The effect of acute hypercholesterolemia on myocardial infarct size and the no-reflow phenomenon during coronary occlusion-reperfusion

PAOLO GOLINO, M.D., PETER R. MAROKO, M.D., AND THOMAS E. CAREW, PH.D.

ABSTRACT  The goal of this study was to determine the effects of acute hypercholesterolemia on the evolution of myocardial infarction in a preparation of coronary occlusion-reperfusion. New Zealand white rabbits were fed a 2% cholesterol-enriched diet for 3 days (plasma cholesterol 329 ± 70 mg/dl), or maintained on the control diet (plasma cholesterol 67 ± 12 mg/dl). Temporary (30 min) coronary artery occlusion was performed in open-chest rabbits with a suture snare. The snare was released to permit reperfusion. When the animals were killed 5.5 hr later, left ventricles were cut into 3 mm slices. Infarct size was determined by planimetry of tetrazolium-stained slices while the area at risk of infarction (hypoperfused zone) was determined by planimetry of the “cold spots” on autoradiograms of the slices that contained 99mTc-labeled microspheres that had been injected 1 min after occlusion. Infarct size, expressed as percent of the hypoperfused zone, was 42.8 ± 1.3% (n = 10) in the control group and was increased by approximately 100% in cholesterol-fed animals to 83.7 ± 2.0% (n = 10, p < .001). To test the hypothesis that vascular obstruction (no reflow) might account for the larger infarct size, thioflavin S was injected immediately before the animals were killed to demarcate perfused myocardium in three additional groups of animals: (1) standard chow–fed rabbits (n = 5), (2) cholesterol-fed rabbits (n = 5), and (3) standard chow–fed rabbits that, in addition, received an infusion of isoproterenol (0.1 µg/kg/min, n = 6), an intervention believed to increase infarct size through a mechanism not dependent on the no-reflow phenomenon. Infarct size (as % of HZ) was again doubled in cholesterol-fed animals compared with controls: 83.5 ± 5.1% vs 38.1 ± 1.4% (p < .001). The infusion of isoproterenol, as expected, also increased infarct size (94.2 ± 5.2% of HZ, p < .001). This increase was similar in magnitude to that in the cholesterol-fed group (p = NS). Despite the large infarcts produced by isoproterenol, the nonreperfused zones (NRZs, expressed as percent of HZ) in control and isoproterenol groups were similar (19.1 ± 3.7% and 28.1 ± 3.0%, respectively). Most importantly, the NRZ was strikingly larger in cholesterol-fed rabbits (86.1 ± 3.8% of HZ, p < .001 vs either control or isoproterenol groups). The NRZ in the cholesterol-fed group was nearly identical in size to the infarcted area. These results imply that while isoproterenol increased infarct size by a mechanism independent of the no-reflow phenomenon (probably by increasing myocardial oxygen consumption), acute hypercholesterolemia increased infarct size by vascular obstruction; i.e., by not permitting effective reperfusion of the myocardium.


Among the numerous recognized risk factors for the development of coronary artery disease (CAD), one of the best known is the association between blood lipids and CAD.1–5 Several prospective studies have established that the risk of cardiac morbidity and mortality is directly related to the concentration of plasma cholesterol.6–8

The most prevalent view is that the increased risk of myocardial infarction associated with elevated plasma cholesterol levels can be adequately explained on the basis of the increase in number and severity of coronary atherosclerotic vascular lesions. The possibility that elevated plasma cholesterol may modify risk status by other mechanisms has in general received less attention, but a number of studies have raised this question explicitly or implicitly. For example, plate-
lets from hypercholesterolemic patients or cholesterol-fed animals are hypersensitive to a variety of aggregating agents in vitro. Moreover, this hypersensitivity to aggregating agents in vitro was also shown by platelets isolated from normal volunteers and incubated in cholesterol-rich media. Likewise, free and lipoprotein-bound cholesterol have been reported to sensitize isolated dog coronary arteries to various vasoconstricting agents. Vasoconstriction in response to ergonovine is augmented in coronary arteries with no visible atherosclerosis from spontaneously hypercholesterolemic rabbits, and is apparently mediated through a serotonergic mechanism. In addition, elevated plasma cholesterol has been reported to potentiate the coronary arterial response to norepinephrine in dogs.

However, there are no studies of the effects of hypercholesterolemia per se on the evolution of acute myocardial infarction. Accordingly, in the present study we examined the effect of diet-induced hypercholesterolemia on infarct size in rabbits subjected to temporary coronary artery occlusion and then reperfusion. In this preparation, rabbits were fed a 2% cholesterol-enriched diet for 3 days, sufficient time to induce moderate hypercholesterolemia (about 330 mg/dl as compared with a control value of 67 mg/dl), but too short a period to produce any detectable atherosclerotic vascular changes.

As described below, cholesterol-fed rabbits had infarcts approximately twice as large as control rabbits. Therefore, a second series of experiments was conducted to determine whether this increase in myocardial damage was due to a failure to reestablish myocardial blood flow after reperfusion.

Methods

Thirty-six New Zealand white rabbits of both sexes weighing 2.5 to 3.5 kg were studied. In the first series of experiments the rabbits were assigned to one of two groups: a control group that received a standard laboratory chow diet, or a cholesterol-fed group that received a 2% cholesterol-enriched diet (Nutritional Biochemicals, Cleveland) for 3 days before the experiment. In the second series of experiments the rabbits were assigned to one of three groups: the two previously described or an isoproterenol group that was fed the standard diet and in addition received an infusion of isoproterenol (0.1 μg/kg/min) throughout the period of coronary occlusion and reperfusion.

The rabbits were anesthetized with a mixture of ketamine (35 mg/kg) and xylazine (5 mg/kg) administered intramuscularly. Anesthesia was maintained during the experiment by intravenous injections of small amounts of pentobarbital (5 mg/kg) sufficient to abolish the corneal reflex. The rabbits were intubated through a tracheotomy and ventilated with a constant-volume respirator (Harvard Apparatus Co., Cambridge, MA). A polyethylene cannula was placed into the aorta through the left carotid artery to monitor arterial pressure. A thoracotomy was performed through the fifth left intercostal space and the heart was suspended in a pericardial cradle. A polyethylene cannula was placed in the left atrium through the left atrial appendage. The large marginal branch of each rabbit’s circumflex coronary artery was temporarily occluded about 0.3 cm from its origin with a surgical suture snare (in rabbits this artery supplies the anterolateral left ventricular wall with a distribution similar to that of the left anterior descending artery in man). Coronary artery occlusion was maintained for 30 min, at which time the ligature was released and reperfusion continued for an additional 5.5 hr. Electrocardiographic lead aVF and systemic arterial pressure (Statham P23 DB pressure transducer) were recorded continuously during the experiment (Gould Instrument). Blood samples were taken from an ear vein on the day of the experiment for determination of plasma cholesterol levels.

Assessment of hypoperfused zone (HZ) after coronary occlusion, blood flow distribution after reperfusion, and infarct size. To permit later assessment of the HZ, that is, the area at risk of infarcting, 2 × 10⁵ ⁹⁹mTc-labeled albumin microspheres (15 μm diameter, activity 0.8 mCi) were injected into the left atrium of each rabbit 1 min after coronary artery occlusion. Several methods have been used to estimate the quantity of myocardium at risk after coronary artery occlusion. Postmortem perfusion of the occluded and nonoccluded coronary artery with dyes or radioopaque material outlines the perfusion bed of the occluded vessel. However, this bed is larger than the area of actual ischemia because it does not take into account the variable inflow of blood from the unoccluded bed through collateral vessels. The area of actual ischemia can be precisely identified by the technique we used since the microspheres injected in vivo distribute with blood only in the normally perfused myocardium. A second advantage of this technique is that the microspheres can be injected before any intervention, thus overwhelming the possibility that the area at risk may change during the experiment. Albumin microspheres have a half-life in vivo of less than 12 hr, which ensures that they remain stable within the myocardium during the experimental period. The variables that determine the density and resolution of an autoradiographic image on exposure to x-ray film include: (1) the injected activity, isotope, and number of labeled microspheres injected, (2) the time before the tissue is placed on the film, (3) the time of exposure, and (4) the stability of labeled microspheres. These factors were constant in all rabbits.

To estimate the distribution of arterial blood flow at death, some animals received an injection of a 6% solution of thioflavin S (1 ml/kg) via an ear vein 10 sec before excision of the heart according to a previously described technique. Thioflavin S is a fluorescent dye with a relatively high affinity for endothelium. Thus, the extent of the capillary bed receiving flow can be visualized in slices of myocardium exposed to the dye when they are viewed under ultraviolet light.

The rabbits were killed by intravenous injection of potassium chloride and the left ventricle was dissected free from all other structures and weighed. The left ventricle was frozen at −70°C for 30 min and cut into eight to 10 slices parallel to the atriocentric groove. Slices were observed first under ultraviolet light, the areas that received blood flow showed a bright white fluorescence. Contours of the fluorescent and nonfluorescent areas (nonperfused zone, NRZ) were traced onto transparent plastic sheets. The total cross-sectional area of the slices and of the NRZs were measured by planimetry.

The slices were next incubated in a 1% solution of triphenyltetrazolium chloride (Sigma Chemical) for 10 min at 37°C. The nonschismic tissue stained dark red (figure 2) and the damaged tissue gray or pale yellow. Again, a transparent plastic sheet was placed over the slices and the contours of these areas were traced; the total cross-sectional area of the slices and the
area of damaged myocardium were measured by planimetry. Thereafter, the extent of the HZ was determined on the same slices. Slices were placed in an x-ray film cassette over a sheet of high-speed x-ray film (Cronex 4, E.I. DuPont) between two medium-contrast enhancing screens. The film was exposed overnight and developed (X-Omat Automatic Processor). To clearly identify the overall contour of the slices, “soft” x-ray (25 kV peak, 100 mA) images were obtained, with the slices held in position by the cassette. The soft x-ray image and autoradiographs were superimposed to permit tracing of the perfused (dark) and hypoperfused (white) areas onto clear plastic overlays. The total cross-sectional area of the slices and the cross-sectional area of HZs were determined by planimetry.

The following variables were calculated: (1) infarct size as percentage of the left ventricle that showed myocardial damage by the TTC staining criterion, (2) the HZ as the percentage of the left ventricle that was hypoperfused 1 min after coronary artery occlusion and therefore that was at risk of infarcting, as determined by autoradiography, (3) the percentage of the HZ that evolved to infarction, which was calculated by dividing infarct size by HZ and multiplying by 100 and represents the percentage of the area at risk that actually evolved to necrosis, (4) the NRZ (area not receiving blood flow at the end of 5.5 hr of reperfusion), as determined by thioflavin S fluorescence, and (5) the percentage of HZ that did not reperfuse, which was calculated by dividing NRZ by HZ and multiplying by 100 and represents the percentage of the HZ that showed the no-reflow phenomenon.

**Statistical analysis.** Results are expressed as mean ± SEM. Comparisons between two means were made by Student’s t test for group observations. In making multiple comparisons among groups, analysis of variance was used. For comparison of heart rate and arterial pressure among groups, an analysis of variance for a design with repeated measures was used (program BMDP-2V).

**Results**

In the first series of experiments the effect of diet-induced hypercholesterolemia on infarct size was tested. Ten rabbits were fed the 2% cholesterol-enriched diet and 10 standard chow–fed rabbits served as controls. On the day of the experiment, plasma cholesterol levels were 329 ± 70 mg/dl in the cholesterol diet group vs 67 ± 12 mg/dl in controls (p < .001). Coronary artery occlusion produced HZ 1 min after occlusion that was similar in both groups (figure 3): 26.0 ± 2.0% vs 27.2 ± 2.8% of the left ventricle in cholesterol-fed and control rabbits, respectively (p = NS). Thus, the area at risk of infarcting in both groups was the same.

After 30 min of coronary artery occlusion and 5.5 hr of reperfusion, however, the percent of the HZ that actually evolved to necrosis was 42.8 ± 1.3% in the control group (n = 10), while it was approximately twice as large in cholesterol-fed animals at: 83.7 ± 2.0% (n = 10, p < .001; figure 4). This increase could not be attributed to any measured differences in hemo-
dynamics; mean arterial pressure and heart rate did not differ significantly between groups at any time during or after occlusion.

To elucidate the mechanisms that may be involved, the hypothesis that vascular obstruction and consequent inability to reperfuse might account for the increased infarct size in the hypercholesterolemic rabbits was tested. Accordingly, another series of experiments were performed in which immediately before death a vital dye, thioflavin S, was injected to demarcate perfused myocardium. Each of 16 rabbits was assigned to one of three groups: (1) control diet (n = 5), (2) cholesterol-enriched diet (n = 5), and (3) control diet plus intravenous isoproterenol (0.1 μg/kg/min) for 6 hr starting immediately after coronary artery occlusion (n = 6). The latter group was included to produce large infarcts comparable in size to those observed after cholesterol feeding, but presumably due to a different mechanism, namely, an increase in myocardial oxygen requirements.

Isoproterenol, as expected, increased heart rate significantly and decreased mean arterial pressure significantly (table 1). Again, there were no significant differences in values for hemodynamic variables in the control and cholesterol-fed rabbits (table 1). The area at risk of infarction, HZ, was similar in the three groups: 31.9 ± 1.0%, 31.4 ± 2.6%, and 30.6 ± 2.9% of the left ventricle in the control, cholesterol-fed, and isoproterenol groups, respectively (p = NS).

As in the first series of experiments, infarct size in the cholesterol-fed group was over 100% larger than in the control group (83.5 ± 5.1% vs 38.1 ± 1.4% of HZ, p < .001; figure 5). In the isoproterenol group, infarct size was similar to that in the cholesterol-fed group at 94.2 ± 5.2% of HZ (p < .001 vs control group and p = NS vs cholesterol-fed group; figure 5). The NRZ in the control group was 19.1 ± 3.7% of the HZ. In the isoproterenol group, despite the much larger infarcts, NRZ was not different from that in the control group at 28.1 ± 3.0% of HZ (p = NS vs control group). The NRZ in the cholesterol-fed group was strikingly larger at 86.2 ± 3.8% of HZ (p < .001 vs control and isoproterenol groups) and included essentially all of the infarcted tissue. Thus, in contrast to the isoproterenol group, in which large infarctions (relative to the area at risk) were not accompanied by an increase in perfusion deficit after release of occlusion, the large infarcts observed in the cholesterol-fed group appeared to be secondary to an impairment of the ability to reperfuse the ischemic zone.

TABLE 1
Hemodynamic variables during 30 min coronary artery occlusion and 5.5 hr reperfusion

<table>
<thead>
<tr>
<th>Time after occlusion</th>
<th>Heart rate (min⁻¹)</th>
<th>Mean arterial pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control¹</td>
<td>Cholesterol¹</td>
</tr>
<tr>
<td>0</td>
<td>167 ± 5</td>
<td>161 ± 6</td>
</tr>
<tr>
<td>15 min</td>
<td>173 ± 4</td>
<td>169 ± 3</td>
</tr>
<tr>
<td>30 min</td>
<td>169 ± 3</td>
<td>170 ± 4</td>
</tr>
<tr>
<td>1 hr</td>
<td>174 ± 4</td>
<td>169 ± 6</td>
</tr>
<tr>
<td>2 hr</td>
<td>172 ± 4</td>
<td>172 ± 5</td>
</tr>
<tr>
<td>3 hr</td>
<td>167 ± 4</td>
<td>165 ± 6</td>
</tr>
<tr>
<td>4 hr</td>
<td>167 ± 5</td>
<td>165 ± 5</td>
</tr>
<tr>
<td>5 hr</td>
<td>170 ± 3</td>
<td>167 ± 5</td>
</tr>
<tr>
<td>6 hr</td>
<td>167 ± 4</td>
<td>168 ± 6</td>
</tr>
</tbody>
</table>

¹Maintained on laboratory chow diet.
²Fed 2% cholesterol-enriched diet for 3 days.
³Maintained on laboratory chow diet, but received intravenous infusion of isoproterenol (0.1 μg/kg/min) beginning at zero time.
⁴All values significantly different by analysis of variance with repeated measurements (p < .001).
Discussion

Myocardial infarction is a dynamic process. The ultimate size of an infarction can be modified by interventions early after coronary artery occlusion, making it possible to either decrease or to increase infarct size.\textsuperscript{21-24} It is especially important to recognize and, if possible, to mitigate factors that may increase infarct size. In this investigation the hypothesis that hypercholesterolemia per se could have a deleterious effect on the evolution of acute myocardial infarction was tested. A rabbit preparation was chosen because of this animal’s extreme sensitivity to dietary cholesterol. It has been shown that as early as 6 hr after the first administration of a cholesterol-enriched diet a significant increase in plasma cholesterol occurs that is associated with an increase in very low-density lipoproteins (VLDL, density < 1.006 mg/ml).\textsuperscript{25} Within days after the initiation of a high-cholesterol diet, the plasma cholesterol level rises more than 10-fold. Most of the excess plasma cholesterol is contained in lipoproteins of a density less than 1.019 mg/ml, which include VLDL and intermediate-density lipoproteins.\textsuperscript{25-27} In comparison with human VLDL, in which triglycerides are the predominant lipid and apolipoprotein B is the major protein, the VLDL particles of cholesterol-fed rabbits contain cholesteryl ester as the major lipid and are markedly enriched in apolipoprotein E.\textsuperscript{26, 27} Because these particles show an abnormal $\beta$-mobility on electrophoresis, they are called $\beta$-VLDL.\textsuperscript{28-30} The $\beta$-VLDL particles are believed to be remnant lipoproteins generated by the action of lipoprotein lipase on triglyceride-rich chylomicrons and VLDL.\textsuperscript{25} In the normal rabbit, cholesterol-rich remnant particles are rapidly cleared by the liver, but in cholesterol-fed animals, hepatic uptake is markedly reduced.\textsuperscript{25, 27}

We therefore point out that some dissimilarities exist between our preparation and the chronic hypercholesterolemia that occurs in humans, particularly with regard to the duration of hypercholesterolemia and composition of lipoproteins.

In our experiments, animals were studied after only 3 days of cholesterol feeding to avoid any possible development of atherosclerotic lesions. In fact, it has been reported that the first coronary lesions occur 1 month after starting a cholesterol-enriched diet.\textsuperscript{31} However, since we did not grossly or microscopically examine the myocardium, the possibility of some very early changes such as migration of circulating monocytes through the endothelium cannot be excluded.

An occlusion-reperfusion preparation was chosen for two reasons. First, in the experimental setting it is known that with permanent, total occlusion of a coronary artery, the eventual extent of myocardial necrosis is nearly equal in size to the zone of initial hypoperfusion.\textsuperscript{32} Thus, while the latter preparation may be appropriate for study of effects of interventions that may decrease infarct size, it would be difficult to detect the effect of an intervention presumed to increase infarct size since the extent of damage would have to extend beyond the HZ. On the other hand, in the occlusion-reperfusion experiments here, infarct sizes in control animals were always considerably smaller than zones of initial hypoperfusion, and thus the likelihood of detection of any adverse effect of hypercholesterolemia was increased. Indeed, in control animals the zone of necrosis in these experiments was approximately 40% of the HZ. Second, occlusion-reperfusion experiments in animals may mimic the situation in patients with acute myocardial infarction undergoing thrombolytic therapy.

The principal results of this study are that (1) hypercholesterolemia in rabbits augments (doubles) infarct size after coronary artery occlusion-reperfusion, and (2) the NRZ assessed at the end of the reperfusion period is significantly larger in hypercholesterolemic rabbits than that in control animals or in isoproterenol-treated animals, although in the latter group infarct size was similar to that in the cholesterol group. Thus, cholesterol feeding augments both the no-reflow phenomenon and the extent of myocardial damage. Indeed, the finding that in this group the NRZs were approximately the same size as the infarct zones suggests that the inability to reperfuse is the cause of the infarct extension in these hypercholesterolemic rab-
bits. In contrast, the increase in infarct size in the isoproterenol group was not accompanied by a similar increase in non-reperfused area, in accordance with the hypothesis that isoproterenol increases myocardial damage by an increase in myocardial oxygen requirements.

Over the last few years there has been intense interest in the concept of treating patients with acute myocardial infarction with thrombolytic agents. In some studies, approximately 80% to 85% of patients treated with intracoronary streptokinase have had successful thrombolysis. This successful thrombolysis was reported to be associated with amelioration of chest pain, improved electrocardiographic findings, and improved thallium-detected perfusion. However, not all studies have demonstrated an improvement in left ventricular function after coronary reperfusion. Moreover, a clinical impression prevails that while some patients show an obvious improvement, including a resolution of cardiogenic shock, many others do not exhibit any measurable improvement. Therefore, there may exist factors that limit the benefit of early coronary artery reperfusion to a different degree in each patient. One factor that may be involved is the inability of blood to reenter all of the vasculature of the previously ischemic myocardium, referred to as the no-reflow phenomenon. It has been demonstrated that the extent of the no-reflow phenomenon is proportional to the time of coronary artery occlusion and a wide variety of mechanisms have been proposed to explain its occurrence. These include capillary compression by parenchymal cell swelling, interstitial edema, obstruction by endothelial cell swelling, aggregation of blood cellular components, thrombosis, and constriction of arterioles. There is as yet no clear indication as to whether any or all of these mechanisms contribute, are coincident with, or are inconsequential sequelae of the death of myocardial cells. The present results suggest that hypercholesterolemia can augment the no-reflow phenomenon and that myocardial damage ensues; if this is true in man, then variation in plasma cholesterol levels among patients may lead to varying degrees of the no-reflow phenomenon and the subsequent extent of myocardial damage.

In considering the results of the present study, it is important to emphasize that the increase in the NRZ in cholesterol-fed rabbits cannot be considered to be a consequence of an increase in the necrotic area; this is demonstrated by the observation that the isoproterenol-treated rabbits that had infarcts similar in size to those in hypercholesterolemic rabbits did not exhibit a substantial increase in the NRZ (figure 5). In fact, this study demonstrates that increases in the extent of myocardial damage may be produced with negligible changes in the area of no-reflow by pharmacologic interventions that increase infarct size by increasing myocardial oxygen requirements. In contrast, however, it is obvious that the final extent of an infarct cannot be smaller than the no-reflow zone. Accordingly, we conclude that the enlarged zone of no reflow is responsible for the augmentation of infarct size observed in the hypercholesterolemic rabbits.

Regarding the possible mechanisms by which acute hypercholesterolemia adversely affects infarct size, preliminary data by our group seem to suggest that platelets play an important role in reducing the efficacy of reperfusion in acute hypercholesterolemic rabbits. It has been reported that a 10-fold increase in platelet accumulation within the myocardial vasculature of hypercholesterolemic rabbits occurs and that the detrimental effect of hypercholesterolemia on infarct size can be completely reversed by lowering the number of circulating platelets.

The present study is the first to demonstrate that a cholesterol-enriched diet in animals can adversely affect infarct size. These results naturally raise the question of whether hypercholesterolemia in man may be a cardiovascular risk factor for reasons other than its role in atherogenesis, perhaps by increasing infarct size and thereby the likelihood of serious sequelae such as cardiac failure or fatal arrhythmias. Also, one might speculate that thrombolytic procedures in patients with acute myocardial infarction may be less beneficial in hypercholesterolemic individuals. Before these questions can be addressed with precision, however, much more will need to be known about the specific mechanisms responsible for the augmented infarct size in the experimental setting. For example, what is the nature of the vascular obstruction? Is it due mainly to an enhanced contribution of the arterial wall or to obliteration of the lumina by blood cells? Do the known effects of hypercholesterolemia on platelet, leukocyte, and red blood cell membrane lipid composition and platelet and leukocyte aggregability play a role in the process? What role, if any, can be attributed to secondary metabolic alterations such as decreased synthesis of prostacyclin by vascular tissue? We gratefully acknowledge the expert technical assistance of Ms. Deborah Moore and Ms. Deborah Evans and the manuscript preparation of Mrs. Patricia Powell.

References

1. Inter-society Commission for Heart Disease Resources: Primary prevention of the atherosclerosis disease. Circulation 42: A55, 1970


The effect of acute hypercholesterolemia on myocardial infarct size and the no-reflow phenomenon during coronary occlusion-reperfusion.

P Golino, P R Maroko and T E Carew

Circulation. 1987;75:292-298
doi: 10.1161/01.CIR.75.1.292

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 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/75/1/292

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/