Clenbuterol Increases Stroke Power and Contractile Speed of Skeletal Muscle for Cardiac Assist

M. Petrou, FRCS; S. Clarke, BSc; K. Morrison, PhD; C. Bowles, PhD; M. Dunn, PhD; M. Yacoub, FRCS

Background—Skeletal muscle assist (SMA) may be limited by loss of power, slowing of contraction and relaxation, and atrophy of the transformed latissimus dorsi muscle (LD). Clenbuterol (clen), a β2-adrenergic receptor agonist, was used to improve the performance of trained skeletal muscle in sheep.

Methods and Results—The following 4 groups were used: A (n = 6), untrained controls; B (n = 6), left LD progressively transformed toward a slow-twitch and fatigue-resistant phenotype by electrical stimulation over 12 weeks (2.5 to 5 V, 240-μsec pulse duration, 35 Hz, 3 to 6 pulses per burst, and up to 40 bursts per minute); C (n = 6), clen-treated (0.5 mg/kg SC) for 12 weeks; and D (n = 6), clen+trained. In a terminal experiment, the mobilized LD was wrapped around a rubber aorta of a mock circulation and stimulated to contract 40 times per minute. Group A had an initial mean pressure augmentation (ΔP) of 24.6 mm Hg and stroke power of 2.28 W/kg, but both fell to <20% of their original values by 15 minutes because of fatigue (P < 0.005). Group B was fatigue-resistant, with a ΔP and stroke power at 60 minutes of 13 mm Hg (70% of initial) and 0.34 W/kg (39% of initial), respectively. The performance of group C was similar to that of controls. In group D, however, the muscles were stronger at all time points than in B, with a ΔP of 23 mm Hg and stroke power of 2.66 W/kg at 60 minutes (P < 0.01). The speeds of contraction (+dP/dt:ΔP) and relaxation (−dP/dt:ΔP) were significantly greater in group D than B. Protein analyses showed group D to have only a trend toward greater abundance of the fast isoforms of myosin heavy chain and sarcoplasmic reticulum Ca2+-ATPase (P > 0.1).

Conclusions—Clen improves the performance of trained skeletal muscle in a model of aortomyoplasty by unknown mechanisms. These findings may have important implications in SMA. (Circulation. 1999;99:713-720.)

Key Words: muscles ■ circulation ■ clenbuterol

Skeletal muscle assist (SMA) is an attractive technique for the treatment of end-stage cardiac failure that uses autologous muscle wrapped around the heart or major vessels and stimulated to contract in systole (cardiomyoplasty) or diastole (aortomyoplasty).1,2 More than 600 cardiomyoplasties have been performed to date, although their efficacy remains to be established.3 The long-term performance of the latissimus dorsi muscle (LD) is vital, yet this is far from optimal. For example, morphological and biochemical abnormalities are known to occur in the skeletal muscle of patients with end-stage heart failure and may persist despite normalization of central hemodynamics.4 Chronic electrical training transforms the LD from a predominantly fast-twitch to slow-twitch phenotype. This induces the fatigue resistance necessary for long-term assist but also produces deleterious effects, such as a fall in stroke power5 and slowing of contraction and relaxation.6 These changes are associated with fast-to-slow transitions in myosin heavy chain (MHC) isoform abundance, although the concomitant changes in the expression of Ca2+-regulatory proteins, such as sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) and phospholamban (PLB), are less well characterized.7 Finally, atrophy and lipomatosis occur as late sequelae of cardiomyoplasty.8 Several approaches have been suggested to compensate for these deleterious changes (reviewed in Reference 9). Androgenic anabolic steroids have been investigated with varying success.10,11 Furthermore, their known serious side effects12 prohibit long-term use.

Chronic administration of β2-adrenergic receptor agonists, such as clenbuterol (clen), induces muscle hypertrophy,13 including untrained LD in the rat.14 Zeman et al15 have shown clen to induce a slow-to-fast fiber type transition and increased tetanic tension and twitch speeds. These changes were most marked in the maturing rat soleus, in which clen retarded the natural fast- to slow-twitch fiber transition seen during the first 8 to 12 weeks after birth. The functional effects of clen on mature fast-twitch skeletal muscle undergoing similar changes in fiber composition due to progressive electrical training, however, are unknown.

We have tested the hypothesis that clen induces beneficial changes in the physiological and biochemical characteristics of trained skeletal muscle.

Received April 22, 1998; revision received September 9, 1998; accepted September 25, 1998.
From Imperial College, National Heart and Lung Institute, London, UK.
Correspondence to Prof Sir Magdi Yacoub, Department of Cardiac Surgery, Royal Brompton Hospital, Sydney St, London SW3 6NP, UK.
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Changes in arterial blood pressure are shown in Figure 1. Anesthetized sheep after 2 mg of clen.

Changes in heart rate and arterial blood pressure in 5 and D were given a challenge dose of 2 mg SC to induce solves in sterile water to 10 mg/mL and stored at 4°C. Animals in C Clen free base (donated by Boehringer-Ingelheim, UK) was dissolved in sterile water. Clen Administration

Clenbuterol in Skeletal Muscle for Cardiac Assist

### Methods

#### Animal Groups

Twenty-four Welsh Mountain ewes with a mean BW of 34 kg (range, 25 to 40 kg) were cared for in accordance with guidelines laid down by the Home Office of Great Britain and Northern Ireland (1986). All were housed in a 12-hour light-dark cycle and given feed and water ad libitum. After 1 to 2 weeks of settlement, they were randomly assigned to 4 groups: group A (n = 6), controls: daily subcutaneous (SC) injections of sterile water 0.5 mL for 12 weeks; group B (n = 6), trained: 2 intramuscular electrodes implanted into the left LD muscle and a myostimulator (Telelectronics Pacing Systems Inc) inserted into a subcutaneous pocket in the interscapular region under general anesthesia. Progressive training was commenced 48 hours later using the stimulation parameters given in Table 1. LD twitch remained visible, and 0.5 mL of sterile water was injected daily; group C (n = 6) was treated with clen 0.5 mg/kg body wt (BW) twice daily SC for 12 weeks; and finally, group D (n = 6), training plus clen administration. All injections commenced on the day the myostimulator was programmed “on.” Only groups B and D were operated on; groups A and C were not subjected to sham operations. Our studies therefore make the assumption that dummy leads within the LD do not significantly alter the structure and function of the LD. This is one important limitation of our study.

#### Clen Administration

Clen free base (donated by Boehringer-Ingelheim, UK) was dissolved in sterile water to 10 mg/mL and stored at 4°C. Animals in C and D were given a challenge dose of 2 mg SC to induce tachyphylaxis, a characteristic property of β-agonists, thus protecting against the subsequent larger treatment doses (0.5 mg/kg BW). Changes in arterial blood pressure are shown in Figure 1.

### Assessment of LD Performance

After 12 weeks, all animals underwent general anesthesia. Muscle relaxants were omitted. The rumenostomach was decompressed with a 12-bore orogastric tube. Intravenous fluid maintenance was in the form of 5% dextrose solution at 100 mL/h. Arterial oxygen saturation was monitored with a pulse oximeter and maintained between 96% and 100%. Core temperature was maintained at 38±1°C with warming blankets. Only the left LD was assessed functionally in all groups.

#### Maximum Linear Isometric Force

An isometric force transducer (Logan-Sinclair Research) was fixed to the head end of the operating table, the abducted-flexed left forelimb was tied securely to it, and the resting tension was set at a baseline of 10 N. This value was determined by measuring the maximum developed force after a brief period of stimulation (5 V, 35 Hz, 6 pulses per burst, 240-μs pulse duration) at different preload tensions (0 to 50 N). This is in agreement with that reported by Kochamba and Chiu.16

#### Wrap Performance on the Mock Circulation System

The design and validation of the mock circulation system (MCS) used was described previously.17 The myostimulator was programmed “off,” and the left LD was mobilized on its intact neurovascular pedicle and tendon and wrapped around the latex aorta of the MCS. The stimulator was programmed “on” at a rate of 40 bursts per minute against a baseline pressure of 70 mm Hg under the stimulation parameters used for the final month of training (see Table 1). Pressure waveforms were recorded at 1, 5, and 15 minutes and every 15 minutes thereafter for a total of 60 minutes. A lethal dose of anesthetic was then administered, and the left and right LDs were dissected out and weighed. Biopsies were taken from standard sites for histological examination and biochemical analyses.

### Data Acquisition, Storage, and Analysis

The pressure-wave signal was amplified and converted from analog to digital form and visualized on a PC loaded with Spike 2 (Cambridge Electronics). This interactive software package allows measurement of pressure augmentation (∆P), stroke volume (SV), contraction time (Ct, ie, time to peak pressure), relaxation time (Rt90, ie, time from peak pressure to 90% decay), and the maximum rates of pressure generation (+dP/dt) and decay (−dP/dt). Mean energy (E) generated over the shortening phase of contraction at 35 Hz, involving the muscle working as a pressure generator against a 70 mm Hg preload, was calculated from the product of mean ∆P and SV. The stroke power was calculated from E/C. Peak energy per contraction was not measured, because it overestimates the true performance (Stanley Salmons, PhD, 1995, personal communication).

The mean and SEM for all these parameters were calculated from 6 to 8 waves at each time point of measurement.

### Immunocytochemistry

Serial cross sections 8 μm thick from frozen LD were cut and mounted. Monoclonal antibodies (Novacastra Laboratories Ltd) for fast (types IIa and IIb; 1:300 dilution) and slow (1:100 dilution) myosin were used to determine the relative frequencies of fast and slow fibers. An antibody for spectrin (1:200 dilution) was used to stain the sarclemma and delineate the cells more clearly. Control slides were treated similarly but with the primary antibody replaced by diluent alone to establish the specificity of immunostaining. Fiber-type frequency and cross-sectional areas were analyzed with the Lucia M Image Analysis System (Nikon, UK, Ltd). Five random fields per section at ×100 magnification were analyzed from LD samples taken from 4 animals in each group. A number ranging from 850 to 2200 cells per group was thus available for statistical analyses.

### Protein Chemistry

Frozen samples of LD stored at −70°C were analyzed for MHC, SERCA, and PLB isoforms. The relative abundance of these is known to change in compensatory overload18 and stretch hyperto-

### Table 1. Stimulation Protocol for Progressive Training of the LD

<table>
<thead>
<tr>
<th>Time</th>
<th>Rate, cpm</th>
<th>Amplitude, V</th>
<th>PPB, No.</th>
<th>Pulse, μs</th>
<th>Burst, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–48 h</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>48 h–1 mo</td>
<td>16</td>
<td>2.5*</td>
<td>3</td>
<td>240</td>
<td>35</td>
</tr>
<tr>
<td>1–2 mo</td>
<td>28</td>
<td>5</td>
<td>6</td>
<td>240</td>
<td>35</td>
</tr>
<tr>
<td>2–3 mo</td>
<td>40</td>
<td>5</td>
<td>6</td>
<td>240</td>
<td>35</td>
</tr>
</tbody>
</table>

*If contractions appeared very weak, then 5 V was used from the outset. cpm indicates contractions per minute; PPB, pulses per burst.

![Figure 1. Changes in heart rate and arterial blood pressure in 5 anesthetized sheep after 2 mg of clen. ■ indicates systolic blood pressure; ▲, diastolic blood pressure; and ○, heart rate.](image-url)
TABLE 2. Body Weight and Muscle Mass

<table>
<thead>
<tr>
<th>Group</th>
<th>BW, kg</th>
<th>BWf, kg</th>
<th>LLD, g</th>
<th>LLDO/RLD</th>
<th>Heart:BW, kg×10⁻³</th>
<th>Heart:BWf, kg×10⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=6)</td>
<td>38±1.64</td>
<td>43±2.1</td>
<td>153±12.1</td>
<td>1.04±0.02</td>
<td>166±4.4</td>
<td>3.56±0.23</td>
</tr>
<tr>
<td>Trained (n=6)</td>
<td>37±0.41</td>
<td>43±2.7</td>
<td>155±14.3</td>
<td>1.05±0.02</td>
<td>183±9.2</td>
<td>3.57±0.15</td>
</tr>
<tr>
<td>Clen (n=6)</td>
<td>28±1.28</td>
<td>41±2.2</td>
<td>147±11.8</td>
<td>1.00±0.02</td>
<td>166±5.9</td>
<td>3.58±0.21</td>
</tr>
<tr>
<td>Clen+Trained (n=5)</td>
<td>28±0.54</td>
<td>37±2.9</td>
<td>145±17.5</td>
<td>1.14±0.70*</td>
<td>174±10.3</td>
<td>3.78±0.29</td>
</tr>
</tbody>
</table>

BW indicates initial body weight; BWf, final body weight; LLD, left latissimus dorsi muscle; and RLD, right latissimus dorsi muscle.

BW was significantly greater for C and D compared with A and C (P<0.001). The LLD:RLD ratio was significantly greater in the clenbuterol (Clen)+trained group than in all the other groups (P<0.05). Values are mean±SEM.

**Phy,** other proteins, such as the troponins, and their calcium-binding properties are also important, although we did not investigate these. Left ventricular and atrial tissues from clen-treated and control animals were analyzed for cardiac atrial natriuretic factor (ANF). Ventricular reexpression of ANF is a useful marker of cardiac hypertrophy.

Crude MHC extracts were prepared and protein concentrations determined according to the method of Bradford. The MHC isoforms were separated by 1-dimensional SDS-PAGE (150×60×0.5 mm) using a modification of the method of Talmadge and Roy. PLB, SERCA, and ANF proteins were separated with 12% wt/vol acrylamide-bis gels (150×160×1.5 mm) according to the method of Ossipow et al.

Western blot membranes were stained, scanned, and analyzed with the Quantity One software package (Protein and DNA Imageware).

**Statistics**

Comparisons between the 4 groups were made by ANOVA, and the Kruskal-Wallis post hoc test was applied for nonparametric data. For comparisons of data from 2 separate time points within the same group, Student’s t test was used. All data are presented as mean±SEM, and results were considered significant at values of P<0.05.

**Results**

**Surgical**

All animals in groups B and D survived surgery and grew normally during the 12-week training period. There were no cases of device failure. One animal in D developed a severe wound infection and was euthanized. The remaining 23 animals were stable during the 3 hours of anesthesia for the assessment of LD performance.

**BW, LD Weight, and Heart Mass**

Table 2 shows that sheep treated with clen (C and D) had a lower initial mean BW than those not receiving the drug (A and B). All were on the same part of the growth curve for Welsh Mountain sheep, but the mean rate of weight gain was significantly greater for C+D than for A+B (920 versus 675 g/wk [P<0.01], respectively). This is in keeping with the known growth-promoting effects of clen.

LD hypertrophy was in proportion to the increase in BW in all groups, ie, the LD/BW ratio was similar (P>0.2). The left and right LDs weighed approximately the same in all groups, with the exception of D, in which the combination of clen and training resulted in the left LD being 18% heavier than the right LD (P<0.05). Interestingly, the exsanguinated heart mass–to-BW ratio was greater in animals that were trained (B and D) than in the nontrained groups (A and C) (P<0.05), and clen did not appear to affect this parameter. Results are summarized in Table 2.

**Linear Performance**

The peak force generated in response to a standard burst stimulation relevant to clinical cardiac assist (ie, 35 Hz, 170 ms) was 86.3±6.7 N/kg in group B and 159.5±12.8 N/kg in A (P<0.001), ie, a 46% reduction with training. C produced only a small increase in linear isometric force (170±17.2 N/kg) compared with controls (P>0.1). In D, however, 145±18.1 N/kg was generated, much higher than with training alone (P<0.05). These data should be interpreted cautiously, however, because the development of maximum force by trained and untrained muscle may require a different duration of tetanic stimulation, a parameter we did not vary.

**Wrap Performance**

**ΔP and Fatigability**

ΔP in group B was 19.9±2.4 mm Hg at the start and 12.5±1.1 mm Hg at 15 minutes, which was maintained throughout 60 minutes, consistent with fatigue-resistant work (see Figure 2A). In contrast, the initial ΔP in A was 24.6±0.9 mm Hg (P<0.05), although this was not maintained beyond 5 minutes because of fatigue. By 15 minutes, ΔP declined to 25% of its initial value (P<0.001). Muscles in group C were more effective than in controls in that ΔP was 32.9±4.2 mm Hg, but they also fatigued within 15 minutes. Group D produced the highest ΔP at all time points and at 60 minutes was 23.6±4.9 mm Hg (P<0.002). In addition, clen did not affect fatigue resistance in this group. Figure 2B shows representative pressure waves.

**Stroke Volume**

There was an acute drop in SV in group A from 21.1±0.7 to 7.9±1.4 mL in the first 5 minutes (P<0.01). A similar drop was observed in C. By 60 minutes, the SV in both groups was <5 mL, compared with B, which gave 10.9±1.35 mL per contraction (P<0.02). However, the wraps from D generated the greatest SV beyond 5 minutes (21.5 to 24.3 mL, P<0.001).

**Stroke Power**

At the start, the stroke power was 61% lower in group B than in the prefatigued controls (P=0.007) (Figure 2C). In group D, the stroke power at 1 minute was 3.79±0.71 W/kg, compared with 0.88±0.2 W/kg in B (P<0.001). This difference persisted so that at 60 minutes, the stroke power done by B was 0.34±0.08 W/kg, compared with the much higher value produced by D of 2.66±0.87 W/kg.

**Contraction**

Ct at the start was 135.1±8.8 and 243.2±6.1 ms in groups A and B, respectively (P<0.001) (see Figure 3A). Similarly, the
+dP/dt;ΔP ratio was more than twice as great in A as B (P<0.01) (see Figure 3B).

C, in group C was similar to that in controls (P>0.2), although the +dP/dt;ΔP was greater in the former at 15 minutes but not beyond (P<0.05; Figure 3B). Group D had faster contractile characteristics than B (see Figures 3A and 3B).

It is noteworthy that both C, and +dP/dt;ΔP did not vary significantly throughout the 60 minutes in all groups.

**Relaxation**

R_{90}, at the start was 115.4±7.2 and 249.2±35.2 ms in groups A and B, respectively (P<0.05) (see Figure 4A). Similarly, the −dP/dt;ΔP was greater in A than B (P<0.05) (see Figure 4B). By 15 minutes, however, coincident with the onset of fatigue, the R_{90} increased significantly in A, to 205.4±13.9 ms (P<0.001) (see Figure 4A), resulting in a fall in the C;R_{90} ratio of 1.20 to 0.64 (P<0.001). In contradistinction, this ratio remained unchanged in the trained muscles throughout the period of testing.

Group C had faster relaxation than A during the first 15 minutes (P<0.01) but had similar R_{90} values thereafter (Figure 4A). In addition, both demonstrated a similar fall in C;R_{90} associated with the onset of fatigue. Quite strikingly, there was a 96% reduction in the R_{90} throughout 60 minutes.

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**Figure 2.** A, Pressure augmentation. Control (C) and clen-treated (†) muscles fatigued within 5 minutes. At 60 minutes, clen-trained (▼) LDs generated greater augmentation per contraction than trained only (●) group. B, Representative pressure waves. C, Stroke power. *P<0.05. Note, logarithmic scale was used on vertical axis.
in group D compared with B (see Figure 4A). Similarly, $-dP/dt:ΔP$ was greater in D than in B ($P<0.001$), as seen in Figure 4B.

The $R_{t90}$ did not change significantly throughout the 60 minutes of hemodynamic augmentation in fatigue-resistant muscles (ie, groups B and D).

**Histology**

In group A, 77% of fibers were immunoreactive to fast myosin, with almost complete fast-to-slow transition with electrical training (group B; see Figure 5A through 5I). The fast fibers had a mean cross-sectional area of $4458 \pm 72 \mu m^2$ in A compared with $1668 \pm 146 \mu m^2$ in B ($P<0.001$). Similarly, the slow fibers measured $6606 \pm 1233 \mu m^2$ in A and $3941 \pm 47 \mu m^2$ in B ($P<0.001$). In C, fast fibers were hypertrophied ($8812 \pm 193 \mu m^2$) compared with A, without a significant change in slow fibers ($5760 \pm 236 \mu m^2$). In D, both fast ($6340 \pm 308 \mu m^2$) and slow ($4647 \pm 74 \mu m^2$) fibers were hypertrophied compared with B ($P<0.001$).
Protein Chemistry

No differences were observed between left LD and right LD samples in group A (P>0.4), and data from these muscles were pooled (see Table 3). In B, only the left LD muscles were subjected to electrical training, with the right LD acting as the internal control. In this group, the right LDs were not significantly different from control group A values for any of the proteins. In C, both left and right LDs were clen-treated and untrained. No significant differences between them were observed, and data from both were pooled. In D, only the left LDs were subjected to clen and electrical training, with the right LDs acting as the internal clen-only controls. The right muscles in D were not significantly different from those in C for any protein studied except SERCA 2A (P<0.05).

Myosin Heavy Chain

Fast MHC was predominant in group A (78.5%) compared with slow MHC (P<0.01); see Table 3. In B, the major isoform in the left (trained) LD was slow MHC (99.6%) compared with the right (untrained) LD (P<0.001). Clen in group C increased the proportion of fast MHC to 89.1% compared with A (P<0.01). In D, the left LD expressed less fast MHC isoform (10.6%) compared with A and C (P<0.001 and P<0.001, respectively). A trend toward a reduced abundance of slow MHC (89.4%) was observed in D compared with B (99.6%).

SERCA

A trend toward higher levels of fast SERCA 1 was observed in group A compared with untrained right LD from B. Training (B,

Figure 5. In control LD, minority of fibers stain positive to slow myosin (a) and majority positive for fast myosin (b). In contrast, almost all cells become negative for fast myosin after 12 weeks of electrical training (c). Clen-treated animals had a significantly reduced proportion of slow myosin type I immunoreactive cells (d) and a concomitant increase in fast type Ila (e) and Ilb (f). Combination of training plus clen resulted in a less than complete transition toward type I predominance (g) and a greater number of type Ila (h) fibers being preserved. There is complete absence of immunostaining for type IIB myosin in muscles that received both training and clen (i).

TABLE 3. Quantitative Changes in Protein Expression

<table>
<thead>
<tr>
<th>Group</th>
<th>%MHC (fast)</th>
<th>%MHC (slow)</th>
<th>SERCA 1</th>
<th>SERCA 2a</th>
<th>PLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control left+right (A) (n=10)</td>
<td>78.53±1.85</td>
<td>21.47±1.85</td>
<td>3.39±0.46</td>
<td>4.31±0.68</td>
<td>0.18±0.03</td>
</tr>
<tr>
<td>Trained left (B) (n=5)</td>
<td>0.36±0.36†</td>
<td>99.64±0.36†</td>
<td>0.45±0.23†</td>
<td>3.85±1.04</td>
<td>0.54±0.09†</td>
</tr>
<tr>
<td>Untrained right (B) (n=5)</td>
<td>81.42±2.89</td>
<td>18.58±2.89</td>
<td>1.61±0.60</td>
<td>4.71±1.32</td>
<td>0.24±0.08</td>
</tr>
<tr>
<td>Clen left+right (C) (n=10)</td>
<td>89.08±3.34*</td>
<td>10.92±3.34</td>
<td>4.23±0.72</td>
<td>1.24±0.41*</td>
<td>0.07±0.04*</td>
</tr>
<tr>
<td>Clen+trained left (D) (n=5)</td>
<td>10.57±9.31</td>
<td>89.43±9.31</td>
<td>1.11±0.39</td>
<td>3.81±0.67</td>
<td>0.30±0.11</td>
</tr>
<tr>
<td>Clen+untrained right (D) (n=5)</td>
<td>89.56±1.66</td>
<td>10.44±1.66</td>
<td>3.33±0.41</td>
<td>2.82±0.59</td>
<td>0.05±0.01</td>
</tr>
</tbody>
</table>

In groups B and D, only the left muscle was electrically trained. The distribution of the fast and slow MHC isoforms is expressed as a percentage of the total MHC, whereas SERCA 1, SERCA 2a, and PLB are expressed in optical density arbitrary units (±SEM). See text for specific comparisons.

*P<0.01; †P<0.001.
left LD) reduced the abundance of the fast SERCA 1 ($P<0.001$) without a significant change in the level of slow SERCA 2a compared with A; see Table 3. In contrast, clen treatment alone (C) had no significant effect on SERCA 1 but resulted in a decrease in SERCA 2A compared with group A ($P<0.01$). The combination of clen and training (D, left LD muscles) significantly reduced the level of SERCA 1 compared with C ($P<0.003$) and A ($P<0.005$). Only a trend toward increased SERCA 1 was observed in B. In D, SERCA 2A was unchanged compared with A and B but higher than C ($P<0.01$).

**Phospholamban**

Group B contained higher PLB levels (0.54 optical density arbitrary units) than group A ($P<0.001$, Table 3). In C, PLB (0.07 optical density arbitrary units) was reduced compared with A ($P<0.05$). The level of PLB in D was intermediate compared with A and B.

**Atrial Natriuretic Factor**

ANF was undetectable in left ventricular extracts taken from groups A and C. Atrial extracts (ANF expression high) from the same animals provided the positive controls. Unfortunately, we have not defined the lower sensitivity of detection of ANF in our system. With a standard loading of 25 μg protein, no signal was observed in any left ventricular samples, whereas the equivalent loading of atrial samples always resulted in a strong positive band. However, we did not do any formal quantification of ANF.

**Discussion**

The addition of a myotrophic stimulus to electrical training is an attractive proposition to enhance the performance of the muscle graft used in SMA. We have shown that the β2-adrenergic receptor agonist clen improves the stroke power of the trained LD during a period of sustained activity on an MCS that mimics the wrap configuration and loading conditions of aortomyoplasty. In addition, significant increases in the rates of tension development and decay were demonstrated. These are potentially desirable effects in the setting of SMA and were induced without any compromise in fatigue resistance.

The mechanism by which cardiomyoplasty provides hemodynamic benefit is unknown, but it includes systolic augmentation of the ventricle, reduction of wall tension, and prevention of ventricular dilatation. All may contribute to various degrees, although we believe the first to be more important and postulate that enhancement of stroke power should translate into hemodynamic benefit. Clinical efficacy, in turn, must depend on long-term muscle structure and function as well as its mechanical coupling to the circulation. The latter is the work transferred from the muscle to the circulation (available stroke work) and is most usefully measured in an in vivo model of aortomyoplasty.

The cause of LD atrophy in cardiomyoplasty patients is multifactorial. Surgical mobilization renders muscle and nerve fibers ischemic, particularly in the distal segment. Once translocated into the mediastinum, the LD is wrapped around the heart with a reduced resting tension, where it remains inactive for 2 weeks to allow its fusion to the epicardium. Atrophy secondary to reduced stretch and disuse may result. Later, further atrophy due to a shift from a predominance of type II (large-diameter) to type I (small-diameter) fibers with electrical transformation may occur. Indeed, up to 60% atrophy may occur 2 to 4 years after cardiomyoplasty.

The growth-promoting effect of clen is not confined to normal skeletal muscle but is also evident in certain states of altered physiology and even pathology. For example, it has been shown to inhibit and reverse denervation and disuse atrophy. It also induces beneficial changes in the mdx mouse. The model used in our study, however, uses in situ training of the LD, believed to be associated with minimal atrophy and lipomatosis. Therefore, the potential benefits of clen need to be studied on in vivo wraps.

Preservation of fast-twitch physiology is theoretically desirable for both cardiomyoplasty and aortomyoplasty. Up to 30% of patients with end-stage heart failure have pure diastolic dysfunction (ie, ejection fraction >40%), and most of the remainder have both systolic and diastolic dysfunction. Wrapping these ventricles with a layer of skeletal muscle that is sluggish to relax may impose a restrictive physiology that may greatly limit the efficacy of SMA. Similarly, effective left ventricular afterload reduction can occur in aortomyoplasty only if the muscle is able to relax rapidly. Our study demonstrates the ability of clen to improve LD performance by significantly increasing stroke power and contractile speed. However, our findings are limited to growing sheep with normal hearts. Because the safety and tolerance of clen in heart failure is unknown, future work should be directed toward evaluating this agent in a model of heart failure.

Fast isoforms of MHC and SERCA were found to be more abundant with clen treatment. These were very modest changes, however, and probably do not account for the much larger increases in power and speed of contraction and relaxation observed. Moreover, they were not significantly changed in group D, indicating that training exerts a more dominant effect on gene expression than the concomitant pharmacological stimulus. Hypertrophy per se could not account for these changes either, because changes in intrinsic characteristics (ie, normalized for mass) were observed. Therefore, other as yet unknown mechanisms may be involved, possibly involving β2-adrenergic receptor activation and associated transduction pathways. The role of β2-adrenergic receptors in the growth-promoting effect of clen, however, remains controversial, although acute administration of other β2-agonists enhances excitation-contraction coupling in isolated skeletal muscle preparations. We can only speculate that a similar effect may be occurring in our chronic in vivo model. Further studies are needed to characterize changes in β-adrenergic receptor density and affinity associated with electrostimulation, as well the transduction pathways that may alter the efficiency of the contractile mechanism.

Several issues need to be addressed before clen can be considered as an adjunct to clinical SMA. For example, although its acute chronotropic effects on the human heart are predictable on the basis of its known β2 effects, the effects of long-term administration are unknown. In a previous study, we observed an increase in heart mass by 18% in the rat, with molecular changes typical of “physiological” hypertrophy, after clen treatment for 21 days. There are no cited reports of clen-induced cardiac hypertrophy in large mammalian
species, including humans, and in the present study we were unable to detect ANF in the ventricles of clen-treated sheep. The effects of clen on the failing heart, however, are completely unknown and need to be investigated in appropriate animal models. It is noteworthy that hearts from trained animals are significantly heavier than those in controls, the mechanism of which is unclear, although it may involve a cardiotoxic mediator elaborated and secreted by exercising skeletal muscle. Similarly, a nonsignificant trend toward a greater abundance of SERCA 1 was observed in control left LD compared with right LD taken from group B animals, in which the left LD was chronically trained. We cannot exclude the possibility that the chronically exercising muscle exerts a systemic effect on other skeletal muscles; this also warrants further investigation.

The limitations of our study include the following. 1. We assume that dummy leads would not significantly alter the integrity of the LD. We found it impractical to use a larger number of animals to include a sham group. Subsequent data from the protein chemistry analyses, however, showed that the right LD (unstimulated) taken from trained animals (ie, left LD electrically trained for 12 weeks) was phenotypically identical to left LD taken from control animals (see Table 3).

2. The changes in contractile properties seen in muscles treated with clen may be associated with alterations in the levels of other proteins, such as the troponins, and their calcium-binding properties, although we did not investigate these.

3. The hemodynamic data from the MCS may not translate clinically and need to be validated in an in vivo model of aortomyoplasty.

4. The acute and chronic effects of clen in animals with heart failure were not investigated, and future work should be directed to this before the potential clinical use of this β2-adrenergic receptor agonist can be considered for improving the results of SMA.

In conclusion, clen improves the biomechanical properties of transformed LD in an ex vivo model of aortomyoplasty. Stroke power was increased by 4-fold, and contraction and relaxation times were reduced by 96% and 105%, respectively. These muscles remained fatigue-resistant despite these alterations. The observed trend toward increased abundance of the fast isoforms of MHC and SERCA probably does not explain the more significant functional alterations. There was no evidence of concomitant cardiac hypertrophy. This study may have important implications for the use of pharmacological agents to optimize the performance of electrically transformed skeletal muscle.

Acknowledgments
This work was supported by Telectronics Pacing Systems Inc. We thank Professor Colin Green and his team at Northwick Park Institute of Surgical Research for excellent technical assistance. A special thanks to Dr Charlotte Maltin for advice with the experimental design.

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Clenbuterol Increases Stroke Power and Contractile Speed of Skeletal Muscle for Cardiac Assist
M. Petrou, S. Clarke, K. Morrison, C. Bowles, M. Dunn and M. Yacoub

Circulation. 1999;99:713-720
doi: 10.1161/01.CIR.99.5.713

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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