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SELECTIVELY RESHAPING A MUSCLE PHENOTYPE: FUNCTIONAL OVERLOAD OF CAT PLANTARSI

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Abstract

Introduction—Functional overload (FO) of the fast plantaris muscle was studied in treadmill exercised (FO-Ex) or sedentary (FO-Sed) adult cats.

Methods—Mechanical, phenotype, and kinematics analyses were performed.

Results—Plantigrade vs. normal digitigrade posture was observed early post-FO. Relative plantaris mass was larger in FO-Sed (10%) and FO-Ex (60%) than controls 12 weeks post-FO. Specific tension was similar across groups, indicating functional hypertrophy. Fiber size was greater, percent slow fibers higher, percent IIA myosin heavy chain (MHC) higher, and IIX MHC lower in FO-Ex than controls. Twitch and half-relaxation times were longer, and the frequency-tension curve shifted towards that observed in slow muscles. EMG and tendon force amplitudes during stepping were larger, and the yield (lengthening) phase occurred at a longer muscle length post- than pre-FO.

Discussion—Reshaping the plantaris phenotype was highly dependent on the overload stimulus, indicating that electrical stimulation paradigms used during rehabilitation should be performed with the muscles under “loaded” conditions.

Keywords

compensatory hypertrophy; *in vivo* muscle tendon forces; EMG; contractile properties; muscle phenotype

INTRODUCTION

Functional overload (FO) of a skeletal muscle by removal of its synergists results in mechanical overload of the remaining musculature. FO of primarily fast muscles produces rapid compensatory hypertrophy and adaptation of the mechanical and metabolic properties towards those normally observed in a larger and “slower” muscle. These adaptations have been characterized extensively in the plantaris muscle of rats^{1–4} and to some degree of mice.^{5,6} A general assumption is that these adaptations are a direct result of the mechanical overload of the muscle, i.e., the increased force demands placed on the overloaded muscle.

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^{3,7} For example, after ~12 weeks of FO the plantaris of rats housed under sedentary cage conditions approximately doubles its mass after removal of both heads of the gastrocnemius and the soleus¹, whereas the larger medial gastrocnemius increases its mass by only 50% after removal of the lateral gastrocnemius, soleus, and plantaris muscles.⁸ This large difference in the amount of hypertrophy is observed although both muscles have similar fiber type distributions and perform similar functions during locomotion, i.e., both muscles are primary plantarflexors. Thus the difference in the amount of hypertrophy between the overloaded plantaris and medial gastrocnemius appears to be directly related to the relative overload on the muscle. In addition, when the FO rats were exercised for 1 hr/day 5 days/week (progressive until 1 mph at a 10% incline) for 10 weeks, the plantaris was ~25% larger than in age-matched non-trained FO rats.⁴ A lack of a hypertrophic response of FO muscles has also been demonstrated in rats that were hindlimb unloaded after the removal of synergists.^{9,10}

The extent of the response of the overloaded muscles may be related to the level of activity of the animal after the surgery. For example, both the rat and mouse are very active animals¹¹ and show high levels of cage activity almost immediately after FO surgery (unpublished observations). Gardiner et al.¹² demonstrated that the amount of activation of the rat plantaris (as determined from mean EMG burst amplitudes and integrals) is elevated significantly for up to 30 days after FO in comparison to control (pre-FO) levels. In contrast, the cat is relatively sedentary in nature,¹³ and this allowed us to titrate the amount of neuromuscular activity experienced by the FO plantaris muscles.

The main purposes of this study were to determine whether 1) the effects of FO on the mechanical and phenotypic properties of the plantaris muscle would be similar in the cat as in smaller and more spontaneously active mammals, i.e., the rat and mouse; and 2) the compensatory hypertrophy of the plantaris was associated with adaptations in the kinetics (increased force production associated with eccentric contractions) and kinematics (enhanced activation as reflected by EMG) patterns while stepping on a treadmill during the early period (1-3 weeks) after the FO surgery. Our working hypothesis was that the adaptations in the mechanical properties of the FO plantaris would be closely related to the level of neuromuscular activity (specifically the muscle loading associated with activation) after the surgery. We, therefore, studied the FO response in a group of cats that were allowed only cage activity and a group that also was exercised on a treadmill briefly 3-4 times per week to increase the level of neuromuscular activity.

Methods

Experimental design

For the determination of the mechanical properties of the plantaris muscle, three groups of adult cats were studied: 1) sedentary, control (control n = 7); 2) FO-sedentary cage activity (FO-Sed, n = 3); and 3) FO plus treadmill exercise (FO-Ex, n = 5). In addition a separate group of control cats (n = 6) was used for determination of the fiber type composition of the plantaris due to some problems with the preservation of the tissues from the initial group of control cats. It should be noted, however, that the fiber type composition of the plantaris in these cats was similar to previously published values.¹⁴

All surgeries were performed under aseptic conditions with the cat under deep anesthesia and with the body temperature maintained using a water circulating heating pad. Each cat first received pre-anesthesia agents (atropine sulfate, 0.9 mg/kg, s.c. and acepromazine maleate, 10 mg/kg, i.m.) and then was anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and maintained at a surgical level with supplemental doses. All cats were housed in spacious cages (4' × 6' of floor space) in groups of 2 or 3. This housing arrangement

maximized the activity of the cats. The cats in the FO-Ex group were allowed 4 to 5 days of recovery prior to being exercised at various speeds (ranging from 0.22 to 2.30 m·s⁻¹) and inclines (0, 10, 20%) on a motorized treadmill for ~5 min/day, 3-4 days/wk depending on the ability of each cat. All FO cats were maintained for ~3 months after the FO surgery. All portions of the study were approved by the Animal Use Committee at the University of California at Los Angeles and followed the American Physiological Society Animal Care Guidelines.

Functional overload surgery

The plantaris muscle was functionally overloaded bilaterally via the removal of the entire soleus and the medial head of the gastrocnemius. In addition, the majority of the lateral head of the gastrocnemius was removed, i.e., all except for a narrow strip bordering the proximal portion of the plantaris where the two muscles are joined by a connective tissue sheath and the major blood supply enters the plantaris. These procedures have been described in detail previously.¹⁵

EMG and tendon force transducer implantation procedures

The plantaris of some of the cats assigned to the FO-Sed (n = 2) and FO-Ex (n = 5) groups was implanted with tendon force transducers and intramuscular EMG recording electrodes ~2 weeks prior to FO surgery. The tendon force transducers were calibrated prior to implantation and then on the tendon during an *in situ* terminal experiment as described in Sherif et al.¹⁶ Force and EMG signals were recorded periodically during treadmill locomotion the week prior to and up to three weeks after FO surgery.

All of the procedures for the EMG and tendon force transducers implants in cats have been described in detail previously.^{16,17} Briefly an amphenol connector was cemented to the skull, and a skin incision was made to expose the plantaris muscle bilaterally. Two multistranded Teflon-insulated stainless-steel wires (15 strands, 50- μ m gauge; Cooner Electronics, Chatsworth, CA) were led subcutaneously to each plantaris muscle. The wires were inserted into the deep (close to the bone) region of the plantaris, i.e., a region with a relatively high proportion of slow fibers.¹⁴ A small notch (~0.5-1.0 mm) of insulation was removed from each wire to form the recording electrode, and each wire was secured with a suture at its entry and exit from the muscle. During the same surgery the distal tendon of the plantaris muscle on both sides was isolated, and an oval-shaped buckle instrumented with two strain gauges and a center bar placed across the oval piece was implanted on the distal portion of the tendon. The lead wires emanated subcutaneously from the same head connector as the EMG wires.

Analyses of EMG, tendon force, and muscle length changes

Recordings of EMG, force, and kinematics were made from each implanted cat during the testing of locomotor ability on a treadmill. During each recording session, each cat was tested at a range of treadmill speeds at which it could perform. Pre-ablation recordings were made after the cats had fully recovered from implantation surgery (~ 1 week post-surgery). Post-ablation recordings were started after one week of recovery, and data were collected between 1 and 3 weeks after synergist ablation. In most cases, the cats could step consistently only at the slowest speeds (1-2 mph) during this early period post-surgery.

Treadmill locomotion was filmed with video (60 fields/sec) or film (100 frames/sec) cameras oriented orthogonal to the direction of locomotion. The hindlimbs of the animals were shaved, and the skin over 8 bony landmarks (iliac crest, greater trochanter, head of the tibia, lateral malleolus, base of calcaneus, head of fifth metatarsal, and first phalanx of the fifth digit) was marked with permanent ink or 5 mm discs of reflective tape.

EMG and force recordings were obtained via a flexible, insulated cable that connected the head connector to a polygraph recorder (Grass Instruments, Model 79D) during the recording sessions. Raw signals were conditioned through a wide band AC differential amplifier (band width of 0.1 Hz to 3 kHz) at a gain of 1000, recorded simultaneously, and stored on an FM tape recorder (TEAC Model XR-510, TEAC Corporation, Montebello, CA). During the recording sessions, the signals were monitored continuously using a storage oscilloscope. A camera and videocassette recorder (Panasonic System Camera, WV D5100; Panasonic AG1280P Panasonic, Cypress, CA) were used to record the video signals. A SMPTE time code generator (Model F30, Fast Forward Video, Irvine, CA) was used to synchronize video frames with the EMG signals recorded on FM tape.

Raw EMG records from FM tape were sampled at 1 kHz using an analog-to-digital (a/d) converter and stored in digital form (DEC PDP 11/23 minicomputer). All records were full wave rectified and processed using computer routines designed to determine EMG burst duration and the integrated area of the rectified burst. A minimum threshold was determined for each pair of recording electrodes to eliminate any EMG baseline noise. For each cat, this threshold was kept consistent for all recording conditions and sessions. The EMG parameters were averaged over 10 to 20 step cycles when the cat appeared to be stepping consistently during each recording session. Mean rectified EMG burst amplitude (mean EMG) was calculated as integrated EMG/burst duration.

Kinematics analyses were performed on selected video sequences containing 8-10 steps at a consistent position on the treadmill belt representing non-accelerating locomotion. The x and y coordinates of all the bony landmarks were digitized on an Amiga 2500 computer using a genlock to overlay video and computer images. A reference point was digitized to assure accurate registration of successive frames. Knee joint positions were corrected by triangulation based on the digitized positions of the greater trochanter, lateral malleolus, and head of the tibia as described by Goslow et al.¹⁸ The time code signal was used to synchronize the frames of video footage with the EMG and force signals recorded on analog tape. The length of the plantaris muscle tendon unit was computed from joint angle data derived from the digitized positions of the bony landmarks. Length was defined as the distance from the origin of the muscle on the femur to the calcaneus marker. Mean peak force production was derived from the same steps analyzed for EMG data.

***In situ* mechanical properties**

For the determination of the mechanical properties of the plantaris, the cats were anesthetized deeply with sodium pentobarbital (35 mg/kg, i.p.) and given supplemental doses (i.v.) of ~20% of the initial dose as needed. Details of the preparation for physiological testing have been published.¹⁹ Briefly, the right plantaris muscle and its associated nerve were isolated carefully with the blood supply left intact. The hindlimb was stabilized by a combination of bone pins, bars and clamps such that each muscle was fixed rigidly and tested in a horizontal position. A reservoir for a mineral oil pool was formed and maintained between 36 and 38° C. A stiff wire was secured around the tendon near the musculotendinous junction. Prior to the testing of the mechanical properties, the tendon force transducer was calibrated *in situ* as described previously.^{16,17}

As previously described (Roy et al., 1984), a modification of a pneumatic isotonic lever was used to measure all isometric and isotonic contractile properties. All contractile events were recorded simultaneously on a polygraph (Grass Instruments model 79D) and a FM tape recorder (Vetter model D instrumentation recorder). Contractions were induced through bipolar silver electrodes with a square-wave pulse (20- μ s duration). Stimulus strength was supramaximal [2-3 times the minimal voltage producing a maximum twitch tension (Pt)]. Average time-to-peak-tension (TPT), half-relaxation time (HRT), and Pt were determined

from ~10 consecutive isometric twitches. Peak isometric tension development was measured at stimulation frequencies of 5, 10, 15, 20, 25, 30, 40, 50, 75, 100, and 200 Hz. The highest tension, regardless of stimulation frequency, was considered the peak isometric tetanic tension (P_o). Isotonic properties were obtained for 15-20 afterloaded contractions (330-ms tetanus at 200 Hz) at various loads less than 30% of P_o . The isometric fatigue properties were determined using 330-ms trains of 40 Hz delivered once/sec for 2 min, and the fatigue index was calculated as a ratio of the tension produced at 2 min divided by the maximum tension measured during the fatigue test.

The mechanical events were recorded and stored on FM tape and then analyzed by computer (Hewlett-Packard MX 2100). Analog-to-digital sampling was performed at a rate of 1 kHz. The voltage deflections recorded on the FM tape were converted into force and velocity (dL/dt). A baseline was established before each contraction by averaging samples of at least 30 ms. The beginning of a contractile event was signaled whenever there were 15 consecutive data samples that increased in amplitude. Isometric twitch TPT consisted of the time from the first of the 15 consecutive increasing samples to peak force production. For each isotonic contraction, peak velocity and the associated force were plotted as $y = (P_o - P)/V$ vs. $x = P$, where P is the measured force. A regression line was fitted to the data and Hill's²⁰ constants a and b were determined. The absolute maximum rate of shortening (V_{max} , $mm.s^{-1}$) was calculated as P_o divided by the y -intercept extrapolated by the regression analysis.

At the end of the mechanical testing, the cats were given an overdose of sodium pentobarbital (100 mg/kg, i.v.). The plantaris muscle was removed, rinsed immediately with saline, cleaned of excess fat and connective tissue, weighed (wet weight), and prepared for subsequent analyses.

Architectural determinations

The contralateral (left) limbs of three control, three FO-Sed, and three FO-Ex cats were used for architectural determinations as described in detail previously.²¹ Briefly, the lower leg was submerged in 10% formalin with the knee and ankle joints fixed at ~90° until they were completely fixed. Muscle length was measured as the distance between the termination of the muscle fibers at their origin and at the insertion. An approximation of the angle of pennation formed by the muscle fibers with respect to the line of pull of the muscle tendon was determined at several locations along the muscle length, and an average value was calculated. The muscle was placed in a 15-20% sulfuric acid solution to macerate the connective tissue, and small bundles of fibers containing 5-10 fibers were dissected from the proximal, middle, and distal regions within each compartment of the muscle. Fiber length was calculated as the average length of these small bundles. Individual fibers from the small bundles were isolated, placed on a glass slide, immersed in glycerin jelly, and the number of sarcomeres counted using a calibrated eyepiece micrometer. At least 25 sarcomeres were measured at three locations (proximal, middle and distal) within each fiber and averaged for each location. A mean of these averages was calculated as the mean sarcomere length for each fiber. An overall mean for all fibers then was calculated and reported as the mean sarcomere length for that muscle. These data then were normalized to a sarcomere length of 2.2 μm to allow direct comparisons across animals and groups. These architectural measurements were used to calculate muscle physiological cross-sectional area (PCSA) as follows:

$$PCSA = (\text{muscle mass}) (\cosine \theta) / (\text{fiber length}) (\text{muscle density})$$

where muscle mass was the muscle wet weight (g); θ was equal to the approximate average angle of fiber pennation in one dimension (°); fiber length was adjusted to a

sarcomere length of 2.2 μm ; and the density of the muscle was assumed to be 1.056 g/cm^3 . Specific tension was calculated as Po/PCSA .

Histochemical and fiber type analyses

A 5-mm block from the mid-portion of the right plantaris from 6 Con, 2 FO-Sed, and 4 FO-Ex cats was mounted on cork in a manner to insure that the fibers were oriented perpendicular to the plane of the cork and quick-frozen in isopentane cooled with liquid nitrogen. Serial cross-sections (10- μm thick) were stained for myosin adenosine triphosphatase (myofibrillar ATPase with alkaline or acid pre-incubation), α -glycerophosphate dehydrogenase, and succinic dehydrogenase. Between 100 and 200 fibers were typed from each of a deep (close to the bone) and a superficial (away from the bone) region of each muscle cross-section. Individual fibers were classified as fast glycolytic (FG), fast oxidative glycolytic (FOG), or slow oxidative (SO) based on the qualitative criteria established by Peter et al.²² The cross-sectional areas of all typed fibers were determined using an image analyzer.

Sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of MHC composition

Isolated myofibrils were prepared from frozen cross sections taken from the mid-belly region of each muscle in the Con and FO-Ex groups used for the histochemical analyses as described by Talmadge et al.²³ The protein concentration of the final myofibrillar suspension was determined according to Bradford.²⁴ Myofibrillar protein was boiled in sample buffer for 2 min at a final concentration of 0.25 mg/ml . MHCs were separated by SDS-PAGE according to Talmadge and Roy.²⁵ The SDS-PAGE gels were stained with Coomassie blue, photographed, and scanned with an Alpha Innotech IS-1000 videoscanning densitometric system (San Leandro, CA) to determine the relative proportions of MHC isoforms in each muscle.

Statistical procedures

The data were analyzed using one-way or two-way analyses of variance, followed by the Tukey post-hoc test to determine significant differences between groups. The 0.05 level of probability was established for statistical significance. Values are reported as means $\pm$ standard errors of the mean.

RESULTS

Adaptations in the kinematics and kinetics of stepping after FO

After FO surgery, the most obvious gross changes observed qualitatively during locomotion was the plantigrade stepping pattern compared to the digitigrade stepping pattern observed pre-FO. The FO cats initially walked “flat-footed” and then recovered to a digitigrade posture after ~3 weeks. In addition, there was some initial compensation at the knee, i.e., the cats generally walked in a slightly more “crouched” position after than before FO surgery. These adaptations were similar in the FO-Sed and FO-Ex groups, but the duration of these effects was shorter in the FO-Ex group.

As stated in the Methods section most (6/8) cats were only able to step at the slowest treadmill speeds after the FO surgery. In general, there was an increase in the magnitude of both the peak force and mean EMG recorded during stepping at the same speed after compared to before FO. The average peak force and integrated EMG for three cats stepping at 1-2 mph were 72 and 60% higher at 1-2 weeks after than before FO. Data for one cat that stepped at multiple speeds after FO are shown in Figure 1. Peak force increased as the speed of the treadmill increased both before and after FO (Fig. 1B), whereas mean EMG

progressively increased with treadmill speed before but not after FO (Fig. 1A). At all comparable speeds, both peak force and mean EMG were higher after than before FO.

Figure 2 illustrates the stance phase changes in length behavior during a typical step cycle before (Fig. 2A) and after (Fig. 2B) synergist ablation. Prior to synergist ablation, the plantaris exhibits a small (~2%) increase in total length at the beginning of stance. The muscle then shortens consistently until the end of stance. In FO cats the initial yield is greater (~4%), and in the early stages after FO the muscle exhibits an overall lengthening throughout the stance phase. The timing of force production relative to the change in muscle length was not different between the pre- and post-conditions, i.e., force production was initiated at the beginning of the yield phase. Since the muscle was at a longer length at the beginning of the yield phase, lengthened throughout the stance phase early in FO, and produced higher forces post-FO compared to pre-FO, the muscle experienced a much more robust eccentric contraction post-FO. Despite the increase in shortening late in the FO period, the large yield phase is maintained, leading to greater eccentric excursions post-FO (data not shown).

Figure 3 further illustrates the nature of the kinematics changes as a function of speed pre-FO and at an early (1-2 weeks) and late (6 weeks) post-FO time point. Figure 3A shows the changes in the maximum length achieved by the plantaris muscle during stance. The maximum length at the early FO time point is at or above the lengths measured pre-FO across the range of speeds tested. By 6 weeks, the maximum length is less than the lengths measured pre-FO. Figure 3B shows the changes in the range of motion experienced by the plantaris during the stance phase. At the early FO time point the plantaris operates at a reduced range of motion across all speeds, whereas at 6 weeks post-FO the total excursion during stance has returned to normal at the speeds measured.

Body mass and adaptations in plantaris mass and architecture

Mean body masses of the control and FO-Ex groups were similar, whereas the mean body mass was 23 and 30% smaller ($P > 0.05$) in the FO-Sed than the control and FO-Ex groups, respectively (Table 1). The absolute plantaris mass was not different in the control and FO-Sed groups, but ~3- and 2-fold larger in the FO-Ex than in the control and FO-Sed groups, respectively. When the mean body mass for each group was considered, the relative plantaris mass was ~40 ($P > 0.05$) and 75% larger in the FO-Sed and FO-Ex than the control group and ~20% larger in the FO-Ex than FO-Sed group. Mean muscle lengths were longer in both FO groups than in the control group, whereas fiber lengths, muscle length/fiber length ratios, and angles of pennation were not different across the three groups. Consequently, the comparisons of mean PCSA across groups were similar to that for relative muscle mass.

Adaptations in mechanical properties

Pt and Po were not different in the control and FO-Sed groups. Pt, however, was ~88 and 61% larger in the FO-Ex than in the control and FO-Sed groups (Fig. 4A). Po was elevated in parallel with the increase in PCSA, i.e., Po was 91 and 84% higher in the FO-Ex than in the control and FO-Sed groups. Consequently, the mean SpT, i.e., Po/PCSA and Pt/Po ratios were not different among the three groups. The absolute and relative frequency-tension relationships were not different in the control and FO groups (Fig. 5). In the FO-Ex group, however, the absolute force was higher than in the control or FO-Sed groups at frequencies of 25 Hz and above (Fig. 5A), and the relative tension was higher than control between 20 and 40 Hz, i.e., at the most physiological rates of stimulation (Fig. 5B and inset). No significant differences in fatigability were observed among the three groups (Fig. 4A).

Mean TPT and HRT were slower in the FO-Ex than in the control group (Fig. 4B). The TPT also was slower in the FO-Sed than the control group ($p > 0.05$ and < 0.10). In contrast, mean Vmax was not different among the three groups.

Adaptations in plantaris MHC composition and in fiber type distribution and size: control vs. FO-Ex

Compared to control values, there was a shift towards the slower MHC isoforms in the FO-Ex group (Fig. 6), i.e., there was a lower percentage of type IIx and a higher percentage of type IIa MHC in the FO-Ex than control group. In general, the adaptations in fiber type composition are consistent with the MHC results, i.e., there was a tendency for a shift towards a slower fiber type composition in the FO-Ex compared to the control group (Fig. 7A and C). The only significant changes, however, were a higher percentage of SO fibers in the deep region of the FO-Ex group and a lower percentage of FG fibers in the FO-Sed group. All fiber types in both regions of the muscle were larger in the FO-Ex than in the control group, whereas only the FOG fibers in the superficial region of the FO-Sed group were larger than in the control group (Fig. 7B and D). In addition, the FG fibers in the superficial region and the FOG fibers in the deep region were larger in the for FO-Ex than FO-Sed group.

DISCUSSION

Adaptations in the mechanical and phenotypic properties of the cat plantaris after FO: Comparison with the rat model

Functional overload of the predominantly fast plantaris muscle in rats^{1,2} results in hypertrophy, a concomitant increase in maximum tetanic tension, a shift in isometric twitch and frequency-tension properties and maximum shortening velocities towards those normally observed in a predominantly slow muscle, a higher fatigue resistance, and a higher percentage of fibers that exhibit slower MHC and high oxidative profiles. In general, the adaptations observed in the cat plantaris were in a similar direction, although in many cases of a lesser magnitude. It should be noted, however, that the effects of FO are being compared at ~12 weeks post-FO in both the cat and the rat. Whether the magnitude of the adaptations in the cat would be similar to those observed in the rat at a later time point post-FO is unknown. In addition, the responses were more robust in the cats that stepped on a treadmill 3-4 times/wk under high-load contractions. The overloaded plantaris in cats that were maintained in their cages and remained sedentary throughout the experimental period, however, showed minimal effects. Thus, it appears that the addition of some high level activity, even for a few minutes per day, provided the stimulus required to increase muscle mass and the shift toward a slower phenotype. In the remainder of the Discussion, comparisons will be between control and FO-Ex cats unless specified.

The most prominent adaptation in an overloaded muscle is the compensatory increase in muscle mass.³ The plantaris muscle in FO rats approximately doubles its force generating capacity in 10-12 weeks.¹ In our study, we show a similar ~2-fold increase in Po in the FO-Ex group 12 weeks post-ablation but no significant change in the FO-Sed group. In addition, the mean cross-sectional area is increased significantly for all fiber types in the plantaris after FO in both rats⁴ and cats (Fig. 6). Absolute tensions are higher in the FO-Ex than in the control animals at all frequencies tested, and there is a shift in the relative frequency-tension curve to the left at the most physiological frequencies of stimulation in both rats¹ and cats. Interestingly, specific tension, as calculated in this study, is maintained at control levels in the cat plantaris after FO, but it decreased in the rat plantaris.¹ One reason for this observation may be related to a species-specific difference in the specific tension of fiber phenotypes. In the rat, the specific tension is significantly lower in predominantly slow

compared to predominantly fast muscles⁸ and in slow vs. fast fibers.²⁶ In contrast, the specific tension is similar in slow and fast muscles²⁷ and slow and fast fibers²⁸ in cats. Thus, with the conversion of fast to slow fibers associated with FO (see below), specific tension would be expected to decrease in rats, but not cats.

The increased activation levels of FO muscles produce a shift in the phenotype and speed-related properties towards those of a “slower” muscle.³ In both exercised and non-exercised rats, this has been shown for both fast and slow muscles, since the overloaded soleus became homogeneously slow.⁸ The decrease in the percent type IIx and increase in type IIa and I (not significant) MHC and the increase in slow oxidative fibers in the deep region of the plantaris in FO-Ex cats also are indicative of it becoming a “slower” muscle. In addition, longer isometric twitch TPT and HRT values were observed after overloading the plantaris in both rats¹ and cats (present data). Vmax, however, was significantly slower in the plantaris of FO than control rats, but it was unaffected in the cat. The reason for this difference in the response of the Vmax is unknown. Vmax is determined primarily by the amount and type of myosin ATPase and by the architectural properties of the muscle, specifically the mean fiber lengths.²⁷ In addition, the fiber type composition of a muscle in rats appears to relate quite closely to its Vmax.²⁹ In both species, the mean fiber length in the plantaris was not different in control and FO groups (Table 1 and ¹). The shift towards a higher percentage of slow fibers with FO was reflected in the slower Vmax in the rat¹, but not the cat (Fig. 4).

Kinematics and kinetics responses of the cat plantaris in the early stages of FO

The plantaris is a two-joint muscle that normally undergoes small length changes at slow rates during all phases of the gait cycle and remains nearly isometric during E3, i.e., the late stage of the stance phase.^{30,31} As the insertion moves to shorten the muscle, its origin moves to lengthen it and vice versa. The distal tendon of the plantaris contributes to the Achilles tendon and then passes around the calcaneus to serve as the origin of the flexor digitorum brevis. The pulley around the calcaneus reaches an anatomical stop and prevents transfer of tension to the distal part of the plantaris-flexor digitorum brevis complex after about 5 mm of motion. In both these studies^{30,31} and our study, the plantaris length measurement was from the knee (origin) to the calcaneus (insertion).

During the early stages after FO surgery, the cats stood and walked plantigrade as opposed to their normal digitigrade posture. Thus, it appears that the remaining plantaris muscle, without the triceps surae, initially could not generate enough force to maintain a normal ankle position. Within about 3 to 4 weeks, however, the plantaris muscle hypertrophied, and the cats resumed a qualitatively normal standing and walking posture. We observed an increase in the maximum length and range of motion of the plantaris muscle in the 1-2 week post-FO period. Maas et al.³² recently reported a similar pattern of length changes at the muscle-tendon unit level in the medial gastrocnemius at 1-2 weeks after denervation of the lateral gastrocnemius and soleus muscle in adult cats. As expected, both the mean EMG and peak force were increased significantly during stepping after the FO surgery. Based on recordings from tendon force transducers, the plantaris produces between 21-38% of the total plantarflexor force exerted by the triceps surae/plantaris complex during quiet standing, walking on a level surface or at an ~10° incline, or trotting in control cats.^{33,34} In this study, the force produced by the plantaris at the same treadmill speeds was ~2-fold higher post- than pre-FO. During this period, the stepping initially was plantigrade and then became more digitigrade, most likely reflecting an increase in the size and force-producing capability of the overloaded plantaris.

An alternate or additional mechanism by which the plantaris could become more efficient with time after the FO surgery could be related to increased reflex activation. In walking

cats, stimulation of extensor group I afferents normally prolongs stance^{35,36} through the opening of a locomotor-dependent reflex pathway.³⁷ After denervating the soleus and lateral gastrocnemius muscle in cats, Whelan and colleagues^{38,39} found an increase in the reflexes from the medial gastrocnemius, indicating increased effectiveness of the medial gastrocnemius group I pathway. Muscle fascicle stretch velocities have been reported to increase during eccentric contractions of the cat medial gastrocnemius after synergist denervation.³² The smaller plantaris muscle most likely would experience similar increases in afferent activation after removal of its synergists. Thus it is possible that a similar increase in the effectiveness of these reflex pathways may be occurring to enhance the activity level of the plantaris during stance in the early stages of FO. This mechanism, however, may be playing a minor role since the Ia connections between the plantaris and its synergists (the triceps surae) are relatively weak.⁴⁰

Perspective

The compensatory hypertrophy associated with overloading a muscle by removing its synergists appears to be loading dependent. After FO surgery, cats that were allowed to be sedentary in their cages showed minimal increases in plantaris mass and force production compared to those cats that were exercised for a few min/day. Consistent with the hypothesis of a loading-dependence effect is the observation that the mean absolute and normalized plantaris masses were 64 and 36% larger in FO rats maintained in sedentary cages vs. hindlimb suspension for 6 weeks after surgery.¹⁰ Since the activation levels of the hindlimb extensor muscles have been shown to return to control levels within about 10 days after suspension⁴¹, the difference in the hypertrophy response can be attributed to the lack of muscle loading in the suspended rats. The observed responses in the cat plantaris after FO with and without exercise, therefore, further support the hypothesis that a mechanism exists by which the loading experienced by a muscle plays a defining role its morphological and mechanical properties. From a clinical perspective, these data indicate that rehabilitative approaches, such as functional electrical stimulation, for maintaining muscle mass after debilitating injuries must consider the loading aspects as well as the activation patterns of the involved muscles. The available evidence is clear that the type, duration, and loading characteristics of rehabilitative interventions can dictate the changes in muscle mass, phenotype, and function. Thus, an effective physical therapy strategy would be to include specific muscle loading and activation patterns that would produce the desired changes.

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ABBREVIATIONS

Ex	exercise
FG	fast glycolytic
FO	functional overload
FOG	fast oxidative glycolytic
HRT	half-relaxation time
Po	maximum tetanic tension
Pt	maximum twitch tension
Sed	sedentary

SO	slow oxidative
TPT	time-to-peak tension

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Figure 1.

Mean peak forces (A) and EMG activity (B) recorded from the plantaris muscle of a functionally overloaded plus exercised cat during stepping at various speeds and at a 0% incline on a treadmill pre-functional overload (FO) and 3 weeks post (FO) are shown. Values are mean $\pm$ SEM for at least 10 steps per condition. *: significantly different from pre-FO at $P < 0.05$.

Figure 2.

Plantaris raw EMG, tendon force, and length recordings in a functionally overloaded plus exercised cat during stepping at 1 mph on a treadmill before (A) and 1 week after (B) synergist ablation are shown. The vertical line denotes the time of visible contact with the treadmill surface by the toes. Note the higher EMG activity, greater peak and rate of force development, and longer initial length of the muscle post-FO compared to pre-FO.

Figure 3.

Plantaris maximum length (A) and range of motion (B) during the stance phase of the step cycle pre-FO (—) and at 1-2 weeks (- - -) and 6 weeks (—) post-FO. At the early stage post-FO the plantaris is operating at a greater length, but over a narrower range of motion. By 6 weeks post-FO the range of motion has returned to pre-FO values. Error bars are SEM. For clarity, only one error bar is presented at each point (negative for pre-FO, and positive for post-FO).

Figure 4.

The force-related (A) and speed-related (B) mechanical properties of the cat plantaris muscle in the functionally overloaded sedentary (FO-Sed), and FO plus exercised (FO-Ex) groups are shown as a percent of control (Con) values (horizontal dashed line). Values are mean $\pm$ SEM. Pt, maximum twitch tension; Po, maximum tetanic tension; Pt/Po ratio; SpT, specific tension (Po/physiological cross sectional area); FI, fatigue index; TPT, time-to-peak tension; HRT, half-relaxation time; Vmax, maximum shortening velocity. *, †: significantly different from Con or FO-Sed, respectively, at $P < 0.05$.

Figure 5.

The absolute (A) and relative (% of maximum tetanic tension) (B) frequency-tension responses of the cat plantaris muscle in the control (Con), functionally overloaded sedentary (FO-Sed) and FO plus exercised (FO-Ex) groups are shown. Values are mean $\pm$ SEM. *, †: significantly different from Con or FO-Sed, respectively, at $P < 0.05$.

Figure 6.

The myosin heavy chain (MHC) composition of the cat plantaris muscle in the control (Con) and functionally overloaded plus exercised (FO-Ex) groups is shown. Values are mean $\pm$ SEM. *: significantly different from Con at $P < 0.05$.

Figure 7.

The fiber type composition (A, C) and fiber cross-sectional area (B, D) of each fiber type of the superficial (top) and deep (bottom) regions of the cat plantaris muscle in the control (Con), functionally overloaded sedentary (FO-Sed), and FO plus exercised (FO-Ex) groups are shown. Values are mean $\pm$ SEM. SO, slow oxidative; FOG, fast oxidative glycolytic; FG, fast glycolytic. *, †: significantly different from Con or FO-Sed, respectively, at $P < 0.05$.

Table 1

Body mass and morphologic properties of the cat plantaris muscle

	Control	FO-Sed	FO-Ex
Body mass (kg)	3.9 ± 0.36	3.0 ± 0.22	4.3 ± 0.24
Absolute muscle mass (g)	6.12 ± 0.96	8.16 ± 0.30	19.18 ± 1.62 [*] , [†]
Relative muscle mass (g/kg)	1.92 ± 0.15	2.72 ± 0.20	3.32 ± 0.17 [*] , [†]
Muscle length (mm)	97.7 ± 0.72	104.9 ± 3.20 [*]	109.9 ± 0.20 [*]
Fiber length (mm)	18.70 ± 1.54	17.83 ± 0.90	17.65 ± 1.15
Fiber length/Muscle length	0.19 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
Angle of fiber pennation (°)	12.0 ± 0	13.0 ± 0.5	13.5 ± 1.5
PCSA (cm ²)	3.86 ± 0.27	4.25 ± 0.29	6.31 ± 0.31 [*] , [†]

Values are mean ± SEM. PCSA, physiological cross sectional area.

^{*} significantly different from control or FO-Sed, respectively, at $P < 0.05$.

[†] significantly different from control or FO-Sed, respectively, at $P < 0.05$.