Evaluation of Sympathetic Nerve Terminals With $[^{11}\text{C}]$Epinephrine and $[^{11}\text{C}]$Hydroxyephedrine and Positron Emission Tomography

Götz Münch, MD; Ngoc T.B. Nguyen, BS; Stephan Nekolla, PhD; Sibylle Ziegler, PhD; Otto Muzik, PhD; Pulak Chakraborty, PhD; Donald M. Wieland, PhD; Markus Schwaiger, MD

**Background**—The goal of the present study was to directly compare the new radiopharmaceutical agent $[^{11}\text{C}]$epinephrine (EPI) with $[^{11}\text{C}]$hydroxyephedrine (HED) through the use of PET.

**Methods and Results**—Seven healthy volunteers and 10 patients were investigated after heart transplantation. PET images of both tracers were of excellent quality in the volunteers. Values for radiolabeled metabolites (measured in percent of blood activity) at 5, 20, and 60 minutes after injection were $\approx 35\%$, $\approx 82\%$, and $\approx 86\%$ for EPI and $\approx 13\%$, $\approx 47\%$, and $\approx 78\%$ for HED, respectively. At 35 minutes, metabolite-corrected mean myocardial retention fraction of EPI ($0.235 \pm 0.022 \text{ min}^{-1}$) was significantly greater ($P < 0.01$) than that of HED ($0.142 \pm 0.012 \text{ min}^{-1}$). Corrected tracer retention fractions of both EPI and HED were significantly reduced in transplant recipients ($0.055 \pm 0.004 \text{ min}^{-1}$, $P < 0.0001$; and $0.050 \pm 0.006 \text{ min}^{-1}$, $P < 0.0001$, respectively) compared with volunteers. Normalization of retention fractions of patients with transplantation within 1 year to volunteers resulted in a value (ratio expressed in percent) of 20.6 $\pm$ 1.8% for EPI, significantly ($P < 0.03$) smaller than 27.8 $\pm$ 0.8% for HED. In patients with transplantation later than 1 year, the values were 26.0 $\pm$ 2.9% for EPI compared with 44.2 $\pm$ 5.6% for HED ($P < 0.014$).

**Conclusions**—Both tracers showed high selectivity for neuronal uptake in the heart, with a significant reduction in tracer retention in transplant recipients compared with volunteers. Compared with HED, EPI showed greater retention in volunteers and a lower retention ratio in transplant recipients, suggesting that EPI may be the superior tracer with higher sensitivity to neuronal abnormalities. Because EPI reflects neuronal uptake, metabolism, and storage, it may be more suitable for the study of neuronal integrity than HED, which primarily traces uptake-1 capacity. (Circulation. 2000;101:516-523.)

**Key Words:** tomography, emission-computed $\bullet$ heart transplantation $\bullet$ epinephrine $\bullet$ hydroxyephedrine

The importance of cardiac sympathetic nerve function under pathological conditions such as ischemic heart disease, cardiomyopathy, and heart transplantation is increasingly recognized.$^{1-3}$ PET in combination with various tracers has been used for the noninvasive assessment of sympathetic nerve integrity. The best characterized PET tracer for presynaptic sympathetic nerve terminal assessment is $[^{11}\text{C}]$hydroxyephedrine (HED).$^{6,9-15}$ HED is a false neurotransmitter with the same neuronal uptake mechanism as norepinephrine, but it is resistant to degradation by monoamine oxidase and catechol-O-methyltransferase.$^{6,16}$ Studies that involved the isolated working rat heart model have shown selective HED uptake via neuronal uptake-1$^{10}$; however, the storage and release characteristics of HED appear to differ from those of the physiological neurotransmitters. Thus, HED is mainly suited for the assessment of uptake-1 function.$^{11}$ Epinephrine (EPI) was developed as an alternative, more physiological tracer for the evaluation of presynaptic sympathetic nerve function with respect to uptake, vesicular storage, and metabolism.$^{5}$ Studies in animals have shown that EPI is avidly transported into the presynaptic nerve terminal via uptake-1 and is stored in vesicles in a similar manner as norepinephrine.$^{16}$

The purpose of the present study was to assess the suitability of EPI as an imaging agent for neuronal function in the human heart and to directly compare the myocardial kinetics of EPI with HED in the same patients. Studies were conducted in volunteers with no history of heart disease and in patients after heart transplantation.

**Methods**

**Subjects**

Seven healthy volunteers served as controls. The absence of cardiac disease was established through history, physical examination, and resting ECG.

Ten patients who had undergone heart transplantation 3.5 to 48 months earlier were studied to evaluate tracer binding in global...
cardiac denervation early after transplantation and in regional denervation several years after surgery. Five patients had heart transplantation <1 year (group A) and 5 patients had transplantation >1 year (group B) before PET studies. Medications taken by transplant recipients included prednisone (5 to 12.5 mg QD), cyclosporin (Sandimmune; 125 to 225 mg BID), azathioprine (Imuran; 75 to 250 mg BID), and acyclovir (200 to 400 mg TID). Some patients were also taking nifedipine (Procardia) and furosemide (Lasix). No signs of transplant rejection were present at the time of the study. None of the patients were taking any medication, such as tricyclic antidepressants, that could interfere with norepinephrine uptake. All subjects provided written informed consent for participation in the study.


HED was synthesized as previously described15 (specific activity >1000 Ci/mmol). Typical radiochemical and chemical purities of the tracer for injection were 95% and 98%, respectively. EPI was also synthesized as previously described4 (specific activity 900 to 2000 Ci/mmol). Radiochemical and chemical purities were 98% and 97%, respectively.

#### Data Acquisition and Analysis

Subjects were studied with the use of a Siemens whole-body scanner (ECAT 931, Siemens/CTI) with a germanium-68 ring source for transmission scans as previously described.11–14 All transplant recipients underwent both EPI and HED PET scanning; however, 1 of the 15 participants was excluded because of technical difficulties. EPI was injected intravenously over 30 seconds, and simultaneous PET image acquisition was initiated for 60 seconds. The scan sequence consisted of 15 frames: 6× 30 seconds, 2× 60 seconds, 2× 150 seconds, 2× 300 seconds, 2× 600 seconds, and 1× 1200 seconds. After 1 hour to allow for 11C decay, the exact imaging procedure was repeated with HED. ECG and blood pressure were monitored before and immediately after injection.

To correct for the contribution of [11C]-labeled metabolites in the blood activity, venous blood samples were drawn at 0, 1, 5, 10, 20, 40, and 60 minutes after the injections. The [11C]-labeled metabolites from EPI were assayed with neutral alumina Sep-Pak cartridges (Waters). Sep-Pak cartridges were rinsed with double-distilled, deionized water; then, 1 mL of the blood sample was applied to a Sep-Pak cartridge with the use of a syringe, and the cartridge was rinsed with 3× 5 mL of 1.5 mol/L Tris-EDTA buffer (pH 8.6). Metabolites were eluted from the cartridge while the intact EPI was trapped. Similarly, the more polar [11C]-labeled metabolites can be separated from intact HED with the use of a solid-phase extraction with C18 Sep-Pak (Waters) cartridges. Blood samples (1 mL) were deproteinized with acid and then adjusted to pH 6 to 7 with 2 mol/L KH2PO4. Each sample was centrifuged, and the supernatant was applied to an activated cartridge and rinsed with 3× 5 mL double-distilled and deionized water to elude labeled metabolites. The radioactivity in the eluents and in the cartridges was measured with the use of a gamma well counter. Relative concentrations of metabolites and intact tracers in blood were then calculated as a percentage of the total activity for each time point. Calculated fractions of intact tracers were plotted as a function of time. This curve was interpolated with the use of a cubic spline function, yielding intact tracer fraction at each time point in the blood time-activity curve generated from dynamic PET data. Metabolite correction was performed through the multiplication of the blood time-activity curve and the intact tracer fractions at the corresponding time points. The corrected blood time-activity curve was subsequently used as the arterial input function for the calculation of myocardial tracer retention.

A semiautomated volumetric sampling procedure was used to generate tissue and blood time-activity curves, polar maps for analysis of homogeneity of myocardial tracer distribution, and retention fractions of tracers. After decay correction, retention fraction (1/min) was calculated as:

\[
\text{Retention} = \frac{C_t(T_1;T_2)}{\int_0^T C_b(t)\,dt}
\]

where \(C_t(T_1;T_2)\) is tissue activity in the image frame between \(T_1\) and \(T_2\) (mCi/mL tissue), and \(C_b\) is blood activity (mCi/mL blood), which was integrated from time \(t=0\) to time \(T_1\).

The clearance of tracers was defined from data between 10 to 60 minutes after injection that were fitted to a monoeponential function and expressed as half-time.

Data are shown as mean±SEM, except for patient and hemodynamic data, which were expressed as mean±SD. HED and EPI retentions from individual patients were compared with the use of a paired Student’s \(t\) test, and data for transplant recipients were compared with those for healthy volunteers with the use of an unpaired Student’s \(t\) test. \(P<0.05\) was considered statistically significant.

### Results

#### Patient and Hemodynamic Data

The control group consisted of 7 male volunteers (mean age 32±5 years, mean weight 71.1±6.9 kg). The mean injected dose was 12.6±0.8 mCi for EPI and 20.4±1.3 mCi for HED. Hemodynamic data are summarized in Table 1. These subjects did not exhibit a hemodynamic response to either EPI or HED. The transplanted group consisted of 10 patients (9 men and 1 woman, mean age 51±8 years, mean weight 68.4±5.9 kg). The interval between transplantation and PET imaging was 3.5 to 48 months. The mean injected dose was 13.9±1.2 mCi for EPI and 20.7±0.7 mCi for HED. Transplanted patients showed a significant increase in heart rate and in systolic blood pressure after EPI injection (Table 1).

#### Determination of [11C]-Metabolites

Figure 1 illustrates the percentage of total blood activity that is intact tracer at 0, 1, 5, 10, 20, 40, and 60 minutes after injection.
injection of EPI and HED in volunteers and transplant recipients. Metabolite assays were performed for each patient except for 1 volunteer and 2 transplant recipients due to technical difficulties. In these individuals, the mean values from the pooled data of the respective group were used to correct the arterial input function. There were no statistically differences in the metabolite data between the transplant recipients and with volunteers. As early as 5 minutes after tracer injection, intact EPI was only approximately 65% of total blood radioactivity. After 20 minutes, <20% was intact EPI, which decreased to 14% after 60 minutes. The appearance of metabolites in blood samples was delayed for HED compared with EPI. Intact HED concentrations were 90% at 5 minutes, 50% at 20 minutes, and 25% at 60 minutes.

Tracer Distribution
Examples of PET images are given for a volunteer (Figure 2) and for a patient 37 months after transplantation (Figure 3). Both tracers gave excellent image quality showing high myocardial uptake in healthy volunteers and patterns of dramatically reduced uptake in transplant recipients. The image displayed in Figure 3 also illustrates an improvement in tracer accumulation in the anteroseptal region compared with other regions of the heart in a patient with transplantation >1 year earlier. In patients with transplant within 1 year, a lack of tracer accumulation in the myocardium was observed.

To evaluate tracer distribution homogeneity, we analyzed tracer retention in 5 myocardial regions (anterior, septal, inferior, lateral, and apical); mean values are given in Figure 4. Both tracers showed comparable tracer distribution in the septal, anterior, and lateral walls. A slight reduction was observed in the inferior wall and the apex in healthy volunteers. The difference between the inferior region and the anterior region was significant for EPI but not for HED.

Tracer Retention
Time-activity curves of tissue and blood for both HED and EPI in a volunteer and a transplant recipient are illustrated in Figure 5. There was a rapid clearance of both tracers from blood and a much slower clearance of activity from the myocardium. As expected, the calculation of tracer clearance from the myocardium resulted in a very long clearance half-time (~10 hours for EPI, ~4 hours for HED; data are given in Table 2). Although EPI showed a trend toward a longer clearance half-time compared with HED, which is consistent with the higher retention fraction of EPI compared with HED, this difference was not statistically significant due to large SDs. A decrease in tissue accumulation of both tracers was observed in transplant recipients (up to 80% in

![Figure 1. Percent total blood activity as non-metabolized tracers at various time points. Filled columns indicate healthy volunteers; hatched columns, transplant recipients.](image)

![Figure 2. Representative PET images and corresponding polar maps of EPI and HED in a healthy volunteer.](image)
denervated areas). Similarly, clearance half-times of both tracers were significantly shorter in transplant recipients than in volunteers. The clearance half-time of EPI was also longer compared with that of HED in transplant recipients.

Myocardial retention fractions of both tracers were calculated with and without metabolite correction. Retention fractions at 5, 15, and 35 minutes (representative of early, mid, and late time points, respectively) are presented in Table 3. After metabolite correction, retention fractions of both tracers in volunteers were significantly greater than retention fractions without metabolite correction at all time points ($P<0.002$).

In a comparison of myocardial retentions of both tracers in volunteers, EPI showed a higher retention than that for HED at all time points ($P<0.01$). Linear regression analysis of retention fractions for EPI versus HED revealed a significant correlation ($P<0.0001$), as shown in Figure 6.

In transplant recipients, the retention of both tracers was also calculated with and without metabolite correction (Table 3). EPI retention fractions in these patients were significantly lower than that in volunteers ($P<0.0001$). Similarly, HED retention fractions were significantly lower in transplant recipients than in volunteers ($P<0.0004$). Patients with heart transplantation were divided into 2 groups: group A (heart transplantation within $<1$ year, $n=5$) and group B (heart transplantation $>1$ year earlier, $n=5$). Although mean retention fractions over the entire myocardium were not significantly different between the 2 groups, there was a significant increase in HED retention in the anterior ($P<0.03$) and septal ($P<0.03$) regions in group B compared with group A. An increase was also found in the lateral wall ($P<0.04$), although it was not as visibly apparent as in the anteroseptal regions. Although differences between the 2 transplant groups in the retention fractions of EPI in the anteroseptal walls did not reach statistical significance, there was an apparent trend toward an increase in group B compared with group A.

To evaluate the specificity of the tracers, retention fractions from whole myocardium and from the anterior wall of transplant recipients were normalized to retention fractions of volunteers, giving ratios of retention fractions expressed as a percentage (Figure 7). The transplant-to-normal ratios for EPI retention in group A, a model of complete denervation (18.9±1.0%), was lower than the ratio in group B (30.4±6.7%) in the anterior wall and in the entire myocardium. However, this difference was not statistically significant. Retention ratios for HED was significantly lower in group A (29.1±6.7%) than in group

![Figure 3. PET images and corresponding polar maps of EPI and HED in a patient who underwent heart transplantation 37 months before PET studies.](http://circ.ahajournals.org/)

![Figure 4. Mean retention fractions in 5 myocardial regions in a polar map display for EPI and HED in healthy subjects, group A (transplantation $<1$ year), and group B (transplantation $>1$ year). $P<0.03$ vs group A.](http://circ.ahajournals.org/)
B (62.6±11.6%), especially in the anterior wall. In addition, transplant-to-normal ratios of HED were significantly higher than those of EPI in group A, and the difference was even greater in group B.

**Discussion**

This study presents the application of 11C-labeled EPI as a new PET tracer for the noninvasive assessment of presynaptic sympathetic neuronal function in direct comparison with the previously developed tracer, HED. Similar to HED, EPI showed excellent myocardial uptake in healthy volunteers (Figure 2) and significant reduction in tracer accumulation in patients after heart transplantation (Figure 3).

The data showed a significant correlation between the retention fraction of EPI and HED. Decay corrected time-activity curves of both tracers (Figure 5) displayed high tissue retention with rapid blood clearance. The results of metabolite analysis in blood samples demonstrated the importance of the correction for 11C-metabolites to prevent underestimation of the calculated myocardial retention of tracers.

Neither tracers showed the pharmacological effects in volunteers. EPI injection, however, caused a significant increase in heart rate and systolic blood pressure in transplant recipients. These hemodynamic alterations may be the consequence of a decrease in the clearance of EPI from the synaptic cleft due to a sympathetic presynaptic dysfunction or to a postsynaptic receptor supersensitivity in patients with heart transplants.17,18

**Metabolite Correction**

In contrast to HED, EPI is sensitive to neuronal metabolism. Rapid degradation of EPI, producing 11C-metabolites in blood, may affect the interpretation of kinetics data, because myocardial tracer retention values are normalized to the integral of the arterial input function. Thus, without the correction for metabolite activity in the arterial input function, the calculated myocardial tracer retention fractions are underestimated. Although HED is not susceptible to neuronal metabolic degradation, it is metabolized by liver tissue, producing 11C-metabolites (Figure 1), albeit much slower than EPI.

The nature of the assay used to detect metabolites in this study does not identify the types of metabolites. It was designed to allow fast clinical application, especially with the short half-life of 11C (20 minutes). For the purpose of correction of the arterial function, however, it is only important to determine the fraction of 11C-labeled metabolite. It is not necessary to identify the metabolites themselves, assuming that they are not substrates for neuronal uptake. In a study with HED in rats, heart tissue was examined, and no significant metabolite accumulation was detected.15 In another study, HPLC analysis of rat heart 5 minutes after injection of EPI detected ≈16% of radioactivity in the form of metanephrine, a metabolite of EPI. However, <2% was detected 30 minutes after injection.16 Because metabolite concentrations detected in blood samples were similar among transplant recipients

<table>
<thead>
<tr>
<th>TABLE 2. Tissue Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>EPI, h</td>
</tr>
<tr>
<td>HED, h</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.05 vs healthy subjects.

†P<0.05 vs EPI.
and healthy volunteers in this study, it is not possible to speculate on the modulation in monoamine oxidase and catechol-O-methyl-transferase activity in transplant recipients compared with volunteers.

**Tracer Homogeneity**

It has been reported that myocardial tracer distribution of HED is dependent on perfusion, but volunteers with no signs of cardiac disease were assumed to have normal perfusion. Therefore, PET perfusion studies were not performed. Nevertheless, data from healthy volunteers showed homogeneous myocardial tracer distribution with a slight reduction in accumulation in the inferior and apical regions of the heart. These regional differences in sympathetic innervation have also been described in studies with the use of MIBG. The difference in retention between the inferior wall and the anterior wall was statistically significant for EPI in uptake in the inferior wall, especially in older male patients. It was hypothesized that the greater retention of EPI compared with HED in the detection of subtle changes in myocardial sympathetic innervation. Further studies are necessary to determine whether age and gender also affect the myocardial distribution of EPI.

### Table 3. Myocardial Retention Fractions With and Without Metabolite Correction

<table>
<thead>
<tr>
<th></th>
<th>EPI</th>
<th>HED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy Volunteers</td>
<td>Transplant Recipients</td>
</tr>
<tr>
<td></td>
<td>Without correction</td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>0.295±0.023</td>
<td>0.075±0.006*</td>
</tr>
<tr>
<td>15 min</td>
<td>0.176±0.016</td>
<td>0.044±0.003*</td>
</tr>
<tr>
<td>35 min</td>
<td>0.103±0.029</td>
<td>0.026±0.002*</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.001 vs healthy subjects.

**Tracer Uptake and Storage**

Evaluations of HED and EPI kinetics in the isolated rat heart revealed selective neuronal uptake via neuronal uptake-1 in that inhibition by desipramine (DMI) resulted in significant blockade of tracer uptake of both tracers. The significantly reduced tracer accumulation in patients who had recently undergone heart transplantation confirms the selectivity of EPI and HED for neuronal uptake. Moreover, the significant correlation between EPI and HED retention fractions further illustrates the similarities between EPI and HED as neuronal markers.

Despite similarities, retention fractions were higher with a longer clearance half-time for EPI than for HED in healthy subjects, suggesting a more efficient retention mechanism for EPI than for HED. Experimental findings in rats showed that the uptake of 3H-EPI was 4-fold higher compared with 3H-metaraminol, which is another catecholamine analog that is structurally similar to HED. One explanation for the higher uptake of EPI compared with its analog is a substrate preference for the physiological tracer, EPI.

Corticoids used as medication for immunomodulation in patients with heart transplantation may be inhibitors of uptake-2 (extraneuronal uptake). Thus, it has been suggested that the greater retention of EPI compared with HED in volunteers, which was not observed in transplant recipients, may reflect greater extraneuronal uptake of EPI in the normal heart. It has been shown that EPI has a greater capacity for extraneuronal uptake compared with norepinephrine in the isolated rat heart, but uptake-2 capacity may differ between animals and humans. Studies in isolated rat hearts showed that both EPI and HED exhibited a relatively small and limited amount of extraneuronal accumulation with DMI, which was reversible as demonstrated by the rapid clearance of both tracers with DMI.

It is important to consider the role of extraneuronal uptake in the kinetics of the tracers, especially under various pathological conditions in which uptake-1 is altered. However, extraneuronal “uptake” is not likely to be the reason for the differences in tracer retention observed between EPI and HED, especially because extraneuronal “retention” is unlikely to contribute to the tracer retention at a late time point.

A major difference between EPI and HED is the neuronal retention mechanism. De Grado et al demonstrated that HED...
can be displaced from the myocardium by DMI in the rat heart. These data suggest that HED is not avidly stored but instead undergoes continuous release and reuptake,10 because the highly lipophilic nature of the HED molecule may allow its passive diffusion across the neuronal plasma membrane. EPI was shown in the same experimental model to be more avidly retained by the myocardium and cannot be displaced by DMI,16 suggesting greater neuronal vesicular storage and better neuronal retention of EPI compared with HED. The dependency of myocardial retention of EPI on vesicular storage was confirmed with the use of the vesicular blocker reserpine. Pretreatment with reserpine showed inhibition of EPI retention by the heart. Although neuronal uptake of EPI is similar to the uptake of HED with respect to its high specificity for the uptake-1 carrier, EPI appears to be a marker for vesicular storage. Therefore, the dependency of HED retention on the reuptake process versus the stable vesicular storage of EPI is more likely the reason for the higher retention of EPI compared with HED in volunteers.

Tracer Retention in Transplanted Heart

The retention of both EPI and HED in the anteroseptal wall in group A (<1 year transplantation) was lower compared with group B (>1 year transplantation), which is in concordance with previously published data suggesting regional and partial recovery in the transplanted heart.14,21,22 The significantly greater retention of HED in the anterior wall of group B compared with group A suggests a partial improvement in patients with earlier heart transplantation. A recent longitudinal study by Bengel et al.,27 who used serial HED-PET studies to evaluate 20 patients (initial PET study at 0.2 to 12 years after heart transplantation, follow-up study 2.4 to 4.2 years later), reported evidence of a time-dependent, progressive reinnervation. EPI retention fractions in the present study also showed an increasing trend in group B compared with group A, but this increase was smaller than the increase observed with HED. In addition, the transplant-to-normal retention ratios of EPI were consistently smaller than those of HED. This difference may indicate that EPI is more sensitive than HED to sympathetic neuronal abnormalities. Furthermore, the difference in the retention ratios between EPI and HED was even greater in group B than in group A, showing a faster recovery in tracer accumulation of HED compared with EPI. An explanation for this observation is a faster improvement in uptake-1 function as shown with HED and a slower recovery of neuronal vesicular storage capacity as reflected with the use of EPI in the transplanted heart. However, this interpretation is only speculative, and studies in a larger patient population are necessary to delineate the integrity of the uptake-1 function and the neuronal vesicular storage in the transplanted heart.

Conclusions

In this study, we presented the first clinical application of the use of EPI as a PET tracer. The data showed that EPI is specific for neuronal uptake. EPI exhibited high myocardial uptake with fast blood clearance, similar to HED, providing excellent image quality. Although both tracers showed comparable qualitative visualization of sympathetic neuronal integrity, the mechanisms in the retention of EPI and HED appear to differ. Neuronal uptake and retention of HED are mediated by the uptake-1 function and a reuptake process. Although neuronal uptake of EPI is also uptake-1 dependent, its retention is dependent on vesicular storage and metabolism. EPI appears to be a superior tracer compared with HED for the study of neuronal integrity.

References

Götz Münch, Ngoc T. B. Nguyen, Stephan Nekolla, Sibylle Ziegler, Otto Muzik, Pulak Chakraborty, Donald M. Wieland and Markus Schwaiger

_Circulation_. 2000;101:516-523
doi: 10.1161/01.CIR.101.5.516

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 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/101/5/516

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/