Influence of the Novel Inotropic Agent Levosimendan on Isometric Tension and Calcium Cycling in Failing Human Myocardium

Gerd Hasenfuss, MD; Burkert Pieske, MD; Maria Castell, MD; Bodo Kretschmann, MD; Lars S. Maier, MD; Hanjörg Just, MD

Background—Levosimendan was shown to increase calcium sensitivity by a novel mechanism and to inhibit phosphodiesterase III activity in animal myocardium.

Methods and Results—We investigated the influence of levosimendan on isometric contractions and calcium transients (aequorin method) in muscle strips from human hearts with end-stage failing dilated or ischemic cardiomyopathy (n=27). Data were compared with the effects of the phosphodiesterase inhibitor milrinone (n=9). The average maximum increase in twitch tension was 47±14% (range, 6% to 150%) at a levosimendan concentration of 0.8±0.3 μmol/L (P<0.01). This was associated with significant increases in maximum rates of tension rise and fall and decreases in times to peak tension, to 50% relaxation, and to 95% relaxation. In aequorin-loaded muscles, levosimendan 10⁻⁶ mol/L increased average tension by 50% (P<0.02), associated with a nonsignificant increase in aequorin light (16%). With milrinone 10⁻⁵ mol/L, average tension increased by 58% and aequorin light by 49% (P<0.05). In those muscle strips with pronounced inotropic effects (>50% increase in tension), there was a comparable and pronounced increase in aequorin light with both agents. However, in muscle strips with weak inotropic responses (<50% increase in tension), the increase in light was significantly higher with milrinone than with levosimendan.

Conclusions—Levosimendan has inotropic and lusitropic actions in failing human myocardium. Comparison with the phosphodiesterase inhibitor milrinone indicates that in case of pronounced inotropic stimulation, the modes of action of the two agents may be similar (phosphodiesterase inhibition), whereas small inotropic effects of levosimendan may result predominantly from calcium sensitization. (Circulation. 1998;98:2141-2147.)

Key Words: levosimendan ■ calcium ■ myocardium ■ heart failure

Recent studies have shown that vasodilators and ACE inhibitors given in addition to diuretics and cardiac glycosides can improve survival in patients with congestive heart failure.1-3 However, even with current state-of-the-art pharmacotherapy, most patients remain symptomatic. Therefore, there is ongoing research for the development of new inotropic agents to be introduced in treatment of congestive heart failure. Two new classes of inotropic agents have recently been studied in clinical trials: (1) agents that increase intracellular calcium, such as the phosphodiesterase inhibitors, which increase cAMP levels to activate protein kinase A; and (2) calcium sensitizers, which increase the response of the myofilaments to calcium. From a clinical point of view, major concerns exist about both classes of inotropes. Agents that increase intracellular calcium mediated by cAMP, although they may be effective in improving hemodynamic parameters, may increase mortality in heart failure patients, as was shown for the phosphodiesterase inhibitor milrinone.4-6 Impaired prognosis may be related to increased energy consumption and arrhythmogenesis due to elevated intracellular calcium concentration. Calcium sensitizers may be harmful because they bear the potential to impair relaxation and diastolic function of the heart.7-10

Levosimendan is a new inotropic agent that was suggested to increase calcium sensitivity by a novel mechanism.11,12 The compound was shown to bind to troponin C, the calcium receptor of myofilaments that triggers activation of contractile proteins. Association of levosimendan with troponin C was suggested to stabilize the calcium-induced change of troponin C conformation, thereby augmenting the effect of calcium binding to troponin C. In addition to this new calcium-sensitizing effect, levosimendan was shown to inhibit phosphodiesterase 3 at high concentrations, which may result in increased intracellular calcium concentration.13,14 Because levosimendan is considered a potential candidate for long-term clinical application in patients with congestive heart failure, further studies are needed to clarify its potential benefits and risks.
heart failure, knowledge of the effects of levosimendan on failing human myocardium is of great importance. In particular, the influence of the agent on intracellular calcium transients and on relaxation of the myocardium is of clinical relevance.

Accordingly, it was the goal of the present study to investigate the effects of levosimendan on isolated myocardium from end-stage failing human hearts stimulated to contract isometrically under physiological temperature and stimulation rates. The influence on intracellular calcium transients was studied by use of the photoprotein aequorin, and the effects of levosimendan on isometric force and aequorin light emission were compared with those of the phosphodiesterase III inhibitor milrinone.

Methods

Patients and Muscle Preparation

Experiments were performed in left and right ventricular myocardi- um from hearts obtained from 27 patients with end-stage failing dilated (n=16) or ischemic (n=11) cardiomyopathy undergoing cardiac transplantation. The mean age of the patients was 52±2 years; 6 were women. The average ejection fraction was 24±1%, cardiac index was 2.1±0.1 L·min⁻¹·m⁻², and pulmonary capillary wedge pressure was 25±2 mm Hg. Previous medication included diuretics (n=24), ACE inhibitors (n=22), cardiac glycosides (n=21), isosorbide dinitrate (n=17), warfarin (n=12), calcium channel blockers (n=5), dobutamine (n=4), dopamine (n=4), β-receptor antagonists (n=3), amiodarone (n=3), enoximone (n=2), and quinidine (n=1).

Ventricular myocardium was dissected from the endocardial surface of the ventricle immediately after cardiacectomy.

The study protocol was reviewed and approved by the Ethical Committee of the University of Freiburg.

Muscle Strip Preparation

The excised myocardium was immediately submerged in a protective solution and oxygenated by bubbling with 95% O₂/5% CO₂. Thin trabeculae or muscle strips were dissected from the endocardial surface of the heart. Myocardial preparations were transferred to the muscle chamber, connected to the force gauge by fine steel hooks (F30 type 372, Hugo Sachs Elektronik), and submerged in normal Krebs-Ringer solution in all levosimendan experiments, including controls. Thereby, maximum DMF concentration increased from 0.5% (control) to 0.77% (milrinone).

Protocol 1: Concentration-Dependent Effects of Levosimendan on Isometric Contractions

During steady-state stimulation (60 min⁻¹, 37°C), isometric contractions were recorded. Thereafter, levosimendan was applied at concentrations of 3×10⁻⁶, 10⁻⁵, 3×10⁻⁵ mol/L, and isometric force signals were recorded under steady-state conditions at each concentration. Experiments were performed in 10 muscle strip preparations from 7 hearts.

Protocol 2: Influence of Stimulation Frequency on the Effects of Levosimendan

Steady-state isometric force-frequency relationship was investigated by recording twitches after stimulation of the muscle strip preparations for 5 minutes at 30, 60, 90, 120, and 150 min⁻¹. Thereafter, levosimendan 10⁻⁶ mol/L was applied, and recording of force-frequency relation was repeated. Experiments were performed in 8 muscle strip preparations from 6 hearts.

Protocol 3: Influence of Levosimendan on Aequorin Light Emission

The Ca²⁺-regulated bioluminescent photoprotein aequorin was mac- roinjected into the quiescent muscle just beneath the endocardium. Light and force signals were recorded (37°C, 60 min⁻¹) and analyzed as described recently during control and after levosimendan was applied at concentrations of 3×10⁻⁶ and 10⁻⁵ mol/L. Fifty light transients were averaged at each experimental step to increase the signal-to-noise ratio. Aequorin light emission was analyzed as the amplitude of the aequorin light signal between the peak systolic light emission and the diastolic baseline values (millivolts of amplifier output). Time from peak light to 50% decline of the light signal was taken from the recordings, and the time constant τ was determined by fitting the decline phase of the aequorin light signal to a monoexponential curve. Experiments were performed in 8 muscle strip preparations from 9 hearts.

Protocol 4: Influence of Milrinone on Aequorin Light Emission

Isometric force and aequorin light were also recorded (37°C, 60 min⁻¹) during control and after application of milrinone at 10⁻⁷, 10⁻⁶, 10⁻⁵, and 3×10⁻⁵ mol/L. Aequorin light signals were analyzed at the same concentrations, the increase in twitch tension was comparable to the effects observed with levosimendan. Experiments were performed in 9 muscle strip preparations from 9 hearts, different from those studied with levosimendan.

Statistical Analysis

Data are expressed as mean±SEM. Differences between control and measurements taken after interventions were compared by paired or unpaired t test if appropriate. If multiple values within 1 group were compared, a t test followed by Bonferroni-Holm transformation was applied. A value of P<0.05 was accepted as statistically significant.

Results

Concentration-Dependent Effects of Levosimendan on Isometric Contractions

Figure 1 shows responses of the individual muscle strip preparations to increasing concentrations of levosimendan. There was a wide range of variation in the response to levosimendan with regard to the maximum effective levosi- mendan concentration as well as the maximum increase in twitch tension. The average maximum increase in twitch
tension was 47±14% at a levosimendan concentration of 0.8±0.3 μmol/L (Table 1). The increase in twitch tension was associated with significant increases in maximum rates of tension rise and fall and significant decreases in time to peak tension, time to 50% relaxation, and time to 95% relaxation (Table 1). Response to levosimendan was similar in muscles from hearts with ischemic and dilated cardiomyopathy.

**Influence of Stimulation Frequency on the Effects of Levosimendan**

Because calcium sensitizers may impair relaxation most pronouncedly at higher heart rates, the influence of levosimendan on isometric contractions was also investigated at higher stimulation frequencies. Without levosimendan, isometric tension tended to decrease with higher stimulation rates (blunted or inverse force-frequency relation in failing human myocardium). Levosimendan (10⁻⁸ mol/L) increased twitch tension by 42%, 35%, and 23% (P<0.05) at 30, 60, and 90 min⁻¹, respectively (Figure 2). There was no frequency-dependent rise in diastolic tension with levosimendan. Time to 50% relaxation was significantly reduced with levosimendan at stimulation frequencies of 60, 90, and 150 min⁻¹ (Figure 2).

**Influence of Levosimendan on Intracellular Calcium Transients**

Figure 3 shows typical original registrations of the effect of levosimendan on isometric force and aequorin light emission.

**TABLE 1. Influence of Levosimendan on Isometrically Contracting Failing Human Myocardium**

<table>
<thead>
<tr>
<th>Levosimendan Concentration, mol/L</th>
<th>Twitch Tension, mN/mm²</th>
<th>+dT/dt, mN/mm²·s</th>
<th>−dT/dt, mN/mm²·s</th>
<th>Time to Peak Tension, ms</th>
<th>Time to 50% Relaxation, ms</th>
<th>Time to 95% Relaxation, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.3±1.4</td>
<td>49±11</td>
<td>33±10</td>
<td>165±8</td>
<td>120±5</td>
<td>336±26</td>
</tr>
<tr>
<td>3×10⁻⁸</td>
<td>5.6±1.6</td>
<td>57±15</td>
<td>39±14</td>
<td>156±6</td>
<td>113±6*</td>
<td>325±28*</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>5.8±1.5</td>
<td>62±16*</td>
<td>41±13</td>
<td>157±7</td>
<td>109±5‡</td>
<td>318±26*</td>
</tr>
<tr>
<td>3×10⁻⁷</td>
<td>6.1±1.3‡</td>
<td>64±14†</td>
<td>42±11‡</td>
<td>152±7*</td>
<td>106±5§</td>
<td>304±25§</td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>6.3±1.3†</td>
<td>64±11†</td>
<td>42±10†</td>
<td>150±7*</td>
<td>104±5§</td>
<td>290±23†</td>
</tr>
<tr>
<td>3×10⁻⁶</td>
<td>5.4±1.0</td>
<td>59±8</td>
<td>35±7</td>
<td>148±6†</td>
<td>104±6§</td>
<td>280±22†</td>
</tr>
<tr>
<td>0.8±0.3×10⁻⁸</td>
<td>+47±14%‡</td>
<td>+48±9%†</td>
<td>+59±16%‡</td>
<td>−7±3%†</td>
<td>−14±2%§</td>
<td>−14±3%‡</td>
</tr>
</tbody>
</table>

+dT/dt indicates maximum rate of tension rise; −dT/dt, maximum rate of tension fall; time to 50% relaxation, time from peak tension to 50% relaxation; and time to 95% relaxation, time from peak tension to 95% relaxation. Percentage values in bottom line give average percentage change at the maximum twitch tension of the different muscle preparations.

*P<0.05; †P<0.01; ‡P<0.005, §P<0.001 vs control.
In the muscle strip shown in the top of Figure 3, the inotropic effect of levosimendan occurred without an increase in peak aequorin light emission. The bottom of Figure 3 shows another muscle strip preparation in which an increase in force by 92% was associated with an increase in light by 47%. Times to 50% and 95% relaxation decreased with levosimendan. The decreases in relaxation times were present in all muscles strips with pronounced and small inotropic effects (Table 2).

Comparison of Levosimendan With Milrinone
Figure 4 shows typical original registrations of the effect of milrinone on isometric force and aequorin light emission. The inotropic effect of milrinone was associated with an increase in the aequorin light emission. At concentrations of $10^{-5}$ and $10^{-4}$ mol/L, milrinone (n=9) increased twitch tension by 58% ($P<0.01$) and 94% ($P<0.005$) and aequorin light emission by 49% ($P<0.05$) and 70% ($P<0.01$), respectively (Figure 5). In comparison, levosimendan in a different group of muscles (n=9) increased twitch tension by 49% ($P<0.02$) and aequorin light emission by 15% ($P=NS$) at a concentration of $3\times10^{-7}$ mol/L and increased twitch tension by 50% ($P<0.02$) and aequorin light emission by 16% ($P=NS$) at a concentration of $10^{-6}$ mol/L (Figure 5). The duration of the aequorin light signal and the diastolic level were not significantly altered with milrinone or levosimendan. Time to 50% decline of the aequorin light signal was 111±16 ms during control and 108±15 ms with milrinone $10^{-4}$ mol/L ($P=NS$); it was 107±15 ms before and 100±17 ms with levosimendan $10^{-6}$ mol/L ($P=NS$). The time constant, $\tau$, of the decline phase of the aequorin light signal did not change significantly with levosimendan ($102±13$ ms at $10^{-6}$ mol/L versus $100±10$ ms).

### Table 2. Influence of Levosimendan on Aequorin Light and Twitch Characteristics

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Twitch Tension, mN/mm²</th>
<th>Aequorin Light, mV</th>
<th>Time to 50% Relaxation, ms</th>
<th>Time to 95% Relaxation, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>Levo</td>
<td>C</td>
<td>Levo</td>
</tr>
<tr>
<td>1</td>
<td>4.7</td>
<td>9.1</td>
<td>354</td>
<td>713</td>
</tr>
<tr>
<td>2</td>
<td>9.2</td>
<td>10.6</td>
<td>146</td>
<td>148</td>
</tr>
<tr>
<td>3</td>
<td>3.3</td>
<td>3.5</td>
<td>348</td>
<td>348</td>
</tr>
<tr>
<td>4</td>
<td>6.6</td>
<td>9.0</td>
<td>620</td>
<td>674</td>
</tr>
<tr>
<td>5</td>
<td>3.6</td>
<td>8.7</td>
<td>556</td>
<td>1030</td>
</tr>
<tr>
<td>6</td>
<td>8.4</td>
<td>19.7</td>
<td>280</td>
<td>676</td>
</tr>
<tr>
<td>7</td>
<td>6.2</td>
<td>8.2</td>
<td>525</td>
<td>435</td>
</tr>
<tr>
<td>8</td>
<td>12.4</td>
<td>13.4</td>
<td>1189</td>
<td>841</td>
</tr>
<tr>
<td>9</td>
<td>4.6</td>
<td>6.7</td>
<td>649</td>
<td>571</td>
</tr>
</tbody>
</table>

*Time to 50% relaxation indicates time from peak tension to 50% relaxation; time to 95% relaxation, time from peak tension to 95% relaxation; C, control; and Levo, levosimendan $10^{-6}$ mol/L.*
during control), whereas it decreased significantly, from 120±22 to 82±14 ms, with 10⁻⁴ mol/L milrinone (P<0.003).

Because the effects of levosimendan varied considerably, the effects of levosimendan and the effects of milrinone were also compared at similar inotropic responses of the different muscle strips. Therefore, muscle strips were arbitrarily divided into 2 groups, those with an increase in tension by <50% with either levosimendan or milrinone (group 1 muscles) and by >50% with either levosimendan or milrinone (group 2 muscles). In group 2 muscles, tension increased by 124±15% (by 7.1±2.1 mN/mm²) with levosimendan (n=3) and by 122±17% (by 6.7±1.8 mN/mm²) with milrinone (n=5). The increase in light with levosimendan was statistically not different from that observed with milrinone.

---

**Figure 4.** Original registrations of aequorin light signals (in mV anodal current) and isometric force signals (in mN) before and after application of milrinone. Arrow denotes time of application of milrinone 10⁻⁵ mol/L. Right, Averaged light and force signals from 50 twitches are given. Top, Registrations from a muscle strip in which isometric force increased by 77% and aequorin light emission by 69%. Bottom, Registrations from a muscle strip in which isometric force increased by 20% and aequorin light emission increased by 17%. Note that recordings were performed during concentration-response experiments; therefore, force and light signals before application of milrinone at 10⁻⁵ mol/L reflect registrations at a milrinone concentration of 10⁻⁶ mol/L.

---

**Figure 5.** Influence of levosimendan (top; n=9) and milrinone (bottom; n=9) on twitch tension (mN/mm²) and aequorin light emission (mV anodal current).

---

**Figure 6.** Effects of levosimendan and milrinone on twitch tension and aequorin light emission. Muscles from levosimendan experiments were divided into 2 groups according to their inotropic response to levosimendan 10⁻⁶ mol/L (Levo, group 1, <50% increase in force (tension); group 2, >50% increase in force). Similarly, muscles from milrinone experiments were divided according to their inotropic response to milrinone 10⁻⁵ mol/L (Mil, group 1, <50% increase in force (tension); group 2, >50% increase in force). Absolute tensions during control were 7.1±1.4 mN/mm² (Levo group 1) and 5.6±1.5 mN/mm² (Levo group 2) (P=NS) and 8.7±3.0 (Mil group 1) vs 7.3±2.9 mN/mm² (Mil group 2) (P=NS).
none (Figure 6). In group 1 muscles, tension increased by $23\pm 7\% \ (1.6\pm 0.5 \ \text{mN/mm}^2)$ with levosimendan (n=6) and by $16\pm 6\% \ (1.0\pm 0.2 \ \text{mN/mm}^2)$ with milrinone (n=4). The increase in light with milrinone was significantly higher than the change in light observed with levosimendan (Figure 6).

**Discussion**

The present study shows that (1) levosimendan exerts a positive inotropic effect in end-stage failing human myocardium; (2) the inotropic effect is associated with an increased rate of relaxation and a reduction in relaxation time; (3) pronounced inotropic effects but not moderate inotropic effects of levosimendan are associated with increased intracellular calcium transients; and (4) in case of pronounced inotropic stimulation, the increase of intracellular calcium with levosimendan is similar to the increase seen with the phosphodiesterase III inhibitor milrinone.

Concentration-response experiments indicate that the inotropic effect of levosimendan in human myocardium can be observed at concentrations of $\geq 3\times 10^{-8} \ \text{mol/L}$. There was a considerable variation between the different preparations with regard to the concentration at which the maximum inotropic effect was reached and the quantity of this effect (Figure 1). This was not due to different causes of the underlying cardiac diseases (ischemic or dilated cardiomyopathy). Furthermore, the inotropic effect in cumulative concentration-response experiments tended to be less pronounced than that observed in the aequorin experiments, in which only 2 different concentrations were used. These observations may be related to the finding that the affinity of levosimendan to troponin C decreases with increasing phosphorylation of troponin I.20 The degree of phosphorylation of troponin I may be different in the various preparations and may be increased during cumulative concentration-response experiments by a phosphodiesterase-inhibiting effect of levosimendan itself. Likewise, the observation that the inotropic effect of levosimendan was diminished at higher heart rates (see Figure 2) may be related to rate-dependent phosphorylation processes.

Interestingly, the positive lusitropic effect of levosimendan was still present at higher rates of stimulation. A positive lusitropic effect has not been observed in previous studies on various calcium-sensitizing agents, in which relaxation time was either unchanged or prolonged.13,21,22 The positive lusitropic effects of levosimendan may suggest that phosphodiesterase inhibition is the dominant mode of action of levosimendan. Phosphodiesterase inhibition by an increase of cAMP and activation of protein kinase A results in phosphorylation of phospholamban, which increases sarcoplasmic reticulum calcium uptake rate, and phosphorylation of troponin I, which decreases calcium affinity of troponin C.23 Both mechanisms favor relaxation. In addition, protein kinase A, by phosphorylation of L-type calcium channels and potentially sarcoplasmic reticulum calcium release channels, increases calcium release substantially.23,24 Inotropic effects mediated through increased cAMP are therefore associated with a pronounced increase in calcium turnover.24

Measurements of intracellular calcium with the photoprotein aequorin showed that calcium transients are not significantly increased with levosimendan when average values are considered (Figure 5). In contrast, the similar average inotropic effect of milrinone was associated with a significant increase in aequorin light emission. This comparison would suggest that calcium sensitization and not phosphodiesterase inhibition is the dominant mode of action of levosimendan. To compare the effects of both agents on intracellular calcium transients at more comparable levels of inotropic responses of the individual preparations, the muscle strips were divided into 2 groups: those muscles exhibiting a small inotropic effect (<50% increase in tension) and those exhibiting a pronounced inotropic effect (>50% increase in tension). In the latter group, the increase in aequorin light was similar and pronounced with both agents. However, in the group of muscles exhibiting small inotropic responses, the increase in calcium was significantly higher with milrinone than with levosimendan, indicating that the modes of action of the two agents are similar at pronounced inotropic effects but different at small inotropic effects. This is in accordance with previous findings of Edes et al14 and Haikala et al25 and strongly suggests that moderate inotropic effects of levosimendan result predominantly from calcium sensitization, whereas phosphodiesterase inhibition may become the dominant mode of action in more pronounced inotropic effects. One may speculate that the quantity of the inotropic response to both agents depends on the function of the cAMP system. In myocardium with impairment of the cAMP system, milrinone may be more effective as a phosphodiesterase inhibitor, whereas levosimendan, which has a weaker phosphodiesterase inhibitory action, elicits the inotropic effect predominantly through myofibrillar calcium sensitization.

With regard to the positive lusitropic action of levosimendan, it is interesting that a decrease in relaxation time was also observed in muscle strips showing only a moderate inotropic response and no rise in aequorin light emission after application of levosimendan (Table 2). This may indicate that smaller degrees of phosphodiesterase inhibition may improve relaxation without significantly influencing calcium transients. In addition, it is tempting to speculate that the novel mechanism of calcium sensitization postulated for levosimendan may be favorable for relaxation. It has been shown that binding of levosimendan to troponin C is calcium dependent.22,23 Therefore, binding of levosimendan to troponin C may be more pronounced during high systolic compared with low diastolic calcium concentrations.23 Accordingly, calcium sensitivity may be increased predominantly during high systolic calcium concentrations.

In summary, the present study in isolated failing human myocardium indicates that moderate inotropic effects of levosimendan occur without increased intracellular calcium transients and therefore result predominantly from myofilament calcium sensitization. Pronounced inotropic effects are associated with increased calcium transients, suggesting that phosphodiesterase inhibition becomes more relevant. Positive lusitropic effects are present independent of the degree of the inotropic effect. Thus, levosimendan in the lower dose range may be a promising agent for treatment of patients with chronic congestive heart failure. Of note, several other inotropic agents that showed favorable effects in experimental studies and short-term clinical trials resulted in increased
mortality of patients during long-term treatment. Therefore, whether or not levosimendan may be beneficial in the treatment of patients with heart failure can only be answered by long-term clinical trials.

Acknowledgments
This study was supported by Deutsche Forschungsgemeinschaft grant HA 1233/3-2 and by a grant from Orion-Farmos.

References
Influence of the Novel Inotropic Agent Levosimendan on Isometric Tension and Calcium Cycling in Failing Human Myocardium

Gerd Hasenfuss, Burkert Pieske, Maria Castell, Bodo Kretschmann, Lars S. Maier and Hanjörg Just

_Circulation_. 1998;98:2141-2147
doi: 10.1161/01.CIR.98.20.2141

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
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:
http://circ.ahajournals.org/content/98/20/2141

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/