Mechanical properties of rabbit latissimus dorsi muscle after stretch and/or electrical stimulation

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IN CLINICAL PRACTICE skeletal muscles can be transposed and used to compensate for muscle disease or malfunction elsewhere in the body. For example, muscles such as the gracilis have been wrapped around the rectum or the urethra to provide a neosphincter to treat fecal or urinary incontinence (30). Similarly, the latissimus dorsi (LD) muscle has been transposed, wrapped around the ventricles, and stimulated to contract in synchrony with systole. The latter procedure is known as dynamic cardiomyoplasty and provides extra power to increase the cardiac output of patients with heart failure (4). However, to perform these new functions the fatigue resistance of these predominantly fast-twitch muscles must be increased. Hence, in the last few years much interest has been expressed in the transformation of fast-twitch, easily fatigued skeletal muscles into fatigue-resistant slow-twitch muscles for potential clinical applications.

The most commonly used model for studies of fiber type transformation has involved chronic low-frequency (10-Hz) electrical stimulation of the tibialis anterior (TA) and/or extensor digitorum longus (EDL) muscles in the rabbit hindlimb (22, 24). These stimulation protocols effectively transform muscles from predominantly fast glycolytic fiber types to dominantly fast glycolytic fiber types to maintain under fentanyl anesthesia. After 3 wk of incrementally applied stretch the LD muscles were 36% heavier, but absolute power output (195 mW/muscle) was not significantly changed relative to that of external control muscle (206 mW).

In contrast, continuous 10-Hz electrical stimulation reduced power output per kilogram of muscle (>75%) after 3 or 6 wk and muscle mass by 32% after 6 wk. When combined, stretch and 10-Hz electrical stimulation preserved or increased the mass of the treated muscles but failed to prevent an 80% loss in maximum muscle power. However, this combined treatment increased fatigue resistance to a greater degree than electrical stimulation alone. These stretched/stimulated muscles, therefore, are more suitable for cardiomyoplasty. Nonetheless, further work will be necessary to find an ideal training program for this surgical procedure.

stretch acts as a powerful anabolic signal to muscle. Stretch, imposed on the TA or EDL of the rabbit by immobilization in plantar flexion, has been shown to result in a 20–30% increase in the mass of these muscles after just 3 days (12). When stretch and 10-Hz electrical stimulation were combined and imposed on the rabbit TA, a 35% increase in muscle weight was induced after 3 days of this treatment (12). This dramatic adaptive growth is associated with very early (1- to 5-h) increases in the expression of the protooncogenes c-fos and c-jun (20) and a subsequent (i.e., 3 days) 40-fold increase in insulin-like growth factor 1 mRNA (12). These changes in gene expression were more marked and occurred earlier than when electrical stimulation or stretch alone was used. This may mean that there is an acceleration of the transformation process, as well as the induction of hypertrophy, when stretch is added to electrical stimulation. However, to date, no long-term studies have been carried out to look at fatigue resistance and power generation after several weeks of such a combined treatment.

The work loop technique involves imposing cyclic length changes on a muscle, during which the muscle is electrically stimulated to produce work throughout the shortening phase of the cycle. The force produced by the muscle throughout such length change cycles can be monitored and plotted against its length. This gives a loop, the area of which represents the net work done by the muscle in each cycle of activity. Multiplying the net work per loop by the cycle frequency gives a measure of the muscle's power output. The work loop technique takes into account factors such as the time needed for activation and relaxation of the muscle as well as shortening deactivation (7, 10) and force enhancement (7, 8). Hence, the work loop technique can produce a realistic simulation of muscle function in vivo (19).

Studies in dogs have shown that the length and breadth of the ventricles change in a roughly sinusoidal manner during the cardiac cycle (23), and similar shape changes have been reported in human hearts (28). During the normal cardiac cycle the muscle fibers of the
ventricular wall of the dog change length by 8–10% (23). In this study we chose the parameters of the length change cycle (i.e., sinusoidal strain of ±5%, i.e., 10% peak to peak) to approximate the strain pattern of the LD muscle when the muscle is wrapped around the heart in cardiomyploplasty.

Although the work loop technique has been widely used to investigate muscle properties (e.g., Refs. 1, 2, 6, 18, 19), most studies have been carried out in isolated muscles in vitro. Such in vitro studies cannot be carried out on large muscles because the central fibers become anoxic, with detrimental effects on muscle performance (27). Therefore, we have adapted this technique to examine the properties of the LD muscle in situ (16), i.e., with its blood supply still intact to prevent the development of an anoxic core.

The work loop technique allows quantification of any changes that might occur in the passive and/or active properties of the muscle during its transformation. Isotonic measurements on LD muscles of the rabbit give an estimated maximum power output that is fourfold greater than that measured in the same muscles by using work loops (16).

When the muscle has been wrapped around the heart in cardiomyploplasty, passive extension of the LD muscle occurs during ventricular diastole, i.e., as the ventricles fill with blood and become distended. Any increased resistance to the extension of the muscle, as might occur due to the deposition of an increased amount of connective tissue in the LD muscle, could compromise cardiac filling. During ventricular systole the wrapped LD muscle is activated via a pacing device to shorten as the ventricles contract. The work loop technique can be used both to measure muscle performance during a specific task (in this case by using suitable parameters to mimic the conditions experienced during cardiomyploplasty) and thus to design suitable training programs to improve fatigue resistance and preserve power output.

In the present study the effects of three muscle-training protocols on the mechanical performance of the LD muscle have been measured and compared. Advantage has been taken of both a novel system that enables the LD muscle to be incrementally stretched at weekly intervals and the work loop technique.

METHODS

Care of Animals and Operative Procedures

All procedures were carried out in accordance with the British Home Office (Animals Scientific Procedures) Act of 1986. Rabbits were allowed free access to food (20% protein; SG1 diet, Burnhills, Cleckheaton, UK) and water and were maintained in a temperature (18 ± 1°C)-controlled room with a 12:12-h (0600–1800) light-dark cycle. Dutch rabbits of either gender, with body weights between 1.0 and 1.4 kg at operation, were used. Operative procedures were carried out under halothane anesthesia, and full aseptic precautions were taken.

Three different training protocols (i.e., stretch, electrical stimulation, and a combination of both treatments) were used, and their effects were studied after 3 or 6 wk.

Incremental static stretch. To stretch the LD muscle, a silicone tissue expander (Nagor, Ashby, UK) was used. An incision was made over the spine, from the level of the point of the scapula, caudally for 4 cm. The skin was raised, and a 3-cm incision was made through the medial border of the left LD and the overlying trapezius muscle 5 mm away from, and parallel to, the spine. A sterile, deflated tissue expander was inserted between the ribs and the LD muscle. The incision in the LD muscle was then sutured around the tube of the expander, and the filler port was located subcutaneously on the animal’s flank. All wounds were then closed.

To avoid damage to the LD muscle, we stretched the muscle incrementally. The degree of stretch applied to the LD muscles was regulated by injecting various quantities of saline into the tissue expander as determined in an earlier study (5). The percentage of stretch produced by injecting 25, 50, or 75 ml of saline into the expander was ~10, 15, or 20%, respectively, beyond the resting length of the LD muscle. At operation, a volume of saline was injected into the expander to give an initial stretch of 10% beyond the resting length. After the muscle was allowed to adapt for 7 days, the stretch stimulus was repositioned by injecting more saline to increase the level of stretch to 15% above the initial resting length. This was further increased to 20% stretch after a further 7 days. In some rabbits this final degree of incrementally applied stretch was then maintained for an additional 3 wk (i.e., a total of 6 wk of treatment) to determine whether the stretch-induced changes could be preserved by simply maintaining the stretch stimulus.

Continuous 10-Hz electrical stimulation. In the rabbits in which the left LD muscle was electrically stimulated, an incision was made on their left side 5 mm from, and parallel to, the scapula. An incision was then made just above the caudal side of the humeral tendon of the LD muscle. This allowed a pair of electrodes, formed from 5-mm-diameter stainless steel washers and temporary pacing wire (Ethicon), to be sutured in position adjacent to the main branches of the thoracodorsal nerve. The wires were passed subcutaneously to a small incision on the animal’s back, where they were connected to an external stimulator (see below). This stimulator was mounted and carried externally on the animal’s back in a fabric jacket, which was designed and made in house.

The portable stimulators used were made in house. The circuit design was based on that described by Salmons and Jarvis (25) but was constructed on circuit board rather than a printed circuit. The finished stimulators were 7 × 4 × 3 cm and weighed 15 g. They delivered stimuli at a frequency of 10 Hz, an amplitude of 4.6 V, and a pulse width of 2 ms.

The 10-Hz electrical stimulation was applied continuously 24 h/day via the thoracodorsal nerve for periods of either 3 or 6 wk.

Combined stretch and 10-Hz electrical stimulation. Some LD muscles were subjected to both the static stretch regime and 10-Hz electrical stimulation (see Incremental Static Stretch and Continuous 10-Hz Electrical Stimulation) concurrently for either 3 or 6 wk.

Neither of the training regimes appeared to cause the animals any discomfort or interfered with their normal forelimb movements or feeding/drinking habits.

Control muscles. Three groups of control LD muscles were studied: those obtained from external (nonoperated) animals, internal controls from the contralateral side of each treated animal, and sham-operated animals. The latter group of control rabbits had tissue expanders and electrodes implanted beneath their LD muscles as described above. However, these expanders were left fully deflated, and the electrical stimulators were switched off. These animals underwent
Values for work were adjusted to control runs as previously described (2, 16).

“Passive” loops were also examined, where length change cycles were imposed on the muscle without electrical stimulation. The work measured in these passive loops represented the net work lost due to the resistance to extension and passive recoil during shortening of the muscle. Power (i.e., passive power loss) was again determined by multiplying the work per loop by the cycle frequency.

Fatigue test. After completion of all other mechanical measurements, the muscles were subjected to an isometric fatigue test. Repeated bursts of 70-Hz electrical stimulation were applied for 0.3 s, with an inactive period of 0.7 s between each burst. The time taken for the force generated to fall to one-half of the initial value was measured ($FT_{1/2}$).

After completion of mechanical tests on each experimental LD muscle, the contralateral muscle was similarly subjected to mechanical testing, thereby acting as an internal control to the treated muscle.

All mechanical tests were completed, animals were killed with an intravenous overdose of Sagatal (pentobarbital sodium).

Statistical Analyses

All statistical analyses were carried out by using a computer statistics package (Instat, Graphpad Software, San Diego, CA). The mechanical properties were statistically analyzed with a one-way analysis of variance with Bonferroni post hoc tests to compare values from experimental, sham-operated, and contralateral muscles to the values of external (nonoperated) control muscles. Data on muscle wet weights, lengths, and fatigue were compared between treated muscles and their internal contralateral controls by using a paired t-test.

RESULTS

Mechanical Properties of Muscles

Sham-operated and internal control muscles. Both the left and right LD muscles of external control animals had their mechanical properties measured. No significant differences were found for any of the parameters measured below, demonstrating that no particular side is favored for weight bearing and that the mechanical performance of the LD muscle is not affected by the prior testing of one side before the other.

Values for tetanic forces, maximum power output, $RT_{1/2}$, and $FT_{1/2}$ were also compared with those of internal (contralateral) control muscles by using a one-way analysis of variance. The only value found to be significantly different from the external controls was the power output (W/kg) measured in the internal control muscles of animals having contralateral LD muscles that had received 3 wk of stretch (Table 1). It was therefore reasonable to conclude that the mechanical properties of the internal control muscles were not generally changed as a result of the various treatment regimes used in this study and that the use of either type of control muscle is valid. Similarly, the properties of the muscles from sham-operated animals were not significantly different from those of the external control muscles (Table 1). Hence, the presence of the expander and the electrodes did not change the mechanical properties of the LD muscle.
Table 1. Mechanical properties of rabbit LD muscles

<table>
<thead>
<tr>
<th></th>
<th>External Control</th>
<th>Sham Op</th>
<th>3-wk Stretch</th>
<th>3-wk Stretch 10-Hz Stimulation</th>
<th>6-wk Stretch</th>
<th>6-wk Stretch 10-Hz Stimulation</th>
<th>6-wk Stretch 10-Hz Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle weight, g</td>
<td>8.0 ± 0.5 (10)</td>
<td>8.7 ± 1.2 (3)</td>
<td>(NS)</td>
<td>7.6 ± 1.6 (5)</td>
<td>7.1 ± 0.7 (5)</td>
<td>7.9 ± 0.7 (6)</td>
<td>8.0 ± 0.8 (4)</td>
</tr>
<tr>
<td>Isometric stress, kN/m²</td>
<td>145 ± 11 (10)</td>
<td>124 ± 6 (3)</td>
<td>NS</td>
<td>158 ± 24 (5)</td>
<td>139 ± 15 (5)</td>
<td>143 ± 16 (5)</td>
<td>138 ± 25 (4)</td>
</tr>
<tr>
<td>Absolute tetanic force</td>
<td>8.8 ± 0.4 (10)</td>
<td>8.6 ± 0.9 (3)</td>
<td>NS</td>
<td>8.5 ± 1.1 (5)</td>
<td>7.5 ± 0.7 (5)</td>
<td>8.1 ± 0.4 (5)</td>
<td>7.9 ± 0.8 (4)</td>
</tr>
<tr>
<td>Maximum power, W/kg</td>
<td>27 ± 1 (8)</td>
<td>28 ± 4 (3)</td>
<td>(NS)</td>
<td>45 ± 9 (4)</td>
<td>26 ± 2 (5)</td>
<td>22 ± 3 (4)</td>
<td>23 ± 0 (2)</td>
</tr>
<tr>
<td>Maximum absolute power, mW</td>
<td>206 ± 15 (8)</td>
<td>250 ± 67 (3)</td>
<td>NS</td>
<td>272 ± 62 (5)</td>
<td>181 ± 23 (4)</td>
<td>168 ± 24 (4)</td>
<td>167 ± 8 (2)</td>
</tr>
<tr>
<td>Power at 3 Hz, mW</td>
<td>150 ± 12 (8)</td>
<td>193 ± 34 (3)</td>
<td>NS</td>
<td>201 ± 40 (5)</td>
<td>154 ± 15 (5)</td>
<td>156 ± 15 (5)</td>
<td>167 ± 2 (2)</td>
</tr>
<tr>
<td>Twitch rise time, ms</td>
<td>31 ± 3 (9)</td>
<td>23 ± 1 (3)</td>
<td>(NS)</td>
<td>37 ± 6 (4)</td>
<td>31 ± 2 (5)</td>
<td>25 ± 1 (6)</td>
<td>30 ± 4 (2)</td>
</tr>
<tr>
<td>Half tetanus relaxation time, ms</td>
<td>22 ± 1 (8)</td>
<td>20 ± 2 (3)</td>
<td>(NS)</td>
<td>23 ± 1 (5)</td>
<td>24 ± 2 (5)</td>
<td>20 ± 1 (5)</td>
<td>23 ± 1 (3)</td>
</tr>
<tr>
<td>Half fatigue time, s</td>
<td>30 ± 3 (9)</td>
<td>36 ± 9 (3)</td>
<td>(NS)</td>
<td>28 ± 3 (5)</td>
<td>46 ± 5 (5)</td>
<td>47 ± 5 (5)</td>
<td>No data</td>
</tr>
</tbody>
</table>

Values are means ± SE; no. of muscles in parentheses. Op, operated; Con, contralateral control; Exp, treated; NS, not significant. Muscles were derived from external control, sham-operated, or rabbits having the left lattisimus dorsi muscles subjected either to stretch, 10-Hz electrical stimulation, or a combination of stretch and stimulation for 3 or 6 wk. No statistically significant differences were found between data from contralateral (internal) and external control muscles. NS, P > 0.05. *P < 0.05; †P < 0.01. ‡P < 0.005 (all by 1-way analysis of variance with Dunnett’s post hoc test).

Statically stretched muscles. After 3 wk of incrementally applied static stretch, decreases were noted in both the isometric stress (35%; P > 0.05) and specific maximum power output (51%; P < 0.05; Table 1). It is clear from the power-cycle frequency curve (Fig. 1) that the specific power output of these stretched LD muscles was decreased at all cycle frequencies studied. However, over the 3 wk that the static stretch was applied, it induced a 36% increase in muscle weight (Table 1). This adaptive growth effectively meant that the absolute tetanic force and power output were not significantly different from the values measured in the smaller internal control muscles (Table 1, Fig. 2). The optimum cycle frequency for power output was not changed in the stretched muscles when compared with control muscles (Figs. 1 and 2). This suggests that there was no significant “slowing” of the muscle’s contractile properties in response to stretch alone. This was supported by the fact that there were no significant increases in either the twitch rise time or the rate of relaxation (i.e., RT₁/₂, Table 1) in these LD muscles after 3 wk of stretch (Table 1).

In some rabbits the incremental stretch stimulus was maintained at its maximum level for an additional 3 wk (i.e., between 3 and 6 wk) to provide 6 wk of treatment in total. This was undertaken to determine whether the adaptive changes induced by 3 wk of incremental stretch were retained, without provision
for any further stretching. The stretch-induced increase in the mass of the muscles was fully maintained or slightly increased (but not significantly) between 3 and 6 wk of treatment (Table 1). Although still remaining lower than the control values, the specific power output of the muscle did not decrease any further over this period (Table 1). However, the absolute power produced by the whole muscle after 6 wk was increased to values greater than those in either the internal or the external control muscles (Table 1) at all cycle frequencies studied (Fig. 3). This stretch protocol also induced changes in the passive properties of the muscles. After 3 wk these were apparent as an increase in the passive power loss at all cycle frequencies investigated, compared with the control muscles (Fig. 4). These changes in the passive properties of the muscles were not retained over the subsequent additional 3 wk of maintained stretch. Indeed, if anything, by 6 wk the passive power losses were now less than those measured in the control muscles at all cycle frequencies examined (Fig. 5).

Electrically stimulated LD muscles. After 3 wk of continuous 10-Hz electrical stimulation, the isometric stress and mass specific power output of the LD muscles had decreased significantly, by 55 and 85%, respectively, compared with the internal control muscles (Table 1). This large loss in power was seen at each of the cycle frequencies examined (Fig. 1). At this time point the weights of the LD muscles were not significantly different from those of the contralateral muscles (Table 1). Hence, the absolute tetanic force and power output were similarly decreased by 51 and 80%, respectively (Table 1, Fig. 2). The shape of the power-cycle frequency curve also changed. Power output peaked at a cycle frequency of ~2 Hz, rather than 5 Hz as in the control muscles (Figs. 1 and 2). This would suggest that the muscle had become slower. This conclusion was supported by consistent but not statistically significant increases in RT1/2 (27%) and twitch rise times (Table 1). FT1/2 of the stimulated muscles increased approximately threefold (Table 1).

After 6 wk of chronic stimulation the muscles had lost 32 and 44% of their weight compared with internal and external controls, respectively (Table 1). All values of tetanic force or power output, whether expressed per kilogram of muscle or per whole muscle, were lower than control muscle values. There was not, however, a dramatic decline in these values as a consequence of prolonging the stimulation from 3 to 6 wk. FT1/2, RT1/2, and twitch rise time were, however, further increased by 6 wk of 10-Hz stimulation compared with 3 wk of treatment (Table 1).

Although after electrical stimulation the passive power losses were larger than those of control muscles, especially at the higher cycle frequencies used (Fig. 4),...
Muscles subjected to 10-Hz electrical stimulation combined with stretch. The purpose of this treatment was to induce muscle hypertrophy (via stretch) rather than atrophy in an attempt to preserve greater absolute power output during transformation by electrical stimulation. Three weeks of combined static stretch and 10-Hz electrical stimulation resulted in decreases in the isometric stress (51%) and mass specific power (81%; Table 1). This decline in power was seen at all cycle frequencies examined (Fig. 1). These muscles were 44% heavier than the control muscles as a result of the stretch component in this combined regime (Table 1). This meant that the absolute power output was slightly greater than that of muscles that had received 10-Hz electrical stimulation alone (Fig. 1). For example, at a cycle frequency of 3 Hz, the mean absolute power output was 23 mW in those muscles that received electrical stimulation alone and 42 mW in those receiving the combined regime (Table 1). However, the absolute power and tetanic force were still substantially decreased by 78 and 46%, respectively, compared with control muscles (Table 1, Fig. 2).

These muscles were 44% heavier than the control muscles as a result of the stretch component in this combined regime (Table 1). This meant that the absolute power output was slightly greater than that of muscles that had received 10-Hz electrical stimulation alone (Fig. 1). For example, at a cycle frequency of 3 Hz, the mean absolute power output was 23 mW in those muscles that received electrical stimulation alone and 42 mW in those receiving the combined regime (Table 1). However, the absolute power and tetanic force were still substantially decreased by 78 and 46%, respectively, compared with control muscles (Table 1, Fig. 2). The power-cycle frequency curve was now flatter, with peak power output being shifted to lie between 2 and 3 Hz (Figs. 1 and 2). This shift was accompanied by a marked increase (145%) in RT1/2 (Table 1), contrasting with the more modest increase (27%) induced by electrical stimulation alone. The twitch rise time was also now significantly elevated by 64% above the internal
By this time point FT1/2 had increased combined stretch and electrical stimulation (Table 1). In addition, the atrophy of the muscle may be accentuated because the stimulated muscles, e.g., in the lower limb of rabbits, this atrophy is explained by the conversion of the fast muscle to slow muscle. In this case, the atrophy is attributed to the conversion of the fast muscle to slow muscle. In this case, the atrophy is attributed to the conversion of the fast muscle to slow muscle. In this case, the atrophy is attributed to the conversion of the fast muscle to slow muscle. In this case, the atrophy is attributed to the conversion of the fast muscle to slow muscle.

After 6 wk of the combined stretch/stimulation, the muscles were only 14% heavier than their contralateral controls (Table 1). This shows that stretch-induced hypertrophy compensates for the atrophy induced by electrical stimulation alone (Table 1). However, despite the preservation of muscle mass, there were still substantial decreases in the absolute tetanic force (57%) and maximum power output (85%) relative to controls (Table 1, Fig. 3). These values were intermediate between the values from muscles that received stretch or electrical stimulation alone but generally much closer to those from LD muscles that had been subjected to electrical stimulation alone for 6 wk (Fig. 3). The absolute power output at a cycle frequency of 3 Hz was greater in those muscles that had received stretch with stimulation than in those subjected to stimulation alone (Table 1). The difference between these two regimes was, however, less marked than that observed after 3 wk of these treatments.

After 6 wk of the combined treatment, the frequency at which the maximum power output was achieved was between 1 and 3 Hz (Fig. 3). The implication is that the mechanical properties were those of a slower muscle. This was confirmed by a further increase in the twitch rise time and a fourfold increase in RT1/2 after 6 wk of combined stretch and electrical stimulation (Table 1). By this time point FT1/2 had increased ∼10-fold above control levels, which was appreciably higher than the changes induced by 6 wk of electrical stimulation alone (Table 1).

The passive power loss after either 3 or 6 wk of the combined treatment was consistently less than in control muscles at each cycle frequency studied (Figs. 4 and 5).

**DISCUSSION**

Chronic low-frequency electrical stimulation has proved effective in modifying the phenotypic properties of skeletal muscles, i.e., in converting fast, glycolytic, and easily fatigued muscles into slow, oxidative, and fatigue-resistant ones (22, 24). This approach can be exploited clinically in situations where muscles may be transposed to undertake new functions but need to have their contractile and/or metabolic properties changed. Chronic, low-frequency stimulation increases fatigue resistance, thereby enabling muscles to work continuously (22, 24). This procedure, however, does cause rapid and marked atrophy (3) with concomitant large losses in force and power generation in both rabbit leg (17) and LD muscles (Table 1, Figs. 1–3). In part, this atrophy is explained by the conversion of the larger fast fibers into smaller slow ones. In certain muscles, e.g., in the lower limb of rabbits, this atrophy may be accentuated because the stimulated muscles often operate at shorter lengths than normal, resulting in additional fiber atrophy (13) over and beyond that caused by the physiological conversion of fiber types. Such large losses in power output could conceivably leave the transformed muscle incapable of generating sufficient power to compress the underlying ventricles, as would be required to provide hemodynamic support in cardiomyoplasty.

We have shown (Table 1) that static stretch induces muscle hypertrophy, with new sarcomeres being added in series and in parallel (5). This rapid growth results from stretch-induced increases in both the rates of protein synthesis and protein breakdown (12), the net effect favoring an accumulation of protein and hence muscle growth. By using our tissue expander to remap the stretch stimulus at weekly intervals, the mass and length of the LD muscle can be progressively increased over 3 wk (Table 1). Up to this point the muscle has adapted to each stage of incremental stretch by adding on more sarcomeres in series. This adaptation enables the overstretched sarcomeres to shorten, providing optimal overlap between the thick and thin filaments (29). In so doing, the muscle also reduces the stretch stimulus. The final maintained, rather than increased, stretch stimulus between 3 and 6 wk was clearly effective in preventing any major reversal of the adaptive growth achieved over the period of incremental muscle loading (Table 1).

When combined with 10-Hz electrical stimulation, the stretch component acts as an anabolic signal that compensates for the atrophy induced by electrical stimulation. The stimulation pattern, however, effectively causes the fiber type conversion. Indeed, static stretch alone, as employed here, does not appear to modify the muscle's phenotypic properties or mechanical performance to any great extent (Table 1, Figs. 1–5). The value of combining stretch with electrical stimulation is discussed below.

More of the muscle mass and length (as assessed by serial sarcomere numbers; data not shown) was preserved in these muscles relative to those subjected to electrical stimulation alone. These muscles would therefore be more suitable to wrap around a hypertrophied failing heart. There was little difference between the maximum power output after either electrical stimulation or the combined stretch and stimulation procedure (Table 1). However, the muscles that had received the combined regime showed better power output at higher cycle frequencies. This was true whether power output was expressed per kilogram of muscle (Fig. 1) or per whole muscle (Fig. 2), the latter being directly influenced by the stretch-induced hypertrophy of the stretch/stimulated muscles (Table 1). In the rabbits studied here, resting heart rate is ∼3–4 Hz, and this would, therefore, be the relevant frequency for cardiomyoplasty. At these cycle frequencies the muscles subjected to the combined regime exhibited a greater power output than those that had only been electrically stimulated. However, this effect was less marked after 6 wk of treatment than after 3 wk. Despite the small differences (Table 1), the slightly greater power output retained in the stretch/stimulated muscles at 3 Hz...
could be important in providing sufficient hemodynamic support to a failing heart.

The maximum power output of a muscle will be influenced by its passive properties. Three weeks of stretch or electrical stimulation alone appeared to increase the passive power loss (Fig. 4). At this time point the proportion of the histological cross sections of the muscle that was made up of collagen (assessed with a Sirius red stain) had increased significantly from 15.5% in the control tissue to 19 and 23% in stretched and stimulated LD muscles, respectively. However, these values were largely restored to the control levels by 6 wk (V. M. Cox and P. Williams, unpublished observations), which correlate with similar values of passive power loss in the control and all trained muscles at this time point. These time-related changes in passive properties probably indicate a slower remodeling of the collagen components, compared with the contractile proteins, in the muscles in response to the individual training regimes.

In the electrically stimulated muscle, the power-cycle frequency curve shifts to the left (Fig. 1). This pattern would be expected with a slowing of contractile characteristics. Innumerable studies have shown that such stimulated muscles change their myosin adenosine triphosphatase isozymes, resulting in a decrease in the velocity of shortening (see Ref. 22 for review). In the stretched/stimulated muscles the shift in the curve was less pronounced, with peak power output at a cycle frequency that was intermediate with respect to that of the control and electrically stimulated muscles (Fig. 1). This would suggest that the contractile characteristics had changed to resemble those of a slow-twitch muscle but to a lesser extent than with stimulation alone. However, this conclusion was actually the reverse of changes measured in the twitch rise times in these muscles (Table 1). Furthermore, preliminary immunocytochemical studies have suggested a greater accumulation of type IIa and type I myosin heavy chains in stretch/stimulated muscles, compared with those that received stimulation at 10 Hz alone (K. Gillott, unpublished observations).

The combined stretch/stimulation treatment over 6 wk increased the fatigue resistance of the muscle, this occurring to a greater extent than with electrical stimulation alone (Table 1). This is consistent with a more rapid onset of the increase in the activity of the mitochondrial enzyme succinic dehydrogenase in stretched/stimulated leg muscles compared with those subjected to 10 Hz alone (13). This would suggest a more rapid increase in the oxidative status of the muscle, one metabolic adaptation associated with an increased resistance to fatigue. The present fatigue test measured isometric force production. Therefore, we are not able to comment on the ability of these trained muscles to sustain work and power in a simulated cardiomyoplasty-like situation. We are presently developing a more physiologically relevant way to test the suitability of these muscles for this procedure.

In cardiomyoplasty, if the rate of relaxation of the LD muscle becomes too prolonged it could compromise the filling of the ventricles during diastole. Therefore, ideally a muscle-training regime should increase fatigue resistance but without appreciably slowing the muscle. Although stretch alone over 6 wk did not increase the relaxation time, electrical stimulation, with or without stretch, did, this effect being slightly more pronounced in the combined treatment at both 3 and 6 wk (Table 1). Overall, the combination of stretch with low-frequency stimulation appears to be more beneficial than either procedure when used alone in training the LD muscle for cardiomyoplasty. These differences were assessed in situ with the muscle operating in a linear configuration. This is clearly very different from the cardiomyoplasty wrap situation, and there are likely to be some differences in muscle performance. The mechanical properties in the latter situation represent the acid test for any muscle training regime and will be the focus of future research.

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