Inhibitory G Protein Overexpression Provides Physiologically Relevant Heart Rate Control in Persistent Atrial Fibrillation

Alexander Bauer, MD; Amy D. McDonald, BS; Khurram Nasir, MD; Leah Peller, BS; Jeffrey J. Rade, MD; Julie M. Miller, MD; Alan W. Heldman, MD; J. Kevin Donahue, MD

**Background**—The need for new treatment strategies for cardiac arrhythmias has motivated our continuing development of gene therapeutic options. Previously, we reported a decreased heart rate in an acute model of atrial fibrillation after atrioventricular nodal gene transfer. Here, we expand those observations to persistent atrial fibrillation and severe heart failure.

**Methods and Results**—After 3 weeks of atrial fibrillation, domestic swine received atrioventricular nodal gene transfer with adenoviruses encoding β-galactosidase (β-gal), wild-type G_{i2} (wtGi), or constitutively active mutant (cGi). Heart rates in awake, alert animals were not altered by β-gal or wtGi. cGi caused a sustained 15% to 25% decrease in heart rate. The wtGi effect became evident with sedation. A tachycardia-induced cardiomyopathy was present before gene transfer. In the β-gal group, cardiomyopathy worsened over time. In the wtGi group, the condition improved slightly, and in the cGi group, ejection fraction was near normal at the end of the study. TUNEL staining results corroborated this finding.

**Conclusions**—cGi overexpression in the porcine atrioventricular node causes physiologically relevant heart rate control in persistent atrial fibrillation. These data advance the development of gene therapy as a potential treatment for common cardiac arrhythmias. (Circulation. 2004;110:3115-3120.)

**Key Words:** arrhythmia ■ gene therapy ■ electrophysiology ■ atrioventricular node ■ fibrillation

Several large clinical trials have associated antiarrhythmic drug therapy with increased mortality, demonstrating a need for other options to treat cardiac arrhythmias. Ablation and implantable devices are useful for several applications, but neither modality fully solves the problem. Radiofrequency ablation can cure focal arrhythmias (eg, atrioventricular [AV] node reentry tachycardia or accessory pathway–mediated tachycardia), but ablation only palliates more diffuse arrhythmias such as atrial fibrillation (AF) or infarct-related ventricular tachycardia. Implantable devices unquestionably save lives, but devices are associated with significant expense, potential complications from implant and replacement procedures, and in the case of defibrillators, pain related to the shock therapy.

AFFIRM and RACE are the most recent clinical trials to refute the antiarrhythmic drug paradigm. These trials compared the strategies of rhythm and rate control for treatment of AF. Like most antiarrhythmic trials, the bias going into these studies was that rhythm control (ie, maintenance of sinus rhythm with drugs or electrical cardioversion) would be proven superior to continued AF with control of the ventricular rate. The ultimate results demonstrated no benefit to the antiarrhythmic drug strategy and a suggestion of harm from the antiarrhythmic drugs, this time a nonsignificant trend toward increased deaths in the rhythm control arm of AFFIRM.

We recently reported a gene therapy strategy for rate control in AF. That proof-of-concept report documented an 18% heart rate reduction during acute AF in instrumented, anesthetized pigs after gene transfer of an inhibitory G protein α-subunit (G_{i2}). Questions arising from those results included the applicability to persistent AF in awake, alert animals and the significance of the observed 18% heart rate reduction. The current work answers those concerns. Using a previously reported model of persistent AF and heart failure, we evaluated the effects of AV nodal gene transfer with wild-type G_{i2} (wtGi) and a constitutively active mutant G_{i2} Q205L (cGi). Because gene expression is known to be limited with first-generation adenoviral (Ad) vectors, the physiologic observations in this report are limited to an 18-day window when Ad-mediated gene expression is known to be stable.

**Methods**

**Adenoviruses**

Ad-β-galactosidase (Adβ-gal) and AdwtGi were provided by Frank Graham (McMaster University, Hamilton, ON, Canada) and Thomas...
Eschenhagen (University Hospital Eppendorf, Hamburg, Germany), respectively. AdECGi was constructed using the Cre-Lox system as previously reported. Quality control of virus stocks included virus concentration determination by DNA absorbance, infective particle titer by plaque assay, transgene expression confirmation by Western blot analysis after transduction of HeLa cells, and absence of replication-competent virus by polymerase chain reaction analysis.

**Chronic AF Model**

Persistent AF was induced using atrial burst pacing as previously described. Domestic swine (18 to 22 kg) were sedated with ketamine 100 mg/kg and anesthetized with pentothal (2 to 5 mL of a 5% solution) and isoflurane (1% to 2%). A pacing lead (Medtronic) was fixed in the right atrial appendage and connected to a pacemaker in the right neck (Medtronic). The atria were paced at 42 Hz for 2-second intervals whenever the atrial rate fell below 180 beats/min. For purposes of this study, persistent AF (as opposed to paroxysmal AF) was defined as continuous AF without any evidence of sinus rhythm on the daily ECG tracings.

Throughout the study, clinical observations and ECG recordings were performed on a daily basis with a 6-lead ECG system. Animals were awake and alert at consistent levels from one reading to the next. Clinical observations included spontaneous activity level, appetite, evidence of dyspnea or edema, and overall appearance. The animals for this study were maintained in accordance with the guiding principles of the American Physiological Society regarding experimental animals. The experimental protocol was approved by the Johns Hopkins Institutional Animal Care and Use Committee.

**Gene Transfer Procedure**

On postpacer day 21 (gene transfer day 0), the animals underwent coronary catheterization for gene delivery. Domestic swine received 325 mg aspirin and 25 mg sildenafil PO and 1000 mg ketamine IM. Anesthesia was induced with 1000 mg IV ketamine and maintained with inhaled isoflurane, 0.5% to 2% in oxygen. The right coronary artery was catheterized, and the AV nodal branch was identified as the vessel tracking in the direction of the AV node originating near the posterior descending coronary artery. All animals in this study had right-dominant coronary systems with a single obvious vessel in the position of the AV nodal branch, so no further maneuvers were undertaken to identify the vessel. A 2.7F infusion catheter was inserted over a guidewire into the AV nodal artery. The following solutions were infused: 10 mL of Krebs' solution containing 5 μg vascular endothelial growth factor, 500 μg nitroglycerin over 3 minutes; 1 mL of Krebs' solution containing 1×10^6 plaque-forming units of Ad and 20 μg nitroglycerin over 30 seconds; and 2 mL of normal saline over 20 seconds. A standard randomization scheme was used to determine which virus was used in each animal. The investigator responsible for clinical observations and ECG measurements was blinded to the virus randomization scheme.

**Echocardiographic Examinations**

Echocardiographic examinations were performed at pacemaker implantation, gene transfer, and on post–gene transfer day 14. All measurements were performed after activation of the pacing protocol for consistent heart rate and irregularity. Left ventricular ejection fraction was calculated from parasternal short- and long-axis views, and chamber sizes were determined from M-mode images. All measurements were made using American Society of Echocardiography criteria.

**Histologic Evaluation**

After euthanization by intravenous KCl overdose in fully anesthetized animals, hearts were removed, and sections for microscopic analysis were fixed in 10% formalin, embedded in paraffin, cut to 7-μm thickness, and stained with hematoxylin and eosin or Masson's trichrome by traditional methods. Terminal dUTP nick end-labeling (TUNEL) staining was performed according to standard protocols. Negative controls were incubated with label solution only, and sections incubated with DNAase I (Sigma) served as positive controls. Sections were examined at ×64 magnification in a random order and blinded fashion by 2 observers. The reported histologic score is the average of these observations. Variables included nuclear enlargement, cellular hypertrophy, cellular myolysis, interstitial fibrosis, and interstitial inflammation. Each sample was graded on a scale of 1 to 5 as previously reported.

**Statistical Analysis**

The data are presented as mean±SEM. Heart rate data were controlled for repeated measurements and to determine correlations between subjects by use of a generalized estimating equation (GEE) with an exchangeable working relation to analyze the longitudinal relations of heart rate to potential use of different modes of therapy. For each pig i, the heart rate measured daily y_i (y_1, y_2, y_3, ... , y_10) was modeled by the XTGEE procedure of the STATA computer package. This method fits a regression model between heart rate measurements and effect of therapy for each pig, taking into account the inherent variability of slope estimates from individual pigs.

For the more straightforward analyses, statistical differences were determined using Student's t test and repeated-measures ANOVA, where appropriate. A probability value <0.05 was considered statistically significant.

**Results**

**Baseline Observations Before Gene Transfer**

Details of the ventricular response rate and resulting tachycardia-induced cardiomyopathy have been previously reported. Like the animals in that report, those in the current study had nonsustained episodes of AF immediately after activation of the burst-pacing algorithm and continuous AF by day 5±1. After developing continuous AF, the animals had no further recurrences of sinus rhythm for the duration of the study. Heart rate was measured once per day in awake, eating animals. During burst pacing and nonsustained AF, the ventricular rate was 281±12 beats/min on the first day after implantation of the pacemaker. Over the first 7 days, the heart rate in AF was 271±4 beats/min, and over the 21-day period before gene transfer, the average rate was 274±4 beats/min (P=NS). There were no significant differences in ventricular rate comparing intervals of burst atrial pacing, paroxysmal AF, and persistent AF).

Immediately after initiation of the burst-pacing protocol, 2 animals developed syncope resulting from the sudden increase in heart rate. These animals recovered without intervention and had no further episodes of syncope. Otherwise, there were initially no apparent behavioral changes resulting from the rapid heart rate. After 14 days, the animals displayed nonspecific signs of lethargy, increased sleep, mild dyspnea with exertion of walking from the pen to the ECG recording/feeding area, decreased play habits, and decreased appetite. Weight gain over the 3-week period averaged 1.3±0.1 kg/wk. In contrast, a set of normal control pigs did not display these behavioral changes, and weight gain averaged 1.8±0.3 kg/wk (P<0.01). Echocardiograms showed significant changes in cardiac dimensions and ventricular contractility comparing day 0 with day 21 (Table). There were no statistically significant differences between groups at the time of gene transfer (day 21).

**Absence of Rate Control From AV Node–Blocking Drugs in the Porcine AF Model**

To evaluate the efficacy of conventional drugs in this model, we tested the effects of digoxin, diltiazem, and esmolol...
administration on ventricular rate. Groups of 5 animals each received sustained-release diltiazem (6 mg/kg) or digoxin (6.3 μg/kg) daily for 5 days. Both doses were chosen because they would be relatively large human doses. Neither agent had any significant effect (Figure 1). On postpacemaker day 21, 4 sedated animals received intravenous esmolol (100 μg/kg), a short-acting β-blocker, given at the weight-based recommended human dose. The reduction in heart rate was negligible after esmolol administration (Figure 1).

In each case, the drug dose was limited by toxicity. Both esmolol and diltiazem worsened heart failure. In the diltiazem-treated animals, dyspnea and edema increased progressively during the 5-day course of therapy. Administration of esmolol caused intractable pulmonary edema and contractile failure leading to death despite aggressive intervention in all 4 animals. None of these animals received AV nodal gene transfer, so they were not included in any further analysis. Digoxin-treated animals had no observable positive or negative effects during drug therapy. Digoxin serum level was found to be in a range considered toxic for humans (3.2 ± 0.3 ng/dL), so the dose was not increased further.

**Differential Effects of cGi and wtGi on Heart Rate Reduction in AF**

On day 21 after pacemaker implantation, 15 animals underwent the AV nodal gene transfer procedure. Groups of 5 animals each received AdwtGi encoding the wild-type rat

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Day of PM</th>
<th>Day of GT</th>
<th>14 Days After GT</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-gal</td>
<td>LA</td>
<td>1.7 ± 0.1</td>
<td>2.6 ± 0.2*</td>
<td>3.4 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td>LVEDD</td>
<td>1.8 ± 0.1</td>
<td>3.6 ± 0.2†</td>
<td>4.1 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td>RVEDD</td>
<td>1.5 ± 0.1</td>
<td>2.2 ± 0.2†</td>
<td>2.7 ± 0.2*</td>
</tr>
<tr>
<td></td>
<td>LVST</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>LVPT</td>
<td>0.8 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>LVEF</td>
<td>72 ± 4</td>
<td>29 ± 2†</td>
<td>22 ± 3*</td>
</tr>
<tr>
<td>wtGi</td>
<td>LA</td>
<td>1.4 ± 0.1</td>
<td>3.0 ± 0.2†</td>
<td>3.5 ± 0.2*</td>
</tr>
<tr>
<td></td>
<td>LVEDD</td>
<td>1.8 ± 0.2</td>
<td>4.0 ± 0.3†</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>RVEDD</td>
<td>1.9 ± 0.3</td>
<td>2.7 ± 0.1†</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>LVST</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>LVPT</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>LVEF</td>
<td>71 ± 4</td>
<td>24 ± 3†</td>
<td>31 ± 3*</td>
</tr>
<tr>
<td>cGi</td>
<td>LA</td>
<td>1.6 ± 0.1</td>
<td>2.4 ± 0.2†</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>LVEDD</td>
<td>1.9 ± 0.2</td>
<td>4.1 ± 0.2†</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>RVEDD</td>
<td>1.9 ± 0.1</td>
<td>2.3 ± 0.1†</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>LVST</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>LVPT</td>
<td>0.8 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.7 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td>LVEF</td>
<td>69 ± 3</td>
<td>34 ± 5†</td>
<td>46 ± 4†</td>
</tr>
</tbody>
</table>

PM indicates pacemaker implantation; GT, gene transfer; LA, left atrial diameter; LVEDD, left ventricular end-diastolic diameter; RVEDD, right ventricular end-diastolic diameter; LVST, left ventricular septal thickness; LVPT, left ventricular posterior wall thickness; and LVEF, left ventricular ejection fraction.

P<0.05.
†P<0.01.
the remainder of the study ($P<0.001$ compared with either wtGi or β-gal). Surprisingly, the wtGi animals had no significant change in heart rate when compared with the Adβ-gal controls ($P=0.1$). All animals remained in AF for the duration of the study, and no heart block or ventricular arrhythmias were noted in any animals during this study. All animals survived to the conclusion of the study, and there were no strokes, myocardial infarctions, or sudden deaths observed.

**Etiology of Reduced wtGi Effects in Chronic AF Compared With Acute AF**

Our observed results in chronic AF contrasted the previous experience in acute AF. To investigate this anomaly, we re-created the conditions used in the previous report by evaluating heart rate during sedation on the seventh day after gene transfer. The results demonstrate that wtGi effects are elicited by sedation (Figure 3). Ad-cGi–infected animals had a $16\pm3\%$ reduction in heart rate when comparing post–gene transfer day 0 with day 7 ($P<0.01$); Ad-wtGi animals had a $12\pm5\%$ reduction ($P<0.01$), and the heart rate increased $5\pm5\%$ in the Adβ-gal animals ($P=NS$).

**Physiologic Responses to Ventricular Rate Control**

At the end of the experiment, Adβ-gal animals had extreme lethargy, obvious dyspnea when walking from the cage to the ECG/feeding area, increased sleep, loss of playful behaviors, and minimal appetites. At the time of sacrifice, these animals had negligible body fat, extensive ascites, and pericardial and pleural effusions.

Both wtGi and cGi groups had continued evidence of heart failure immediately after gene transfer, but the symptoms improved during the 18 days after gene transfer. The cGi group had no symptoms at the end of the study; they had normal activity and appetite. At the time of sacrifice, there was negligible extravascular fluid present. Of the animals in the wtGi group, 3 had mild heart failure symptoms with lethargy and mild dyspnea with exertion but a resumption of normal sleep and play patterns and no suppression of appetite. The other 2 animals had no detectable symptoms, but all wtGi animals had evident ascites and effusions at sacrifice.

Echocardiographic parameters were correlated with the clinical observations in the post–gene transfer period (the Table). β-Gal animals had increasing chamber sizes and worsening left ventricular function. The wtGi animals had modest improvements in left ventricular function and stabilization of ventricular chamber size but continued increases in left atrial size. The cGi group had near-normalization of left ventricular function, stabilization of left atrial and right ventricular chamber size, and a reduction in left ventricular size. At the time of sacrifice, the hearts were examined for signs of intracardiac thrombus. One animal in the Adβ-gal group had a well-developed thrombus in the left atrium. No other animals in any group had intracardiac thrombus.

**Histologic Findings After Gene Transfer**

All hearts were subjected to histologic analysis with hematoxylin and eosin, Masson’s trichrome, and TUNEL staining. The extent of cellular disorganization was more evident in ventricular tissue of control animals than in cGi animals, but signs of cellular hypertrophy and interstitial inflammation were similar for all groups. Left ventricular fibrosis was more extensive in β-gal and wtGi pigs than in cGi pigs (Figure 4). No significant differences were found between left ventricular samples of control and wtGi pigs, and no significant differences were noted for right ventricular samples. Despite the modest reduction in atrial size on the echocardiograms, no changes in any atrial histologic parameters were noted between groups.

Figure 3. Relative changes in heart rate after sedation. Comparison is made of heart rate on day of gene transfer and on post–gene transfer day 7. *$P<0.01$, day 7 vs day 0. Abbreviations are as defined in text.

Figure 4. Results from hematoxylin and eosin and Masson’s trichrome staining of cardiac samples. See text for details of scoring system. *$P<0.05$. LV and RV indicate left and right ventricle, respectively. All other abbreviations are as defined in text.
By guest on April 15, 2017

AF and severe chronic heart failure.

...model reduced heart rate to a level similar to that seen in the basal ventricular septum. We cannot definitively exclude the possibility that gene transfer to this small region affected global ventricular function, but the temporal association between gene transfer, heart rate control, and functional improvement and the global nature of this improvement supports the idea that rate control is the primary factor in the reversal of tachycardiomyopathy. As such, our observed improvement in left ventricular function documents the physiologic relevance of the achieved level of ventricular rate control.

Discussion

Development of gene therapy as a viable treatment for cardiac arrhythmias has occurred at a disappointing rate. To date, there are only 3 in vivo examples of the concept: our original report of AV nodal gene transfer in sedated pigs with acute AF and 2 illustrations of genetic pacemaker activity. In the current report, we continue the process of developing gene therapy as a potential option for cardiac arrhythmias. In a stepwise fashion, we first showed rate control in an acute model of AF, and now we demonstrate rate control in a physiologically relevant model of persistent AF and severe chronic heart failure.

Relevance of the Model

Our original report of AV nodal gene therapy for ventricular rate control gave proof to the concept that gene therapy can treat common cardiac arrhythmias. A problem with that report was the somewhat contrived nature of the model. The pigs were anesthetized and instrumented at the time of heart rate measurement. The AF was created by short-term burst pacing and was self-limited in duration. The current report strives to test the hypothesis in a more clinically relevant model.

In patients, AF typically appears secondary to some other form of heart disease (eg, coronary artery disease, hypertension, valvular disease, or left ventricular dysfunction). Similar to the human condition, our porcine model had AF in the setting of cardiac dilation, dysfunction, and severe heart failure symptoms. Also as in many severe heart failure patients, conventional drug therapy did not adequately control the ventricular response rate in our pigs. Another similarity is the tachycardia-induced left ventricular dysfunction, which occasionally plays a role in the human situation. Major differences between the porcine model and the human condition are the extraordinarily fast ventricular rate and rapid development of cardiomyopathy in the porcine model, although it could be considered that the drug-refractory fast rate and severe left ventricular dysfunction make this an appropriately rigorous model for testing this new therapy.

Physiologically Relevant Rate Control With cGi Gene Transfer

cGi gene transfer to the AV node resulted in a 15% to 25% reduction in heart rate, resulting in reversal of the clinical symptoms, a moderate recovery of cardiac function, and the beginnings of cardiac structural remodeling. Both ejection fraction and left ventricular end-diastolic diameter improved significantly, whereas left atrial and right ventricular diameters stabilized. Most histologic parameters lagged behind the functional measurements, although the decrease in TUNEL-positive cells in the cGi group suggests that structural remodeling might have continued beyond the limited follow-up of this report. The exact mechanism for reversal of the tachycardiomyopathy is unclear and probably complex. In our original report, gene transfer was largely confined to the AV node, but there was a small area of gene transfer in the basal ventricular septum. We cannot definitively exclude the possibility that gene transfer to this small region affected global ventricular function, but the temporal association between gene transfer, heart rate control, and functional improvement and the global nature of this improvement supports the idea that rate control is the primary factor in the reversal of tachycardiomyopathy.

Combination of Heart Failure and Arousal Overcome the wtGi Effect

The response of wtGi in the persistent AF model was disappointing, given our previous report. The response does shed some insight into the interactions between physiologic status of the animal and transgene effects. During sedation, wtGi overexpression in the chronic AF/chronic heart failure model reduced heart rate to a level similar to that seen in the adrenergically stimulated animals of the acute AF study. This result is consistent with the hyperadrenergic state previously documented in the AF/severe chronic heart failure model, which also potentially explains the inefficacy of conventional AV node-blocking drugs in our model. When the chronic AF/chronic heart failure animals were awake, heart rate control was completely lost. This behavior is reminiscent of the effect seen with digoxin in humans, in whom rate control is adequate at rest but quickly lost during activity or adrenergic stimulation. The interaction between adrenergic stimulation and wtGi overexpression suggests that the protein integrates normally into the G-protein receptor-coupled system. Obviously, further study is needed to inves-
tigate the mechanism on a molecular and cellular level, but the response to wtGi gene transfer provides encouragement that the gene transfer effect is caused by normal processing and placement of the transgene protein.

Conclusions

The current study continues the development of gene therapy as a potential option for this common arrhythmia. Our model is comparable to the clinical scenario of severe left ventricular dysfunction, persistent AF with a rapid ventricular response, and both inefficacy and intolerance of rate-controlling medications. Currently, treatment of such human patients requires ablation of the AV node and permanent dependence on a pacemaker. In our model of awake, fully functional animals, gene therapy–induced rate control was sufficient to reverse the tachycardia-induced cardiomyopathy. Further study with long-term expression vectors (eg, adenovirus20 or helper-dependent adenovirus23) and cardiac-specific promoters is needed, but this report advances the concept of using gene therapy as a future treatment option for common cardiac arrhythmias.

Acknowledgments

This study was supported by the National Institutes of Health, the American Heart Association, and the Deutschen Forschungsgemeinschaft. Pacemakers, leads, and the burst-pacing program were donated by Medtronic, Inc, Minneapolis, Minn.

Disclosure

Dr Donahue has ties to Excigen, a gene therapy company. None of the authors have any relationships to disclose. The observations made and measurements reported in the article were performed by Dr Bauer, who has no relationship to Excigen or any other gene therapy company. The statistical analyses were performed by Dr Nasir, who has no relationships to disclose.

References

Inhibitory G Protein Overexpression Provides Physiologically Relevant Heart Rate Control in Persistent Atrial Fibrillation
Alexander Bauer, Amy D. McDonald, Khurram Nasir, Leah Peller, Jeffrey J. Rade, Julie M. Miller, Alan W. Heldman and J. Kevin Donahue

Circulation. 2004;110:3115-3120; originally published online October 25, 2004; doi: 10.1161/01.CIR.0000147185.31974.BE
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 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/110/19/3115

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/