Effects of Carbon Monoxide on Myocardium

ULTRASTRUCTURAL CHANGES IN RABBITS AFTER MODERATE, CHRONIC EXPOSURE

By Knud Kjeldsen, Henrik Klem Thomsen, and Poul Astrup

ABSTRACT

Sixteen rabbits were placed in exposure chambers: eight were continuously exposed to 180 ppm of carbon monoxide and eight were exposed to atmospheric air for 2 weeks. The myocardial ultrastructure of all the rabbits was examined. In rabbits exposed to carbon monoxide, local areas of partial or total necrosis of the myofibrils and degenerative changes of the mitochondria were found. Extra- and intracellular edema, increases in the number of ribosomes and lipofuscin granules, and reparative fibrotic changes also occurred. Varying degrees of injury were noted in the blood vessels. Capillary edema, but never total stenosis, was seen in some areas. Stasis and occasional small perivascular hemorrhages were typical on the venous side; on the arterial side the characteristic picture was one of endothelial swelling, formation of subendothelial edema, and degenerative changes of the myocytes. The present study supports the hypothesis that chronic exposure to low levels of carboxyhemoglobin can produce myocardial damage and account for the increased risk of myocardial infarction and sudden death seen in heavy cigarette smokers.

KEY WORDS  sudden death  myocardial infarct  hypoxia  secondary myocardiopathia  smoking  cardiac edema  myofibril necrosis

Light, chronic carbon monoxide exposure produces arterial injury that is histologically indistinguishable from early atherosclerosis (1) in animals; moreover, it greatly enhances aortic and coronary atheromatosis in rodents (2) and primates (3). Ultrastructural studies indicate that carbon monoxide exposure damages arterial endothelium, thereby facilitating the influx of plasma components into the subintima and media. Furthermore, carbon monoxide exposure in human volunteers and in dogs causes abnormal leakage of the vascular system (4–6).

The medical importance of these findings derives from the frequent occurrence of high carboxyhemoglobin levels in smokers (7), indicating that the carbon monoxide content of tobacco smoke is an important etiological agent in the development of arterial disease in smokers. Because sudden heart death, often without demonstrable coronary thrombosis at autopsy, is not uncommon in middle-aged heavy smokers, we decided to investigate the effect of light, chronic carbon monoxide exposure on the myocardial ultrastructure.

Methods

ANIMALS AND DIET

The experiment was carried out on 16 castrated male albino rabbits of the Danish country breed. The rabbits were about 5 months old and weighed between 3 and 3.5 kg. All rabbits were purebred, and they were all obtained from the same source. All were fed standard rabbit pellets and tap water.

EXPOSURE TO CARBON MONOXIDE

All rabbits were placed in exposure chambers; eight were exposed to carbon monoxide and the other eight served as controls which were kept in atmospheric air. The experimental rabbits were continuously exposed for 2 weeks to 180 ppm of carbon monoxide in atmospheric air as described earlier (2, 7). Carboxyhemoglobin concentrations were measured in blood samples taken from an ear vein once a week; the mean value was 16.7 ± 1.2% (SE). The concentration of carbon monoxide in the chamber air was measured twice a day with a Beckman 215A infrared analyzer; the mean value was 184 ± 18 ppm.

PREPARATION OF TISSUE FOR MICROSCOPY

After 2 weeks the rabbits were killed by a blow on the head. The thorax was opened, a cannula was immediately inserted into the left ventricle, and 0.5 liters of 4% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4) and 0.2M sucrose was infused under light pressure; infusion time was 4–5 minutes. Thereafter, the heart was excised, opened, and washed in physiological saline. Specimens were obtained from the subendocardial area of the posterior papillary muscle of the left ventricle and from the subepicardial area of the left ventricular wall near the apex.
Results

MACROSCOPIC FINDINGS

No macroscopic changes were seen in the hearts of control or experimental rabbits. There was no evidence of clots, hemorrhages, or fibrotic areas.

LIGHT MICROSCOPIC FINDINGS

Sections from the experimental rabbits were markedly different from those from the control group. Pronounced edema and irregular splitting of the muscle cells were evident in the hearts of the experimental rabbits. In some areas stasis of veins and venules occurred, and occasionally small accumulations of erythrocytes were noted adjacent to venules, probably representing petechiae. Otherwise, no significant changes were found in blood vessels or intracellular organelles at this level of magnification.

ULTRASTRUCTURAL FINDINGS

In sections from the control rabbits, the ultrastructural appearance (Fig. 1) was similar to that described in various laboratory animals by earlier investigators (10, 11); therefore, it will not be repeated in this paper.

Myocardium from the experimental rabbits showed a number of pathological changes compared with that from the control group. The pathological findings were identical in severity and distribution in the subendocardium of the papillary muscle and the subepicardium of the apex of the left ventricle; therefore, they will be described together.

Interstitial Tissue.—Marked edema with subsequent widening of the interstitial space occurred in the experimental rabbits. Lipid droplets were noted in the histiocytes (Fig. 2), and the cytoplasm of the fibroblasts contained many polysomes and a dilated endoplasmic reticulum, enclosing granular electron-dense material. Numerous bundles of formed collagen fibers were found throughout the interstitium and adjacent to the fibroblasts.

Sarcolemma.—In areas showing maximal contraction of the myofibers, scalloping became so pronounced that the arcades assumed a papilliferous appearance. In a few places the damage had proceeded into total rupture with a subsequent outpouring of intracellular organelles—especially mitochondria—into the interstitial space. The number of subsarcolemmal vesicles was not increased, but a few giant vesicles, reaching the size of an "arcade," were noted.

Intercalated Disk.—In most of the disks no changes were observed. However, fragmentation of the dense areas (macula adhaerens + fascia adhaerens) of the disks occasionally occurred. In some disks a prominent widening of the intercellular space developed in the undifferentiated regions of the disk (Fig. 3).

Myofibrils.—The myofibrils exhibited a broad spectrum of changes ranging from an almost normal appearance to total necrosis. In some areas the only change was a widening of the Z bands, which also had a blurred demarcation (Fig. 4A). In other areas a thinning out of the filaments was noted; this change often progressed into rupture of the fiber at one or more places, most often at the I band. In these fibers the Z band often became considerably fragmented (Fig. 4B). In still other areas myofibrils were considerably contracted, giving the impression of almost complete homogenization. The only remaining substructures were degenerated Z bands (Fig. 5). In a few scattered, localized areas necrosis was present with a resulting complete disintegration of substructure (Fig. 4C). Scattered, scarlike reparative changes, mainly consisting of bundles of collagen fibrils, were seen in a few sections (Fig. 6).

Mitochondria.—The mitochondria showed several pathological changes. The most consistent finding was swelling and ballooning of the mitochondria (Fig. 4B). Another characteristic change was condensation of the cristae which progressed into more or less complete homogenization of the mitochondrial matrix (Fig. 7). Quite often mitochondria were seen regenerating from the outer membranes by building concentric layers of cristae from the outside toward the middle. Clusters of densely packed mitochondria, most of which were circular in form, were often found dispersed in the myocytes (Figs. 5, 8). Finally, the mitochondria in some of the myocytes were transformed into myelin bodies (Fig. 9) which were usually situated near the sarcolemma and consisted of extremely electron-dense material arranged in either concentric irregular membranes or homogeneous circular bodies.

Sarcoplasmic Reticulum.—No changes in the sarcoplasmic reticulum were seen in the hearts of the experimental rabbits. The only change was a widening of the Z bands, which also had a blurred demarcation (Fig. 4A). In other areas a thinning out of the filaments was noted; this change often progressed into rupture of the fiber at one or more places, most often at the I band. In these fibers the Z band often became considerably fragmented (Fig. 4B). In still other areas myofibrils were considerably contracted, giving the impression of almost complete homogenization. The only remaining substructures were degenerated Z bands (Fig. 5). In a few scattered, localized areas necrosis was present with a resulting complete disintegration of substructure (Fig. 4C). Scattered, scarlike reparative changes, mainly consisting of bundles of collagen fibrils, were seen in a few sections (Fig. 6).

Mitochondria.—The mitochondria showed several pathological changes. The most consistent finding was swelling and ballooning of the mitochondria (Fig. 4B). Another characteristic change was condensation of the cristae which progressed into more or less complete homogenization of the mitochondrial matrix (Fig. 7). Quite often mitochondria were seen regenerating from the outer membranes by building concentric layers of cristae from the outside toward the middle. Clusters of densely packed mitochondria, most of which were circular in form, were often found dispersed in the myocytes (Figs. 5, 8). Finally, the mitochondria in some of the myocytes were transformed into myelin bodies (Fig. 9) which were usually situated near the sarcolemma and consisted of extremely electron-dense material arranged in either concentric irregular membranes or homogeneous circular bodies.

Sarcoplasmic Reticulum.—No changes in the sarcoplasmic reticulum were seen in the hearts of the experimental rabbits. The only change was a widening of the Z bands, which also had a blurred demarcation (Fig. 4A). In other areas a thinning out of the filaments was noted; this change often progressed into rupture of the fiber at one or more places, most often at the I band. In these fibers the Z band often became considerably fragmented (Fig. 4B). In still other areas myofibrils were considerably contracted, giving the impression of almost complete homogenization. The only remaining substructures were degenerated Z bands (Fig. 5). In a few scattered, localized areas necrosis was present with a resulting complete disintegration of substructure (Fig. 4C). Scattered, scarlike reparative changes, mainly consisting of bundles of collagen fibrils, were seen in a few sections (Fig. 6).

Mitochondria.—The mitochondria showed several pathological changes. The most consistent finding was swelling and ballooning of the mitochondria (Fig. 4B). Another characteristic change was condensation of the cristae which progressed into more or less complete homogenization of the mitochondrial matrix (Fig. 7). Quite often mitochondria were seen regenerating from the outer membranes by building concentric layers of cristae from the outside toward the middle. Clusters of densely packed mitochondria, most of which were circular in form, were often found dispersed in the myocytes (Figs. 5, 8). Finally, the mitochondria in some of the myocytes were transformed into myelin bodies (Fig. 9) which were usually situated near the sarcolemma and consisted of extremely electron-dense material arranged in either concentric irregular membranes or homogeneous circular bodies.

Sarcoplasmic Reticulum.—No changes in the sarcoplasmic reticulum were seen in the hearts of the experimental rabbits. The only change was a widening of the Z bands, which also had a blurred demarcation (Fig. 4A). In other areas a thinning out of the filaments was noted; this change often progressed into rupture of the fiber at one or more places, most often at the I band. In these fibers the Z band often became considerably fragmented (Fig. 4B). In still other areas myofibrils were considerably contracted, giving the impression of almost complete homogenization. The only remaining substructures were degenerated Z bands (Fig. 5). In a few scattered, localized areas necrosis was present with a resulting complete disintegration of substructure (Fig. 4C). Scattered, scarlike reparative changes, mainly consisting of bundles of collagen fibrils, were seen in a few sections (Fig. 6).

Mitochondria.—The mitochondria showed several pathological changes. The most consistent finding was swelling and ballooning of the mitochondria (Fig. 4B). Another characteristic change was condensation of the cristae which progressed into more or less complete homogenization of the mitochondrial matrix (Fig. 7). Quite often mitochondria were seen regenerating from the outer membranes by building concentric layers of cristae from the outside toward the middle. Clusters of densely packed mitochondria, most of which were circular in form, were often found dispersed in the myocytes (Figs. 5, 8). Finally, the mitochondria in some of the myocytes were transformed into myelin bodies (Fig. 9) which were usually situated near the sarcolemma and consisted of extremely electron-dense material arranged in either concentric irregular membranes or homogeneous circular bodies.

Sarcoplasmic Reticulum.—No changes in the sarcoplasmic reticulum were seen in the hearts of the experimental rabbits. The only change was a widening of the Z bands, which also had a blurred demarcation (Fig. 4A). In other areas a thinning out of the filaments was noted; this change often progressed into rupture of the fiber at one or more places, most often at the I band. In these fibers the Z band often became considerably fragmented (Fig. 4B). In still other areas myofibrils were considerably contracted, giving the impression of almost complete homogenization. The only remaining substructures were degenerated Z bands (Fig. 5). In a few scattered, localized areas necrosis was present with a resulting complete disintegration of substructure (Fig. 4C). Scattered, scarlike reparative changes, mainly consisting of bundles of collagen fibrils, were seen in a few sections (Fig. 6).

Mitochondria.—The mitochondria showed several pathological changes. The most consistent finding was swelling and ballooning of the mitochondria (Fig. 4B). Another characteristic change was condensation of the cristae which progressed into more or less complete homogenization of the mitochondrial matrix (Fig. 7). Quite often mitochondria were seen regenerating from the outer membranes by building concentric layers of cristae from the outside toward the middle. Clusters of densely packed mitochondria, most of which were circular in form, were often found dispersed in the myocytes (Figs. 5, 8). Finally, the mitochondria in some of the myocytes were transformed into myelin bodies (Fig. 9) which were usually situated near the sarcolemma and consisted of extremely electron-dense material arranged in either concentric irregular membranes or homogeneous circular bodies.
Myocardium from a normal rabbit (longitudinal section). Note the regular appearance of the myofibrils, the regular chromatin pattern, and the short distance between the capillaries and the myocytes. N = nucleus; Z, A, and I = Z, A, and I bands; IS = interstitial space; C = capillary; M = mitochondria; ID = intercalated disk. Bar indicates 1 μm.

Myocardium from an experimental rabbit (longitudinal section). Note the edematous interstitial space (IS) and the histiocyte (H) containing lipid droplets. V = venule and E = erythrocyte. Bar indicates 1 μm.

Myocardium from an experimental rabbit. A cross section through the intercalated disk (ID) shows dehiscence in the undifferentiated parts of the disk (arrows). Bar indicates 1 μm.
Myocardium from an experimental rabbit. Photomontage showing different grades of myofibrillar injury. A: Z bands are widened and have a washed out appearance. To the left the myofibrils are intact, but to the right they are breaking up between the Z bands. Note also the accumulation of ribosomes close to the mitochondria. B: Most of the filaments between the Z bands have disappeared. The mitochondria are circular with a homogeneous matrix. C: Myofibrils are broken into small pieces and the normal structure is disorganized. Bars indicate 1 μm.

CATALOG OF FIGURES

342 KJELDSEN, THOMSEN, ASTRUP

FIGURE 4

experimental rabbits.

Nucleus.—Most nuclei were swollen and had an abnormally clear appearance due to clumping and peripheral arrangement of the chromatin (Fig. 5). In the light areas clusters of ribosomes were regularly found.

Sarcolemma.—Intracellular edema was present in all myocytes, but it was always much less pronounced than it was in the interstitia. A constant finding was an increased number of polysomes; these structures were scattered throughout the cell, but they were particularly numerous around the nucleus and close to the mitochondria (Fig. 7). In most areas the number of glycogen granules was diminished, but in a few micrographs the sarcoplasm of the single cell was completely packed with granules (Fig. 8). Rather few lipid droplets were seen, but in contrast to the controls lipofuscin granules were a regular finding (Fig. 5).

Arterioles.—The endothelial cells appeared to be swollen and they often contained large vacuoles. Occasionally fluid-filled blisters were noted near the basement membrane. In a few micrographs the interendothelial junctions were distended between the "attachment belts." In all specimens the subendothelial space was considerably widened by prominent edema progressing between the myocytes of the media. The myocytes often showed either homogenization or vesiculation. The pathological changes were similar to those described earlier in the aorta after carbon monoxide exposure (1). However, as a rule, the edema of the subendothelial space was less pronounced, probably because the absence of an internal elastic membrane allowed an easier propagation of edematous fluid into the interstitial space.

Venules.—The venules were always surrounded by edema. The perivascular area occasionally contained small accumulations of erythrocytes and thrombocytes, probably representing petechiae. Apart from swelling of the endothelium, no changes were found in the vessel wall. Some venules were dilated, obviously due to stasis; the wall in these vessels appeared thin and stretched.

Capillaries.—Occasionally the endothelium of capillaries appeared swollen and distorted and was surrounded by edema, but in most areas no or only slight changes were noted. No correlation was observed between the degree of stenosis and the degree of tissue damage in the same area. In all vessels the number of pinocytotic vesicles seemed equal in control and carbon monoxide-exposed rabbits.
Myocardium from an experimental rabbit (longitudinal section). The two nuclei (N) have lumpy, peripherally arranged chromatin and appear slightly swollen. The myofibrils are thinned out and ruptured (below to the right). To the left the myofibrils are contracted (arrows). Note the large number of mitochondria and lipofuscin granules (LP). Bar indicates 1μ.

Myocardium from an experimental rabbit, illustrating reparative changes. Between bundles of collagen fibers (CF), poorly defined structures, probably projections from fibroblasts (arrows), are seen. Bar indicates 1μ.
Discussion

Morphological Changes in the Myocardium after Exposure to Carbon Monoxide and to Hypoxia.—Light microscopic changes in the heart due to acute carbon monoxide poisoning have been described a number of times in the literature (7, 12), but very few studies have dealt with chronic mild carbon monoxide exposure in animals. Ehrich et al. (13) exposed dogs to a carbon monoxide concentration of 100 ppm for 11 weeks, and Wanstrup et al. (14) exposed rabbits to 90 ppm for 12 weeks. Both investigators found significant myocardial damage similar to that found in the present study with edema, degenerative muscle changes, and fibrosis.

The ultrastructural myocardial changes demonstrated in the present study were identical not only to the findings in many studies concerning the effects of acute hypoxia (11, 15) including studies in which the oxidation processes were inhibited by cyanide (16), malonate (17), and carbon monoxide (18), but also to the findings in the surprisingly few studies of myocardial ultrastructure in animals exposed to chronic hypoxia for a few weeks or a few months. Sulkin and Sulkin (19) exposed rats to severe grades of hypoxia for up to 6 weeks. According to these authors, pathological changes only developed below 6.5% O₂ in the inspired air. In later studies, these investigators (20) showed that myocardial lesions developed earlier and at higher oxygen levels in older rats than they did in younger rats. Bischoff et al. (21) investigated myocardial ultrastructure in dogs, rabbits, and rats maintained at an altitude of 4,300 m for 5 months; the lesions in dogs and rabbits were similar to those found in cattle with high mountain disease (22), but the rats appeared to be more resistant and only developed minor changes. In principle the lesions were identical in these studies: the main abnormalities in the muscle cells consisted of intracellular edema, dilatation of sarcoplasmic reticulum, mitochondrial swelling and damage, separation of myofilaments and intercalated disks, increase in lipid droplets, lipofuscin granules, and glycogen, and, in severe hypoxia, degeneration of myofilaments and scattered, isolated areas with complete loss of substructure. The endothelial cells often showed swelling or hyalinization.

The character of the lesions demonstrated in the present study indicated a superimposition of reparative changes, apparently reflecting an interplay between continuing hypoxia and adaptive responses of acclimatization. Preliminary results indicate that early pathological myocardial changes...
occur in rabbits after only a few hours of exposure to 180 ppm of carbon monoxide, but at present very little is known about threshold limits or time course in the development and regression of carbon monoxide-induced myocardial damage. According to Jennings et al. (23), mild early ischemic changes of the myocardium are reversible, although later changes such as muscle necrosis and pronounced mitochondrial injury are irreversible. The pathological changes seen in the present study such as myelin body formation, homogenization and fragmentation of myofibrils, and severe mitochondrial injury with loss of the limiting membrane were clearly irreversible and led to the formation of fibrotic scarlike areas.

**Mechanisms Involved in the Oxygen-Depriving Effect of Increased Carboxyhemoglobin Concentrations.**—The pronounced morphological changes seen in this study indicate that the myocardium suffered a severe deprivation of oxygen due to an impairment of the oxygen transport function not only of hemoglobin but also of myoglobin, which has a higher affinity for carbon monoxide than does hemoglobin, leading to approximately 2–3 times higher carboxymyoglobin concentrations at relatively low carboxyhemoglobin levels. The displacement to the left of the oxygen dissociation curves of the two pigments also impairs the availability of oxygen and leads to a decrease in myocardial oxygen tension, which under normal conditions is relatively low due to the extraction of as much as 70–75% of the oxygen from arterial blood. This impairment of oxygen transport and oxygen delivery is only partly adjusted by the increase in coronary blood flow induced by carbon monoxide, since the coronary sinus oxygen tension
decreases during carbon monoxide exposure (24). It should furthermore be kept in mind that the diffusion distance for oxygen is considerably lengthened due to extracellular and intracellular edema and in some areas also due to edema of the capillary endothelium.

Furthermore, carbon monoxide competes with oxygen for the various heme-containing enzymes. The competition depends on the relation between PCO and PO₂ and it is more deleterious at low oxygen tensions. The pronounced changes of the mitochondria demonstrated in this study indicate a severe effect of carbon monoxide exposure on the cytochrome system. This effect is probably intensified by the low oxygen tensions occurring in the heart at rest, since anoxia in in vitro experiments potentiates the carbon monoxide–induced delay in reaction velocity of the cytochrome system (25).

Most likely the carbon monoxide–induced functional changes occur in morphologically intact mitochondria, since it has been demonstrated that intact mitochondria from ischemic heart muscle in the postischemic period can have grossly deficient function (23). The severe morphological changes...
demonstrated in this study after exposure to 16–18% carboxyhemoglobin indicate that the function of the mitochondria must have been greatly damaged.

Possible Association between Carbon Monoxide-Induced Myocardiopathy and Myocardial Infarction without Thrombus Formation.—Friedberg and Horn (26) and Büchner (27) first drew attention to the fact that acute myocardial infarction without a demonstrable acute coronary occlusion was a common finding at autopsy. These observations, which later were confirmed by others (28–30), led Spain and Bradess (29) to doubt the correctness of the traditional concept of a myocardial infarct as "an area of coagulation necrosis in a tissue due to local anemia resulting from obstruction of circulation to the area" (31), and they pointed out that thrombus formation could well be secondary to the infarct. They gave evidence for this hypothesis by demonstrating that in patients dying within 1 hour after the acute attack, a thrombotic occlusion was found in only 16% of the cases. If the patients survived 1–24 hours, a demonstrable thrombosis was seen in 37% of the autopsies, and after 1 or more days in the hospital the percent increased to 54%.

Further support for this concept was provided by Baroldi (32) who demonstrated that in a large number of autopsies of acute, recent infarcts and sudden deaths a coronary thrombosis could not be demonstrated in more than 50% of the cases. Baroldi concluded that myocardial infarction probably is triggered by metabolic disturbances in myocardium furnished by an artery with a critically low blood flow and that thrombus formation later takes place where the artery is partially occluded by atherosclerotic processes.

We want to emphasize that myocardial damage may lead to abnormalities of repolarization and that ventricular fibrillation is probably generated in the periphery of a myocardial infarct, where necrotic and viable, but hypoxic, tissues are intermingled (30). In this connection it should be mentioned that acute carbon monoxide exposure in humans may lead to abnormal electrocardiograms and the development of arrhythmia (33).

In man there is evidence that myocardial fibers in many myocardial infarcts are subjected to ischemia which falls just short of producing irreversible damage (34). In heavy smokers high carboxyhemoglobin saturations could therefore play a role in both determining the size of a myocardial infarction and—in connection with nicotine (35, 36)—triggering ventricular fibrillation, thereby contributing to the understanding of the high frequency of sudden unexpected deaths in middle-aged heavy cigarette smokers. These effects are aggravated when coronary blood flow is decreased due to atherosclerotic obliterations, the occurrence of which in smokers is also enhanced by inhaled carbon monoxide (1–3). We feel justified in concluding that the carbon monoxide in tobacco smoke is a toxic factor of major importance, being mainly responsible for the increased risk of coronary atherosclerosis, myocardial infarction, and sudden heart death in heavy smokers.

Acknowledgment

We wish to express our gratitude to Mrs. Bodil Hviid for technical assistance, to Mr. H. M. Olsen for valuable help with the animal experiments, and to Mr. Erik Jensenius for the photographic work.

References

12. SHAFER, N., SMILAY, M.G., AND MACMILLAN, F.P.: Primary...


Effects of Carbon Monoxide on Myocardium: Ultrastructural Changes in Rabbits After Moderate, Chronic Exposure

KNUD KJELDSEN, HENRIK KLEM THOMSEN and POUL ASTRUP

Circ Res. 1974;34:339-348
doi: 10.1161/01.RES.34.3.339

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1974 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/34/3/339

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research 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 Research is online at:
http://circres.ahajournals.org/subscriptions/