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SARCOMERE NUMBER ADAPTATION AFTER RETINACULUM TRANSECTION IN ADULT MICE

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Summary

Skeletal muscle has been shown to adjust serial sarcomere number in response to chronic static length changes. However, the adaptive responses to alterations in the dynamic environment are less well defined. The adaptations of the adult mouse tibialis anterior (TA) muscle to altered length and excursion were investigated by surgical transection of the flexor retinaculum. TA moment arm and muscle excursion increased by 38±7 % (mean ± S.E.M.) and fully extended (plantarflexed) muscle length was decreased by 8 % after flexor retinaculum transection. In spite of the significant shortening of the muscle in full plantar- and dorsiflexion, serial sarcomere number decreased by 10±1 % after 2 weeks of recovery. Gait analysis of these transected animals revealed a 14±3 % decrease in dorsiflexion angular velocity after transection. The decrease in angular velocity was less than the increase in moment arm and, as a result, muscle velocity was calculated to increase by 20±4 %. These data suggested that the muscle adapted in response to the underlying change in length, irrespective of the altered excursion or velocity.

Key words: fiber length, sarcomere number, immobilization, muscle, mechanics, mouse.

Introduction

Sarcomeres act as length- and velocity-dependent force generators (Edman, 1979; Gordon *et al.* 1966) in striated muscles. The mechanical properties of a sarcomere are scaled to the whole muscle on the basis of their arrangement within the muscle. Fiber length, or the number of sarcomeres in series, influences both the force–length and force–velocity characteristics of a muscle. Similarly, the length and velocity ranges over which a muscle is capable of generating force are determined by the resting length of its fibers.

Biological regulation of fiber length can be appreciated from the relative lack of variation in the fiber length of a particular muscle. Within a functional group, fiber length and moment arm are closely correlated (McClearn, 1985). Thus, during normal movement, muscle fibers within a functional group undergo similar relative length changes, which suggests that fiber length may be influenced by fiber excursion. Between functional groups, fiber length varies systematically and consistently, suggesting that fiber length is matched to functional demand (Burkholder *et al.* 1994; Friederich and Brand, 1990; Lieber *et al.* 1990, 1992*a*; Sacks and Roy, 1982; Wickiewicz *et al.* 1983). For example, Burkholder *et al.* (1994) demonstrated that each functional muscle group of the mouse hindlimb was associated with a stereotypical fiber length. In muscles with stereotypical functions, such as those involved in jumping (Lutz and Rome, 1994), swimming (Rome and Sosnicki, 1991) and walking (Walmsley *et al.* 1978), a specific combination of fiber length, moment arm and joint kinematics permits near-peak muscle force and power production. In these cases, optimal myofilament overlap results in maximum force production while sarcomere number is such that each sarcomere shortens at a velocity yielding maximum power. These studies imply that fiber length is tightly regulated.

Numerous experimental results have demonstrated that fiber length, or serial sarcomere number, is highly plastic. This phenomenon is observed most clearly using immobilization models, where a muscle is held in a chronically lengthened or shortened position (Tardieu *et al.* 1979; Williams and Goldspink, 1978). In as little as 2 weeks after immobilization begins, sarcomere number changes such that maximal force and optimal sarcomere length are produced at the position of immobilization. This result, along with results obtained using other models of chronic length change, has led to the concept that sarcomere length is optimized to a particular joint angle, presumably to maximize force production. However, the difficulty in interpreting results from immobilization studies is that it is impossible to identify the particular signal to which sarcomere number adapts (Herring *et al.* 1984). Sarcomere number may change within a muscle fiber so that optimal sarcomere length is determined by resting muscle length, by resting muscle tension, by an extreme of muscle length or even by the length of most frequent or maximal muscle activation.

Chronic length change models cannot distinguish between these possibilities.

Serial sarcomere number is also sensitive to dynamic stimuli (Alder *et al.* 1959; Crawford, 1954; Matano *et al.* 1994). For example, if the tibialis anterior moment arm of young rabbits is increased by surgical transection of the crural ligament, muscle excursion is dramatically increased and whole-muscle length, and presumably fiber length, increases in proportion to the increased excursion. Unfortunately, the interpretation of these experiments is confounded by the rapid growth rate of these animals, which results in increased fiber length. Serial addition of sarcomeres is inhibited in immobilized, growing mice (Williams and Goldspink, 1978)

Thus, there is evidence to suggest that muscle fiber length is matched to the static and dynamic force demands placed upon that muscle. It is also clear that muscles can adapt in response to static length changes and that young muscles can respond to alterations in dynamic length changes. It is not known whether adult muscles are also capable of remodeling in response to alterations in the dynamic biomechanical environment.

In the light of the functional importance of fiber length and the general lack of understanding of the mechanical and biological factors that regulate fiber length, the purpose of the present study was to develop a model in which the demands placed upon the muscle were altered so that sarcomere number could adapt to either length or excursion in a way that could be differentiated. We therefore developed a minimally invasive model whereby the ankle pretibial flexor retinaculum was surgically transected and the tibialis anterior (TA) muscle was permitted to move away from the center of rotation of the ankle joint (Fig. 1). By transecting the ankle flexor retinaculum, the TA muscle–tendon unit (MTU) takes a more direct line from knee to foot, increasing the TA moment arm and decreasing MTU length as the foot dorsiflexes. The mechanical effect on the muscle can be viewed as a chronic shortening as well as an increased excursion and velocity. The resultant sarcomere number changes definitively point to sarcomere number regulation in adult mice by length as opposed to excursion or velocity. A brief version of this report has been presented previously (Burkholder and Lieber, 1996*a*).

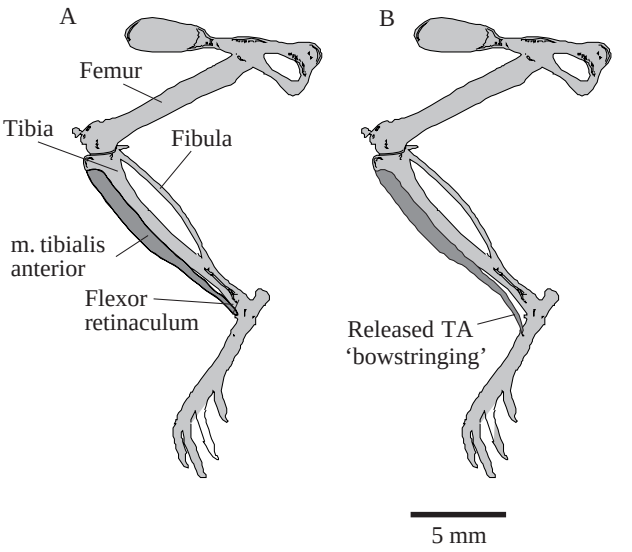

Fig. 1. Schematic depiction of the surgical procedure used to alter mouse tibialis anterior (TA) moment arm (A). The normal TA muscle passes beneath the crural ligament (the flexor retinaculum) and acts as a pulley. (B) After transection, the TA is allowed to elevate away from the ankle axis center of rotation, thus 'bowstringing' and increasing muscle excursion. The dark area represents the TA muscle.

Materials and methods

Surgical procedure

Thirty-four female Swiss-Webster mice were randomly divided into one of three groups: control (C, N=10), 1-week retinaculum transection (N=12) or 2-week retinaculum transection (N=12). Mice undergoing retinaculum transection were sedated with an intraperitoneal cocktail of Rompum (10 mg kg^{-1}), Ketamine (90 mg kg^{-1}) and Acepromazine (1.5 mg kg^{-1}). With the aid of a dissecting microscope, a small (2 mm) incision was made on the lateral surface of the foot, exposing the flexor retinaculum (crural ligament). The ligament was transected with fine scissors and the TA tendon elevated from its normal path, resulting in a more direct line to the insertion site (and thus in the muscle length changes reported below). The wound was then closed with cutaneous glue (Nexaband, Phoenix, AZ, USA). Transected mice were permitted normal cage activity immediately upon recovery. The entire procedure was typically completed in 3–5 min. Animals behaved normally immediately upon recovery, indicating the minimally traumatic nature of this surgical procedure.

Skeletal muscle architecture

At the conclusion of the experimental period (1 or 2 weeks), passive muscle length was measured with dial calipers *in situ* with the ankle fully plantarflexed (130 °), neutral (90 °) and fully dorsiflexed (40 °). The tibialis anterior was then removed, pinned under slight tension and fixed overnight in 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.0). Muscles were rinsed in PBS and cleaned of extraneous tissue before weighing. Muscle length (L_M) was measured with dial calipers under a dissecting microscope. Small fiber bundles (5–50) were teased from the lateral, medial, superficial and deep regions of the TA. Results from the different regions were not statistically different and were combined. Fiber bundle length (L_F) was measured using a digital filar eyepiece (Lasico, model 112983, Los Angeles, CA), and sarcomere length (L_S) was measured using laser diffraction in at least three regions of each fiber bundle (Lieber and Blevins, 1989; Lieber *et al.* 1984). Serial sarcomere number (S) was calculated for each bundle by dividing mean fiber bundle length by mean sarcomere length measured by diffraction. Physiological cross-sectional area (PCSA in mm^2) was calculated according to the formula:

$$\mathrm{PCSA} = M_M/(\rho \times 0.0025S)\,,$$

where M_M is muscle mass (mg) and ρ is muscle density (1.06 g cm^{-3}). Calculation of fiber length in the equation from sarcomere number (L_F=0.0025S) corrected for any variations in muscle length that occurred as a result of fixation at slightly different lengths. Normalization to a sarcomere length of 2.5 μm was used because this is assumed to be the optimal sarcomere length in rats (Walker and Schrodt, 1973).

Force–velocity measurements

To measure isotonic muscle properties and to estimate maximum shortening velocity (V_{max}), animals (N=6) were anesthetized as described above. The TA was exposed and attached to a servomotor (model 6650, Cambridge, MA, USA) by a short (5 mm) length of 5-0 gauge silk suture. The tibia and muscle origin were fixed rigidly to the testing apparatus using transcortical pins. The compliance of the system, including lever arm and suture, was approximately 1 μm g^{-1}. Muscles were activated by direct, supramaximal 100 Hz stimulation of the peroneal nerve using a stimulator (model S-88, Grass Instruments, Quincy, MA, USA). Muscle length was adjusted to the length (L_0) that produced maximum tension (P_0). Force–velocity trials consisted of a 400 ms tetanus during which the muscle was held at L_0 for 300 ms. The servo was then switched to force control, and the muscle was allowed to shorten against a constant, predefined fraction of P_0 for 100 ms. Shortening velocity was calculated from the slope of the muscle length trace 15 ms after the initiation of shortening to permit length transients to settle yet without allowing excessive shortening. Force transients settled within 10 ms. Rest periods (2 min) were imposed between trials at 80, 60, 40, 30, 20, 15 and 10 % of P_0 in the order stated. These data were fitted to the Hill equation (Hill, 1938) and extrapolated to zero load to yield V_{max}.

Tibialis anterior moment arms

Moment arms were measured in operated and contralateral limbs after 2 weeks of recovery in separate subjects (N=4) (mean ± S.E.M. body mass 23±1 g). Surgery was performed as described above. The musculature above the knee was removed and the Achilles tendon transected. The skin around the ankle was left intact. A 5-0 gauge silk suture was tied to the TA tendon proximal to the retinaculum and threaded through a metal guide loop at the TA origin. The suture was then tied to an inductive length transducer in series with a small weight. The foot was fixed to a plate and mounted on a potentiometer so that foot rotation was coaxial with the potentiometer. Tendon length and ankle angle were recorded as the foot was rotated through its range of motion (Fig. 2). The limb was removed from the testing apparatus between each of the three or more measurement repetitions. Potentiometer output was linear with angular rotation (19 mV degree^{-1}; r^2=0.98, P<0.001) and was calibrated to goniometer measurements of ankle angle (defined as the angle between the foot and tibia) with each repetition.

To eliminate constant-length offsets, tendon excursion–joint angle data (Fig. 2) were centered on the Cartesian coordinate system by the addition of a constant. Shifted data were then fitted to arbitrary polynomials using stepwise linear regression (Burkholder and Lieber, 1996*b*) and the polynomial returned to the initial coordinate system. The moment arm as a function of joint angle was then calculated by differentiating these polynomials. In the case where mean moment arms were calculated, the data were fitted to a line, and the slope of this line taken to be the mean moment arm.

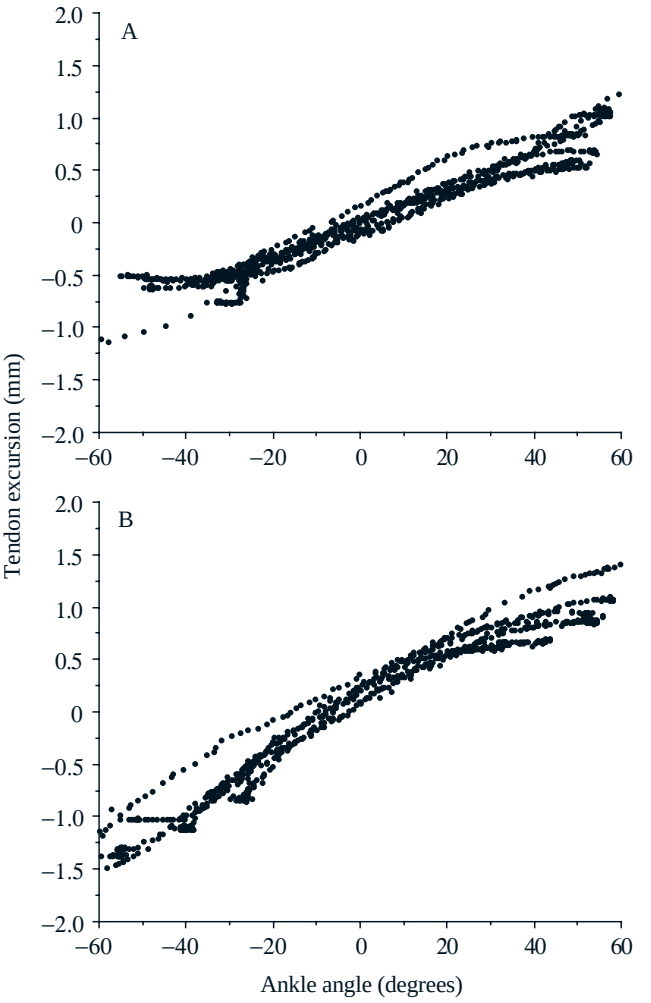

Fig. 2. Ankle angle–tendon excursion data used for moment arm determination. These data were acquired and differentiated as described in Materials and methods to yield moment arm as a function of joint angle. (A) Control unoperated limb. (B) Limb 2 weeks after flexor retinaculum transection.

Swim training after retinaculum transection

To determine whether muscle changes observed were dependent on activation level, 2-week post-transection mice (N=4) were trained to swim for 30–60 min per day during the 2-week post-transection period. Swimming has been shown to increase dramatically the activation of the anterior compartment muscles (Roy *et al.* 1985) and ensured that the level of TA use was greater than that observed during normal cage activity.

Gait analysis

A separate group of mice (N=4) was video-taped at 500 frames s^{-1} during unrestrained walking to measure joint angular velocity occurring prior to and after retinaculum transection. Two operated (2 weeks following transection) and two non-operated mice were allowed to move freely in a long, narrow acrylic chamber. Only complete, uninterrupted steps were included in the analysis. Frames were digitized every 10 ms from the moment the toe left the ground to full dorsiflexion (3–6 frames per step) using a Macintosh Quadra 840AV (Apple Computer, Cupertino, CA, USA). The angle between the foot and the ventral surface of the tibia was digitized (NIH Image version 1.60, Bethesda, MD, USA) and the angular velocities over each step were averaged. Twenty-eight steps from four animals were analyzed.

Mathematical modeling

Muscle static and dynamic force production were estimated using a mathematical model implemented in Mathematica (Wolfram Research, Champaign, IL, USA). Briefly, two-dimensional muscles were created from the measured muscle length, fiber length and pennation angle (Burkholder *et al.* 1994). The area of these muscles was taken to be constant, based on the contractile and morphometric data of Zuurbier and Huijing (1992). Using this assumption, it was possible to calculate fiber and sarcomere length as a function of muscle length. These sarcomere lengths were used to estimate isometric and isotonic force production. V_{max} for the TA was taken to be 9.2 L_0 s^{-1}, as reported below on the basis of direct measurements. The model was used to generate all force and power results and to estimate sarcomere lengths at muscle lengths other than the length of fixation.

To estimate muscle length over the range of motion, values were linearly interpolated between measurements of passive muscle length at ankle angles of 40°, 90° and 130°. Forces throughout the range of motion were estimated by calculating the force each muscle could produce at these interpolated muscle lengths.

Power as a function of joint velocity was calculated by predicting the muscle shortening velocity. Joint angular velocity was multiplied by the mean moment arm to produce muscle shortening velocity, which was then used to estimate the isotonic force and power capacity of each muscle.

Statistical analyses

Mean sarcomere number was compared between groups using one-way analysis of variance (ANOVA) with significance level set to 0.05 (StatView 5.0, Abacus Concepts, Berkeley, CA, USA). Differences between groups were determined using Fisher's least-squared differences *post-hoc* comparisons. Numerical values are reported in the text and figures as means ± S.D unless otherwise stated.

Results

Moment arm, integrated over the range 40–130°, increased from 0.95±0.3 to 1.4±0.4 mm (P<0.05) 2 weeks after retinaculum transection (Table 1). Retinaculum transection also changed the relationship between the moment arm and joint angle (Fig. 3). Prior to transection, the moment arm was relatively constant throughout the range of motion, as would be expected since the TA tendon passed beneath the flexor retinaculum. After transection, there was a notable peak during dorsiflexion because the altered geometry allowed the TA to

Table 1. *Musculoskeletal properties of mouse tibialis anterior muscle*

Variable	Experimental group		
	Control (N=10)	Transected (1 week, N=12)	Transected (2 weeks, N=12)
Body mass (mg)	27±4	26±4	28±4
Sarcomere number	3200±200	3100±200	2900±200*
PCSA (mm^2)	5.5±0.9	5.5±0.5	5.3±0.5
Integrated moment arm (mm) (N=4 for both groups)	0.95±0.3		1.4±0.4*
Dorsiflexion muscle length (mm)	11.7±0.5	10.8±0.3*	11.0±0.7*
Plantarflexion muscle length (mm)	13.4±0.7	12.8±0.5*	13.0±0.5*
Predicted joint angle for peak tension (degrees)	89±37	102±48	88±49
Muscle length (mm)	12.4±0.5	12.3±0.9	12.0±0.6
Fiber bundle length (mm)†	8.0±0.5	7.9±0.6	7.2±0.4*
Passive muscle excursion (mm)	1.6±0.5	2.0±0.4	2.0±0.5
Preferred dorsiflexion angular velocity (degrees s^{-1}, N=4)	2200±300		1900±400*
Predicted relative power at preferred gait speed (% maximum)	92±2		74±5*

Values are means ± S.D.
*Indicates significant difference from the control value (P<0.05).
†Fiber bundle lengths normalized to sarcomere length of 2.5 μm.
PCSA, physiological cross-sectional area.

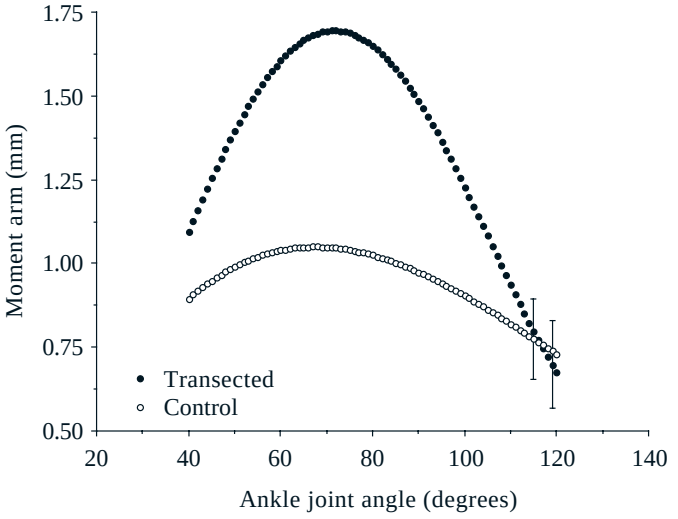

Fig. 3. Moment arm of the tibialis anterior of control (○) and retinaculum-transected (●) mice *versus* ankle joint angle. The greatest differences between the two groups are found during dorsiflexion (joint angles less than 100 °) where the tibialis anterior is permitted to elevate away from the tibia. Values are means (N=4). Error bars represent the mean S.E.M. across the data set. Data are plotted at intervals for clarity; the actual data set represents a continuous function calculated from length and angle records as described in Materials and methods.

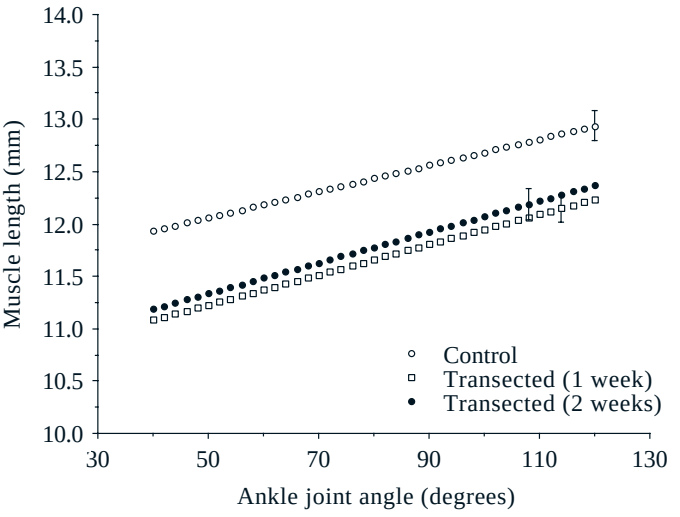

Fig. 4. Muscle length–ankle angle relationships calculated using measured moment arm and sarcomere number for the tibialis anterior of control (○, N=10), retinaculum-transected for 1 week (□, N=12) and retinaculum-transected for 2 weeks (●, N=12) mice. Both transected groups yield significantly shorter muscle lengths (P<0.05) with slightly steeper slopes than do control animals. Values are means, and error bars represent the mean S.E.M. across the data set.

'bowstring' in operated animals. The major effect of transection was to increase the moment arm at joint angles of less than 100 °. On the basis of moment arm measurements, average MTU excursion was calculated to increase from 1.4 mm in control animals to 1.9 mm in the transected groups over the full range of motion (50–150 °).

The altered moment arm resulted in significant changes in *in situ* muscle lengths (Fig. 4). Fully plantarflexed (maximum) measured muscle length decreased slightly but significantly from 13.4±0.7 mm in controls to 13.0±0.5 mm in the 2-week transection group (P<0.05) (Table 1) because the TA took a more direct, medial path to the insertion site. Fully dorsiflexed (minimum) measured muscle length also decreased significantly from 11.7±0.5 mm in controls to 11.0±0.7 mm in the 2-week transection group (P<0.005). The TA tendon visibly buckled in the transected groups when the joint was placed in full dorsiflexion, so minimum muscle length was slightly overestimated in these groups.

Muscle architectural properties are summarized in Table 1. Despite the increase in muscle excursion and significant decrease in minimum muscle length, sarcomere number decreased significantly over the 2-week experimental period from 3200±200 in control animals to 2900±200 in 2-week transected mice (P<0.005). Similar results were obtained after 2 weeks of swim training following retinaculum transection (2950±170, N=4). PCSA was not significantly altered. Whole-muscle V_{max} was essentially unchanged, ranging from 9.3±2.5 $L_F\,s^{-1}$ in controls to 9.2±1.9 $L_F\,s^{-1}$ in 2-week transected mice,

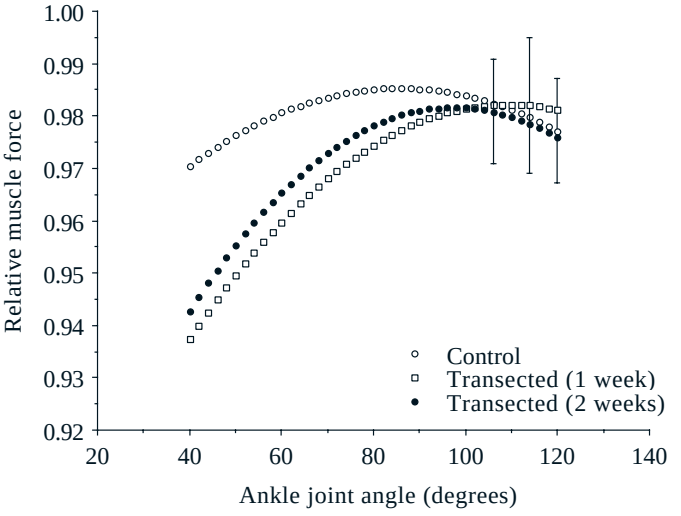

Fig. 5. Predicted muscle force–joint angle relationships for the tibialis anterior of control (○, N=10) retinaculum-transected for 1 week (□, N=12) and retinaculum-transected for 2 weeks (●, N=12) mice. Both control and 2-week transected mice have peak force near the neutral joint angle (approximately 80 °), while 1-week transected peak force is noticeably shifted to higher angles, has lower values throughout most of the range of motion and peaks during plantarflexion. Values are means. Error bars represent the mean S.E.M. across the data set.

probably indicating no significant change in fiber-type composition.

Using the combined muscle architecture data and moment arms, the relative muscle force–joint angle curves could be calculated (Fig. 5). One week after retinaculum transection (prior to the significant change in sarcomere number, Table 1),

the joint angle corresponding to peak force shifted from the control value of 89 to 102°, although this change was not statistically significant (Fig. 5; Table 1). In contrast, 2 weeks after retinaculum transection, optimal joint angle had returned to 88° (Fig. 5; Table 1).

Video analysis of stepping in normal and transected mice revealed that 2-week transected mice preferentially dorsiflexed at slightly slower and more variable rates. Unoperated mice (N=2) dorsiflexed fairly consistently at 2200±300° s^{-1}, which was significantly faster than 2-week transected mice, which dorsiflexed significantly more slowly (1900±400° s^{-1}, N=2, P<0.02). Despite this 14% decrease in angular velocity after transection, the 30% increase in moment arm and significant reductions in dorsiflexion and plantarflexion muscle lengths will result in a calculated approximately 20% increase in the velocity of TA shortening during dorsiflexion, from 2.7 to 3.3 L_0 s^{-1}. The effect of altered sarcomere number is also reflected in the decreased power production calculated at the preferred gait speed. Whereas control mice were calculated to generate 92±2% of peak TA muscle power, 2-week transected mice were calculated to produce only 74±5% of their peak TA muscle power.

Discussion

The purpose of the present study was to investigate the mechanical factors that regulate sarcomere number in adult mouse skeletal muscles. Our objective was to study this phenomenon using a model in which muscle motion was permitted so that the relative influences of excursion and length could be determined. This is in contrast to the numerous reports of sarcomere number adaptation obtained using immobilization models.

The immediate effect of retinaculum transection was to alter muscle length at full plantar- and dorsiflexion. The mean moment arm increase of 38% from 0.95 to 1.4 mm 2 weeks following transection had two main effects: the first was a non-significant increase in muscle excursion by approximately 25% from 1.6 to 2.0 mm; the second was a decrease in calculated sarcomere length at most joint angles. Our main interest was in determining the factor(s) to which the muscle responded.

From a theoretical point of view, the effect that this intervention might have on sarcomere number can be predicted by making assumptions about the mechanical factors that regulate sarcomere number. For example, if sarcomere length at a particular joint angle were the dominant signal responsible for adjustment of sarcomere number, then, after retinaculum transection, a decrease in sarcomere number would restore sarcomere length to the value present at that joint angle before surgery. However, if excursion were the dominant signal, then sarcomere number would increase in proportion to the increase in moment arm to restore sarcomere excursion to the pre-operative range. Finally, if shortening velocity or power production dominated the signaling, then sarcomere number would increase in proportion to the change in muscle shortening velocity (which need not be the same as the moment arm change).

First, we consider the possibility that sarcomere number is regulated to maintain a constant sarcomere or muscle fiber excursion. This mechanism was suggested by the results of Crawford (1954) and T. J. Koh and W. Herzog (in preparation). The strong correlation reported previously between fiber length and moment arm (McClearn, 1985) also indicates that fiber excursion might be regulated. However, there is considerable variability in literature reports of functional sarcomere length ranges in different organisms of as low as 0.10 μm in fish (Lieber *et al.* 1992*b*) and as high as 3.4 μm in humans (Cutts, 1988), and which encompass the ascending (Dimery, 1985; Rack and Westbury, 1969), plateau (Cutts, 1989; Lieber *et al.* 1992*b*) and descending (Lieber and Boakes, 1988; Lieber *et al.* 1994; Tardieu *et al.* 1977*a*) regions of the force–length curve. If sarcomere excursion were a critical regulatory signal, then sarcomere length range might be expected to be highly conserved across muscles and species. Even within *Rana pipiens*, Lieber and Brown (1993) reported sarcomere length changes per degree of joint flexion that ranged from a minimum of 3.7 nm $degree^{-1}$ for the cruralis muscle acting at the knee to a maximum of 12.5 nm $degree^{-1}$ for the semitendinosus muscle acting at the hip. Sarcomere excursion does not seem to be constrained to a particular range or region of the length–tension curve and, therefore, is probably not tightly regulated. In the present study, the calculated muscle fiber and sarcomere length range increased by approximately 30%. An increase in sarcomere number of approximately the same magnitude would restore the sarcomere length range of operated animals to control levels, which was not observed. Thus, this mechanism can be rejected.

An alternative strategy for the TA after retinaculum transection would be the restoration of average sarcomere velocity or muscle power. This might be considered *a priori* to be a more likely response compared with restoring optimal muscle length since it is known that muscle velocity is a more potent determinant of tension across the physiological range than is muscle length. In the present study, we measured a 14% decrease in dorsiflexion angular velocity after transection. In spite of this decrease, the 38% increase in measured moment arm would result in an increased calculated muscle velocity of 20%, which would decrease the force-generating ability of the muscle. Given the highly nonlinear nature of the force–velocity relationship, this velocity increase is predicted to decrease isotonic force production by 39% and to decrease power production by 20% at the preferred gait ankle angular velocity (Fig. 6). On the basis of this prediction, the muscle could increase sarcomere number by approximately 20% to restore sarcomere velocity to normal. This was not the case. The fact that sarcomere number decreased by 2 weeks following transection provides no support for the regulation of sarcomere number to maintain optimal power or normal excursion in adult mouse skeletal muscle.

The third and final possible strategy is that changes in sarcomere number occur to maintain an optimal sarcomere

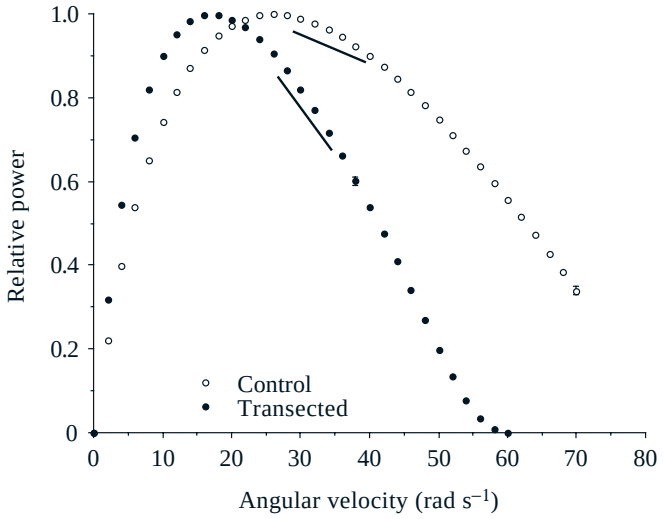

Fig. 6. Predicted power *versus* ankle angular velocity for control (○, N=10) and 2-week transection (●, N=12) groups. Error bars represent the mean S.E.M. across the data set. The lines represent preferred gait speed measured from video recordings.

length at a given joint angle. Our data support this contention. Our calculations indicate that the joint angle corresponding to peak tension in the TA was approximately 90° in control animals. One week after transection, presumably because of the increased moment arm, this angle corresponding to optimal muscle length showed a tendency to be shifted towards plantarflexion by approximately 13°. To maintain the optimal sarcomere length near the neutral joint angle, a decrease in sarcomere number would be required. We calculate that a decrease in sarcomere number by approximately 10% would restore the relationship between optimal length and neutral joint angle, which was the value obtained experimentally. This decrease in sarcomere number of 10% results in shorter sarcomere lengths when the ankle is in greater plantarflexion. It should be noted that this calculated sarcomere length change would not be accompanied by a large change in calculated muscle isometric force potential. For example, in the control group, muscles would generate 98±2% P_0 at the neutral joint angle, whereas in the 1-week transection group, in spite of the 13° shift in optimal joint angle, the muscles generated 97±3% P_0 at this angle. Thus, optimizing force *per se* does not appear to provide the driving force behind the adaptation of sarcomere number.

Other investigators have presented data suggesting that muscle excursion is a more powerful regulator of sarcomere number, unlike the results obtained in the present study. Crawford (1954) and T. J. Koh and W. Herzog (in preparation) studied the excursion of the TA in young rabbits after transection of the ankle flexor retinaculum. They measured an increase in fiber length and serial sarcomere number that compensated for the altered excursion. The reason for the difference between their studies and ours may be the different time courses (they investigated changes after 4 weeks) or may represent the use of different strategies by muscles depending on the age of the animal. The differences may also simply reflect species differences. To investigate whether our findings represented an adaptation endpoint, four mice were studied 8 weeks following transection. These animals showed essentially the same pattern as the 2-week post-transection animals (serial sarcomere number 2850±90).

Age-related differences have been reported previously using immobilization models. For example, the sarcomere number of immobilized adult muscles increases if they are held in a lengthened position or decreases if they are held shortened (Spector *et al.* 1982; Tabary *et al.* 1972; Williams and Goldspink, 1978). In either case, remodeling results in the optimal sarcomere length and peak force production occurring at the length of immobilization. Young animals, while also producing peak force at the length of immobilization, have a reduced sarcomere number whether the immobilized muscle is held lengthened or shortened (Tardieu *et al.* 1977*b*). All of these models support the contention of Williams and Goldspink (1973) that, while all muscles are sensitive to length variation, young muscles may be more sensitive to variation in the range of muscle lengths.

One other factor that might account for the differences observed between adult mice and young rabbits in the above-cited studies is that their activity levels may differ. If adult mice are significantly more sedentary than young rabbits, mouse muscle may not be sufficiently exposed to excursion or velocity signals. While we did not measure cage activity or TA muscle electromyographic activity directly, we did test for this possibility indirectly by swim-training some mice after transection. It is known for rats that swimming results in greater activation of the TA as the toes are dorsiflexed against the inertial forces of the water prior to the kicking power stroke (Roy *et al.* 1985). Our use of this enforced activity also ensured that the muscles of transected mice were exposed to the full, dynamic impact of the surgical intervention. Under these conditions, the change in sarcomere number was essentially identical to that observed in the absence of swim training. Thus, we reject the hypothesis that the mice failed to receive an adequate velocity or excursion signal.

In summary, we report a reduction in serial sarcomere number in adult mice in response to retinaculum transection. The mature muscle appears to respond to this intervention by restoring the joint angle at which peak force occurs to control values by a decrease in sarcomere number in spite of the accompanying reduction in force and power production. Our data provide no support for the adaptation of adult muscle based on dynamic increases in muscle excursion or velocity. Clearly, the cellular structures and mechanisms responsible for our findings remain to be identified.

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