Mechanical properties of the latissimus dorsi muscle after cyclic training

GRAHAM N. ASKEW,1 VALERIE M. COX,2 JOHN D. ALFRINGHAM,1 AND DAVID F. GOLDSINK3

1School of Biology, University of Leeds, Leeds LS2 9JT; 2School of Natural and Environmental Sciences, Coventry University, Coventry CV1 5FB; and 3Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool L3 2ET, United Kingdom

Received 14 March 2002; accepted in final form 16 April 2002

Askew, Graham N., Valerie M. Cox, John D. Altringham, and David F. Goldsink. Mechanical properties of the latissimus dorsi muscle after cyclic training. J Appl Physiol 93: 649–659, 2002. First published April 19, 2002; 10.1152/japplphysiol.00218.2002.—Cardiomyoplasty is a procedure developed to improve heart performance in patients suffering from congestive heart failure. The latissimus dorsi (LD) muscle is surgically wrapped around the failing ventricles and stimulated to contract in synchrony with the heart. The LD muscle is easily fatigued and as a result is unsuitable for cardiomyoplasty. For useful operation as a cardiac-assist device, the fatigue resistance of the LD muscle must be improved while retaining a high power output. The LD muscle of rabbits was subjected to a training regime in which cyclic work was performed. Training transformed the fiber-type composition from approximately equal proportions of fast oxidative glycolytic (FOG) and fast glycolytic (FG) fibers to one composed of almost entirely of FOG with no FG, which increased fatigue resistance and resulting rapid contraction kinetics. Muscle mass and cross-sectional area increased but power output decreased, relative to control muscles. This training regime represents a significant improvement in terms of preserving muscle mass and power compared with other training regimes, while enhancing fatigue resistance, although some fiber damage occurred. The power output of the trained LD muscle was calculated to be sufficient to deliver a significant level of assistance to a failing heart during cardiomyoplasty.

SKELETAL MUSCLE HAS THE POTENTIAL to provide power that can be used to provide cardiac assistance for patients in end-stage heart failure (3). Skeletal muscle assistance can take a variety of forms. All involve stimulating the muscle to contract to assist blood flow but differ in the way in which the muscle is used. For example, dynamic cardiomyoplasty and aortomyoplasty are surgical procedures that involve wrapping the patient’s own latissimus dorsi (LD) muscle around the heart or aorta, respectively, to assist the heart in actively pumping blood (3, 8). Alternatively, the LD muscle can be used to power hydraulic actuators that transfer the work performed by the muscle into hydraulic energy to facilitate circulation (24, 31). The human LD muscle is less resistant to fatigue than the myocardium because of predominance of fast fibers in the LD muscle (29). For useful operation as a cardiac-assist device, the fatigue resistance of the LD muscle must be improved. Current procedure for dynamic cardiomyoplasty involves a postoperative period of 2 wk, during which the muscle remains unstimulated, followed by a progressive transformation by electrical stimulation (9). Thus a considerable time elapses before significant cardiac assistance is attained. To avoid this, some attention has been placed on training the muscle before the operation such that the patient receives significant functional benefit shortly after the operation. The training procedure must yield a muscle that can develop significant power output (for useful cardiac assistance), be rapid contracting (slow relaxation could impair ventricular filling during diastole), and be highly fatigue resistant.

Several different muscle transformation models have been employed, most of which involve electrical stimulation, passive stretch, or a combination of electrical stimulation with static stretch. Chronic, low-frequency electrical stimulation at 10 Hz transforms predominantly fast muscle fibers into slow fibers, over a period of 6–8 wk (25, 27). This transformation renders the muscle highly resistant to fatigue, but unable to generate high power outputs, because of a reduction in the maximum shortening velocity and substantial reductions in the fiber cross-sectional area (16, 17, 26). Lower frequencies of stimulation (2.5 Hz) over 12 wk result in transformation to a muscle consisting of predominantly fast oxidative glycolytic (FOG) fibers (18, 23, 30). Loss of muscle mass when 2.5-Hz stimulation was used was not as great as when 10-Hz stimulation was used, and, in addition, the reduction in maximum shortening velocity was approximately one-half of that observed with 10-Hz stimulation. As a result, the maximum power output during isotonic contractions was 55% higher after the 2.5-Hz stimulation regime com-

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.jap.org
pared with 10-Hz stimulation, but it was reduced to less than one-half of that measured in contralateral control muscles (30). Some training protocols have involved intermittent bursts of stimulation. Intermittent stimulation produces a muscle comprising predominantly FOG fibers after 6–12 wk. These trained muscles show improved fatigue resistance compared with control muscles and generate an isotonic power output that is 40–100% that of control muscles (10, 21).

Passive stretch of skeletal muscle leads to muscle enlargement, predominantly because of the addition of sarcomeres to the ends of the muscle fibers (13, 32). Combining stretch with continuous 10-Hz stimulation results in a hypertrophied, fatigue-resistant muscle. However, transformation to a slow fiber type limits the power output and slows the contraction kinetics, rendering it unsuitable for cardiomyoplasty (13, 16).

To date, there has been only limited success in developing a transformation regime suitable for preoperative training of the LD muscle for cardiomyoplasty, and substantial improvements can undoubtedly be made. In addition, previous studies have ignored the fact that whatever transformation regime is used to precondition the LD muscle, the LD muscle will effectively be retrained by the work it performs when contracting around the heart. Previous work in our laboratory (16) has used silicone tissue expanders to impose static stretch on the LD muscle in combination with continuous low-frequency stimulation. In the present experiments, by cyclically inflating and deflating a silicone tissue expander implanted beneath the LD muscle and phasically stimulating the muscle, we trained the rabbit LD muscle by subjecting it to cycles of work. This training regime is certainly the most relevant because it simulates the conditions that would be imposed on the muscle during cardiomyoplasty. The work loop technique (19) was used to assess the performance of the muscle after transformation by imposing cyclic length changes and phasic stimulation on the muscle. The work loop technique allows power output to be measured under conditions that simulate the way in which many muscles operate in vivo and integrates many factors that determine performance that are not adequately assessed under isotonic or isovelocity techniques. Histochemical analysis was carried out to determine the fiber-type composition of control and trained muscles.

METHODS

All experimental procedures were carried out in accordance with the British Home Office Animals (Scientific Procedures) Act of 1986. Dutch rabbits were housed in a temperature-controlled room (18 ± 1°C) with a 12-h light (0600–1800) and 12-h dark cycle, with free access to food and water. The animals had a mean body mass of 1.13 ± 0.04 kg (12), an estimated resting heart rate of 230 beats/min (4 Hz), and a maximum heart rate of ~420 beats/min (7 Hz), based on scaling equations (4, 20, 28).

Surgical procedure. All surgery was carried out under halothane anesthesia, and full aseptic technique was employed at all times. Hair was removed between the spine and left scapular and also above the humeral insertion of the LD muscle. An incision was made through the skin, above the spine. By cutting parallel to the muscle fibers, a second incision was made through the left LD muscle and overlying trapezius muscle, ~5 mm from the spine. A sterile, deflated silicone tissue expander (75 ml; Nagor, Ashby, UK) was inserted beneath the LD muscle. The incision made in the muscle was closed around the filling tube of the tissue expander, leaving the tube protruding through the skin.

A pair of stimulating electrodes, constructed from pacemaker wire (Ethicon), was implanted in close proximity to the main branches of the thoracodorsal nerve near the humeral insertion of the LD muscle. The electrode wires were passed subcutaneously to the incision on the animal's back. The skin wounds were then closed, leaving the electrode wires and tissue-expander filling tube protruding. Elasticated bandaging (Elastoplast) was wrapped around the skin wounds to minimize the risks of infection and reopening of the wounds. The electrodes and filling tube were housed in the pocket of a custom-made fabric jacket, which passed over the front legs and around the belly of the rabbit. A period of 6 h post surgery elapse before training was initiated. During training, rabbits were free to move around a large enclosure (1.0 × 0.7 m).

Establishment of an appropriate cyclic work training protocol. The LD muscle will be cyclically stretched when it is wrapped around the ventricles during cardiomyoplasty. To simulate these length changes, a custom-built pneumatic pump (Oak Medical) was used to inflate and deflate the silicone tissue expander via its filling tube. The pump had controls that allowed both the duration and the rate of inflation and deflation to be adjusted. These controls were adjusted such that the strain imposed on the LD muscle simulated the strain that would be produced if the muscle were wrapped around the heart. The strain imposed on the muscle was calibrated in a freshly killed rabbit by using sonomicrometry (model 120-1000, Triton Technology). Three pairs of crystals (each comprising a transmitting and receiving crystal) were inserted into the proximal, medial, and distal region of the LD muscle ~10 mm apart and aligned along the muscle fibers to determine the variation in the applied stretch over the length of the muscle.

By varying the rate and duration of inflation, the degree of stretch imposed on the LD muscle could be varied. In a series of pilot experiments, we varied the inflation volume (15 or 25 ml) and the training duration (1, 2, or 4 h per day). Training was carried out for 4 days, after which the animal was killed and the muscle assessed for any damage after staining with hemotoxylin and eosin (see Histochemical analysis below).

The length of the muscle was cycled at a frequency that was approximately equal to the resting heart rate of the animals used in the experiment (4 Hz). The deflated silicone tissue expander imposed a 5% increase in initial muscle length. Cyclic inflation-deflation with 15 ml of air produced a further 12% increase in initial muscle length, and 25 ml produced an additional 17% increase in initial muscle length. Thus the total degree of stretch relative to the initial muscle length was 17 and 22% for inflation volumes of 15 and 25 ml, respectively. No muscle fiber damage was observed with an inflation volume of 15 ml. However, fiber damage was severe (at least 50% of muscle fibers) with an inflation volume of 25 ml. A thick connective tissue layer was observed beneath the expander with an inflation volume of 25 ml and with an inflation volume of 15 ml for training durations above 1 h.

Training protocol. Our selection of an appropriate cyclic work training protocol was based on a preliminary set of training experiments described above. An inflation volume of 15 ml for a period of 1 h/day was used because no fiber
damage was observed and only a thin layer of connective tissue developed between the surface of the muscle and the tissue expander over a period of 4 days. In humans, the diameter of the left ventricle during diastole is \(37^\circ\text{C}\) greater than during systole (5). Exactly how these length changes relate to the circumferential strain is unclear; however, it seems likely that the strains we imposed on the LD muscle during training (\(-17\%\) stretch) are relevant to those that the muscle would experience when wrapped around the ventricles. The inflation volume was kept constant over the course of the training duration so that the degree of stretch given above is relative to the muscle length before training. If the muscle increases in length over the training period, the actual strain will decrease.

In rabbits with implanted tissue expanders and stimulating electrodes, cyclic length changes were imposed on the LD muscle at a frequency of 2 Hz. However, each inflation-deflation cycle lasted for only 275 ms; i.e., there was a 225-ms period between each cyclic length change. The frequency of each length change cycle was 3.6 Hz, which approximately coincided with the resting heart beat frequency of the rabbits. The "rest" period between each inflation-deflation cycle acted as a safety factor to avoid the possibility of any residual air being left in the tissue expander before the subsequent inflation cycle and approximately simulated having an active cycle on alternate heartbeats.

With each length change cycle, a single trigger signal was emitted from the pump. This was used to synchronize bursts of electrical stimulation to the LD muscle with the length change. An external programmable cardiomystimulator was used to deliver bursts of stimuli to the LD muscle (model SP1005, Medtronic). A stimulation duration of 125 ms delivered at a frequency of 65 Hz was used, with the onset of stimulation timed to coincide with the start of muscle shortening. The stimulation amplitude was 8 V, which was \(-56\%\) of that which yielded the greatest twitch force, determined from preliminary experiments. The amplitude was reduced to this level to reduce the possibility of nerve damage that might result over a prolonged period of training and also to reduce stress to the animal. A pulse width of 0.45 ms was used.

Muscles were subjected to this regime for 1 h/day throughout the training period. Training lasted for either 3 or 6 wk, with training occurring on each of 5 consecutive days followed by 2 nontraining days per week. Two nontraining days were incorporated into each week in an attempt to allow recovery from any muscle damage that might result from the training.

Mechanical assessment of the LD muscle. Details of the methods used to assess the mechanical properties of the rabbit LD muscle have been previously published (16); a brief description is outlined here. Before mechanical testing, rabbits were given a premedication of Hypnorm (3.5 \(\mu\)g/kg body mass of fentanyl citrate and 1 mg/kg body mass of flumazenil) and 0.5 mg/kg body mass of midazolam base, administered by subcutaneous injection. Subsequent intramuscular injection of Hypnorm and subcutaneous injection of midazolam maintained anesthesia. The body temperature of the rabbit was maintained at \(37 \pm 0.5^\circ\text{C}\) by a heated table. The mechanical properties of both the trained (left) and untrained (right) LD muscle were determined.

The LD muscle was exposed through the skin and dissected free of its connections to the spine and surrounding fascia, leaving the humeral tendon and the thoracodorsal nerve and blood vessels intact. The free, spinal origin of the muscle was sutured onto a plastic frame and fastened to an isometric force transducer (RDP electronics), which was attached to the arm of a servomotor. Throughout the experiments, the muscle was irrigated with physiological saline at \(37^\circ\text{C}\). The electrodes used during training were used to deliver the stimulation during the mechanical testing of the left LD muscle; electrodes were sutured to the right LD muscle, as described previously in Surgical procedure. The rabbit was secured firmly to the experimental table to minimize external compliance.

Initially, isometric properties of the LD muscle were determined with the servo-arm stationary. The muscle length was adjusted to approximately in situ length, and the stimulation amplitude was adjusted to that which yielded peak twitch force. A series of isometric twitches was used to find the length of the muscle at which peak force was obtained \((L_0)\). The times to peak twitch force and half relaxation were recorded at \(L_0\). A series of isometric tetani (300 ms duration) were used to ascertain the fusion frequency of the muscle (typically 70 Hz). The peak tetanic force was recorded and was used to calculate the maximum isometric stress (i.e., force/cross-sectional area). Muscle cross-sectional area was calculated by dividing wet muscle mass (determined at the end of the experiment) by muscle density (1,060 kg/m\(^3\)) and \(L_0\). A period of at least 2 min was allowed between twitches and tetani to prevent fatigue.

The work loop technique has been used to assess the mechanical properties of muscles under conditions that simulate the way they operate in vivo (1, 22). It is equally applicable to assess the potential postoperative role of a trained muscle for use in cardiomyoplasty (16). Sinusoidal length changes were imposed on the muscle while it was phasically stimulated. The position of the servo-arm was controlled by using a computer-generated wave. A stimulation wave was synchronized to the length wave, but it could be offset by using a phase shift. Force and length were recorded by using a PC microcomputer, using custom-written software. Muscles were subjected to four cycles of work over a range of cycle frequencies (1–7 Hz) that encompasses the cycle frequency (3.6 Hz) that was used to train the muscles and the resting and stressed rabbit heart rates. A constant strain of 0.1 \(L_0\), distributed symmetrically about \(L_0\), was used in all of the work loop experiments. The timing and duration of stimulation were systematically optimized to give the maximum net power output at a particular cycle frequency. Force was plotted against length, generating a work loop. Net work per cycle was determined by integrating force with respect to length, and net power was calculated by using an average of the work generated in cycles 2 and 3 multiplied by cycle frequency. To monitor any change in muscle performance, control runs were carried out every fourth or fifth run. We assumed a linear change in muscle performance between control runs and corrected our experimental runs to account for the expected decline in power output. In some cases, muscle performance improved slightly over the course of the experiment. Experiments were terminated if the control power output fell below 80% of the maximum control power. A period of \(-5\) min was allowed between work loop measurements for metabolic recovery. In vitro, the pattern of force generation during a cyclic contraction, and hence the shape of the work loop, is determined by the physiological properties of a muscle and the lengthening-shortening pattern imposed on the muscle. Work loop shape was compared in the control and trained muscles to provide insight into differences or similarities in their physiological properties.

The final mechanical assessment was the isometric fatigue resistance of the muscles. This was carried out by subjecting the muscles to 300-ms isometric tetani (stimulation frequency 65 Hz) at a repetition frequency of 1 Hz. The time

\[ J \text{ Appl Physiol} \quad \text{VOL 93} \quad \text{AUGUST 2002} \quad \text{www.jap.org} \]
652 MECHANICAL PROPERTIES OF CYCLICALLY TRAINED LD MUSCLES

taken for force to fall to 50% of the maximum was recorded. Although fatigue during cyclic contractions (2) would more accurately simulate the performance of the muscle in situ around the heart, we selected an isometric fatigue regime to compare with earlier studies of this muscle (16).

After the mechanical testing, the rabbit was killed with a lethal injection of 180 mg/kg body mass of Sagatal (pentobarbital sodium). Each LD muscle was dissected from the animal. Fat and connective tissue attached to the surface of the LD muscle was removed, and the wet weight was recorded.

Histological analysis. Full details of the histological methods used on these muscles have been previously published (8, 12). Two strips of muscle, ~3 mm wide, were cut from the cranial and caudal regions of each muscle. From each of these strips, two histology blocks were taken (from the medial and lateral areas). We sampled from different regions of the muscle to account for differences in the fiber composition of the lateral and medial areas of the rabbit LD muscle (12). Each histology block was supported between two pieces of liver tissue on cork disks, with the fibers oriented vertically. They were covered with OCT compound (Tissue-Tek) and frozen in 2-methylbutane, chilled in liquid nitrogen. Each tissue sample was stored at −70°C.

Transverse 10-μm sections were cut and mounted on slides from each block of muscle by use of a cryostat at −20°C. After being thawed for 10 min at room temperature, sections were stained either with hematoxylin and eosin or Sirius red, or they were stained to demonstrate the presence of succinate dehydrogenase (SDH) or myosin ATPase (with an alkaline preincubation) activity. For SDH and myosin ATPase, positive and negative staining was recorded in ~300 muscle fibers from each sampling region. The percentage of fibers that showed damage was determined by using sections stained with hemotoxylin and eosin. Fibers were regarded as being damaged if they exhibited any disruption of their cytoplasm or central nuclei, marked eosinophilia, a large increase in size with a shift from polygonal to a round shape, or any signs of invasion by mononuclear cells (8).

Statistical analysis. The mechanical properties and physical characteristics of the LD muscles from the different groups of animals were compared by using one-way ANOVA with Student-Newman-Keuls post hoc tests where statistical differences were found.

RESULTS

Animals and muscle size. There were no statistically significant differences between the body masses of the rabbits from the external control and experimental groups (P = 0.26). The LD muscles from the external and contralateral control groups were not significantly different in mass. In contrast, LD muscles trained for 3 or 6 wk were ~60% heavier than the control muscles.

Table 1. Mechanical properties of rabbit latissimus dorsi muscles

<table>
<thead>
<tr>
<th>Muscle mass, g</th>
<th>External Control</th>
<th>3-wk Cyclical Training Regime</th>
<th>6-wk Cyclical Training Regime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>5.4 ± 0.3(25)</td>
<td>4.7 ± 0.4(4)</td>
<td>6.5 ± 0.9(4)</td>
</tr>
<tr>
<td>Exp</td>
<td></td>
<td>7.4 ± 0.7(4)</td>
<td>10.4 ± 1.3(4)††</td>
</tr>
<tr>
<td>Muscle length, cm</td>
<td>11.8 ± 0.2(25)</td>
<td>10.8 ± 0.1(4)</td>
<td>12.2 ± 0.2(4)</td>
</tr>
<tr>
<td>Con</td>
<td></td>
<td>11.3 ± 0.6(4)</td>
<td>13.4 ± 0.6(4)§§</td>
</tr>
<tr>
<td>Exp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle cross-sectional area, mm²</td>
<td>42.5 ± 2.4(25)</td>
<td>41.3 ± 3.4(4)</td>
<td>52.6 ± 9.6(3)§</td>
</tr>
<tr>
<td>Con</td>
<td></td>
<td>61.8 ± 3.2(4)</td>
<td>73.5 ± 9.8(4)§§</td>
</tr>
<tr>
<td>Exp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Twitch rise, ms</td>
<td>28.7 ± 2.3(16)</td>
<td>14.5 ± 3.1(2)</td>
<td>20.5 ± 2.7(4)</td>
</tr>
<tr>
<td>Con</td>
<td></td>
<td>18.3 ± 2.2(4)</td>
<td></td>
</tr>
<tr>
<td>Exp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to half twitch relaxation, ms</td>
<td>37.0 ± 2.5(13)</td>
<td>27.0 ± 1.0(2)</td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td></td>
<td>33.8 ± 6.4(3)</td>
<td>25.4 ± 2.0(4)</td>
</tr>
<tr>
<td>Exp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum tetanic force, N</td>
<td>7.1 ± 0.3(25)</td>
<td>6.4 ± 0.5(4)</td>
<td>7.6 ± 0.5(3)</td>
</tr>
<tr>
<td>Con</td>
<td></td>
<td>6.0 ± 0.9(4)</td>
<td>5.3 ± 0.4(4)</td>
</tr>
<tr>
<td>Exp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum isometric stress, kN/m²</td>
<td>176.2 ± 10.1(25)</td>
<td>157.0 ± 14.7(4)</td>
<td>155.9 ± 31.2(3)</td>
</tr>
<tr>
<td>Con</td>
<td></td>
<td>96.2 ± 13.9(4)★</td>
<td>77.5 ± 15.7(4)★</td>
</tr>
<tr>
<td>Exp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum power output, mW</td>
<td>216.9 ± 16.1(15)</td>
<td>171.5 ± 30.3(4)</td>
<td>235.2 ± 22.5(3)</td>
</tr>
<tr>
<td>Con</td>
<td></td>
<td>134.0 ± 13.0(4)</td>
<td>121.0 ± 36.6(4)</td>
</tr>
<tr>
<td>Exp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum power output, W/kg</td>
<td>41.2 ± 4.5(15)</td>
<td>35.7 ± 3.5(4)</td>
<td>36.2 ± 5.5(3)</td>
</tr>
<tr>
<td>Con</td>
<td></td>
<td>18.2 ± 1.1(4)★</td>
<td>12.6 ± 3.4(4)★</td>
</tr>
<tr>
<td>Exp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency for maximum power, Hz</td>
<td>6.1 ± 0.3(15)</td>
<td>6.0 ± 0.4(4)</td>
<td>5.7 ± 0.9(3)</td>
</tr>
<tr>
<td>Con</td>
<td></td>
<td>5.3 ± 0.5(4)</td>
<td>5.5 ± 1.0(4)</td>
</tr>
<tr>
<td>Exp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to half fatigue, s</td>
<td>38.0 ± 2.0(4)</td>
<td>35.5 ± 7.6(4)</td>
<td>31.5 ± 4.5(3)</td>
</tr>
<tr>
<td>Con</td>
<td></td>
<td>64.0 ± 2.9(3)</td>
<td>55.5 ± 15.1(3)</td>
</tr>
<tr>
<td>Exp</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; no. of muscles are in parentheses. Exp, experimental muscles in rabbits trained for 3 or 6 wk; Con, untrained contralaterals in trained rabbits. Data were analyzed with 1-way ANOVA and Student-Newman-Keuls for pairwise multiple comparisons where significant differences were found with ANOVA. Statistical significance was tested at the 5% level. Statistically significant difference (P < 0.05): ★ compared with external controls; † compared with 6-wk contralaterals; ‡ compared with 3-wk experimental group.
In the trained muscles, \( L_0 \) was 5% longer after 3 wk and 10% longer after 6 wk of training than their respective contralateral control muscles (Table 1; \( P < 0.001 \) for 6-wk cyclically trained muscles compared with external controls). In the trained muscles, \( L_0 \) was 5% longer after 3 wk and 10% longer after 6 wk of training than their respective contralateral control muscles (Table 1; \( P = \) not significant). The difference in \( L_0 \) between the two trained groups was significant (\( P = 0.011 \)) and for the 6-wk trained muscles compared with the external control group (\( P = 0.007 \)).

**Isometric contraction kinetics, force, and stress.** The twitch rise and relaxation times of the trained muscles were not significantly different from those of the contralateral controls (Table 1). Differences in the force generated during isometric tetanic contractions in the external and contralateral control muscles were entirely due to differences in physiological cross-sectional area. Thus the isometric tetanic stress for these muscles was almost identical (\( \sim 160 \text{ kN/m} \)). However, both the absolute force and isometric stress developed by the trained muscles were lower than the controls (Table 1). After 6 wk of training, the maximum absolute tetanic force was 30% lower and the isometric stress 50% lower than in contralateral control muscles (Table 1).

**Mechanical performance of muscles during cyclic contractions.** The maximal absolute net power output (mW) of the control muscles (external and contralaterals) was \( \sim 200 \text{ mW} \) (Table 1). The trained muscles generated considerably less power (i.e., 22 and 49% lower than the controls after 3 and 6 wk of training, respectively; Fig. 1, Table 1). However, these values were not significantly different from the controls.

For cardiomyoplasty, it is important that the muscles are capable of generating a high power output at a frequency equivalent to the heart rate. Figure 1 shows the relationship between the absolute net power output and cycle frequency for external control, contralateral control, and trained muscles. Peak power output was at a cycle frequency of 6–7 Hz for all control muscles. Reductions in power were seen at all cycle frequencies after 3 and 6 wk of training. The frequency at which the maximal net power output was obtained was not significantly affected by training, being \( \sim 5–6 \text{ Hz} \) in all of the muscle groups (Table 1). Note that this frequency was calculated as the average of the optimum frequency for each individual muscle and hence differs from the frequency for maximum power output in Fig. 1, which is based on the average power at each cycle frequency. The trained muscles also generated a lower maximal, muscle mass-specific net power output than the controls (Fig. 2). The peak power output decreased to 49% after 3 wk and to 35% after 6 wk of that generated by the contralateral control muscles (Table 1). The power generated by the contralateral and external control muscles was not significantly different (\( \sim 40 \text{ W/kg} \); Table 1).

**Shape of the work loops.** Figure 3 shows typical work loops recorded in the five groups of muscles at three different cycle frequencies. These encompass most of the range over which the muscles produce positive work (Figs. 1 and 2). Work generated per cycle de-
creased as cycle frequency increased, reflecting the decline in average force with increasing mean shortening velocity (force-velocity effects). The work loops for the muscles from the control groups were larger than for the trained muscles because of the higher stresses developed. However, the overall shape of the work loop for the different muscle groups remained largely unaltered at each of the cycle frequencies.

Fatigue resistance. Resistance to fatigue was assessed by using repeated bouts of isometric tetanic contractions and monitoring the force. After training, fatigue resistance was improved, with an approximate doubling in the time to half fatigue after both 3 and 6 wk of training (Table 1).

Histological analysis. Myosin ATPase staining of sections after alkaline preincubation allows fibers to be classified as either fast [i.e., FOG and fast glycolytic (FG)] or slow [slow oxidative (SO)]. There were statistically significant differences between the relative number and relative cross-sectional area of type FOG...
and FG fibers between the external (12) and contralateral control muscles \( (P < 0.001) \). This increase in the oxidative capacity of the contralateral muscles may be due to a compensatory increase in weight bearing (i.e., postural support) on the unoperated side. It could also reflect the increased exercise the trained rabbits had during the training period, when they were free to hop around quite a large enclosure, in contrast the external control animals that were caged (12). The 3-wk trained muscles had the same relative number of SO fibers (5–7%). In contrast, a large change was seen in the number of fibers that stained positive for the oxidative enzyme SDH. After 3 wk of training, all of the fibers stained positively for SDH (types SO and FOG), whereas in the contralateral control muscles 50% of the fibers showed no evidence of the enzyme and were assumed to be type FG. This shows a marked increase in the oxidative capacity of the muscle with cyclic training.

Muscle fiber damage and collagen content. There was very little fiber damage to the contralateral muscles (Fig. 4, top left). Training resulted in localized damage to some of the muscle fibers of the experimental muscle, largely in the area adjacent to the silicone tissue expander (Fig. 4, top right and bottom). The amount of damage was highly variable (compare Fig. 4, top right vs. bottom), with very low values in some muscles and considerable damage in others, with a mean of 20.7 ± 10.6% (3).

Histological sections were stained with Sirius red (see METHODS), which stains collagen red. A marked increase was seen in the amount of connective tissue surrounding individual muscle fibers and also surrounding bundles of muscle fibers. There was a large increase in the thickness of the epimysium. In addition, a collagenous sheath was frequently observed along the surface of the muscle, adjacent to the tissue expander (Fig. 4, top right and bottom). This easily peeled away from the muscle at dissection and appeared to be separate from the epimysium.

DISCUSSION

Effects of the implanted tissue expander on the properties of the LD muscle. When deflated, the implanted tissue expanders used in this study impose a passive stretch of ~5%. This means that all experimental LD muscles underwent this degree of stretch continuously for 3 or 6 wk, in addition to a further 12% when cyclic training for 1 h/day. The implanted part of the tissue expander was identical to that used previously in our laboratory to study the effects of continuous passive

Fig. 4. Transverse section from the medial region of rabbit latissimus dorsi muscle stained with hematoxylin and eosin in contralateral control (top left) and 3-wk trained (top right) muscles. Bottom: section from a 3-wk trained muscle showing a collagenous sheath that was typically formed adjacent to the silicone tissue expander and the extensive muscle fiber damage and replacement with collagenous tissue. E, epimysium; C, collagenous sheath; D, damaged fiber layer.
The muscle mass-specific power output was reduced by ~65% after 6 wk of cyclic training (Fig. 2, Table 1). Because the strain was kept constant in the work loop experiments, the decrease in power can be attributed entirely to a reduction in force generation. However, the reduction in mass-specific power was greater than the decrease in isometric tetanic stress. Work loop shape does not show any significant variation with training (Fig. 3), indicating that the force-velocity characteristics of the muscle and the kinetics of activation and deactivation (see also Table 1) have not changed. The decrease in the maximum, absolute net power output was not as great as the reduction in muscle mass-specific power output relative to control muscles simply because of the muscle hypertrophy that occurred during cyclical training.

The training regime also had marked effects on the oxidative capacity of the muscle. This is shown by the greater time taken for the force to decline by 50% in the fatigue test (Table 1) and by the increase in the percentage of fibers that stained positively for SDH (Table 2). No significant changes in the twitch contraction kinetics indicate that the muscle had remained fast-twitch in type. This is supported by the myosin ATPase staining, with no shift in expression to slow-type myosin.

Assessment of the training regime in relation to cardiomypoplasty. Significant hemodynamic benefits generally have not been observed in patients in whom cardiomypoplasty has been performed (6). Indeed, in some cases, impaired ventricular diastolic function has been observed (7). It seems likely that muscle inactivity during the postoperative delay results in atrophy, loss of power, and decreased contractile speed (14). Probably a better course of action would be to precondition the LD muscle before cardiomypoplasty. This would enable almost immediate activation of the LD muscle after surgical reconditioning. If training regimes are to be suitable for cardiomypoplasty, they must induce high power output, fatigue resistance, and rapid contraction kinetics.

A number of different training regimes have been investigated so far. These include electrical stimulation combined with passive stretch of the muscle (16) as well as training by allowing the LD muscle to cyclically generate work (present study; Ref. 15). Electrical stimulation combined with passive stretch transforms the LD muscle into a slow-contracting, fatigue-resistant muscle with a low power output (16). Cyclic training has proved more successful, yielding a fast-contracting, fatigue-resistant muscle that is capable of generating greater power (15). To assess the suitability of the muscle to cardiomypoplasty, the muscle’s properties must be measured in an appropriate way. We measured the mechanical power output of the trained muscles by using the work loop technique, which simulates the cyclic contractions the LD muscle performs during cardiomypoplasty.

Trained muscles were capable of generating high power outputs and maintained rapid contraction kinetics relative to control untrained muscles (Table 1).
However, to assess the suitability for potential use in cardiomyoplasty, the mechanical properties of the trained muscles must be related to the mechanics of the heart. The left ventricular pumping power is equal to the product of blood pressure, stroke volume, and heart frequency. On the basis of scaling equations (4, 20, 28), it can be calculated that the power output of the left ventricle of rabbits (mass of 1.13 kg as in the present study) is \( \approx 0.1 \, \text{W} \) at rest and is \( 0.4 \, \text{W} \) during maximal exercise. The power output of the LD muscle after 6 wk of cyclic training at 4 Hz (resting rabbit heart rate) is \( \approx 0.1 \, \text{W} \) and at 7 Hz (during exercise) 0.09 W (Fig. 1). Thus, allowing for some loss of work in elevating the heart (10), it can be concluded that the LD muscle trained in this way could still deliver significant power to the heart during cardiomyoplasty, both at rest and during exercise. Similarly, in skeletal muscle ventricles dynamically trained for 3 mo, power outputs up to three times greater than that of the heart at rest have been achieved (15). Training of the LD muscle by performance of cyclic contractions is a significant improvement over previous preconditioning regimes. For example, rabbit LD muscles trained in this way were able to generate only 5% of the left ventricular power output at cycle frequencies corresponding to resting heart rate and were unable to generate any power at frequencies equivalent to maximal exercise (16).

In addition to preserving power output (50% of untrained muscles) and maintaining rapid twitch kinetics, trained muscles exhibited greater fatigue resistance. For useful operation as a cardiac-assist device, the LD muscle must be able to sustain work indefinitely. We determined the fatigue resistance of the LD muscle by repeated performance of isometric tetani. Although the fatigue resistance was improved, it was not as great as that observed by James et al. (16) after continuous electrical stimulation combined with passive stretch. Unlike in many other training regimes that have been applied to rabbit LD muscle, in these experiments training was not continuous. It is unlikely that the properties of the muscle have reached a steady state, and the differences in fatigue resistance may reflect this. The assessment of fatigue resistance (i.e., isometric contractions) is not the way in which the LD muscle will operate during cardiomyoplasty, so we are unable to ascertain the level of power that can be sustained during such contractions. Future experiments will determine fatigue resistance as a decline in work during cyclic contractions (2). Only when this has been determined will it be known what level of assistance the muscle can deliver to the heart and whether this can be delivered with every heartbeat or less frequently due to muscle fatigue.

One adverse effect of the training procedure was a variable, but significant, amount of fiber damage. Damage was primarily localized adjacent to the tissue expander and may have been caused by abrasion, perhaps as a result of the initial training being too demanding. Fiber damage and partial replacement of

Fig. 5. Effects of cycle frequency on absolute mechanical power output (A) and muscle mass-specific mechanical power output (B) of rabbit latissimus dorsi muscles. C: relationship between maximum muscle mass-specific power output and time to half fatigue during an isometric fatigue test. Data are shown for external control (Ext), contralateral controls (control), and 3- and 6-wk cyclically trained (Cyc) muscles from experiments in this study and from muscles that have received 3 or 6 wk of continuous 10-Hz stimulation (Stim), static stretch (Str), or a combination of continuous 10-Hz stimulation with static stretch (SS) from an earlier study (Ref. 16 as indicated in C). Values are means \( \pm \text{SE} \) for 4–6 muscles. Lines for plots A and B represent third-order polynomials and for plot C an exponential, all fitted by using a least-squares regression.
muscle fibers by fibrous and fatty tissue have been observed after long-term use of LD muscles in goats for cardiomypoplasty (11). Experiments currently underway are attempting to reduce the amount of damage produced.

Implications for cardiomypoplasty and comparison with other training regimes. This study has shown that, although the loss of muscle power was substantial, it was still less than that seen when muscles are treated with continuous 10-Hz electrical stimulation (Fig. 5, A and B; Ref. 16). A power output of ~60% of the maximum control power output could be maintained over a cycle frequency range from 3 to 7 Hz (Figs. 1 and 5, A and B). This is in contrast to muscles trained with continuous 10-Hz electrical stimulation, which could not produce any power at a cycle frequency >5 Hz (Fig. 5 A and B; Ref. 16) and produced a maximum power output of only 19% of the control muscles. Although stretch alone preserved ~80% of the maximum control power, there was no improvement in fatigue resistance (16). Combined stretch with stimulation yielded a high level of fatigue resistance, but power declined to only 20% of the maximum control value.

There appears to be a trade-off between maximum absolute power that the muscle can generate and its resistance to fatigue during repeated tetanic contractions (Fig. 5C). The highest resistance to fatigue was observed in those muscles that had been subjected to static stretch combined with electrical stimulation, but these muscles also generated the lowest power output compared with other training regimes. It may be that the level of cardiac assistance is severely impaired by either limited resistance to fatigue or low power output.

Conclusions about the effects of cyclic training on power output and fatigue resistance should, of course, be tempered by the fact that the properties of the muscle may change if durations and intensities of training regime were increased. Only then can a thorough evaluation of this training method be made with respect to its suitability for cardiomypoplasty, aortomyoplasty, or other forms of skeletal muscle assistance.

We thank Dr. R. S. James (Coventry University, Warwick, UK) for providing us with data for the rabbit LD muscle power output after 10-Hz electrical stimulation, static stretch, and a combination of the two treatments (used in Fig. 5).

This work was supported by a Wellcome Prize Studentship (to G. N. Askew) and the Jules Thorn Charitable Trust (to V. M. Cox).

REFERENCES

MECHANICAL PROPERTIES OF CYCLICALLY TRAINED LD MUSCLES


