Clenbuterol-Supported Dynamic Training of Skeletal Muscle Ventricles Against Systemic Load
A Key for Powerful Circulatory Assist?

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Background—The profound loss of power that occurs in skeletal muscle after electrical conditioning has been the major limiting factor in its clinical application. This study investigates a 3-fold approach for chronic conditioning of skeletal muscle ventricles (SMVs) combining electrical transformation, dynamic training against systemic load, and pharmacological support with clenbuterol.

Methods and Results—In 10 adult male goats, SMVs were constructed from latissimus dorsi muscle wrapped around an intrathoracic training device with windkessel characteristics. SMVs were stimulated electrically and trained dynamically by shifting volume against systemic load. Group 1 goats were controls (n=5), and group 2 goats (n=5) were supported with clenbuterol (150 µg 3 times a week). SMV dynamics were recorded weekly over 5 to 8 months: peak pressure (P_max), stroke volume (SV), volume displacement per minute (VD), stroke work per day (SW/d), and maximum rates of pressure generation, +dP/dt_max, and decay, −dP/dt_max. In group 1, after 149.5±2.7 days (n=4), data were P_max=70.8±4.7 mm Hg, SV=3.2±1.2 mL, VD=62.3±21.1 mL/min, SW/d=0.8±0.4 kJ, +dP/dt_max=64±13 mm Hg/s, and −dP/dt_max=156±32 mm Hg/s. These parameters were significantly improved (P<0.007) in the clenbuterol-treated group 2 after 151±2.7 days: P_max=176.2±43.8 mm Hg, SV=23.3±6.1 mL, VD=568.2±186.1 mL/min, SW/d=9.1±2.2 kJ, +dP/dt_max=1134±267 mm Hg/s, and −dP/dt_max=1028±92 mm Hg/s. In 2 SMVs of group 2, VD increased to 1090 and 1235 mL/min after 202 and 246 days of training, respectively. At termination, myosin heavy chains were totally transformed into myosin heavy chain-1 in all SMVs.

Conclusions—This clenbuterol-supported dynamic training provides powerful SMVs that may have important clinical implications for the treatment of end-stage heart failure by muscular blood pumps. (Circulation. 2000;101:2213-2219.)

Key Words: muscles ■ electrical stimulation ■ contraction ■ circulation ■ clenbuterol

The incidence of heart failure is increasing in western countries.1 Medical treatment and heart transplantation are the most accepted therapies. As adjunct or even alternative treatments, biomechanical circulatory assist using the autologous skeletal muscle as in cardiomyoplasty or aortomyoplasty or as skeletal muscle ventricles (SMVs) are highly attractive approaches and have been investigated experimentally and clinically for several decades.2-12 The profound loss of power that occurs in skeletal muscle after electrical conditioning has been the major limiting factor in its clinical application.13,14

A 2-fold approach to skeletal muscle conditioning, including a gentle stimulation protocol in combination with dynamic training, has been shown to improve muscle performance.15-17 A power-increasing effect of the β2-adrenergic receptor agonist clenbuterol in electrically conditioned latissimus dorsi muscle (LDM) was recently demonstrated over an evaluation period of 60 minutes in growing sheep.18 Theoretically, a 3-fold approach to skeletal muscle conditioning combining electrical transformation, dynamic training, and pharmacological modulation by clenbuterol may further enhance mechanical performance of muscle-powered circulatory assist.

Methods

Animals
Experimental investigations were carried out in 10 adult male Boor goats, 3.7±1.1 years old, with a weight of 77.6±6.0 kg. They were castrated 4 weeks before the operation to keep them together and avoid injuries between them. The experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. They were...
supervised by a representative of the District President of the local Society for Prevention of Cruelty to Animals.

**Myostimulators and Electrodes**

Commercially available myostimulators were used (Medtronic model 7420/7424 and Telectronics model 7220). An epimysial electrode 30 mm long (custom-made, Medtronics, Bakken Research Center) was attached to the muscle close to the branches of the nervus thoracodorsalis. On the opposite side of the muscle, an electrode 60 mm long (Medtronics SP5951–500-60-NMS) was placed subfascially.

**Training Device**

The training device (Figure 1) was made of silicone rubber (Q3, Dow Corning) and has been described in detail before. Briefly, it consists of a central chamber and 2 compliant side bladders filled with saline solution. The barrel-shaped central chamber and the side bladders have volumes of 150 mL and 50 mL each, respectively. The side bladders are constructed with a compliance of 1.0 to 1.3 mL/mm Hg, simulating the windkessel characteristics of the arterial system in normal subjects with 1.07 mL/mm Hg.

**Operative Procedure**

The operation was performed under general anesthesia as described before. In brief, the left LDM was dissected free, folded to a double layer, and wrapped around the central chamber of the training device. The SMV was transferred into the thorax and fixed at the thoracic wall.

**Training Procedure**

The training procedure was a combination of an electrical conditioning by burst stimulation and a dynamic training with contractions against a constant filling pressure of 60 to 70 mm Hg within the elastic training device. Each SMV contraction shifted volume into the expanding side bladders, generating a pressure increase. After muscle contraction, the expanded side bladders shifted the fluid back to the pumping chamber before the next contraction cycle could commence. Electrical conditioning was performed as shown in Table 1 and described in detail before. Dynamic training is characterized by auxotonic contractions with simultaneous increase of muscle tension and decrease of muscle length.

**Experimental Groups and Clenbuterol Medication**

The 10 male goats were assigned alternately to the different groups without remarkable differences in age and weight (groups 1/2: age, 3.5 ± 1.0/3.8 ± 1.0 years and body weight, 77.2 ± 6.4/78.0 ± 6.3 kg). Animals in group 1 were controls (n = 5), and animals in group 2 (n = 5) were treated with clenbuterol (Boehringer-Ingelheim). A dosage of 150-μg capsules 5 times a week for the first 2 weeks was administered orally, followed by the same dosage 3 times per week.

**Data Acquisition**

With the goats unsedated, pressure changes inside the training device induced by SMV contractions were measured once a week by piercing of a subcutaneous vascular access port, which communicated with the lumen of the training device via a 5F, 40-cm-long catheter (Figure 1). The piercing needle (Surecan, Braun) was connected with a 100-cm stiff plastic tube to an electromechanical pressure transducer (Hewlett Packard, model 1290 C). Pressure data were recorded and stored in a PC (AT 486, 33 MHz; resolution, 12 bit; sampling rate, 200 Hz). At each measurement, the filling pressure was adjusted to 60 mm Hg by addition of usually <3 mL of 10% hyperosmolar saline solution via the subcutaneous vascular access port. Thereafter, 5 series of couples of pressure traces were recorded. Then, with the myostimulator switched off, the compliance of the side bladders was determined as described in detail elsewhere.

**Analysis of Compliance**

The compliance of the side bladders was compared between the 2 groups after the first week and after 1, 3, and 5 months after surgery. As a representative value for the compliance, the pressure increase after injection of 30 mL of saline solution into the training device in addition to the load of 70 mm Hg was defined as ∆P (PaC).

**Analysis of Fluid Dynamic Parameters**

SV was assessed relating the pressure increase during a muscular contraction to the determined compliance curve of the side bladders of the device as described before. In vitro, it was shown that this method of indirect SV evaluation was valid, relying on a nonexpandable central chamber (R = 0.996). In vivo, it was demonstrated that the compliance curve is solely an expression of the side bladders into which the SV was expelled. After a volume of 100 mL had been added into the training device, it was shown by x-ray examination that there was no change in diameters of the central chamber (n = 4). For an approximate control of the shifted volume, the difference of the radii of the 2 side bladders was determined by x-ray examination.
Therefore, the side bladders were regarded as spheres and the volume shift as the difference of sphere volumes before and after contraction. This SV validation by x-ray examination was performed in test situations with a volume shift between 14 and 83 mL and was found to be comparable to the indirect SV calculation described above.22

Stroke work, W, was evaluated by means of numerical integration of the pressure-volume curve of the training device determined at each measurement15,22

\[
W = \sum_{i=m}^{n_{\text{max}}} \left[ (A_i + A_{i+1}) = \sum_{i=m}^{n_{\text{max}}} \left( V_i - V_{i-1} \right) \left( \frac{P_i + P_{i-1}}{2} \right) \right],
\]

with \( n_{\text{min}} \) and \( n_{\text{max}} \) bordering the slices of minimal and maximal volume. \( V_i \) and \( P_i \) represent the volume and pressure value at slice number \( i \).

Stroke work was standardized to LDM weight, which was then called specific stroke work (J/kg). Stroke work per day was approximated by multiplying stroke work by the number of contractions per day. Maximum rate of pressure development (\( +dP/dt_{\text{max}} \)) and maximum rate of pressure decay (\( -dP/dt_{\text{max}} \)) were calculated by the first derivation of pressure trace, filtered with a low pass of 30 Hz. Time to peak pressure, \( t_{\text{Pmax}} \), and relaxation half-time, \( t_{\text{relax}} \), were standardized to SV to neutralize the influence of SV variability.

Analysis of MHCs

Muscle samples for analysis of myosin baseline composition were taken intraoperatively from the free wall of the SMV opposite the muscle pedicle. Further samples of the muscle were harvested after training from the same location of the SMV and from the corresponding contralateral part of the LDM. Muscle samples were frozen under liquid nitrogen, stored at \(-80^\circ\text{C}\), and pulverized, then used for myosin extraction by the method of Ho et al23 and brought to electrophoresis on acrylamide gel for a separation for 20 hours at 150 V. The gel was fixed for 24 hours with methanol. All gels underwent a silver staining by the method of Oakly et al.24 The gel electrophoresis was photo documented, and after a computer scanning, was quantified by Gel-Pro Analyzer version 3.0 for Windows software.

Statistics

Data are presented as mean±SD. A Mann-Whitney \( U \) test was performed for comparison between groups. Differences were considered significant at values of \( P<0.05 \). All statistical calculations were performed with Winstat 3.0 software (Kalmia Co).

Results

Animals and Operative Procedure

All 10 male Boor goats survived the operative procedure. There was no failure in the myostimulators and electrodes. In 1 animal of group 1, an elastic training device failed as a result of leakage on postoperative day 49, and the goat was excluded from the study. The average training time of group 1 (\( n=4 \)) was 149.5±2.7 days and that of group 2 (\( n=5 \)), 151±2.7 days. Two goats of group 2 were observed up to 202 and 246 days, respectively. Mean body weight of group 1 decreased during the training period minimally from 77.2±5.9 to 76.5±9.5 kg, and it increased significantly in the clenbuterol-treated group 2 from 78.0±6.3 to 87.8±9.3 kg (\( P=0.02 \)) after 151±2.7 days of training. There was no significant difference in body weight at the beginning of the training between groups 1 and 2 (\( P=0.23 \)).

Table 1. Stimulation Protocol for Progressive Training of SMVs

<table>
<thead>
<tr>
<th>Time</th>
<th>cpm</th>
<th>Amplitude, V</th>
<th>PpB, n</th>
<th>Pulse Width, µs</th>
<th>Burst Frequency, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–24 h</td>
<td>0.2</td>
<td>1.5–2.5</td>
<td>3</td>
<td>120</td>
<td>33</td>
</tr>
<tr>
<td>24–48 h</td>
<td>0.2</td>
<td>5</td>
<td>3</td>
<td>120</td>
<td>33</td>
</tr>
<tr>
<td>48 h–1 wk</td>
<td>0.2–0.3</td>
<td>5</td>
<td>6</td>
<td>120</td>
<td>33</td>
</tr>
<tr>
<td>1–4 wk</td>
<td>0.3–1</td>
<td>5–7</td>
<td>6–14</td>
<td>120</td>
<td>33</td>
</tr>
<tr>
<td>1–2 mo</td>
<td>1–3</td>
<td>5–7</td>
<td>14</td>
<td>120</td>
<td>33</td>
</tr>
<tr>
<td>2–4 mo</td>
<td>3...42</td>
<td>5–7</td>
<td>14</td>
<td>120–210</td>
<td>33</td>
</tr>
</tbody>
</table>

\( \text{cpm} \) indicates counts per minute; \( \text{PpB pulses per burst} \).

Figure 2. Pressure curves during dynamic training from SMV of group 1 without \( \beta_2 \)-stimulation (A) and group 2 supported by clenbuterol (B). Clenbuterol-supported SMVs of group 2 maintained pressure (function) at a high level over time. Stimulation pattern is shown with an increasing number of pulses per burst.
Pressure
In group 1 (4 animals; average follow-up, 149.5±2.7 days), an increased peak pressure, \( P_{\text{max}} \), was observed during the first 60 days, followed by a continuous decline corresponding to a poor fluid dynamic outcome (Figures 2 and 3; Table 2). SMVs of group 2 (n=5) showed much better mechanical performance (Figures 2 and 3, Table 2) with maintained or increased peak pressure, \( P_{\text{max}} \), during the first 151±2.7 days of training. In group 2, in accordance with the time course of pressure, \( P_{\text{max}} \), the maximum rate of pressure decay was low, 64\( \pm \)13 mm Hg/s, and in group 1, \( dP/dt_{\text{max}} \), was high, with a \( dP/dt_{\text{max}} \) of 1134\( \pm \)267 mm Hg/s (\( P<0.007 \)). +dP/dt_{max} of the 2 goats after 202 and 246 days of training resulted in 1027 and 1102 mm Hg/s, respectively.

Compliance
During the training period, compliance decreased remarkably, as demonstrated by an increased \( \Delta P_{30 \text{ mL}} \) value. \( \Delta P_{30 \text{ mL}} \) after the first postoperative week was: group 1, 33.0\( \pm \)10.4 mm Hg; group 2, 37\( \pm \)12.0 mm Hg (\( P=0.31 \)); after 1 month: group 1, 44.3\( \pm \)25.3 mm Hg; group 2, 64.2\( \pm \)30.3 mm Hg (\( P=0.29 \)); after 3 months, group 1, 79.6\( \pm \)52.7 mm Hg; group 2, 87.3\( \pm \)84.2 mm Hg (\( P=0.44 \)); and after 5 months: group 1, 158.0\( \pm \)126 mm Hg; group 2, 192.3\( \pm \)146 mm Hg (\( P=0.02 \)). No significance was found between groups up to the third postoperative month. After 5 months of dynamic training, compliance was significantly different between the 2 groups.

Stroke Volume
In group 1, in accordance with the time course of pressure, stroke volume (SV) increased slightly and dropped to a low level. SV after 149.5±2.7 days of training was 3.2\( \pm \)1.2 mL per beat, corresponding to a volume displacement (VD) of 62.3\( \pm \)21.1 mL/min.

In group 2, the SV time course characteristics showed a more pronounced increase at the beginning, followed by a relatively rapid decline, reaching an individual plateau that was maintained during further training. Maximum SV was 2 times higher at the beginning (type II fibers) than at the end (100% type I fibers) of training. This effect was observed in all cases in group 2. SV per beat was 23.3\( \pm \)6.1 mL after 151±2.7 days of training, corresponding to a VD after 5 months of training of 568\( \pm \)186 mL (Table 2). Differences from group 1 were significant (\( P<0.007 \)). In 2 goats, after a training of 202 and 246 days, respectively, continuous pumping capacity of \( >1 \) L/min (1090 and 1235 mL/min) was obtained (Table 2).

Stroke Work
Stroke work per day in group 1 was poor, achieving 0.8\( \pm \)0.4 kJ after 149.5±2.7 days of training. In group 2, in accordance with the time course of pressure, SV, and contraction frequency, stroke work per day increased continuously up to 9.1\( \pm \)2.2 kJ (\( P<0.007 \)). End-stage stroke work of the 2 goats after 202 and 246 days of training was 26.8 and 27.8 kJ/d, respectively.

Contraction
In group 1, after 149.5±2.7 days of training, maximum rate of pressure rise, \( +dP/dt_{\text{max}} \), was low, 64\( \pm \)13 mm Hg/s, and in group 2, it was high, with a \( +dP/dt_{\text{max}} \) of 1134\( \pm \)267 mm Hg/s (\( P<0.007 \)). +dP/dt_{max} of the 2 goats after 202 and 246 days of training resulted in 1027 and 1102 mm Hg/s, respectively.

Relaxation
The maximum rate of pressure decay was low in group 1 with a \( -dP/dt_{\text{max}} \) of 156\( \pm \)32 mm Hg/s and high in group 2 with a \( -dP/dt_{\text{max}} \) of 1028\( \pm \)92 mm Hg/s (\( P<0.007 \)).

The relaxation half-time, \( t_{\text{relax}} \), was measured as 54.4\( \pm \)12.5 ms/mL in group 1 and 6.0\( \pm \)1.7 ms/mL in group 2 (\( P<0.007 \)).
After 202 and 246 days of clenbuterol-supported dynamic training, $-\frac{dP}{dt_{\text{max}}}$ was evaluated at 923 and 901 mm Hg/s, respectively, and the corresponding relaxation half-time normalized to SV was evaluated at 6.0 and 6.8 ms/mL.

**Morphology**

The muscle morphology of the SMVs was related to its power delivery. A thin muscle wall (Figure 4A) with low power in group 1 showed histologically severe muscle damage, with a fiber substitution by fat cells. In the thick muscles of group 2, supported by clenbuterol (Figure 4B) with high power delivery, muscle fibers were mainly preserved, fewer fat cells were integrated, and there were obvious signs of hypertrophy and less connective tissue. This muscular tissue has to undergo further morphometric analysis.

**Myosin Heavy Chains**

Myosin heavy chain (MHC) composition of prospectively harvested LDM was similar in the 2 groups: group 1, 22.1±6.5% MHC-1 and 77.9±6.5% MHC-2 and in group 2, 28.8±7.7% MHC-1 and 71.2±7.7% MHC-2. SMVs after training of both groups showed a total transformation into 100% MHC-1.

**Discussion**

To the best of our knowledge, this study provides the first evidence that a 3-fold approach to skeletal muscle conditioning by a combination of electrical stimulation, dynamic training, and pharmacological modulation with clenbuterol provides a power source with a high potential for effective circulatory assistance. In our training device, which mimics systemic loading conditions, the addition of clenbuterol to electrical transformation and dynamic training resulted in a remarkable improvement in muscular performance, with a continuous pumping capacity of $>1$ L/min with a 300-g muscle, which has not been demonstrated before in adult muscular tissue. LDM in patients usually weighs ≈600 g. Therefore, a higher pumping capacity may be expected, even considering that 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.

**Dynamic Training**

The contraction power needed for dynamic training against a load of 60 to 70 mm Hg from the beginning was much higher than under a low load of 5 to 10 mm Hg tested before.15,21 That is why 2 additional conditions were established to increase contraction power. First, muscle mass was doubled by the use of male goats with an LDM weight of 300 g instead of female goats with an LDM weight of 150 g, which were used before.21 Second, the stroke power was supposed to be increased by application of the $\beta_2$-stimulator clenbuterol, which was shown elsewhere to be power-generating in nonfatiguing type I fibers.

**$\beta_2$-Adrenergic Receptor Agonist Clenbuterol**

Several drugs are known to improve muscle hypertrophy and function.25–28 Clenbuterol, a selective $\beta_2$-adrenergic receptor agonist, induces skeletal muscle hypertrophy and power in the rat25 and also cardiac hypertrophy with
preservation of certain physiological features, such as systolic and diastolic cardiac function. Clenbuterol is the most frequently used and most potent of the β2-agonists, because it results in a 10% to 20% increase in mass of skeletal muscle in 8 to 14 days as well as an increase in myocardium of ~18%. A dosage of clenbuterol of 150 μg/d was chosen to test quantities that are expected to apply clinically later on. The time intervals of application were calculated to try to avoid an accumulation of this drug, considering an elimination half-time of 34 hours.

Myosin Heavy Chains
The fiber composition of LDM preoperatively was not significantly different before dynamic training of both groups and thus could not have influenced the outcome of training. MHC-1 was found in 100% of group 1 goats as well as in the clenbuterol-supported group 2 goats after chronic stimulation and pumping. Thus, the hypothesis of preserved MHC-2 resulting from increased power delivery could not be verified.

Compliance
In normal subjects, the total compliance of the arterial system was found to be 1.07 mL/mm Hg. Values of 0.9 mL/mm Hg were assumed in advanced age because of hypertension and arteriosclerosis. In our compliant training device in the clenbuterol-supported group 2 animals, compliance varied from 0.08 to 0.34, with a stroke work per day of 5.5 to 11.8 kJ. These low, nonphysiological compliance values (Table 1) are regarded as the main limitations of this study. The less compliant device is found to be combined with the lowest stroke work per day and the most compliant bladders with the greatest energy delivery (Table 2). This impressive high power is thought to be even more favorable if dynamic training is performed against physiological compliance of the arterial system.

Application
The positive effect of clenbuterol observed on the power development of SMVs during dynamic training against systemic load (Figure 2, right, and Figure 3, right) may be sufficient to speculate that this β2-stimulation may strongly improve the hemodynamic efficacy of other muscle-powered cardiac assist procedures. Indeed, in dynamic cardiomyoplasty, LDM surrounding dilated hearts should require more power than in SMVs with small diameters (Laplace’s law).

Muscle contraction and relaxation were faster in clenbuterol-treated ventricles than in those without drug supply (Table 2). Preservation of a high +dP/dtmax of 1134±267 mm Hg/s and −dP/dtmax of 1028±92 mm Hg/s is a sign of powerful SMVs with remarkably good SVs. Relaxation of −dP/dtmax of human left ventricle levels off at 2000 mm Hg/s. That is why the relaxation characteristics in our study also indicate that this kind of conditioning may predispose the skeletal muscle more to a counterpulsator application as SMVs or aortomyoplasty than a direct cardiac assist in the form of a cardiomyoplasty.

As shown before, dynamic training against increasing load results in increasing muscular power. However, this training procedure would imply a 2-stage surgical procedure with primary training up to a systemic load and a subsequent integration into the circulation. A 2-step operation as shown elsewhere may not be practicable for patients with end-stage heart failure. A successful training against systemic load, however, combined with a 1-step operation, may be a more practical approach for clinical application of muscular blood pumps.

To summarize this experimental work, clenbuterol-supported dynamic training may be regarded as a key for powerful muscular blood pumps. SMVs trained as described are expected to become clinically effective as muscular blood pumps performed in a 1-step operation and trained within circulation.

Acknowledgment
We thank Boehringer-Ingelheim, Germany, for the gift of clenbuterol. A special thanks to P.A. Grandjean, MSc, Bakken Research Center, Maastricht, Netherlands, for his comments and to Britta Keding for her excellent support and evaluations.

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Circulation. 2000;101:2213-2219
doi: 10.1161/01.CIR.101.18.2213

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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