Magnetic Resonance Imaging of Chronic Myocardial Infarcts in Formalin-Fixed Human Autopsy Hearts

Jordan C.M. Hsu, MD; G. Allan Johnson, PhD; William M. Smith, PhD; Keith A. Reimer, MD, PhD; Raymond E. Ideker, MD, PhD

Background In post-myocardial infarction patients, three-dimensional structure of the infarct as well as infarct size are likely to be important factors affecting mortality, cardiac function, and arrhythmias. Current morphological methods for determining three-dimensional infarct structure in autopsied hearts are inexact and time consuming. The cardiac magnetic resonance imaging techniques used in living patients have shown potential in determining infarct size and structure but have limited resolution for morphometric postmortem studies. The recent development of magnetic resonance microscopy raises the possibility that three-dimensional infarct structure can be quantified at microscopic levels in autopsied hearts. The purpose of this study was to determine the ability of magnetic resonance imaging at different spatial resolutions to differentiate infarcted from noninfarcted myocardium.

Methods and Results Magnetic resonance imaging was performed at 2.0 T on cross sections taken from 10 autopsied hearts containing old myocardial infarcts. T1 was derived from six images with repetition times (TRs) for each image ranging from 100 to 3200 milliseconds. T2 was derived from multiecho images with echo times (TEs) ranging from 10 to 60 milliseconds. Resolution was approximately 400×400 μm in 2-mm-thick slices. Sites of infarcted and noninfarcted tissue were identified from histological sections taken from each slice, and the T1 and T2 values of these sites were obtained. Microscopic images were acquired with voxels of 100×100×625 μm, representing tissue volumes more than 1000-fold smaller than conventional clinical images. In all cases, T1 of infarcted tissue (459±266 milliseconds, mean±SD) was greater than that of noninfarcted tissue (272±163 milliseconds). Also, in all cases, T2 of infarcted tissue (49±14 milliseconds) was greater than that of noninfarcted tissue (35±8 milliseconds).

Conclusions T1 and T2 values for infarcted tissue are significantly different from those of noninfarcted tissue (P<.001). Based on these findings, it should be possible to develop techniques to perform three-dimensional imaging and quantitation of infarcts with a resolution of 400 μm or less. When volumetric three-dimensional imaging was performed using a T1-weighted sequence, the resulting 2563 arrays supported isotropic resolution at 400 μm (voxel volume, 0.064 mm3). Subsequent volume rendering using a compositing algorithm clearly shows the infarcted areas in three dimensions. The techniques demonstrate the potential for quantitative three-dimensional cardiac morphometry using magnetic resonance imaging. (Circulation. 1994;89:2133-2140.)

Key Words • infarction • magnetic resonance imaging • morphometry

Myocardial infarct size has been shown to be an important prognostic factor affecting mortality, cardiac function, and subsequent arrhythmias. Current morphological methods of postmortem infarct sizing, including subjective estimation, weighing of infarct photomicrographs, determination of circumferential infarct extent, point counting, and computer-assisted planimetry, are inexact or time consuming. In addition to infarct size, it is also likely that the three-dimensional structure of the infarcted and surrounding noninfarcted tissue influences mortality, cardiac function, and arrhythmias. Very little is known about the three-dimensional structure of myocardial infarcts in autopsied hearts because the morphometric techniques to determine it are much more tedious and time consuming than those of just estimating infarct size. Thus, the development of imaging modalities that can determine the anatomic structure of an infarct in a relatively short amount of time is highly desirable. Magnetic resonance microscopy raises the possibility that three-dimensional infarct structure can be quantified at microscopic levels if the technique can detect infarcted tissue in postmortem hearts. The goal of this study is to evaluate different methods for differentiation of infarcted and noninfarcted tissue in formalin-fixed, human heart autopsy specimens using magnetic resonance imaging techniques.

Methods

Patient Selection

Ten deceased patients whose hearts were examined by the Duke University Cardiovascular Pathology Laboratory between November 1982 and February 1991 were included in this study. All had a clinical history of myocardial infarction of 6 months or longer before death and a fibrotic infarct found in the heart during pathological examination.

Heart Preparation

The standard protocol of the Duke Cardiovascular Pathology Laboratory was followed in the examination of all the

Received September 30, 1993; revision accepted December 3, 1993.

From the Departments of Pathology (J.C.M.H., K.A.R., R.E.I.) and Medicine (W.M.S., R.E.I.), Duke University School of Medicine, Duke University Medical Center; Center for In Vivo MR Microscopy (G.A.J.), Duke University Medical Center; and Engineering Research Center for Emerging Cardiovascular Technologies of the School of Engineering (G.A.J., W.M.S., R.E.I.), Duke University, Durham, NC.

Correspondence to William M. Smith, PhD, Box 3140, Duke University Medical Center, Durham, NC 27710.
hearts. This protocol consisted of (1) injecting the coronary arteries with micropaque gelatin, (2) photographing and radiographing the entire heart, (3) examining the coronary arteries every 5 mm and noting the degree of narrowing, (4) fixing the heart by immersion in 3.7% buffered Formalin solution for at least 2 days, (5) slicing the ventricles transverse to their long axis into five or six cross sections, each approximately 2 cm thick, and (6) photographing and radiographing the cross sections. The hearts in this study had been stored in 3.7% buffered Formalin for 1 to 9 years after this examination. One cross section was selected for magnetic resonance scanning by gross inspection based on the presence of a solid, transmural infarct in one portion of the cross section and the presence of noninfarcted myocardium in another portion of the cross section. It was required that the cross section contain a volume of scar large enough that two areas within solid infarct could be selected for relating magnetic resonance imaging data to anatomic characteristics. All hearts for which these criteria were met were included in the study. Before scanning, each cross section was rinsed with water for 10 minutes and patted dry. The right ventricle was removed from each specimen so that the cross section would fit into the 10-cm-diameter bore of the radiofrequency coil. The heart cross section was next placed into a 100 x 15-mm polystyrene disposable petri dish into which holes were drilled to accommodate eight CuSO₄ solutions with known T1 and T2. These CuSO₄ standards were approximately 53 mm in length and 5 mm in diameter. The petri dish was filled with bovine agar (10 g/100 mL H₂O) that was allowed to gel. Fig 1 is a schematic drawing of the sample preparation.

All scans were performed on a GE 2.0-T model CSI Omega system dedicated to magnetic resonance microscopy, using a 10-cm birdcage radiofrequency coil. Single (two-dimensional) transverse image slices, 2 mm thick, were taken of the left ventricle cross section. For regions selected as corresponding to histologically identified infarcted and viable tissue, the spin-lattice relaxation time, T1, was calculated from six images with repetition times (TRs) of 100, 200, 400, 800, 1600, and 3200 milliseconds, respectively, using a least-squares fit to the following equation:

\[ S = N(H)[1 - (1 + f)e^{-(TR/T1)}] \]

(1)

where S is the measured signal, N(H) is the proton density, and f is the negative cosine of the flip angle, which accounts for variations in local radiofrequency fields. T1, N(H), and f are the fitted parameters. Spin-spin relaxation time, T2, was calculated from multiecho images with echo times (TEs) of 10, 20, 30, 40, 50, and 60 milliseconds using a least-squares fit to the following equation:

\[ S = N(H)e^{-(TE/T2)} \]

(2)

All scans required two signal excitations in a 256³ voxel matrix with a field of view of 100 mm. Imaging times ranged from 2 to 54 minutes.

An additional cross section from one of the hearts, 20 mm thick, was imaged using 16 signal excitations per view in a 1024³ voxel matrix with a TR of 200 milliseconds, a TE of 10 milliseconds, a resolution of approximately 100 µm, and a field of view of 100 mm. Six Teflon tubes were positioned in an area of old infarct in this heart cross section (four perpendicularly through the slice and two diagonally through it) to aid in the alignment of the image slice with the histological sections described below.

Two additional infarcted human hearts were imaged while they were still intact, before they were cross sectioned. Three-dimensional images were acquired with routine three-dimensional Fourier encoding (3DFT). For these images, the acquisition array size was expanded to a 256³ volumetric array that permitted isotropic encoding at 400-µm resolution (0.064 mm³ voxel volume). Because the data are isotropic, resolution is maintained in all planes; thus, it was possible to view the volume data set from any direction or through any plane without loss of spatial resolution.

**Histological Examination**

Verification of infarcted and noninfarcted tissue was performed by microscopic examination of histological sections. For cross sections scanned in the 256³ matrix, two regions of solid infarct and two regions of noninfarcted myocardium, each approximately 1 mm³ in area, were identified. Histological sections through the depth of the cross section were taken at 500-µm intervals from the infarcted region and at 1-mm intervals from the noninfarcted region. The sections were stained with Masson stain. The areas in the magnetic resonance images that corresponded to the regions of infarcted and noninfarcted tissue in the histological sections were identified by comparing and aligning the cardiac anatomic features in the sections and the images. The T1 and T2 relaxation times for the two infarcted regions were compared with those of the two noninfarcted regions in each cross section. For the heart scanned in a 1024³ matrix, step sections were taken throughout the cross section at 100 µm and at 1-mm intervals for the infarcted and viable tissue, respectively. The histological slides were then matched with the magnetic resonance images as before.

**Three-dimensional Display**

Volume images were constructed on a Silicon Graphics VGX320 workstation using Voxel View Ultra (Vital Images). The software uses a compositing algorithm that allows interactive volume rendering with an adjustable transparency-opacity mapped to the signal intensities of all voxels in the volume. In this case, signal intensity corresponding to infarcted tissue (long T1) was mapped to a low transparency,
thus appearing bright in the resulting volume-rendered images.

Statistical Analysis

Statistical significance comparing mean T1 and T2 values for infarcted and noninfarcted myocardium was calculated using Student's paired t test. A value of \( P < .05 \) was considered statistically significant.

Results

Infarcted tissue could be differentiated from normal in both T1- and T2-weighted images (Fig 2). The longer T1 of infarcted tissue results in reduced signal in the T1-weighted image. The longer T2 results in brighter pixels in the T2-weighted image. The two images are at the same level of one of the specimens included in the study. The images were acquired without moving the specimen, so that they are directly comparable. The difference between infarcted and viable tissue is more easily visualized in the T1 image than in the T2 image. On the T1-weighted image, there is an island of viable tissue visible between the orientation holes.

Fig 3A is a 1024\(^2\) T1-weighted image from a 625-\(\mu\)m slice cross section adjacent to that shown in Fig 2. The orientation holes and the structure of the infarct are much more easily appreciated than in the lower-resolution image. The shading in the image arises from \( B_1 \) inhomogeneity in the coil. Fig 3B is a magnified portion of the slice in Fig 2A, with arrows pointing toward the orientation holes. Fig 3C is a histological section chosen to match the orientation and level of the images in Fig 3A and 3B. The slice has been stained with Masson stain, so that the infarcted tissue is light and the viable tissue is dark in the photomicrograph. The match between the magnetic resonance image and the photomicrograph is well within the limits imposed by distortion of the tissue caused by dehydration, paraffin infusion, and sectioning and by the fact that the histological section is in a plane slightly rotated with respect to the magnetic resonance image plane. Fig 3 illustrates that magnetic resonance imaging, at least at higher resolutions, can qualitatively distinguish between infarcted and viable tissue.

Fig 4 shows the values of T1 (Fig 4A) and T2 (Fig 4B) for each patient in the study. The values are those derived from the fits to Equations 1 and 2. The error bars are the standard deviations of the T1 and T2 values as estimated from the range of the two measurements\(^{14} \) for each heart in each tissue type. The precise and accurate measure of T1 and T2 from magnetic resonance images is plagued by systematic errors arising from variations in radiofrequency excitation, field homogeneity, gradient effects, and noise in the images. The standards in each image returned T1 and T2 values that varied less than 10% over all of the studies, ensuring that the T1 and T2 calculations are precise to this degree. The "true" relaxation times were measured using spectroscopic (nonimaging) techniques. The T1 and T2 values measured from the images agreed with the spectroscopic measures within the 10% precision, ensuring this level of accuracy.

In every case, T1 and T2 were longer for infarcted tissue than for noninfarcted tissue. This fact provides a basis for developing algorithms for automatically distinguishing between the two tissue types. The Table shows the mean and SD values of the T1 and T2 values of the two areas of noninfarcted myocardium and two areas of infarcted myocardium for all 10 of the patients included in the study. The values of T1 and T2 were significantly longer for fixed, infarcted tissue than for fixed, noninfarcted tissue.

Fig 5 is a plane from the three-dimensional magnetic resonance image of one of the intact hearts. This is a sagittal view, looking from the left ventricle to the right ventricle, at the level of the aortic root and the posterior papillary muscle, which are clearly seen. Again, the overall signal intensity variation is due to \( B_1 \) inhomogeneity in the coil. In this T1-weighted image, the infarct is visible as a dark streak in the posterior left ventricular wall. There is viable tissue in both the endocardium and the epicardium on either side of the infarct. Fig 6 is a ray-traced three-dimensional rendering of the T1-weighted image of the same heart shown in Fig 5. In this figure, the gray scale has been inverted so that the infarcted tissue is lighter than the viable myocardium. The image is rendered by assigning opacities to each voxel that is related to the signal strength in that voxel. The left ventricular cavity is visible as a light gray structure within the much darker myocardial wall, and the light infarct is in the posterior left ventricle. The view in the upper left corner is approximately postero-inferior, and successive frames are rotated by 20° in a counterclockwise direction when viewed from the base to the apex. The lower right panel shows the heart facing the left ventricular free wall.

For the three-dimensional encoding schemes used here, the total acquisition time is determined by the number of excitations per view (two), the number of phase encoding steps along two of the dimensions, and TR:

\[
\text{Total Time} = 2 \times 256 \times 256 \times \text{TR}
\]

Thus, the short TR used for the T1-weighted images (TR=200 milliseconds, TE=10 milliseconds) in Figs 5 and 6 allows the acquisition of the three-dimensional data set in 8 hours. The signal-to-noise ratio in the image is clearly adequate to allow much shorter acquisition. Because these scans were acquired overnight, there was no need to shorten the acquisition time.

Comparison of the magnetic resonance image and the histological sections in the heart slice scanned with a 1024\(^2\) matrix indicated that infarcted and noninfarcted tissue could also be detected with a resolution of approximately 100 \( \mu\)m (Fig 3). Images of the intact hearts indicated that the fine detail of infarct structure could be seen in the intact heart (Fig 5) and that the images could be manipulated to allow appreciation of the three-dimensional structure of the infarct (Fig 5). Because of the improved contrast between tissue types and the reduced acquisition time, T1-weighted images were superior for detecting old myocardial infarcts in Formalin-fixed cardiac tissue.

Discussion

This study compared T1 and T2 relaxation time characteristics of infarcted and noninfarcted myocardium in Formalin-fixed human hearts. T1 and T2 values of infarcts were from 1.3- to 2.5-fold and from 1.1- to 1.8-fold greater than those of viable tissue, respectively. The significant difference found in relaxation times
Fig 2. A, T1-weighted image of a slice from one of the 10 hearts. Infarcted tissue with longer T1 values than noninfarcted tissue is clearly seen. The image was obtained at 85 MHz with a resolution of 256^2. The number of excitations was two, repetition time was 200 milliseconds, and echo time was 10 milliseconds. Light circles to the left of the heart slice are CuSO_4 standards. B, T2-weighted image of the same slice shown in A. Infarcted tissue is less well seen. Repetition time was 1600 milliseconds.
allows identification of the three-dimensional location and extent of old infarcts in postmortem magnetic resonance studies, particularly in T1-weighted images as illustrated in Figs 5 and 6.

Current methods of quantifying myocardial infarcts in autopsied hearts are time consuming and tedious. All current methods require cutting the heart into a number of cross sections by slicing the heart perpendicular to its long axis. In one method, a series of infarct estimations is made from the cut surface of each slice and finally from the entire heart by summing the amount of infarct in all slices. This is achieved by estimating the gross amount of infarct in each cross section and recording this estimate on diagrams of different cross sections of the ventricles. Each cross section is next cut into six blocks from which histological sections are taken and microscopically examined to improve accuracy of infarct estimation on the cross-sectional diagrams. The amount of infarct is then estimated to the nearest 25% on the corrected diagrams. The percent infarct of the total ventricular mass within a given segment is determined by multiplying the percent infarct in that segment by the...
average amount of ventricular mass previously found to be represented by that segment.

Infarct estimation using photomicrographs involves cutting each photomicrograph into infarcted and noninfarcted parts and weighing the respective parts to the nearest milligram. The percent infarction is determined by dividing the weight of the infarcted portion by the weight of both portions.

Point counting as a method of infarct estimation involves counting the number of evenly spaced points on a grid placed over infarcted and noninfarcted myocardium. Again, the percent infarct is determined by dividing the number of points over the infarcted area by the number of points over infarcted and noninfarcted areas.

Computer-assisted planimetry involves the use of a digitizing pen to trace ventricular epicardial and endocardial outlines and borders of infarcted regions from enlarged photographs of the top cut surface of each cross section. Areas within each outline are calculated, and the area of ventricular muscle is taken as the epicardial area outline minus the endocardial outline. The amount of infarct in a slice is calculated by dividing the area of infarcted tissue by the calculated area of ventricular muscle. Total percent of infarct is calculated using the percent infarct of the top of each cross section and the volume of each slice.

Although all of these techniques furnish estimates of infarct size, they all include errors introduced by tissue shrinkage during dehydration and paraffin infusion dur-

Mean and SD Values of T1 and T2 of Noninfarcted and Infarcted Tissue From the 10 Specimens Included in Study

<table>
<thead>
<tr>
<th>T1</th>
<th>Infarcted Tissue</th>
<th>Noninfarcted Tissue</th>
<th>T2</th>
<th>Infarcted Tissue</th>
<th>Noninfarcted Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, milliseconds</td>
<td>459</td>
<td>272</td>
<td>49</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>SD, milliseconds</td>
<td>266</td>
<td>163</td>
<td>14</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>
ing preparation of the histological slides. Furthermore, only the computer-assisted techniques allow visualization of the heart and infarct geometry.

In contrast to the limitations found in the present techniques for quantifying infarcts just discussed, magnetic resonance imaging has the ability to provide direct visualization of the three-dimensional anatomic extent of old infarcts postmortem through T1 and T2 displays. It can also show heart geometry including, for example, regional wall thinning caused by infarction. Another advantage of magnetic resonance imaging is its ability for three-dimensional reconstruction, which allows detection and display of infarcts and other heart structures with a resolution as high as 100 μm as shown in Fig 3. In addition, the ability to view the heart image from multiple angles, including transverse (Fig 3A), axial, sagittal (Fig 5), and oblique, as well as the ability to serially change the spatial orientation in steps (Fig 6) allows visualization of the heart and infarcts from any direction. Because magnetic resonance microscopy is a noninvasive technique, it has the added advantage of being nondestructive. Thus, at the conclusion of magnetic resonance imaging, the heart remains intact so that histological sections, counting of radio-labeled microspheres, or other diagnostic procedures can still be performed. Magnetic resonance techniques for quantifying myocardial infarct size are also considerably less time consuming and tedious than current histological methods. A scan of a single slice of the type shown in Fig 2 requires a total acquisition time of 1.7 minutes, whereas a three-dimensional volumetric scan of an entire heart consisting of 256 such slices would entail a total acquisition time of 7.3 hours. Recent improvements in pulse sequences using fast-spin echoes can easily reduce the acquisition to less than 1 hour.

Previous clinical studies in cardiac ischemic disease have used magnetic resonance to image acute and old myocardial infarcts in living patients. Resolution is limited in such studies because of motion caused by the beating heart and by respiration and because of interference from the thoracic cage. In dogs, magnetic resonance analysis reveals increased T1 and T2 relaxation times in acutely infarcted myocardium compared with viable muscle.20-22 These increased relaxation times are most likely due to interstitial edema.20-22 Analysis of old infarcts has shown decreased signal intensity, ventricular wall thinning, and shorter T2 values of infarcts (28.7±9.9 milliseconds) than of noninfarcted myocardium (45.4±9.9 milliseconds) in living patients.23 T2 values return to values similar to that of noninfarcted myocardium as long as 7 months after infarction, particularly in anterior infarcts.24 The low-intensity and decreased T2 values observed in patients with a history of old infarction are consistent with the explanation that those areas represent myocardium that underwent substantial replacement by fibrous scar. Explanations for prolonged or normal T2 elevation in some of the infarcts, including cellular infiltration, T2 changes nonspecific for irreversibly injured myocardial tissue, or infarcted areas containing residual existing myocardium, are speculative as no histological evaluation could be performed in these studies.

Short-term magnetic resonance studies of fixation with 10% formaldehyde of various tissues, including rat muscle, liver, and spleen and human brain, have shown consistently that T1 values decrease with the duration of fixation.25-27 These studies also showed an initial increase in T2 values with formaldehyde fixation, which decreased with time. However, T1 and T2 measurements were carried out to only 48 hours after fixation, whereas the hearts in this study had been fixed for at least 1 year.

T1 and T2 values change as tissue structure and molecular mobility change.26 Formalin may form covalent and ionic links within tissues, particularly with amino groups.25 This process may cause denaturation of protein, dehydration of tissue, and restriction of molecular mobility. Thus, fixation of tissue may lead to structural changes, which may result in dissipation of energy from proton spins and, subsequently, decrease T1 and T2 times.25

The data in this study show a wide range of relaxation times for both noninfarcted and infarcted tissue among different samples, probably reflecting a number of variables, including fixation technique, length of storage, specimen age, and biological variability.28 The most significant aspect of the T1 and T2 data, however, is that despite this wide variation, infarcted tissue consistently had longer T1 and T2 values than did noninfarcted tissue. Thus, the ability to differentiate infarcted from noninfarcted tissue was preserved regardless of the specimen age or the specimen preparation. The optimal threshold values for T1 and T2 for distinguishing between infarcted and noninfarcted myocardium can be determined by examination.
of the image histogram or by histological verification in a few representative sections.

It is possible that the injection of micropaque gelatin into the coronary arteries of the hearts in the study could have affected the relaxation times measured. However, because micropaque is not paramagnetic, its effect would be at the bulk level. That is, the difference in micropaque and Formalin in the vascular spaces would constitute the source of changes in T1 and/or T2. As the vascular spaces constitute less that 3% to 5% of the total volume in any tissue element, a 40% change in T1/T2 of this space translates to less than a 10% to 15% change in overall T1/T2. Therefore, it is unlikely that this effect influenced the results appreciably.

Significance and Application

Magnetic resonance microscopy may become a valuable tool in the three-dimensional reconstruction of the morphology, geometry, and size of infarcted and noninfarcted tissue in Formalin-fixed hearts. Not only should it be less tedious and time consuming than present postmortem infarct-quantitating techniques, but magnetic resonance microscopy also may add increased resolution and noninvasiveness to infarct measurement.

Most previous morphological studies of autopsied hearts have concentrated on identifying the relation of infarct size with cardiac function and mortality. Other infarct characteristics, such as circumferential extent, transmural extent, dimensions of any spared subendocardial or subepicardial myocardium, amount and location of infarcted tissue interdigitated with viable muscle (patchiness of the infarction), and irregularity of the infarct borders, may also significantly affect cardiac function, mortality, and the incidence and type of arrhythmias. Examples of studies that could be performed using this technique include determining if a consistent, prototypical morphology exists in patients at risk for ventricular arrhythmias and determining the effect of reperfusion on infarct size and structure.

Acknowledgments

This work was supported in part by National Institutes of Health research grants HL-42760, HL-44066, HL-28429, HL-33637, and RR-05959 and The National Science Foundation Engineering Research Center Grant CDR-8622201. We acknowledge Gary Cofer, Steve Suddarth, and Mark Martin for assistance in magnetic resonance scanning and image reconstruction and Pat Cruz and Tim Young for assistance in collecting and preparing the autopsied hearts. This article was based in part on material presented at the 15th Annual International Conference of the Engineering in Medicine and Biology Society of the IEEE.

References

Magnetic resonance imaging of chronic myocardial infarcts in formalin-fixed human autopsy hearts.
J C Hsu, G A Johnson, W M Smith, K A Reimer and R E Ideker

_Circulation_. 1994;89:2133-2140
doi: 10.1161/01.CIR.89.5.2133

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 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/89/5/2133

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/