Differences in rat skeletal muscles after incline and decline running

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Differences in rat skeletal muscles after incline and decline running. J. Appl. Physiol. 85(1): 98–104, 1998.—Rats were trained for 5 days by running on either an inclined or declined treadmill. Three days later, the rats were anesthetized, and angle-torque curves were plotted for the vastus intermedius muscles. The maximum active torque was generated at significantly greater muscle lengths for muscles from decline-trained rats compared with incline-trained rats. Sixteen muscles were then fixed and acid digested, and fiber lengths and sarcomere lengths were measured. The estimated average number of sarcomeres in series was greater in muscle fibers from decline-trained animals. Fourteen other muscles underwent a test series of lengthening contractions, all from the same knee angle. Torque fell less and the optimum angle shifted less for muscles from decline-trained animals, showing that the decline-trained muscles were more resistant to changes in mechanical parameters that indicate damage. These results support but do not prove the proposal that the lesser damage from a series of eccentric contractions seen in muscles trained by prior eccentric contractions is due to a greater number of sarcomeres in series.

ec eccentric; muscle damage; sarcomeres; delayed-onset muscle soreness

It is common experience that use of muscles in situations in which they are repetitively forced to lengthen while generating active tension, known as “eccentric” contractions, leads to pain and tenderness that peak 1–2 days after the exercise. This “delayed-onset muscle soreness” is accompanied by a series of changes to the mechanics, histology, and biochemistry of the muscle, collectively referred to as “damage” from eccentric contractions (reviewed in Refs. 1, 3, 8). Similar changes can also be shown in whole animals and in isolated muscles subjected to a series of forced active lengthenings. Such a series of contractions, whether voluntary in intact conscious animals or stimulated in isolated muscles, is referred to as “eccentric exercise.”

Schwane and Armstrong (26) showed that a prolonged bout of decline running by untrained rats produced biochemical changes in muscles, particularly the vastus intermedius, the deep red layer of the knee extensors, indicative of cell damage. They concluded that these postural muscles undergo eccentric contractions while lowering the animal down the decline, acting as brakes, but undergo concentric contractions during incline running. They also showed that training, consisting of 30 min of running on a declined treadmill daily for 5 days before a prolonged test bout of decline running, greatly reduced the apparent damage from the test bout, compared with otherwise similar incline training. Neither they nor others were able to explain the mechanism by which the different training exercises produced the different levels of protection (1, 3, 8).

Lynn and Morgan (19) showed that rats similarly trained on a declined treadmill averaged 12% more sarcomeres in series than did rats that had done the same period and speed of running on an incline, as shown by measuring the fiber lengths and sarcomere lengths of fibers from fixed, acid-digested muscles. They postulated that the greater number of sarcomeres reduced the sarcomere length at a given knee angle, confining operation during decline running, assuming unchanged gait, to the ascending limb of the sarcomere length-tension curve and avoiding the plateau and descending limb with its associated instability of a near-uniform distribution of sarcomere lengths. These instabilities have been proposed as the first step in the process by which damage occurs (21). A previous study (27) has shown that the amount of damage is strongly dependent on muscle length. Increased shortening velocity after training that involved lengthening contractions (see Fig. 1 of Ref. 9) is compatible with an increased number of sarcomeres, although many other factors may be involved. Because the acid digestion removed the tendinous material, Lynn and Morgan presented no evidence concerning possible differences in the tendinous attachments of the muscle fibers after the different exercises.

Such differences in the number of sarcomeres in series would be expected to result in differences in the length-tension relationships of the muscles, which is the prediction to be tested in the present paper. Difficulties in accurately measuring muscle length and relating it to the physiological range can be overcome by leaving the muscle with its insertion and origin intact and by measuring the torque generated as a function of joint, in this case knee, angle. Because the moment arm of the knee is relatively independent of knee angle, the angle-torque curve should be very similar to a length-tension curve. This method is also potentially able to measure adaptation of the effective tendon length.

The training effect measured as extra sarcomeres is predicted to be correlated with the effects of a test bout of eccentric contractions. These effects can be assessed by many different parameters: histological, perceptual, and biochemical as well as mechanical. A simple and widely used mechanical measurement is the fall in tension measured at the length that was optimum before the contractions (see references in Discussion, Measurement of damage). However, because eccentric contractions lead to a shift in the length-tension curve (4, 16, 29), the drop in tension at constant length is at least partly due to the shift in the length-tension curve and not a real decrease in tension-generating capabil-
ity. In this study, the comparison of shift in optimum angle, the drop in torque at the pre-eccentric contraction testing (ECT) optimum length, and the drop in torque when the length is readjusted to the post-ECT optimum was undertaken.

METHODS

Twenty mature male Long Evans rats (Rattus norvegicus), mostly littersmates, were selected for uniform age (all animals were born within a 3-day period) and body weight at the time training began. Brief treadmill-running tests were used to select the 16 most willing runners. These were divided into four batches of four rats, two for incline training and two for decline training per batch. The batches were trained and tested in succeeding weeks. The age at surgery varied from 156 to 184 days, but incline- and decline-trained groups had equal numbers of rats of each age, with near-equal average body weights.

At surgery, the optimum knee angle for torque generation was determined for both vastus intermedius muscles from each rat (see Surgery for details). Within each batch, one incline-trained and one decline-trained animal were then used for fixation and fiber length determination (10 of 16 muscles completed the protocol; the other 6 produced estimates of sarcomere count but not of optimum angle). The other animals were used for ECT (see ECT; 14 of 16 muscles completed the protocol). The losses were due to premature death of the anesthetized animals.

Training. The method for training the rats has been described previously (19). Briefly, rats were trained over 5 days. The only difference between the training groups was the slope of the treadmill, either inclined or declined by 16° to horizontal. This ensures that differences are due to the slope of the treadmill and not to other exercise parameters. Training consisted of the rats running at a velocity slightly above the walk-trot gait transition speed, −16 m/min, for a period of 15 min the first day, building by 5 min/day up to 35 min on the final days, with a 1.5-min rest break included every 5 min. Each animal was started at a slower speed on the first day to accustom it to the procedure. No stimuli were used to encourage running except for a jet of air applied to the tails of animals on the first day if necessary. Incline- and decline-trained animals were housed together, with water and food freely available but with no exercise facilities.

Surgery. All data collection occurred 3 days after the last training session. The animals were anesthetized with intraperitoneal urethane (−2.0 g/kg), with supplementary doses (1 g/ml) as required to maintain deep anesthesia. For each leg, the femoral nerve was freed to enable stimulation. The vastus intermedius was mechanically isolated by dividing away the overlying vasti and cutting their insertions on the patella tendon. The femoral nerve had all branches not innervating the vastus intermedius cut. Thus the vastus intermedius was the only muscle generating torque about the knee. Connective tissue was removed from both sides of the muscle, with care being taken to maintain blood supply. For testing, the animal was in a supine position on a heating blanket, with its pelvic girdle and the base of the tail clamped while the lower leg (tibia and fibula) was secured to the equipment (see Angle-torque curves). The femur was positioned so that its axis was vertical and the lower limb was set initially at a knee angle of 90°. The muscle and nerve were immersed in paraffin while retained in skin flaps.

Stimulation. Muscle activation was accomplished by stimulation of the femoral nerve proximal to the femur at 90 pulses/s, which was determined to be supramaximal stimulation during preliminary work. Bipolar platinum electrodes driven by a Devices type 2533 isolated stimulator were used with a voltage that was just supramaximal, between 1 and 1.2 V. Pulse duration was 0.2 ms, and the period of activation was 350 ms or just enough to produce a maximal response. The rest period between contractions during measurement of length tension curves was 2 min.

Angle-torque curves. All results were measured as knee angle and torque generated by the muscle while it was still attached to the bones, rather than as length and tension of an isolated muscle. This avoids difficulties that we have experienced in attaching tendons to equipment, particularly slippage and the difficulty of preserving the in vivo muscle length with sufficient accuracy to show differences in optimum. Provided that the lever arm of the muscle about the joint does not change significantly with angle, these measurements are equivalent. Our unpublished observations support this assumption.

The angles reported are the angles included at the knee, between lines drawn through the articulation points of hip, knee, and ankle. Larger angles correspond to extension of the knee and shortening of the muscle. The knee angle was controlled by using a linear electromagnetic muscle puller connected to a fine steel cable, the other end of which was wound around a cable drum. The drum was fitted with a torsion spring to keep the cable taut at all times, so that movements of the puller were faithfully transmitted to the drum. Connected to this drum was a spring steel beam, with a pair of wire resistance strain gauges bonded on opposite faces near its fixed end. Fine waxed linen thread was used to firmly secure the lower leg into two notched supports attached to the beam. The knee was aligned with the axis of the drum so that movement of the puller produced pure rotation of the lower leg about the knee. The strain gauges gave a measure of the torque exerted at the fixed end of the beam, with no errors due to bearing friction. After calibration, knee angle was read from the position signal of the puller.

The interval between torque measurements in angle-torque curves was 9°. The compliance in the apparatus, measured by computer imaging before and during isometric contraction, was found to be −0.5° for maximum isometric activation of vastus intermedius.

The increase in torque on activation was plotted against knee angle. To find the optimal length, a Gaussian curve was fitted to points with torque above 80% of maximum torque. The Gaussian was chosen as a simple smooth curve, with the position of the peak specified by a parameter of the curve so that both the position of the peak and its SE are provided by the fitting program. It was found to be a generally good fit for points above 80%, although the fit became worse as lower points were included. Other methods of estimating the peaks were tried and gave similar results.

Sarcomere counting. Both vastus intermedius muscles from four incline-trained and four decline-trained rats were used for sarcomere counting. The methods used and the tests undertaken to validate them are described by Lynn and Morgan (19). The muscles were fixed, while still attached to the femur and the lower leg, in formaldehyde-saline buffered at pH 6.9, at maximum flexion of the knee for at least 1 wk. The fixed legs were imaged by using a video camera, and the femur length of each leg and the included angle of each knee were then calculated by using the image-processing program IPLab (Signal Analytics, Vienna, VA). The femur length measurements were used to ensure that relevant body dimensions were well matched between training groups. The muscles were then digested in 70% nitric acid and dispersed in an ultrasonic bath, and the suspension was serially diluted to
provide a truly random sample of 24 fibers per muscle by elimination of any choosing of fibers by the experimenter. The use of ultrasound dispersion reduced the duration of digestion needed and greatly reduced the number of broken fibers seen. Fiber lengths were measured by computer imaging and sarcomere lengths were measured by laser diffraction to produce an estimate of the average number of sarcomeres in series in the muscle fibers.

Fibers from the muscles used for ECT were not used for sarcomere counting because of reports of sarcomere damage in fibers from muscles subjected to eccentric contractions (2, 18, 24, 25, 29).

ECT. Both vastus intermedius muscles from eight animals were used for ECT. The ECT consisted of 20 tetanic contractions, spaced 1 min apart, that included the muscle's being lengthened due to a 27° decrease in included knee angle in 33 ms, starting from 90°.

Data processing. Statistical processing used Stat-View (Abacus Concepts, Berkeley, CA). All values are means ± SE. Incline-decline comparisons are unpaired two-tailed t-tests. Igor (Wavemetrics, Lake Oswego, OR) was used for data processing and fitting of Gaussian curves for optimum angle estimation.

RESULTS

Length-tension relationship. Figure 1 shows two length-tension plots, one for a muscle from an incline-trained rat and one for a muscle from a decline-trained rat. The fitted Gaussian curves, used to find the optimum angles, are also shown. Smaller included angles, corresponding to greater muscle lengths, are shown in Fig. 1, right. Note that the optimum occurred at longer muscle length (smaller included angle) in the muscle from the decline-trained animal.

Figure 2 shows the histograms of optimum knee angles for torque generation for all muscles successfully measured, 13 from decline-trained and 11 from incline-trained rats. The distributions are nonoverlapping, and optimum length for muscles from decline-trained animals occurs at longer muscle length than for muscles from incline-trained rats. The means are 43.6 ± 1.1° (SE) for decline-trained and 57.3 ± 1.3° for incline-trained animals (P < 0.0001).

Sarcomere counts. The number of sarcomeres in series per fiber was estimated for 24 fibers from each of 16 muscles, 8 each from decline and incline-trained rats, as described by Lynn and Morgan (19). The overall means, 3,475 ± 6 for the 192 fibers from eight decline-trained rats and 3,193 ± 67 for the 192 fibers from eight incline-trained rats, were significantly differ-
Fig. 3. Mean number of sarcomeres in series in muscle fibers compared with optimum knee angle for torque generation. Vertical error bars are SE for the 24 fibers measured from each muscle. Horizontal error bars are estimated error (SD) of position of peak of fitted Gaussian curve for the same muscle, as supplied by the fitting algorithm in Igor (see Data processing). Sarcomere counts are clearly dependent on training and strongly correlated with the optimum angle. Line has slope predicted if all muscles had the same tendon lengths and skeletal dimensions. Vertical position of the line is arbitrary. By eye, it is apparent that the best-fit line to the points would be steeper than this prediction, implying that the change in sarcomeres was more than enough to account for the change in optimum angle. The regression line for the muscle averages shown in Fig. 3 is steeper than the prediction but not significantly so. However, the slope of the regression line for the corresponding plot of all the individual fibers was $-28.3$ sarcomeres per degree (SE of $\pm 3.5$). The probability of the slope being more shallow than the predicted slope of $-17.5$ sarcomeres per degree is $<0.0025$. This implies that $38\%$ of the difference in sarcomere numbers ($95\%$ confidence limits of $14$ and $62\%$) does not appear as a difference in optimum angle, suggesting that it has been taken up by shortened tendons.

A direct comparison of fiber length differences ($5.5\%$ longer at maximum knee flexion for decline training) with sarcomere count differences ($8.8\%$ more sarcomeres for decline training) leads to the same general conclusion, although it is subject to considerable additional inaccuracies, particularly due to variability in fixation angle.

ECT. Six muscles from incline-trained rats and eight from decline-trained rats completed the protocol comprising initial measurement of optimum angle, ECT, and post-ECT measurement of optimum angle. Several measures were made of the effect of the ECT. The first was the shift in optimum from before to after the series of eccentric contractions (Fig. 4). Negative angles of shift imply smaller included knee angles and longer muscle lengths for maximum torque after ECT, as predicted. The mean shift in optimum angle resulting from ECT was significantly less ($P < 0.0001$) for the muscles from decline-trained rats ($-1.4 \pm 0.25^\circ; n = 8$) than for those from incline-trained rats ($-12.1 \pm 0.75^\circ; n = 6$).

A second measure of the effect of the ECT was the torque at the post-ECT optimum angle, representing the drop in force other than that due to the shift in optimum (Fig. 5A). Despite more scatter than with the shift in optimum length, there is again a clear difference between the two groups. Means were $82.8 \pm 5.0\%$ of pre-ECT torque at optimum length for muscles from decline-trained rats and $58.8 \pm 4.9\%$ for muscles from incline-trained rats ($P = 0.006$). This difference in
significance levels, 0.0001 for shift in optimum due to ECT and 0.006 for torque, would suggest that the torque after the ECT is more variable than shift in optimum length.

When torque was measured at the pre-ECT optimum angle instead of the post-ECT optimum (Fig. 5B), the means were 77.4 ± 6.3% of pre-ECT torque for decline-trained and 39.3 ± 4.5% for incline-trained rats (P = 0.006), consistent with being the combined effects of drop in torque at the post-ECT optimum length and the shift in the optimum angle. Note that the one low torque value in Fig. 5 makes a major contribution to the uncertainty of the decline group. Note also that muscles from decline-trained animals typically lost <20% of their peak tension because of fatigue and deterioration.

Morgan (21) predicted that disruption of sarcomeres and damage to fibers will be greater when more of the lengthening occurs on the descending limb of the sarcomere length-tension curve. Because the eccentric contractions were all performed over the same range of knee angles, as is normal testing of the effects of eccentric exercise, this becomes a prediction of more damage in muscles with a shorter optimum muscle length, that is, a larger optimum angle. An overall correlation between all measured parameters and the pre-ECT optimum knee angle is clear in Figs. 4 and 5, but any correlation within either training group is not significant. Linear regression of the shift in optimum due to eccentric contractions for all muscles gave a correlation coefficient of r = -0.87 and a probability of nonnegative slope of P < 0.0001. For the percent torque remaining after eccentric contractions measured at the post-ECT optimum angle, r = -0.61 and P = 0.02. If torque was measured at the pre-ECT optimum length, r = -0.69 and P = 0.006, again consistent with contributions from both the true decrease in tension and the shift in optimum angle.

**DISCUSSION**

Measurement of damage. While the most direct evidence of muscle damage is probably histological evidence, this is very difficult to quantify because of the extreme sampling difficulties and the continued development of histological changes for days and even weeks after eccentric contraction exercise (10, 14). A fiber that has a single half sarcomere of a single myofibril disrupted immediately after the exercise may be necrotic along its whole length some hours later. For this reason, most studies use other parameters, including drop in tension (see Mechanism of training), lack of fusion at low stimulation frequencies (5, 7, 23) and shift in the length-tension relationship (16, 29), as well as nonmechanical measures such as pain and tenderness levels of muscle and circulating proteins and other measures (14), to quantify damage. The shift in length-tension relationship has the advantages of being fully apparent at the end of the exercise, simply measured, largely independent of fatigue, and readily understood in terms of our theory (29) as evidence of the first stage of the process that leads to histologically observable damage.

Figures 4 and 5A show that the shift in length-tension relationship correlated well with the more commonly used decrease in tension, even when this was measured at the new optimum, eliminating any contribution of the shift. Both depended on training and correlated with pre-ECT optimum length, but the shift in optimum appeared to be the better measure in that it gave a clearer difference between muscles from incline- and decline-trained rats.

Tension measured after ECT at the pre-ECT optimum length (Fig. 5B), a more commonly used measure (5–7, 12, 13, 15, 17, 20, 22, 28), was found in these experiments to decrease because of both true decreases in tension-generating capacity and shift in the length-tension curve. The difference between the two measure-
ments depended on the training. For decline-trained rats, the mean difference was 5.4% of the pre-ECT tension. For incline-trained rats, the difference was 19.5%. Thus the contribution of shift to the apparent decrease in torque measured at fixed angle was greater for the muscles showing the greater decrease in torque.

Choice of controls. The choice of controls for experiments of this type has been considered previously (19). The conclusion was that the best comparison is between incline- and decline-exercised rats, rather than between unexercised and decline-exercised rats, to control for the effects of exercise such as and the probable variability in the amount of exercise undertaken spontaneously by nominally unexercised animals. This decision does make it impossible to answer the question, “Which exercise group changed?” Previous sarcomere counts indicated considerable variability, but on average both incline- and decline-trained groups did (19).

Mechanism of training. The existence of a rapid adaptation or training of muscle to suit eccentric or concentric exercise, such as that which angled treadmill running provides for the vasti, has been shown by many workers in many different animals, muscles, and exercises (3, 5, 6, 9, 26). They all showed reduced evidence of damage from a test bout of eccentric contractions after training involving eccentric contractions, but none showed any structural differences in the muscles that might explain the difference in susceptibility to damage. Morgan (21) suggested that the structural difference could be the number of sarcomeres in series in the fibers, increased by eccentric contraction training and/or decreased by concentric contraction training. To take the rat example, this would cause the contractions involved in the test bout of decline running to occur at shorter sarcomere lengths in muscles from decline-trained rats than in the muscles from incline-trained rats. This would avoid operation on the plateau and descending limb of the sarcomere length-tension curve and hence the sarcomere length instabilities that are to be expected there during lengthening and are postulated to be the first stage in the process leading to damage from such exercise (21). A previous study by Talbot and Morgan (27) confirmed this. It was found that more damage occurred to isolated toad muscles when the stretch occurred on the descending limb of the length-tension curve as opposed to the ascending limb. The reduced strain on sarcomeres for a given joint movement would also provide a contribution to protection. Such a difference in the number of sarcomeres in series has been reported (19) by using fixation and acid digestion. The present experiments confirm those results by the completely independent method of measuring optimum knee angle as well as by repeating those experiments and showing a strong correlation between the two methods when all the muscles are considered. There would seem to be no possible source of error that could produce such strongly correlated differences in such independently measured parameters.

The lack of apparent correlation between damage measures and pre-ECT optimum angle within the training groups leaves open the possibility that the difference in the optimum angles is not the only, or even perhaps the main, mechanism for the different effects of ECT. It is clear that eccentric contraction training produces greater optimum angles because of more sarcomeres in series and also produces protection against changes indicative of damage from eccentric contractions, but a correlation does not necessarily infer cause and effect. However, the differences in the number of sarcomeres in series in fibers must now be considered the most probable cause of the lesser damage in muscle accustomed to eccentric contractions.

Changes in tendon. The finding of different sarcomere numbers raises the question of whether the tendon length changes to partially compensate for differences in the number of sarcomeres. If it did not, then tension generation at short muscle lengths (extreme knee extension) would be compromised after eccentric contraction training. The results (Fig. 3) suggest that some adaptation of the tendon does occur within the 1-wk time span investigated, but the magnitude of the change is subject to considerable uncertainty.

Conclusion. We conclude that eccentric contraction training of muscles, compared with concentric contraction training, leads to muscles with more sarcomeres connected in series and more resistant to damage from eccentric test contractions. This rapid adaptation of the number of sarcomeres to the pattern of use of the muscle is in accord with the observations of Goldspink (11) and the predictions of Morgan (21), adding further credibility to the underlying proposal that lengthening of active muscle at long length is distributed very nonuniformly, with a few sarcomeres that are stretched to beyond filament overlap accounting for most of the length change in each myofibril.

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