Successful and Reproducible Myocardial Opacification During Two-dimensional Echocardiography From Right Heart Injection of Contrast

Flordeliza S. Villanueva, MD; William P. Glaasen, PhD; Jiri Sklenar, PhD; Ananda R. Jayaweera, PhD; and Sanjiv Kaul, MD

Background. Myocardial contrast echocardiography currently involves intra-arterial injection of contrast. For this technique to have a broader application, it is necessary that myocardial opacification be achieved from a venous injection of contrast.

Methods and Results. To achieve myocardial opacification after right-side injection of contrast, two groups of open-chest anesthetized dogs were studied. Group 1 included nine dogs in whom microbubbles of various sizes, concentrations, and volumes were injected into the left atrium to determine microbubble characteristics that influence myocardial opacification. Group 2 included eight dogs in whom the effect of the combination of microbubble characteristics and myocardial blood flow on myocardial opacification was evaluated after right atrial injection of contrast. Background-subtracted time-intensity plots were generated from the myocardium to measure peak videointensity. In the group 2 dogs, digital subtraction and color coding were used to further highlight the contrast effect. The number, concentration, and size of the microbubbles all independently affected (p<0.01) peak myocardial videointensity after left atrial injection of contrast on multivariate analysis. Highly concentrated microbubbles (4.4 to 5.1 billion/ml) given during dipyridamole-induced coronary hyperemia was most frequently (88%) associated with myocardial opacification after right atrial injection of contrast and was the best predictor of this result on multivariate analysis (χ²=9.01, p=0.003). No changes were noted in left atrial, left ventricular, and pulmonary artery pressures despite injection of large numbers of microbubbles into the right atrium.

Conclusions. Successful and reproducible myocardial opacification can be achieved during myocardial contrast echocardiography after right atrial injection of contrast. These findings could have far-reaching implications in the use of myocardial contrast echocardiography in acute and chronic ischemic syndromes in humans. (Circulation 1992;85:1557–1564)

KEY WORDS • echocardiography • myocardial opacification • microbubbles

Although the utility of myocardial contrast echocardiography (MCE) in the assessment of myocardial blood flow has been established, this technique currently involves intra-arterial injection of contrast.1–3 Despite the achievement of left ventricular (LV) cavity opacification after pulmonary capillary wedge and peripheral venous4–10 injection of contrast, myocardial opacification from injection at these sites has remained elusive. Applications of MCE have thus been limited to the invasive settings of the cardiac catheterization laboratory11–14 or the operating room.15,16 For this technique to have broader applications, it is necessary that myocardial opacification be achieved from a venous injection of contrast.

The first goal of the current study was to determine microbubble characteristics that influence myocardial opacification in vivo. Accordingly, left atrial (LA) injection of contrast was performed using various volumes, sizes, and concentrations of microbubbles. The second goal was to assess the effects of bolus dispersion during transpulmonary passage and of myocardial blood flow on myocardial opacification after right atrial (RA) injection of contrast. To achieve this goal, RA injection of contrast was performed while the composition of the bolus of injection, cardiac output, and myocardial blood flow were manipulated. Because myocardial opacification from RA injection of contrast can be subtle, the final goal of this study was to develop image-processing algorithms that would make this effect more apparent and quantifiable.
Methods

Group 1 dogs (n=9) were used to determine the microbubble characteristics that influence myocardial opacification from LA injection of contrast. Group 2 dogs (n=8) were used to determine the effect of the combination of microbubble characteristics, transpulmonary bubble passage, and myocardial blood flow on myocardial opacification after RA injection of contrast. The protocols were approved by the Animal Research Committee at the University of Virginia.

Animal Preparation

The dogs were anesthetized with sodium pentobarbital (30 mg/kg) (Abbott Laboratories, North Chicago, Ill.), intubated, and ventilated with a respirator pump (Model 607, Harvard Apparatus, Natick, Mass.). Additional anesthesia was given during the experiment as needed. An 8F catheter was placed in the right femoral vein for intravenous administration of fluids and drugs. A similar catheter was placed in the right femoral artery for monitoring of arterial pressure and measurement of blood gases. The tip of a 7.5F Swan-Ganz catheter (Baxter, Irvine, Calif.) was placed in the main pulmonary artery (PA) via the right internal jugular vein for the determination of cardiac output in the Group 2 dogs. This catheter was also used to measure PA pressures.

A left lateral thoracotomy was performed, and the heart was suspended in a pericardial cradle. An 8F catheter was placed in the LA for measurement of LA pressure and for the injection of contrast in the group 1 dogs. In the group 2 dogs, a similar catheter was placed in the RA for injection of contrast. In this group of dogs, a micromanometer-tipped catheter (Model PC-484B, Millar Instruments, Houston, Tex.) was inserted via a left ventriculotomy into the LV cavity for the measurement of LV end-diastolic pressure and the first derivative (dP/dt) of LV pressure.

Hemodynamic Measurements

The arterial, LA, and PA catheters were connected to a multichannel physiological recorder (Model 4568C, Hewlett-Packard Co., Everett, Mass.) via fluid-filled transducers (Model 1280C, Hewlett-Packard). Baseline pressures were recorded before each injection in all dogs. In group 1 dogs, arterial and LA pressures were recorded 30 seconds after LA injection of contrast. In group 2 dogs, arterial, PA, and LV end-diastolic pressures and LV dP/dt were recorded at 30 and 180 seconds after RA injection.

Two-dimensional Echocardiography

Two-dimensional echocardiography (2DE) was performed with a phased-array system (RT5000, General Electric Medical Systems, Milwaukee, Wis.) with a 5-MHz transducer. Images were obtained at the mid-papillary muscle short-axis level. Maximal dynamic range of 72 dB was used, and the gain and power output settings were initially optimized during each experiment and kept unchanged throughout. A saline bath acted as an acoustic interface between the heart and the transducer. The images were recorded on 0.5-in. VHS videotape with a high-fidelity videotape recorder (Panasonic Model AG6200, Matsushita Electrical Co., Japan).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Physical Characteristics of Microbubbles Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bubble size* (μm)</td>
</tr>
<tr>
<td>Group 1 dogs</td>
<td>5.8±0.1</td>
</tr>
<tr>
<td></td>
<td>5.8±0.2</td>
</tr>
<tr>
<td></td>
<td>5.6±0.2</td>
</tr>
<tr>
<td></td>
<td>5.4±0.3</td>
</tr>
<tr>
<td></td>
<td>4.4±0.1</td>
</tr>
<tr>
<td></td>
<td>4.2±0.3</td>
</tr>
<tr>
<td>Group 2 dogs</td>
<td>5.3±0.1</td>
</tr>
<tr>
<td></td>
<td>4.4±0.3</td>
</tr>
<tr>
<td></td>
<td>4.0±0.1</td>
</tr>
<tr>
<td></td>
<td>3.9±0.3</td>
</tr>
</tbody>
</table>

*Values are mean±SD.

Contrast Agent

Sonicated microbubbles made from 5% human albumin were custom designed for these experiments by Molecular Biosystems, Inc., San Diego, Calif. The bubble sizes and concentrations are depicted in Table 1. One-half to 2 ml of these bubbles were hand-injected into the LA over 1 second in the group 1 dogs. The peak pressures generated in the 22-cm-long 8F catheter placed in the LA during these injections were measured in one dog and were 25, 35, and 65 mm Hg for injection volumes of 0.5, 1.0, and 2.0 ml, respectively. Ten milliliters of bubbles were hand-injected into the RA over 2 seconds in the group 2 dogs. The peak pressure generated in the catheter placed in the RA during this injection, as measured in one of the dogs, was 210 mm Hg.

Image Analysis

2DE images were analyzed on an off-line computer (Mipron Image Processing System, Kontron Electronics, FRG). Images from videotape were transferred to video memory in a 244×244×8-bit format. Consecutive end-diastolic frames, starting from just before injection of contrast until 8–10 seconds after injection, were selected for analysis. The selected frames were aligned by a previously described cross-correlation technique implemented in our laboratory. Three approaches were then used for determining the presence of contrast in the myocardium.

The first approach was used in both groups of dogs and involved the generation of time–intensity plots from the myocardium as previously described. A region of interest comprising at least 600 pixels was placed over the anterior myocardium, and the average videointensity (0–255 grey level) in this region was measured. The average precontrast videointensity value within the region of interest was subtracted from the mean videointensity in each subsequent end-diastolic frame to generate background-subtracted time–intensity plots. Peak videointensity was derived from these plots.

The second and third approaches were used only in group 2 dogs and were developed to depict myocardial perfusion in a manner easily discernible to the eye. The first of these two approaches used digital subtraction. Three to five aligned pre- and postinjection end-diastolic frames were averaged to enhance the signal-to-noise ratio. Digital subtraction was performed on the two averaged frames. Our particular approach categorized...
the pixel intensities obtained after subtraction according to the grey-level change: increased contrast effect and no change/decreased contrast effect (attenuation). All intensities in the former category appeared in shades of grey proportional to the increase in pixel intensities in the subtracted image, and all intensities in the latter category appeared as black. This strategy allowed optimal appreciation of myocardial contrast enhancement in the resulting subtracted image.

The digitally subtracted images were then used to develop the third approach, which used pseudo-color-coding of the myocardial pixels to visually highlight myocardial opacification further. Such an approach was selected because, whereas the human eye can discern only limited shades of grey, it can discriminate between many more hues of color. Only the pixels within the digitally subtracted images that demonstrated an increase in peak intensity between preinjection and postinjection images were assigned a color-coding scheme. To use the full dynamic range of this scheme, the grey level values in the subtracted image were expanded to a 128 grey-level scale. The color map chosen was that representing the spectrum of color changes occurring in a progressively heated object, with red representing the least contrast, orange and yellow more contrast, and white the most contrast. The LV cavity was masked out. In this manner, we were able to highlight the spatial flow patterns within the myocardium.

All images were read blinded to bubble characteristics and physiological state (baseline, during dobutamine, or after dipyridamole). For contrast enhancement after RA injection to be present in the group 2 dogs, two criteria had to be met: 1) an increase of five or more grey levels on the background-subtracted time–intensity plots and 2) evidence of contrast in digitally subtracted grey scale or pseudo–color-coded images.

**Protocol**

In group 1 dogs, boluses of various microbubble sizes, concentrations, and volumes were injected into the LA in each dog. The ventilator was stopped for 10 seconds during the injection to minimize cardiac translation and changes in acoustic properties of the anterior myocardium induced by movement of the heart in relation to the transducer. Arterial and LA pressures were measured at each stage.

In group 2 dogs, 10 ml of each concentration and size of microbubbles was injected during three different conditions: baseline, during intravenous infusion of dobutamine (15 µg/kg/min) to increase cardiac output and reduce transpulmonary transit time of the bubbles, and after intravenous administration of dipyridamole (0.56 mg/kg) to selectively increase myocardial blood flow. The ventilator was stopped for 30 seconds during 2DE imaging. Arterial, PA, and LV end-diastolic pressures, LV dP/dt, and thermodilution cardiac output were measured at each stage.

**Statistical Analysis**

Data were analyzed with RS/1 (Bolt, Beranek and Newman, Cambridge, Mass.) and BMDP (University of California, Los Angeles, Calif.). Differences in means were compared by Student’s t test or ANOVA, and differences in proportions were compared with the χ² test. Independent correlates of myocardial opacification (peak videointensity) on multivariate analysis were determined by stepwise multiple linear or logistic regression analysis. Statistical significance was defined as *p*<0.05.

**Results**

**Group 1 Dogs**

*Results of MCE.* Figure 1 is an example of videointensity data from one of the group 1 dogs after LA injection of microbubbles. At a size of 5.6 µm and a concentration of 1.12 billion bubbles/ml, increasing volumes of bubbles resulted in greater contrast effect (Figure 1A). Similarly, at a size of 5.6 µm and volume of 2 ml, a concentration of 1.12 billion bubbles/ml produced the most opacification and 0.55 billion/ml produced the least, while 0.7 billion bubbles/ml produced intermediate opacification (Figure 1B). Finally, at a concentration of 1.12 billion bubbles/ml and volume of 2 ml, peak videointensity was higher when larger (5.6-µm) than when smaller (4.2-µm) bubbles were used (Figure 1C).

Figure 2 summarizes the results from 112 LA injections in the nine group 1 dogs. At all bubble sizes and concentrations, a larger volume of injectate resulted in a
significantly higher peak videointensity. Similarly, larger bubbles produced the greatest opacification at most bubble concentrations and volumes, and the most concentrated bubble solution produced the greatest myocardial opacification for most volumes and sizes. The number (volume of injectate), concentration, and size of the microbubbles all independently (p<0.01) correlated with peak videointensity on multivariate analysis with F values of 17.8, 15.2, and 13.0, respectively.

**Hemodynamic results.** Slight increases were observed in arterial (105±31 versus 107±32 mm Hg, p=0.05) and LA pressures (7.8±1.8 versus 7.9±1.8 mm Hg, p=0.02) 30 seconds after LA injection of contrast.

**Group 2 Dogs**

**Results of MCE.** By the criteria described above, myocardial opacification occurred in 40 of 85 RA inject-
tions (47%). Figure 3 is an example of successful opacification in one Group 2 dog. In Figure 3A, contrast can be seen in the right ventricle and has not yet appeared in the LV. In Figure 3B, contrast can be seen in the LV with posterior wall attenuation. Subtle contrast effect is noted in the anterior, medial, and lateral walls compared with the precontrast image in Figure 3A. When digital subtraction is performed, the contrast effect is much more apparent (Figure 3C). Regions that were brighter in Figure 3A than in Figure 3B, such as the right ventricular cavity and the posterior LV wall, appear black in the subtracted image. Similarly, regions that were brighter in panel B than in Figure 3A, such as the LV cavity and LV myocardium, appear to have contrast effect in the subtracted image. When frames later in the injection sequence are digitally subtracted, the initially attenuated posterior region also demonstrates contrast enhancement (Figure 3D). The overall contrast effect, however, is smaller than that in earlier frames immediately after appearance of contrast in the LV cavity. In no instance was contrast noted in the myocardium in the absence of marked posterior wall attenuation after contrast appeared in the LV cavity.

When the digitally subtracted frames in this same dog are color-coded, the contrast effect becomes even more visually apparent (Figure 4). This effect is noted in the appearance of yellows in the anterior, medial, and lateral walls early after injection (Figure 4C), which corresponds to Figure 3C. This effect is also noted in the posterior wall after resolution of posterior wall attenuation (Figure 4D), which corresponds to Figure 3D. The background-subtracted time–intensity plot for this stage also shows contrast enhancement (Figure 5).

The combined data from the 85 RA injections are summarized in Figure 6. Because of the narrow variability in the range of bubble sizes used, this characteristic was not included as a separate variable for this analysis. For bubbles ranging in concentration from 1.47 to 1.68 billion bubbles/ml, 40% of injections opacified the myocardium at baseline. Neither the addition of dobuta-

Figure 4. Pseudo–color-coding of images corresponding to those in Figure 3. These images are (panel A) before appearance of contrast in the left ventricle, (panel B) early after contrast appearance in the left ventricle showing subtle contrast effect in the myocardium, (panel C) a color-coded digitally subtracted image showing contrast in the myocardium except for the posterior wall, which is attenuated corresponding to the grey-scale image in panel C in Figure 3, and (panel D) a color-coded digitally subtracted image from frames later in the cardiac cycle showing myocardial opacification without posterior wall attenuation corresponding to the grey-scale image in panel D in Figure 3.

Figure 5. Background-subtracted time–intensity plot obtained from the anterior myocardium after right atrial injection of contrast in the same dog whose images are depicted in Figures 4 and 5.
mine nor dipyridamole at this concentration improved the success rate of opacification (43% and 36%, respectively, p=NS).

In comparison, the addition of dobutamine at bubble concentrations of 4.41 to 5.04 billion bubbles/ml increased the opacification rate from 25% at baseline to 50%, although this increase was not statistically significant. The administration of dipyridamole resulted in the greatest likelihood of myocardial opacification at this bubble concentration (88%, p<0.01). By multivariate analysis, the combination of dipyridamole and highly concentrated bubble solutions was independently associated with successful myocardial opacification (χ²=9.01, p=0.003). Cardiac output, which was a significant univariate predictor of myocardial opacification (being 7.3 l/min with successful opacification and 5.9 l/min with unsuccessful opacification, p=0.004), was not a predictor on multivariate analysis. Likewise, other combinations of drug and bubble concentrations were not independent predictors of myocardial opacification on multivariate analysis.

**Hemodynamic results.** As expected, cardiac output increased significantly during dobutamine infusion, from 5.1±1.8 l/min at baseline to 8.8±1.8 l/min (p<0.001), whereas it did not change significantly after dipyridamole infusion (5.8±0.6 l/min). Although dobutamine resulted in increases in PA pressures and LV dP/dt, RA injection of contrast did not produce any additional changes (Table 2). The arterial pressure decreased after dipyridamole infusion. For all stages, arterial pressure increased by 30 seconds after RA injection of contrast and decreased to baseline at 180 seconds (Table 2).

**Table 2. Hemodynamic Results in Group 2 Dogs**

<table>
<thead>
<tr>
<th></th>
<th>Preinjection</th>
<th>Postinjection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP (mm Hg)</td>
<td>111.0±17.9</td>
<td>121.8±24.7*</td>
</tr>
<tr>
<td>PAP (mm Hg)</td>
<td>10.3±4.9</td>
<td>10.9±5.7</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>11.6±4.1</td>
<td>12.1±2.8</td>
</tr>
<tr>
<td>LV dP/dt</td>
<td>2,504±512</td>
<td>2,711±561</td>
</tr>
<tr>
<td><strong>During dobutamine infusion (15 μg/kg/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP (mm Hg)</td>
<td>105.9±16.0</td>
<td>118.1±20.9*</td>
</tr>
<tr>
<td>PAP (mm Hg)</td>
<td>17.1±6.9</td>
<td>16.8±7.0</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>12.6±5.9</td>
<td>13.2±6.5</td>
</tr>
<tr>
<td>LV dP/dt</td>
<td>5,727±1,009</td>
<td>5,889±966</td>
</tr>
<tr>
<td><strong>After dipyridamole infusion (0.56 mg/kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP (mm Hg)</td>
<td>84.8±15.4</td>
<td>96.2±20.0*</td>
</tr>
<tr>
<td>PAP (mm Hg)</td>
<td>10.9±3.8</td>
<td>11.2±4.1</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>10.7±5.2</td>
<td>11.6±5.3</td>
</tr>
<tr>
<td>LV dP/dt</td>
<td>2,498±777</td>
<td>2,606±807</td>
</tr>
</tbody>
</table>

AP, mean arterial pressure; PAP, mean pulmonary artery pressure; LVEDP, left ventricular end-diastolic pressure; LV, left ventricular; dP/dt, first derivative of pressure.

*p<0.05 by ANOVA.

**Discussion**

This study demonstrates, for the first time, that it is possible to reproducibly achieve myocardial opacification after RA injection of contrast. It was found that highly concentrated bubble injections administered into the RA during coronary hyperemia consistently resulted in myocardial opacification without producing significant adverse hemodynamic effects. This new finding sets the stage for the possibility of myocardial opacification after venous injection of contrast in humans.

**Factors Influencing Myocardial Opacification**

As exemplified by the results from our group 1 dogs, of the several factors studied, the number, size, and concentration of microbubbles all independently influence myocardial opacification. Since the absolute number of bubbles entering the LV cavity determines how many are accessible to the coronary circulation, both volume and concentration should be important. Furthermore, because the reflectivity of a bubble is related to the sixth power of its radius, bubble size should also have an important effect on opacification. It is important, however, to achieve a balance between bubble...
reflectivity and its ability to traverse the capillaries. In this regard, we believe that a bubble size of 6 μm is optimal.

The extent of microbubble loss or entrapment in the lungs would affect the number of bubbles arriving in the LV cavity. At physiological conditions, the lungs appear to filter microbubbles beyond a certain size.20–22 Interestingly, this filtering capacity appears to diminish when the lungs are bombarded with extreme numbers of bubbles or when drugs like aminophylline are administered.20 Using microbubbles similar in size to ours, a previous study estimated a 60% transmission rate of microbubbles under basal hemodynamic conditions.7

Bubbles contained in a compact bolus might be better able to opacify the myocardium than the same number of bubbles dispersed in a larger volume. Thus, prolonged transpulmonary transit, a function of cardiac output, or a large injectate volume with less concentrated bubbles might result in a smaller number of bubbles entering the coronary circulation at any time. Our results suggest that injection of more concentrated bubbles in the RA is more successful in achieving myocardial opacification and that this effect is more likely to be seen at higher cardiac outputs.

The contrast effect of bubbles crossing the lungs might be influenced by LV systolic pressures. When bubbles are injected into the RA, opacification within the LV cavity has been reported to be less during LV systole.8,23 The cause of this effect is not known but may be related to either bubble destruction or reduction in bubble size at high LV pressures. Although we did not notice reduced contrast effect within the LV cavity during systole in our dogs, the number of bubbles injected was so large that, had bubble destruction or alteration occurred, enough bubbles would still have survived these outcomes to opacify the myocardium. We achieved very high pressures within the catheters during injection of the bubbles in the RA, which could have resulted in their alteration. Slower injection, however, would have resulted in a larger and more dilated bolus in the right heart.

Under resting conditions, the myocardium receives only 4–5% of cardiac output.24 Thus, selective increases in myocardial blood flow would allow more bubbles to enter the coronary circulation. We achieved the highest success rate with myocardial opacification when we increased myocardial blood flow by administering dipyridamole without appreciably changing cardiac output. We also used image-processing algorithms that increased our ability to appreciate the presence of contrast within the myocardium. Other factors that might influence the success of myocardial opacification, such as echo instrumentation, were not examined.

Safety of Injecting Large Numbers of Bubbles in RA

Even intracoronary injection of sonicated albumin microbubbles has been found to be safe.25,26 These bubbles do not cause coronary hyperemia and have minimal effects on left heart and systemic pressures and regional function. In our group 1 dogs, we found minimal change in LA and arterial pressures 30 seconds after LA injection of contrast. In our group 2 dogs, we found no changes in PA and LV pressures and LV dp/dt 30 and 180 seconds after RA injection of contrast. We did, however, note an increase in arterial pressure 30 seconds after injection that abated by 180 seconds, which we feel is related to transient hypoxia from our temporarily shutting off the respirator. Therefore, we believe that in dogs, injecting large numbers of bubbles into the right heart does not produce major changes in pulmonary and systemic hemodynamics.

In addition to the effect on cardiopulmonary function, the possible neurological effects of bubbles are of concern. In a previous study, we injected large numbers of bubbles into rabbits and found no pathological effects on the brain on histological examination.25 The safety of bubbles in the numbers and concentrations required to produce myocardial opacification from RA injection, however, needs to be evaluated in clinical studies.

Study Limitations

Because the primary goal was to achieve transpulmonary myocardial opacification, the injections were performed from the RA. Further studies are required to determine whether peripheral venous injections of contrast will similarly opacify the myocardium. It also remains to be seen whether subtle contrast effects can be detected by current imaging systems using less optimal acoustic windows than were used in our study. Finally, because the present protocol used short-axis views of the heart, the initial intense contrast effect in the LV cavity resulted in early attenuation of posterior regions of the heart. Alternative imaging planes, such as apical views, can be used to assess the contrast effect in these regions.

Clinical Implications

The potential clinical applications of MCE using peripheral injections of sonicated microbubbles are far reaching. Our data suggest that concentrated bubbles given during pharmacologically induced coronary hyperemia would result in the greatest likelihood of myocardial opacification. Such an approach would also help define critical coronary stenoses and is not different from dipyridamole20TI imaging.27 It is anticipated that improvement in 2DE systems and contrast agents will facilitate the broad application of this perfusion imaging modality.

References


23. Mottley J, Everbach EC, Schwarz KO, Schleich B, Meltzer R: Decay of ultrasound integrated backscatter from a saccharide contrast agent is accelerated by increased pressure (abstract). *Circulation* 1990;82(suppl III):II-28


Successful and reproducible myocardial opacification during two-dimensional echocardiography from right heart injection of contrast.

F S Villanueva, W P Glasheen, J Sklenar, A R Jayaweera and S Kaul

*Circulation.* 1992;85:1557-1564
doi: 10.1161/01.CIR.85.4.1557

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 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/85/4/1557

**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/