Biomechanical Hearts
Muscular Blood Pumps, Performed in a 1-Step Operation, and Trained Under Support of Clenbuterol

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Background—As shown previously in goats, clenbuterol increased the power of electrically conditioned skeletal muscle ventricles (SMVs) of clinically relevant size (150 mL), which were constructed around a mock system. They pumped against a pressure of 60 to 70 mm Hg immediately during surgery and up to several months after, finally at >1 L/min. SMVs without clenbuterol administration failed. Thus, we expected that clenbuterol-supported SMVs might become integrated into the circulation by a 1-step operation instead of the 2-step procedure required up to now.

Methods and Results—In adult Boer goats (n = 5), latissimus dorsi muscle was wrapped around a polyurethane chamber of 150 mL that was connected to the descending aorta. This muscular flow-through pumping chamber containing a stabilizing inner layer (called a biomechanical heart [BMH]) was formed and immediately made to work against a systemic load with the support of clenbuterol (5 \times 150 \mu g/wk). During surgery, the mean stroke volume of BMHs was 53.8 \pm 22.4 mL. One month after surgery, in peripheral arterial pressure, the mean diastolic (P_{MD}) and minimal diastolic (P_{min}) pressures of BMH-supported heart cycles differed significantly from unsupported ones (P_{MD} = -2.4 \pm 0.9 mm Hg [P < 0.04], P_{min} = 2.9 \pm 1.1 mm Hg [P < 0.04]). After BMH-supported heart contractions, the subsequent maximal rate of pressure generation, dP/dt_{max}, increased by 20.5 \pm 8.1\% (P < 0.02). One BMH, catheterized 132 days after surgery, shifted a volume of 34.8 mL per beat and 1.4 L/min with a latissimus dorsi muscle of 330 g. Depending on duration of training, the percentage of myosin heavy chain type 1 ranged between 31\% and 100\%.

Conclusions—Under support of clenbuterol, BMHs of a clinically relevant size can be trained effectively in the systemic circulation after a 1-step operation and offer the prospect of a sufficient volume shift and probably unloading of the left ventricle. (Circulation. 2001;104:717-722.)

Key Words: muscles n electrical stimulation n circulation n clenbuterol n heart-assist device

The function of failing native hearts can be substituted in part or in whole by biological, mechanical, and biomechanical therapies. Biological substitution (cardiac transplantation) is limited worldwide to \approx 3000 cases per year, or <5\% of the potential recipients. The most common role of mechanical devices is not as a permanent alternative to transplantation but as a bridge to transplantation or to recovery of native cardiac function if reverse remodeling can occur. For the overwhelming majority of patients with end-stage heart failure, no definitive therapy is currently available. Biomechanical therapies such as dynamic cardiomycoplasties were not clinically successful. After electrical conditioning, resulting in a tremendous decline in muscle power, they were not able to squeeze failing hearts, with their large diameters and wall stress. Muscular blood pumps, however, with small ventricular diameters, need less power to contract and are expected to be a successful treatment option in muscle-powered cardiac assistance.

In previous studies, skeletal muscle ventricles (SMVs) were connected to the circulation by a second operation after a vascular delay and an electrical conditioning period. They pumped for up to 4 years in the circulation. These SMVs (sack ventricles) in dogs pumped with low stroke work because of their small chamber volume of 25 mL. To simulate a stroke volume of >1 L/min, smaller SMVs had to be built. SMVs in goats built around a training device with a chamber volume of 150 mL, pumping against a load of 60 to 70 mm Hg without any drug administration, however, were not successful. Thus, drugs were tested to generate more muscular power.

A power-increasing effect of the \beta_2-adrenergic receptor agonist clenbuterol in electrically conditioned latissimus
dorsi muscle (LDM) was recently demonstrated in growing sheep. By use of an elastic device, dynamically trained intrathoracic SMVs without vascular delay and supported by clenbuterol were able to contract against a constant load of 60 to 70 mm Hg immediately after construction, and they pumped >1 L/min continuously after several months of training. Relevant side effects of clenbuterol are not in question, because it has been in clinical use for the treatment of chronic pulmonary obstruction for years in a similar dosage. Furthermore, the well-known cardiac arrhythmogenic effects of β₂-receptor stimulators in general should be negligible for clenbuterol. This motivated us to build a new muscular blood pump consisting of a muscular wrap and a stabilizing inner pumping chamber and to train it within the circulation. This blood pump was able to contract against a load of 60 to 70 mm Hg immediately after construction and to pump >1 L/min continuously after several months of training.

### Table 1. Stimulation Protocol for Experimental Biomechanical Hearts

<table>
<thead>
<tr>
<th>Time</th>
<th>Burst PpB, n</th>
<th>PW, μs</th>
<th>Ampl, V</th>
<th>BF, Hz</th>
<th>BpM</th>
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<tr>
<td>0–21 d</td>
<td>5–10</td>
<td>120</td>
<td>2.0–5</td>
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<td>0.3–3 Unsynchronized</td>
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<tr>
<td>1st mo</td>
<td>10</td>
<td>120</td>
<td>5</td>
<td>33</td>
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<td>Mode 1:20</td>
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<td>R-Delay [ms] 350</td>
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<td>BpM 3–4.5</td>
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<td>2nd mo</td>
<td>12</td>
<td>120</td>
<td>5–7</td>
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<td>Mode 1:16 1:12</td>
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<td>R-Delay [ms] 350 240</td>
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<td>BpM 3.8–5.6 7.5–9.2</td>
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<td>3rd mo</td>
<td>12</td>
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<td>Mode 1:10 1:8 1:6</td>
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<td>R-Delay [ms] 350 240 160</td>
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<td>BpM 6–9 11.3–13.8 18.3–21.7</td>
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<tr>
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<td>12</td>
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<td>Mode 1:6 1:5 1:3</td>
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<td>R-Delay [ms] 350 240 160</td>
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<td>BpM 10–15 18–22 36.7–43.3</td>
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</table>

PpB indicates pulses per burst; PW, pulse width; Ampl, stimulation amplitude; BF, burst frequency; BpM, burst per minute; and bpm, beats per minute. Stimulation started unsynchronized up to day 21 followed by a heartbeat-synchronized pattern, in a 1:20 to 1:3 mode depending on levels of heart rates (60–90, 90–110, and 110–130 bpm) and training duration with minimally 3 to maximally 43.3 bpm.
pump is called a biomechanical heart (BMH). These BMHs, with a pumping chamber of 150 mL, were tested in a long-term large-animal study evaluating both the hemodynamic effects and the myosin heavy chain (MHC) composition of the muscle of the BMH wall.

Methods

Animals
Experiments were carried out in 5 adult male Boer goats, 3.3±1.2 years old, with a mean weight of 79±6.6 kg. They were castrated 4 to 6 weeks before operation; they were kept together, and injuries among them were prevented. The study was supervised by a representative of the Society for the Prevention of Cruelty to Animals of the District Administration.

Myostimulator and Electrodes
Commercially available myostimulators were used (Medtronic model Itrel II 7424, Telectronics model 7220). An epimysial electrode 30 mm long (custom-made, Medtronic, 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 (Medtronic SP5591-500-60-NMS) was placed subfascially. For the synchronization of the muscle contraction (BMH) with the action of the heart, a cardiac screw-in electrode (model 4951, Medtronic) was inserted into the apex of the left heart (Figure 1).

Pumping Chamber
The stabilizing pumping chamber consisted of a double-layered polyurethane membrane and was barrel-shaped, with a maximum inner diameter of 6 cm, containing a volume of 150 mL. The wall of the polyurethane chamber was reinforced by a cage with 16 metal springs, as shown in Figure 2. Each spring had a width of 4 mm and was 0.5 mm thick. The spring cage was located between the 2 polyurethane membranes. The membrane material was the same as that used clinically to manufacture the pumping chambers of the MEDOS left heart assist device. Two ring-reinforced vascular prostheses (Impra Medica GmbH) with a diameter of 16 mm were connected to the pumping chamber.

Operative Procedure
General anesthesia was induced by 100 mg xylazine hydrochloride (Rompun 2%, BayerVital GmbH) and 1 mg atropine sulfate (Atropinsulfat, Fresenius) before orotracheal intubation and maintained with 10 to 30 mL/h propofol 2% (Disoprinvan 2%, GlaxoWellcome). Blood pressure was monitored via the ear artery and kept between 80 and 120 mm Hg systolic.

The construction of the BMH was effected by mobilizing the LDM totally. Its tendon was cut. Nerve and vessel were untouched. Two stimulating electrodes were anchored, and the muscle was folded into a muscular tube and wrapped around the pumping chamber (Figure 1). The BMH was inserted into the left cranial thorax after partial resection of the third and the fourth ribs. Then it was connected to the descending aorta by ring-armed vascular prostheses through a caudal thoracotomy from the bed of the 5th rib. The aorta was ligated between the 2 anastomoses (Figure 1). Intensive care was carried out for 24 to 48 hours.

Stimulation Protocol and Medication
The electrical stimulation protocol was based primarily on our own former experience.5,13,17 During the first 3 weeks, the muscular wrap was stimulated by a myostimulator that was not synchronizable with the heart (Itrel II, Medtronic model 7424), delivering initially 1 contraction per 3 minutes up to 3 muscle contractions per minute after 3 weeks of training (Table 1). The unsynchronized Itrel II was used because no available ECG-triggered myostimulator could deliver these low beat frequencies. To stimulate the BMH synchronized with the heart, the Itrel II was substituted by a myostimulator.

Figure 3. Peripheral arterial blood pressure curves with BMH-assisted heartbeats. A, During dynamic training of BMHs within circulation over time. Pressure curves of BMH (second pressure peak) are modulated by an increased number of pulses per burst and recorded on postoperative days 2, 21, and 28. B, BMH-assisted heartbeat has an increased diastolic pressure, followed by $P_{aw}$ (afterload reduction). Thereafter, contractility $dP/dt$ of myocardium is increased vs a situation without circulatory assist. Pressure traces from animal 4 on postoperative day 20 immediately after changing Irrel to Telectronics for synchronization of BMH contraction to heart action. C, Pressure trace of 2:1 augmentation visualizes polyurethane pumping chamber with metal spring of BMH of goat 5 on postoperative day 409.

Figure 4. Pressure-volume loops performed during surgery for a BMH in goat 5, with stroke volume of 87 mL.
from Telelectronics 3 weeks after surgery (Telelectronics model 7220) with a stimulation protocol shown in detail in Table 1.

Dynamic training was supported with a $\beta_2$ stimulation drug (clenbuterol, Boehringer-Ingelheim) at a dosage of one 150-$\mu$g capsule 5 times a week for 2 weeks. Thereafter, clenbuterol was administered orally and continued 3 times per week. For anticoagulation, 2 tablets per day of 100 mg acetylsalicylic acid (Aspirin 100 N, Bayer) were given.

**Analysis of Hemodynamics**

The pumping capacity of the BMHs was evaluated during surgery (n=4) and 4 months after surgery (n=1) by the conductance catheter method18–20 with a 7F conductance catheter (Leycom, Cardio Dynamics). The multiple electrode catheter was placed inside the stabilizing inlay of the BMH along its long axis. Stroke volume was determined from the difference of maximal and minimal volume as documented in the pressure-volume loop of the ventricle. To determine stroke work, the area included by the pressure-volume loops was calculated.

In addition, during a stimulation period up to 414 days, the hemodynamic effects of the BMHs (n=5) were documented by measuring the peripheral blood pressure invasively once a week via the ear artery (Figure 3). Pressure curves of BMH-supported heartbeats were used to evaluate mean diastolic ($P_{\text{m}}$) and minimal diastolic ($P_{\text{min}}$) pressures. Maximal rate of pressure generation, $dP/dt_{\text{max}}$, after a BMH-supported heart contraction was determined by a computer (Figure 3B).

**Myosin Heavy Chains**

Muscle samples for analysis of myosin baseline composition were taken during surgery from the free wall of the BMH opposite the muscle pedicle. Further samples were harvested after training from the same location of the muscular wrap and from the corresponding contralateral part of the LDM. MHCs were analyzed and quantified as described before.13

**Statistics**

Data are presented as mean±SD. A Wilcoxon test was performed for comparison between groups of paired samples (n=5, Table 2A). Differences were considered significant at values of $P<0.05$. All statistical calculations were performed with the software Winstat 3.0 (Kalmar Co).

**Results**

**Animals**

All Boer bucks (n=5) survived the operative procedure. Outcomes and complications differed. Goat 1 died on post-operative day 36 of anastomotic bleeding. In goat 2, kinking of the cranial vascular prosthesis led to a severe stenosis with thrombosis of the total device and malperfusion of the caudal part of the animal, leading to its death on day 32. The death of goat 3 was also caused by kinking of the prosthesis, which was followed by thrombosis on day 71. Goat 4 was lost because of an intestinal embolism on postoperative day 59.
The most successfully trained BMH was in goat 5. Its arterial pressure was documented up to postoperative day 409 (Figure 3B and 3C). It died on postoperative day 414. Necropsy showed that the BMH cavity was thrombosed as a result of perforation of the inner polyurethane membrane by a broken metal spring. This led to a thrombus between the 2 polyurethane layers that occluded the ventricular outflow tract.

**Mechanical Performance**

**Pressure**

Intraoperative pressure within the ventricular cavity of the contracting BMH (n = 4), measured via the conductance catheter, resulted in a maximal pressure (P_{BMH\text{max}}) of 131 ± 14 mm Hg and a minimal pressure (P_{BMH\text{min}}) of 19.4 ± 6.2 mm Hg. Pressures measured in goat 5 after 132 days of training resulted in P_{BMH\text{max}} of 119 ± 9 mm Hg and P_{BMH\text{min}} of 42 ± 3 mm Hg (Table 3).

Data 1 month after surgery as measured via the ear artery showed an increase of P_{MD} of supported beats by 2.9 ± 1.1 mm Hg (P < 0.04), whereas P_{min} decreased by 2.4 ± 0.9 mm Hg (P < 0.04) (Table 3). Thus, BMHs were hemodynamically effective.

As shown in Table 2B, final evaluated pressure values of each goat of augmented P_{MD} as well as P_{min} were comparable to those 1 month after training.

**Stroke Volume**

Determined by the conductance catheter method, the intraoperative stroke volume of BMHs was 53.8 ± 22.4 mL (n = 4, Table 3 and Figure 4) and 34.8 ± 1.6 mL per beat (Figure 5), respectively, 1.4 L/min at 40 contractions per minute in goat 5, after 132 days of training. The 1.4 L/min was evaluated during heart catheterization under synchronization of BMH contractions at 80 bpm in a 1:2 mode, a situation similar to the elected stimulation protocol during a heart rate of 110 to 130 bpm in a stimulation mode of 1:3 as shown in Table 1. Small volume changes within the BMH chamber induced by the heart action can be recognized in Figures 4 and 5 (better in Figure 5) as a PV coil in the range between 136 and 142 mL.

**Stroke Work**

Calculation of intraoperative stroke work of BMHs (n = 4) by pressure-volume loops resulted in 0.72 ± 0.32 J. After 132 days of training (and fiber transformation), the BMH of goat 5 showed a reduced stroke work of 0.2 ± 0.07 J.

**Contraction**

In peripheral arterial pressure curves that followed BMH contractions (Figure 3B), the maximal rate of pressure generation, dP/dt_{max}, increased significantly, by 20.5 ± 8.1% (P < 0.02), 1 month after surgery. Corresponding relative values of the last measured dP/dt_{max} were similar to those found 1 month after training (Table 2B).

**Morphology**

Macroscopically, the morphology of the muscle was well preserved in all 5 muscular wraps. After 414 days of pumping, the muscular wall in goat 5 showed the largest muscular thickness, ≈18 mm, with a macroscopically and microscopically well-preserved muscular structure (Figure 2B).

**Myosin Heavy Chains**

MHC compositions of prospectively harvested LDM were 21.3 ± 5.9 % MHC 1 and 78.7 ± 5.9% MHC 2. In 5 goats, the LDM of the BMHs after training showed a myosin transformation from MHC 2 to MHC 1 depending on duration of training: goat 1, 36% (36 days); goat 2, 31% (32 days); goat 3, 49% (71 days); goat 4, 34% (59 days); and goat 5, 100% (414 days).

**Discussion**

At present, these BMHs are experimental devices, created to demonstrate that muscular blood pumps could be constructed in a clinically favorable 1-step operation and could be trained effectively under support of clenbuterol within the circulation.

**Pumping Chamber**

The pumping chamber was used mainly for 3 reasons: first, to stabilize the ventricular pump cavity with improved flow characteristics to minimize thromboembolic complications; second, to prevent muscle damage by overstretch-induced ischemia; and third, to prevent a ventricular chamber rupture. Fibrin coating of the blood contacting the polyurethane membrane as well as minor blood deceleration in a flow-through ventricle in contrast to a sack ventricle might have helped to prevent major thrombus generation.
The failure of the inlay in goat 5 was due to a broken metal spring. This complication can be avoided by use of a monolayered polyurethane wall without metal springs.

Small experimental SMVs with a cavity of 25 mL with less wall stress (Laplace’s law), without power-increasing drug support, and without a pumping chamber were able to work up to 4 years, but it is still to be proved whether these types of sack ventricles will work in larger sizes of ~100 to 200 mL without any drug support.

**Operative Procedure**

A 1-step operation to create a muscular blood pump in comparison with a 2-stage procedure might have less operative mortality as well as fewer minor bleeding complications during and after reoperation.

**Stimulation Protocol**

This stimulation protocol was based primarily on our own former experience. In contrast to stimulation protocols of other blood pumps, it starts during the first (only) operation with bursts with prolonged time intervals followed by an increasing number of bursts per minute, including an increasing number of pulses per burst.

**Mechanical Performance**

In this experimental setting, BMHs induced only small changes of peripheral arterial pressure values such as $P_{\text{md}}$, $P_{\text{min}}$, and $dP/dt$ but reasonable shifted volumes. The 1.4 L/min operation, contracting immediately upon operation while long-term autologous muscular blood pump built in a 1-step support, and without a pumping chamber were able to work up to 4 years, but it is still to be proved whether these types of sack ventricles will work in larger sizes of ~100 to 200 mL without any drug support.

We think that BMHs trained within the circulation and supported by a $\beta_2$-stimulator have the potential to become a clinically relevant treatment option for patients with end-stage heart failure.

**Acknowledgments**

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**References**

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