaneous needle electrodes (type E2, Grass Instrument Co., Quincy, MA) were placed subcutaneously 1 cm inferior and distal to the fibular head, where the nerve enters and innervates muscles of the anterolateral compartment that are the dorsiflexors of the hindpaw (Fig. 3). The skin was tattooed at the electrode insertion sites to ensure reproducible placement throughout the period of study. The electrodes were connected to a stimulator (model S88, Grass Instrument Co., Quincy, MA). Stimulation of the peroneal nerve produced isometric dorsiflexion of the hindpaw, which was quantified by the load cell and force transducer.
FIG. 1. Muscle function apparatus. a: Dog positioned with hindlimb in muscle function apparatus. b: Close-up view of hindlimb with foam support (f.s.) positioned under thigh. Nerve stimulator is connected to nerve electrodes (n.e.) to stimulate common peroneal nerve that innervates the anterolateral compartment (ALC) to produce dorsiflexion which is measured by the force transducer. Measured force vector of isometric contraction is perpendicular to clamped paw and transducer support. c: Muscle function apparatus with fixed L-shaped arms at an angle $\theta$ with respect to horizontal. Note that force transducer is perpendicular to L arms and at a distance $r$ from axis of rotation at malleolar clamps. d: Strip-chart recorder for monitoring muscle function parameters.

Testing Protocol

Isometric twitch torque was elicited in a voltage range from 1 to 6 V, frequency of 2 Hz, and duration of 3 ms. Using these stimulation parameters, torque values were maximized while torque variabilities were minimized. Time to peak tension and one-half relaxation time were recorded for isometric twitch force as previously described (3). After a 2-min recovery period, stimulation frequency was increased to 50 Hz and isometric tetanic torque (10 s duration) was measured. Separated by 5-min recovery periods, endurance was then tested using partially fused tetani at 15 and 10 Hz, and the time required for decay to one-half of the initial force was determined.

Statistical Analyses

Data were expressed as means ± SD or ± SE for all muscle function parameters. Differences between means were evaluated by ANOVA procedures for repeated measures and by Student's $t$ test. Statistical significance was set at the 95% confidence level. Coefficients of variance were calculated as SD/mean × 100%. Muscle function parameters were expressed as a function of the weight of the dog and analyzed by linear regression. According to the formula $t = r(\sqrt{n} - 2)/\sqrt{1 - r^2}$, $t$ values were derived from the linear regression analyses. Standard Student’s $t$ tests were then used to determine the confidence of correlation (95% confidence level corresponds to $p < 0.05$).

RESULTS

In four hindlimbs of two dogs the coefficients of variance [(SD/mean) × 100%] for isometric twitch torque and isometric tetanic torque (tested repeatedly on a given day) were 3 and 4%, respectively. If the animal was not removed from the apparatus between tests, the reproducibility was within the
resolution of our apparatus to measure force (20 g for twitch force and 40 g for tetanic force).

In four hindlimbs of two dogs, isometric twitch torque (Fig. 4) and isometric tetanic torque (Fig. 5) were measured at various tibiotarsal angles between 145 and 195° without removing the dog from the apparatus. Although maximum forces were achieved at a tibiotarsal angle of 145° (the angle of maximum attainable dorsiflexion, corresponding to approximately 20° dorsiflexion in the human ankle), this angle produced the greatest variation in measured forces (coefficient of variance of 4%). At this angle it was also technically difficult to maintain correct alignment of the hindpaw in the muscle function apparatus. The tibiotarsal angle of 175° gave the most reproducible data (coefficient of variance of 1%). Even though dorsiflexion torques of the anterolateral muscles were not maximal, the tibiotarsal angle of 175° was selected for subsequent studies since it allowed the most reliable comparisons. This angle corresponded to an apparatus angle θ of 5° (Fig. 1c).

In 37 hindlimbs of 20 dogs the coefficients of variance for isometric twitch torque and isometric tetanic torque on interday testing up to 2 months were 7% for both parameters (n = 179 tests for twitch torque, n = 175 tests for tetanic torque). Time to peak tension (range from 30 to 50 ms) and one-half relaxation time (range from 35 to 55 ms) had coefficients of variance of 13% (n = 174 tests) and 11% (n = 174 tests), respectively. Most of this variance resulted from the 5 ms time resolution of our recording system and was not due to interday changes in intrinsic contractile properties of anterolateral muscles. Endurance times at 10 Hz ranged from 12.0 to 20.6 min for the left leg and 9.6 to 17.6 min for the right leg. Times at 15 Hz were too short to give statistically meaningful fatigue results.

Muscle-function parameters (isometric twitch torque, isometric tetanic torque, time to peak tension, and one-half relaxation time) were expressed as a function of dog weight and analyzed by linear regression according to the formula \( y = A + Bx \).

In both the right and left legs, isometric twitch torque (Fig. 6) and isometric tetanic torque (Fig. 7) showed excellent linear correlation with increasing...
body weight (confidence of correlation was at the 99.5% level for both torque parameters). Isometric twitch torque and isometric tetanic torque of the left leg were consistently stronger than in the right leg. Time to peak tension \( (r = 0.33) \) and one-half relaxation time \( (r = 0.08) \) correlated poorly with body weights of the dogs. Nevertheless, these latter two parameters provide important indices of intrinsic muscle properties.

For the two dogs of near-equal weight tested at weekly intervals for a period of 2 months (seven tests per dog), maximum isometric twitch torque was \( 1.77 \pm 0.10 \text{ Nm (mean \pm SD)} \) and maximum isometric tetanic torque was \( 3.30 \pm 0.20 \text{ Nm (mean \pm SD)} \). Thus, these torque parameters had coefficients of variance of 6%. Time to peak tension was \( 41 \pm 2.5 \text{ ms} \) and one-half relaxation time was \( 39 \pm 7.0 \text{ ms} \).

Following tourniquet application for 6 h, isometric twitch torque decreased from \( 2.31 \pm 0.08 \text{ (base line)} \) to \( 0.99 \text{ Nm 2 days after application of tourniquet and recovered to 1.69 Nm after four weeks (Fig. 8). Isometric tetanic torque fell from} \)
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4.59 ± 0.15 Nm (base line) to 1.92 Nm 2 days post-tourniquet and recovered to 2.82 Nm 4 weeks post-tourniquet (Fig. 9). Torques also dropped slightly in the control leg, perhaps indicating a systemic effect of the 6 h tourniquet application.

DISCUSSION

Many pathological conditions involving myoneural dysfunction are managed by the orthopaedic surgeon. Central nervous system derangements such as stroke, poliomyelitis, and amyotrophic lateral sclerosis affect skeletal muscle function. Peripheral nerve disorders such as neuritis, entrapment syndromes (e.g., carpal tunnel syndrome), and direct injury to nerve with secondary neuroapraxia, axonotmesis, or neurotmesis produce muscle dysfunction. Myopathic disorders including muscular dystrophies and polymyositis present challenging orthopaedic problems related to skeletal muscle dysfunction.

In this study a reliable, noninvasive method of measuring long-term myoneural function in a canine hindlimb was developed. In two dogs of equal weight, torque measurements were reproducible to within 6% over a period of 2 months. Complete removal and re-entry of a dog in the apparatus yielded reproducibility to within 4% on a given day. Exercise, diet, and other physiological conditions that change daily may account for half of the 7% interday variation that we determined for the 20 dogs included in this study. The technique was able to quantitate the time course of muscle dysfunction after tourniquet ischemia.

Torques Versus Tibiotarsal Angle

Isometric twitch and tetanic torques correlated negatively with tibiotarsal angle (Figs. 4 and 5). This probably reflects the dependence of force on muscle length which is well established for whole muscles and single fibers (12). It is interesting that torques are maximized when the ankle is fully dorsiflexed and are decreased as the ankle is plantarflexed. In a recent study of human dorsiflexion, torque associated with voluntary contraction is maximized when the foot is approximately in a neutral position (15). Generally, however, torques for human ankles are higher in plantarflexion than in corresponding angles of dorsiflexion. In both humans and dogs, however, torque is maximized at the approximate joint angle observed in the standing (bipedal and quadrupedal, respectively) position. As measured by X-ray films with the dog in a normal standing position, tibiotarsal angle is 135°. In the standing human, tibiotarsal angle is 90°. In this study, angles were usually measured using a goniometer and therefore were accurate only to ±5°. With respect to angles of dorsiflexion and plantarflexion, the aforementioned differences in torque between humans and dogs probably relate to differences in gait between the two species. Angular dependence of torque in the dog may be due to increased sarcomere length of all anterolateral
muscles during plantarflexion, or may represent a complex interaction between the various muscle groups within the anterolateral compartment.

**Torques Versus Dog Weight**

Correlations of torque and dog weight are highly significant ($p < 0.005$) and should be valuable for future investigations using this apparatus. Thus, in this noninvasive setting, it appears that force can be normalized in terms of force per kilogram body weight. The mass of the muscle within the anterolateral compartment (1) is approximately 0.2% of the body mass of the dog ($1.96 \pm 0.13 \text{ g/kg, } n = 10 \text{ dogs}$). The positive correlation between body mass and isometric torques (Figs. 6 and 7) probably represents a proportionality between muscle mass and cross-sectional area.

From the data in Fig. 7, the isometric tetanic torque of a 23 kg dog is about 2.75 Nm at a tibiotarsal angle of $175^\circ$. Using data from Fig. 5, maximum torque of the hindpaw is 2.93 Nm. Based on the geometry of muscle origins and insertions (as measured from X-ray films), the maximum muscle force developed is 140 N (calculated for an insertion angle of $15^\circ$, 8 cm away from the pivot point). For a 23 kg dog, average cross-sectional area of the anterolateral compartment is 4.5 cm$^2$ (calculated for a 10 cm long compartment and a total compartmental volume of 45 cm$^3$). The normalized force is therefore 31 N/cm$^2$, which agrees well with data from isolated single fibers (12), fiber bundles (9), and whole muscle (9). This force is consistent with the data of Garfin and colleagues (10), who measured a tetanic force of 60 N for the exposed tibialis cranialis. Their value represents 43% of the maximum force measured in this study for the entire anterolateral compartment, a result compatible with the fact that the tibialis cranialis comprises 46% of the mass of the compartment.

**Physiological Properties of Anterolateral Muscles**

Isometric tetanus-to-twitch torque ratio averaged 2–3 in this study. This ratio is characteristic of isolated mammalian muscles composed of predominantly fast muscle fibers (6). Using the data of Armstrong and co-workers (1), the weighted average fiber type within the anterolateral compartment was calculated to be approximately 30% Type I and 70% Type II. The average time to peak twitch tension of 40 ms can be converted to a maximum contraction velocity of about 5 muscle lengths/s, using the relationship established by Close (6). This value agrees well with the theoretical value of 41 ms, which is obtained using the model of Biscoe and Taylor (4), for muscle with a fast fiber percentage of 70%. The average half-relaxation time of 39 ms coincides with data obtained from isolated, predominantly fast muscle (9,16). As expected, time to peak tension and half-relaxation time correlated poorly with body weight because they reflect intrinsic properties of muscle (6).

**Endurance**

Average endurance times measured at 10 Hz are considerably less than those expected for a muscle composed of 70% fast oxidative and 30% slow oxidative fibers (5). This discrepancy is probably a result of our stimulation protocol that allowed only 100 ms rest between successive stimuli. The decrease in force we observed with this protocol was probably due to neural fatigue or fatigue at the neuromuscular junction (8). Studies are now underway using a stimulation protocol that allows more complete rest between successive stimuli, thus permitting neural and neuromuscular junction recovery while revealing muscle fatigue.

**Peroneal Nerve Stimulation**

Placement of the electrodes into subcutaneous tissue near the common peroneal nerve is the most invasive aspect of this technique. Although higher stimulation voltages are necessary, transcutaneous stimulation could be used if a totally noninvasive technique is required. Secondary nerve injury and subsequent decrease of torque and endurance may occur with time, although our torque results indicate that this did not occur. A neural deficit, however, could be masked by a training effect of repeated studies on the muscle. To avoid nerve injury and to reduce experimental errors involved with placement of the electrodes, chronically implanted electrodes might improve the present technique.

Our technique allows repetitive measurements without apparent damage to the neuromuscular unit. Previously described techniques are more invasive and allow only single day determinations of muscle function. Patterson and associates used an isolated tendon preparation to determine muscle
function in the quadriceps and gastrocnemius/soleus complex of adult cynomolgus monkeys after tourniquet application (18). They determined muscle function at 1, 6, or 7 days after tourniquet application but were limited to a single measurement in each monkey because of the invasive nature of their technique. Our technique allows multiple determinations over a period of months.

Applications of the Technique

Muscle function data obtained in this noninvasive manner are consistent with data established for isolated muscle as discussed above. This technique allows long-term quantitation of processes that affect neuromuscular systems. Studies of the effects of multiple factors such as exercise, diet, surgery, and drugs on muscle function are now possible. Our results demonstrate that this muscle function apparatus yields reliable and quantitative results noninvasively. With little modification the design is applicable to other muscle groups and animals as well.

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