Vasodilator Responses of Coronary Resistance Arteries of Exercise-Trained Pigs

Judy M. Muller, PhD; Paul R. Myers, PhD, MD; M. Harold Laughlin, PhD

**Background**

The purpose of this study was to test the hypothesis that vasodilator responses of porcine coronary resistance arteries are increased by exercise training.

**Methods**

Yucatan miniature swine were randomly divided into groups of exercise-trained (ET) and sedentary (SED) control pigs. ET pigs were placed on a progressive treadmill training program lasting 16 to 20 weeks, and SED pigs remained inactive during the same time period. Coronary resistance arteries 64 to 157 μm in diameter were isolated for in vitro evaluation of relaxation responses to the endothelium-independent dilators sodium nitroprusside (1×10⁻⁶ to 1×10⁻⁴ mol/L) and adenosine (1×10⁻⁶ to 1×10⁻⁵ mol/L) and to bradykinin (1×10⁻⁸ to 3×10⁻⁷ mol/L), an endothelium-dependent agent. Relaxation responses to adenosine and sodium nitroprusside were not altered by exercise training. Endothelium-dependent relaxation to bradykinin was enhanced in coronary resistance arteries from ET pigs (IC⁵₀ ET, 0.07±0.02 nmol/L; SED, 1.59±0.09 nmol/L). To determine whether prostanoids and/or the nitric oxide synthase pathway were involved in the ET-induced changes in bradykinin-induced vasodilation, responses to bradykinin were examined in coronary resistance arteries from both ET and SED pigs in the presence of indomethacin and in the presence of nitro-monomethyl L-arginine (L-NMMA). Both indomethacin and L-NMMA produced significant inhibition of the bradykinin-induced relaxation in vessels from both groups. Despite decreased bradykinin-induced relaxation after indomethacin, bradykinin-induced vasodilation was still enhanced in vessels from the ET group. L-NMMA caused greater inhibition of the bradykinin-induced relaxation in coronary resistance arteries from ET pigs relative to arteries from SED pigs and eliminated the training-induced enhancement of the bradykinin responses.

**Conclusions**

These results suggest that exercise training enhances bradykinin-induced vasodilation through increased endothelium-derived relaxing factor/nitric oxide production by the L-arginine/nitric oxide synthase pathway. (Circulation. 1994;89:2308-2314.)

**Key Words**

- endothelium
- muscle, smooth
- L-arginine
- nitroprusside
- adenosine
- bradykinin
- indomethacin

**Experimental Animals**

Adult female miniature swine weighing 25 to 40 kg were obtained from the breeder (Charles River) in five lots of eight animals each. Each group of eight pigs was randomly divided into a group of four ET pigs and four sedentary control (SED) pigs. The ET pigs were placed on a progressive treadmill training program similar to that described by Tipton et al for dogs, and the SED pigs were confined to their pens during the training period, 16 to 22 weeks. Data were obtained from resistance arteries from a total of 20 ET and 20 SED pigs.
All animals used in this study were housed and maintained according to standards set forth by the American Association for Accreditation of Laboratory Animal Care. Animals were euthanized by methods approved by the department of Laboratory Animal Medicine, University of Missouri, Columbia.

Training Program

In week 1 of training, ET pigs ran on the treadmill at 8.0 km/h for 15 minutes (sprint) and at 4.8 km/h for 20 to 30 minutes (endurance). The intensity and duration of exercise bouts increased steadily so that by week 12 of training, the pigs ran 85 minutes a day, 5 days a week. Training bouts of 85 minutes included a 5-minute warm-up at a speed of 4.0 km/h, a 15-minute sprint run at speeds of 9 to 13 km/h, a 60-minute endurance run at speeds of 6 to 10 km/h, and a 5-minute cooldown at a speed of 3.3 km/h. Ranges are given for running speeds because actual running speed during training depended on the ability of each pig.

Treadmill Performance Test

At the beginning and again at the end of the training period, treadmill performance tests were administered to both ET and SED pigs to evaluate exercise tolerance. The treadmill performance test consisted of four stages of exercise. In stage 1 the pigs ran 5.0 km/h with 0% grade for 5 minutes. In stage 2 the pigs continued to run at 5.0 km/h for 10 minutes and the grade was increased to 10%. In stage 3 speed was increased to 6.9 km/h while the grade remained at 10%. The pigs ran for 10 minutes in this stage. In the final stage, stage 4, the pigs ran up a 10% grade at 9.6 km/h until exhaustion was reached. ECG monitoring of heart rates was performed continuously throughout the test, and total exercise times were recorded.

Preparation of Coronary Resistance Arteries

After completion of exercise training or the period of sedentary confinement, pigs were sedated with ketamine (30 mg/kg IM) and anesthetized with sodium pentobarbital (30 mg/kg IV). A left thoracotomy was performed, and hearts were rapidly removed and placed in cold Krebs' solution (4°C) previously aerated with 95% O2/5% CO2. Subepicardial resistance arteries 70 to 160 μm in intraluminal diameter and approximately 1 mm in length were isolated from the left anterior descending coronary artery distribution with the aid of a Zeiss dissecting microscope. Resistance arteries were placed in a Flexiglas chamber maintained at 4°C and filled with Krebs' solution oxygenated with 95% O2/5% CO2 at pH 7.4. Each end of the vessel was cannulated with a glass micropipette (40 to 50 μm in diameter) and secured with 10-0 Ethicon nylon suture. The vessel was then superfused at 10 ml/min with Krebs' solution at 37°C. Vasoactive agents were added to the superfusate reservoir. The reservoir solutions were changed to wash out drugs. The cannulated vessel was viewed through a Nikon inverted microscope (Nikon Diaphot TMD). The microscope was coupled to a video camera (Panasonic WV 1500X) and TV monitor (Panasonic TR930B). An image of the vessel was displayed on the TV monitor, and intraluminal diameter measurements were made continuously with a video tracking device (Living Systems) similar to that described by Halpern et al.14 The tracking device produced a DC signal that was recorded on a computer data acquisition system (MacLab, World Precision Instruments).

Intraluminal pressure was maintained constant in the absence of intraluminal flow with the use of a servo syringe perfusion pump apparatus. Each cannulating pipette was connected to a pressure transducer (Sorensen Transpac II) and a syringe perfusion pump (Technical Resources Core, Dalton Research Center, University of Missouri, Columbia). The vessel was pressurized by setting one of the perfusion pumps to the desired pressure and closing off perfusion at the opposite end of the vessel. Vessels were used only if their intraluminal diameter was smaller than 160 μm at a distending pressure of 40 mm Hg and there was no evidence of leaks as indicated by inability to maintain intraluminal pressure.

Oxidative Enzyme Capacity

After the pigs were killed, samples were taken from the middle of the triceps brachii muscles, frozen in liquid nitrogen, and stored at −70°C until processed. Citrate synthase activity was measured in the samples by the spectrophotometric assay described by Srere.15

Experimental Design

This study consisted of two series of experiments. The objective of the first series of experiments was to compare vasodilator responses of coronary arteries with endothelium-dependent and endothelium-independent vasodilators in ET and SED pigs. In these experiments, vasodilator responses to bradykinin (endothelium-dependent11) and to sodium nitroprusside and adenosine (endothelium-independent agents16) were examined. Results from this initial series of experiments indicated that responsiveness to bradykinin was enhanced in coronary resistance arteries from ET pigs. Therefore, the second series of experiments was designed to determine whether the enhanced bradykinin-induced relaxation observed in vessels from ET pigs was due to alterations in the L-arginine/nitric oxide (NO) synthase pathway and/or the cyclooxygenase pathway. Vasodilator responses to bradykinin were determined in the presence of nitro-monomethyl-L-arginine (L-NMMA), an inhibitor of the L-arginine/NO synthase pathway,17-19 and indomethacin, a cyclooxygenase inhibitor.16

Experimental Protocols for Determining Vasodilator Responses to Adenosine, Sodium Nitroprusside, and Bradykinin

Vessels were allowed to equilibrate at an intraluminal pressure of 40 mm Hg for 1 hour, during which time the temperature of the superfusate was warmed to 37±1°C. We chose to use an intraluminal pressure of 40 mm Hg because Chilian et al10 reported this as the normal pressure for coronary arteries of this diameter. Vasodilator responses were examined in arteries after a steady-state level of preconstriction had been obtained by addition of endothelin sufficient to produce a constriction of the vessel to 35% to 60% of maximal vessel diameter at this pressure. Since variable levels of spontaneous tone were present, varying concentrations of endothelin (0 to 2 nmol/L) were needed to produce this level of preconstriction in individual vessels. Some vessels developed this level of constriction spontaneously, eliminating the need for administration of endothelin. Previous work indicated that porcine coronary microvessels that develop spontaneous tone may require less endothelin to reach a desired level of preconstriction.20 Vasodilator responses were similar in microvessels preconstricted with endothelin and those that developed spontaneous tone.20 In the present study, concentration-response relations for vasodilators were determined by cumulative addition of adenosine (1x10^-10 to 1x10^-5 mol/L), sodium nitroprusside (1x10^-10 to 1x10^-4 mol/L), and bradykinin (1x10^-13 to 3x10^-7 mol/L) in half-log increments to the superfusate.

Experimental Protocol for Determining the Effect of Indomethacin on Bradykinin-Induced Relaxation

After equilibration and after a steady level of preconstriction was obtained, an initial concentration-response relation for bradykinin (1x10^-13 to 3x10^-7 mol/L) was obtained. The vessel was then washed for 30 minutes. After the wash period, 5 μmol/L indomethacin was added to the superfusate, and the vessel was incubated for 20 minutes. If the vessel was not adequately preconstricted after wash and incubation, endothelin was administered to achieve a level of preconstriction similar to that achieved before the first concentration-response
Experimental Protocol for Determining the Effects of L-NMMA, D-NMMA, and L-NAME on Bradykinin-Induced Relaxation

After equilibration at a steady level of preconstriction, an initial concentration-response relation for bradykinin (1 x 10^-13 to 3 x 10^-7 mol/L) was obtained. The vessel was then washed for 30 minutes. After the wash period, 3 mmol/L L-NMMA was added to the superfusate, and the vessel was incubated for 20 minutes. If the vessel was not adequately preconstricted after wash and incubation, endothelin was administered to achieve a level of preconstriction similar to that achieved before the first concentration-response relation was generated. A second bradykinin concentration-response relation was then generated. This protocol was also followed to determine the effects of N^0-nitro-L-arginine methyl ester (L-NAME) (300 μmol/L) and D-NMMA (3 mmol/L) on bradykinin-induced relaxation.

Solutions and Drugs

Krebs' buffer contained (in mmol/L) NaCl 118.3, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25, and glucose 11.1. pH was adjusted to 7.4, and the solution was aerated with 95% O2/5% CO2. Stock solutions of all drugs were prepared in distilled, deionized water prepared fresh daily. Sodium nitroprusside, adenosine, indomethacin, and L-NAME were purchased from Sigma Chemical Co. Bradykinin was purchased from BaChem. D-NMMA and endothelin were purchased from Peninsula Laboratories. L-NMMA was a gift from Abbott Laboratories.

Data Analysis

Citrate synthase activities, heart weight/body weight ratios, and exercise times were compared by Student's unpaired t tests. Vasodilator responses were expressed as a percent of the total relaxation achieved in response to 100 μmol/L sodium nitroprusside. IC50 values were defined as the concentration of the drug that produced 50% relaxation of the endothelin-induced preconstriction. These values were determined with a linear regression computer program (Basica IC50). IC50 values were expressed as logarithms and compared between ET and SED groups by Student's unpaired t tests. To determine whether L-NMMA and indomethacin produced a significant change in the IC50 for bradykinin, the IC50 value was determined in vessels in which bradykinin responses were determined both before and after treatment with the inhibitor in the same vessel and then compared within ET and SED groups by Student's paired t tests. For all statistical analyses, significance was defined as P<0.05.

Results

Indexes of Training

At the beginning of the training program, ET pigs became fatigued after running approximately 35 minutes on the treadmill. Their tolerance for treadmill exercise increased throughout the training so that by the end of the training period, ET pigs were completing 85-minute training bouts consistently. The effectiveness of the training program was also evident in that ET pigs had heart rates significantly lower than those of SED pigs during the moderate stages of posttraining treadmill performance tests. Heart rates were 14% and 11% lower in ET pigs than in SED pigs during stages 2 and 3 of the treadmill performance tests, respectively. In addition, citrate synthase activity of the triceps brachii muscles was increased (ET citrate synthase activity in

Characteristics of Isolated Resistance Arteries

Baseline intraluminal diameters (measured at a distending pressure of 40 mm Hg and in the presence of 100 μmol/L sodium nitroprusside) in vessels from ET pigs ranged from 65 to 153 μm, with a mean resting diameter of 117±3 μm. Baseline diameters were not different in vessels from SED pigs, ranging from 64 to 157 μm, with a mean resting diameter of 114±3 μm. The mean level of preconstriction was similar in arteries from ET and SED pigs (56.0±1.8% and 55.7±1.8% in ET and SED, respectively). The concentration of endothelin necessary to achieve this level of preconstriction was significantly less in ET pigs, 1.3±0.3 mmol/L, compared with SED pigs, 2.1±0.4 mmol/L (n=20 in each group).

Vasodilator Responses to Adenosine and Sodium Nitroprusside

Adenosine produced concentration-dependent relaxation of vessels from both ET and SED pigs (Fig 1). There were no significant differences in the concentration-response curves, maximal responses, or IC50 values between ET and SED groups. The concentration-response curves for sodium nitroprusside are presented in Fig 2. There were no significant differences between SED and ET IC50 values or maximal relaxation induced by sodium nitroprusside.

Responses to Bradykinin

Sensitivity to bradykinin was markedly enhanced in vessels from the trained group, as indicated by the leftward shift in the ET concentration-response curve (Fig 3). The IC50 for bradykinin in the ET group was
20-fold lower than that of the SED group. Bradykinin produced 100% relaxation in vessels from both groups.

**Effects of Indomethacin**

Incubation of endothelin-preconstricted vessels in 5 μmol/L indomethacin did not produce significant changes in vessel diameter (<1% change in diameter in vessels from both ET and SED pigs). Indomethacin inhibited bradykinin-induced relaxation in vessels from both SED pigs and ET pigs, as reflected in the increases in IC50 values (Fig 4). Although treatment with indomethacin caused the greatest inhibition of bradykinin-induced relaxation at low concentrations of bradykinin, bradykinin still produced 100% relaxation after indomethacin treatment. As shown in Fig 4, indomethacin treatment did not eliminate the differences in the bradykinin responses found between ET and SED groups.

**Effects of L-NMMA, D-NMMA, and L-NAME**

Incubation of preconstricted vessels in 3 mmol/L L-NMMA caused further constriction in vessels from both ET and SED pigs. In vessels from ET pigs, diameter decreased 40.9±11.6%, whereas diameter in vessels from SED pigs decreased 38.4±8.7%. These changes in diameter produced by L-NMMA were not significantly different between groups.

L-NMMA produced significant inhibition of the bradykinin-induced relaxation in vessels from both ET and SED pigs. Similar to the effects of indomethacin, L-NMMA treatment did not affect maximal response but caused a significant increase in the IC50 value in vessels from both groups of pigs (Fig 5). Treatment with L-NMMA caused greater inhibition of the bradykinin-induced relaxation in vessels from ET pigs relative to inhibition of the response of vessels from SED pigs. Treatment with L-NMMA eliminated the difference in the IC50 values between groups with no significant difference between ET and SED concentration-response curves for bradykinin (Fig 5). The optical isomer D-NMMA had no effect on bradykinin-induced relaxation in vessels from ET or SED pigs. L-NAME also inhibited bradykinin-induced relaxation in vessels from both ET and SED pigs. As with L-NMMA,
treatment with L-NAME produced greater inhibition of the response to bradykinin in vessels from trained pigs.

Discussion

This study was designed to determine whether vasodilator responses of isolated porcine coronary resistance arteries are altered by chronic exercise training. Both endothelium-independent (adenosine and sodium nitroprusside) and endothelium-dependent (bradykinin) vasodilator responses were evaluated in isolated resistance arteries, in the absence of confounding mechanical, neural, and humoral influences that may also be altered by exercise training. The results reveal that exercise training did not alter the responses of coronary resistance arteries to adenosine or nitroprusside. In contrast, bradykinin-induced vasodilator responses were dramatically enhanced in vessels from trained pigs. The enhanced responsiveness to bradykinin found in coronary resistance arteries from ET pigs was eliminated by inhibition of NO synthase with L-NMMA and L-NAME.

Vasodilator Responses to Adenosine and Sodium Nitroprusside

Our finding that vasodilator responses to adenosine and sodium nitroprusside are not altered in coronary resistance arteries from ET pigs surprised us because Oltman et al7 reported increased sensitivity to adenosine and blunted relaxation to sodium nitroprusside in isolated epicardial arteries from ET pigs. In the initial experiments in this study, resistance arteries were obtained from the same hearts as used for the study of Oltman et al. It is possible that exercise training produces changes in the coronary vasculature that are linked to the functional and anatomic role of the vessels of different sizes. Collectively, these data suggest that exercise training produces nonuniform changes in the responsiveness of the coronary vasculature.

The results of three previous studies stimulated our hypothesis that coronary resistance arteries would exhibit increased sensitivity to adenosine and sodium nitroprusside. Laughlin et al3,4,6 reported that coronary blood flow was greater in the hearts of ET pigs during maximal vasodilation with adenosine. Also, measuring changes in coronary blood flow velocity in response to intracoronary infusion of pharmacologic agents, DiCarlo et al6 reported that sensitivity of coronary resistance vessels to adenosine was enhanced in ET dogs. The cause of the difference in our results and what was expected on the basis of the reports of Laughlin et al3 and DiCarlo et al6 cannot be established at this time. However, it may be because our experiments were performed in isolated arterioles where neural, mechanical, and humoral influences could be eliminated. Also, in the in vivo preparations, infusion of adenosine may have increased intracoronary flow and altered the distribution of resistance in the microcirculation. As a result, resistance vessels may be exposed to higher flow and altered perfusion pressure. We have preliminary evidence that responses to changes in both intraluminal pressure and flow are altered in coronary resistance arteries from trained pigs.22,23 Thus, enhanced responsiveness to adenosine reported in hearts of trained animals in vivo may be due in part to enhanced flow-induced vasodilation. Possibly, administration of adenosine in vivo produced vasodilation, which increased coronary blood flow, triggering further dilation caused by increased flow.

Responses to Bradykinin

In contrast to the results obtained with direct smooth muscle vasodilators, relaxation to bradykinin was enhanced in coronary resistance arteries from ET pigs. Bradykinin-induced dilation has been shown to be a receptor-mediated, endothelium-dependent response in porcine coronary resistance vessels.12 Additionally, preliminary data indicate that flow-induced vasodilation, which has been shown to be an endothelium-dependent response in porcine coronary arteries,11 is also enhanced in resistance arteries from ET pigs.22 In other studies of the effects of training on vasodilator responses in the coronary circulation,7,8 changes in endothelium-dependent responses have not been apparent. Rogers et al10 reported that responsiveness of isolated dog conduit coronary arteries to substance P and α1adrenergic agonists were unchanged by training. Also, Oltman et al7 reported that removal of the endothelium had no effect on increased responsiveness to adenosine in large, conduit coronary arteries from trained pigs.

Bradykinin stimulates endothelial release of both prostacyclin24,25 and NO.17,18,26 Therefore, we chose to investigate the possibility that bradykinin-induced release of either NO or prostacyclin was increased in coronary resistance arteries from ET pigs. Indomethacin produced significant inhibition of bradykinin-induced dilation in vessels from both groups, indicating that at least part of the bradykinin response is mediated through release of a prostanooid vasodilator. Inhibition of prostaglandin synthesis appeared to cause a greater shift in the IC50 value for bradykinin in vessels from ET pigs, suggesting that the increased responsiveness to bradykinin may be mediated...
in part by the cyclooxygenase pathway. However, treatment with indomethacin did not eliminate the difference in bradykinin responses between ET and SED groups, indicating that most of the increased responsiveness to bradykinin was a result of a mechanism other than the cyclooxygenase/prostacyclin pathway. Since indomethacin had no effect on diameter in passively distended vessels, there appears to be no basal release of a prostanooid vasodilator from these vessels. Further, since indomethacin had no effect on diameter in vessels preconstricted with endothelin, our results indicate that there was no endothelin-induced synthesis of vasoactive prostaglandins.

L-NMMA, an inhibitor of NO synthase, also produced inhibition of bradykinin-induced relaxation in vessels from ET and SED pigs. Kuo et al. reported that L-NMMA did not inhibit bradykinin-induced relaxation in porcine coronary arteries. It is not clear why our results differ from those of Kuo et al. It is possible that these represent age-related effects. Kuo et al. conducted their experiments with vessels from immature farm pigs, whereas the present study was conducted with adult miniature swine. The possibility that the difference between findings of Kuo et al. and present findings is due to an age difference is supported by a recent report of Zellers and Vanhoutte, in which they found that endothelium-dependent relaxation responses increased with maturation in porcine lung vessels.

L-NMMA produced greater inhibition of bradykinin-induced relaxation in coronary resistance arteries from ET pigs. Before treatment with L-NMMA, the bradykinin concentration-response curve for the ET group was shifted to the left with respect to the curve for the SED group. After treatment with L-NMMA, the bradykinin concentration-response curve for the ET group was shifted slightly to the right of the curve for the SED group, although no significant difference existed between the curves. Thus, treatment with L-NMMA eliminated the difference in sensitivity to bradykinin found between ET and SED groups. D-NMMA had no effect on the response to bradykinin in vessels from either group. Bradykinin responses were also determined in vessels from both ET and SED pigs in the presence of L-NAME. L-NAME caused greater inhibition of bradykinin-induced relaxation in vessels from ET pigs, much like L-NMMA. These results support the conclusion that inhibition of bradykinin-induced relaxation produced by L-NAME and L-NMMA is due to direct inhibition of NO synthase and not to nonspecific effects of these compounds.

Treatment with L-NMMA produced significant constriction in vessels that had been preconstricted with endothelin. This may be a result of basal endothelin-derived relaxing factor (EDRF) release in the presence of endothelin or of endothelin-stimulated EDRF release. The additional constriction produced by L-NMMA did not differ between ET and SED groups, indicating that stimulation of EDRF release by endothelin was not proportionately altered in the vessels from ET pigs relative to SED animals.

The concentration of endothelin necessary to produce 50% constriction of arteries from ET pigs was statistically less than that necessary for arteries from SED pigs (1.2 versus 2.1 nmol/L). This raises the possibility that the differences between bradykinin responses of ET and SED pigs are caused by differences in endothelin concentrations. However, the difference in endothelin concentrations is small. Also, endothelin was used to preconstrict all vessels, and only responses to bradykinin were different between ET and SED pigs, whereas responses to adenosine and sodium nitroprusside were not different between groups. Further, L-NMMA treatment produced similar amounts of constriction in arteries from ET and SED pigs, suggesting that basal release of EDRF/NO was not different under these conditions. Finally, L-NMMA treatment eliminated the enhanced responsiveness to bradykinin in arteries from ET pigs. These considerations led us to conclude that the enhancement of bradykinin-induced vasodilatation is a result of increased EDRF/NO release via the L-arginine/NO synthesis pathway.

In conclusion, exercise training produced an enhanced bradykinin-induced relaxation in porcine coronary resistance arteries. Vasodilator responses to adenosine and sodium nitroprusside were not altered by training. Further, the enhancement of bradykinin-induced relaxation appears to be caused by increased EDRF/NO release. Increased EDRF/NO release could be due to alterations in bradykinin receptor/signal transduction or in the L-arginine/NO synthesis pathway. Because resistance arteries in this size range are important in determining total coronary vascular resistance, enhanced endothelium-dependent relaxation may contribute to altered control of vascular resistance and increased coronary blood flow capacity in the hearts of trained animals. It should be noted, however, that a change in the response to bradykinin may not imply a physiologically important change in response to exercise.

Acknowledgments

This study was supported by National Institutes of Health (NIH) grant HL-36531 to Dr. Laughlin and NIH training grant HL-07004 to Dr. Muller. The authors wish to thank Miles A. Tanner, Pam Thorne, and Tammy Knox for important technical contributions to this work. We gratefully acknowledge that the L-NMMA used in this study was provided by Abbott Laboratories, Abbott Park, Ill.

References


J M Muller, P R Myers and M H Laughlin

Circulation. 1994;89:2308-2314
doi: 10.1161/01.CIR.89.5.2308

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/89/5/2308

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/