Gap Junction Remodeling and Spironolactone-Dependent Reverse Remodeling in the Hypertrophied Heart

Jiaxiang Qu,* Frank M. Volpicelli,* Luis I. Garcia, Nefthi Sandeep, Jie Zhang, Lucrecia Márquez-Rosado, Paul D. Lampe, Glenn I. Fishman

Abstract—Pressure overload is a common pathological insult to the heart and the resulting hypertrophy is an independent risk factor for sudden cardiac death. Gap junction remodeling (GJR) has been described in hypertrophied hearts; however, a detailed understanding of the remodeling process and its effects on impulse propagation is lacking. Moreover, there has been little progress developing therapeutic strategies to diminish GJR. Accordingly, transverse aortic banding (TAC) was performed in mice to determine the effects of progressive pathological hypertrophy on connexin (Cx)43 expression, posttranslational phosphorylation, gap junction assembly, and impulse propagation. Within 2 weeks after TAC, total and phospho-Cx43 abundance was reduced and incorporation of Cx43 into gap junctional plaques was markedly diminished. These molecular changes were associated with progressive slowing of impulse propagation, as determined by optical mapping with voltage-sensitive dyes. Treatment with the aldosterone receptor antagonist spironolactone, which has been shown to diminish sudden arrhythmic death in clinical trials, was examined for its effects on GJR. We found that spironolactone blunted the development of GJR and also potently reversed established GJR, both at the molecular and functional levels, without diminishing the extent of hypertrophy. These data suggest a potential mechanism for some of the salutary electrophysiological and clinical effects of mineralocorticoid antagonists in myopathic hearts. (Circ Res. 2009;104:365-371.)

Key Words: gap junction  spironolactone  arrhythmias  optical mapping  mouse  connexin

Hemodynamic overload is a commonly encountered pathological cardiovascular stimulus, and the associated hypertrophy has long been considered an independent risk factor for cardiac mortality, including sudden cardiac death.1–6 The effects of hypertrophic stimuli on gap junction expression and function have been studied in a number of experimental systems, including cultured neonatal myocytes, animal models, and human pathological specimens (reviewed elsewhere7,8). In neonatal cardiac myocytes, connexin (Cx)43 gap junction protein expression increases in response to acute administration of hypertrophic agents such as angiotensin II and cAMP.9,10 However, in vivo, both in experimental animals and in humans, prolonged hemodynamic overload is more commonly associated with significant downregulation of Cx43 expression, as well as lateralization of gap junctional protein away from the intercalated disks, ie, with gap junction remodeling (GJR).11–14 Interestingly, in a study of volume overload–induced hypertrophy, a transient upregulation of Cx43 was observed within hours after imposition of the hemodynamic load, followed by significant reduction in myocardial Cx43 content.15 Taken together, these in vitro and in vivo data suggest that GJR may be biphasic, although the second phase, characterized by diminished density of junctional channels, may be more clinically relevant.

In the present study, we used the well-characterized transverse aortic constriction (TAC) model to evaluate GJR and impulse propagation during progressive cardiac hypertrophy in intact hearts. Our results suggest that GJR significantly diminishes conduction velocity, although not sufficiently to support inducible arrhythmias. Altered post-translational phosphorylation of Cx43 was observed within days after imposition of TAC, suggesting that aberrant processing of this gap junctional protein is an essential element in the remodeling process. We found that treatment with the mineralocorticoid antagonist spironolactone blunts the development of pathological GJR and also potently reverses established GJR, both at the molecular and functional levels, without diminishing the extent of hypertrophy.

Materials and Methods

Animal Model

All animal procedures were carried out in accordance with Public Health Service Guidelines for the Care and Use of Laboratory Animals and approved by the New York University School of Medicine.

Original received July 24, 2008; revision received December 8, 2008; accepted December 10, 2008.

From the Leon H. Charney Division of Cardiology (J.Q., F.M.V., L.I.G., N.S., J.Z., G.I.F.), New York University School of Medicine; and Molecular Diagnostics Program (L.M.-R., P.D.L.), Fred Hutchinson Cancer Research Center, Seattle, Wash.

*Both authors contributed equally to this work.

Correspondence to Glenn I. Fishman, MD, Leon H. Charney Division of Cardiology, New York University School of Medicine, 522 First Ave, Smilow 801, New York, NY 10016. E-mail glenn.fishman@med.nyu.edu

© 2009 American Heart Association, Inc.

Circulation Research is available at http://circres.ahajournals.org

DOI: 10.1161/CIRCRESAHA.108.184044

365
Medicine Institutional Animal Care and Use Committee. TAC was performed on C57Bl6 male mice (3 to 4 months of age) as previously described.16 Mice received either standard chow diet (AIN-76A, Research Diets, New Brunswick, NJ) or standard chow supplemented with spironolactone (50 mg/kg per day).

**Echocardiography**

Left ventricular dimensions and function were assessed by echocardiography as previously described using an ATL 5000CV Ultrasound System (Philips Medical, Bothell, Wash).17

**Fibrosis Index**

Formalin-fixed, paraffin embedded sections were stained with Mason’s trichrome and analyzed with Image-Pro Plus 5.0 software (Media Cybernetics, Bethesda, Md) as previously described.18

**Western Blot Analysis**

Total protein lysates and Triton X-100 insoluble pellet fractions were prepared from the apical two-thirds of the ventricle as previously described.19 Primary antibodies included rabbit polyclonal 18B, which recognizes all forms of Cx43 and is directed toward an epitope from the carboxyl terminus; mouse monoclonal Cx43NT1, which also recognizes all forms of Cx43 but is directed toward an epitope from the amino terminus; rabbit antipS325/328/330-Cx43 and rabbit antipS365-Cx43 antibodies, which recognize specific phosphorylated forms of Cx43.19–21 Equivalency of protein loading was verified by probing for vinculin for total lysates or by Ponceau staining of the membrane for Triton X-100 pellets. Signals were visualized and quantified using the Odyssey Imaging System (Li-Cor, Lincoln, Neb).

**Immunohistochemistry**

Staining was performed on formalin-fixed, paraffin-embedded hearts using either fluorescein isothiocyanate or peroxidase-conjugated secondary antibodies as previously described.19,22

**Heart Isolation and Optical Mapping**

High-resolution optical mapping experiments were performed as previously described, using either a charge-coupled device camera (Dalsa, CAD-128, Waterloo, Calif) or, in more recent experiments, a newer generation CMOS video camera (Ultima-L; SciMedia Inc.).17,23 Studies were performed in the absence of any pharmacological or mechanical motion–reduction techniques. Epicardial conduction velocity (CV) measurements were obtained from the left ventricular surface, with only those pixels residing between 1 and 3 mm from the stimulation site being included in the analyses.22,24 Because of the improved robustness of the signal, we focused primarily on measurements of CVmin although CVmax values are reported as well. For studies of pharmacological gap junction uncoupling, after baseline parameters were determined, hearts were perfused with 18α-glycyrrhetinic acid (αGA) (7.5 μmol/L) for 15 minutes (Sigma-Aldrich), and CV measurements were verified by probing for vinculin for total lysates or by Ponceau staining of the membrane for Triton X-100 pellets. Signals were visualized and quantified using the Odyssey Imaging System (Li-Cor, Lincoln, Neb).

**Results**

**Progressive Hypertrophy With TAC**

TAC resulted in an initial phase of cardiac hypertrophy followed by the development of left ventricular dilatation and deterioration in contractile function, as determined by serial echocardiographic studies. The gross appearance of the heart at 2, 4, and 8 weeks after aortic constriction is shown in Figure 1A. Progressive cellular hypertrophy was also observed; by 8 weeks after TAC, there was an increase of 31% in cell length and an increase of 56% in cell width. These data are summarized in Tables 1 and 2.

**GJR and Altered Cx43 Phosphorylation Status**

To determine whether cardiac hypertrophy was associated with GJR, including changes in posttranslational phosphorylation.

**Table 1. Gross and Cellular Hypertrophy in TAC Mice**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Four Weeks</th>
<th>Eight Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight, mg</td>
<td>139 ± 5 (12)</td>
<td>282 ± 11 (13)†</td>
<td>324 ± 9 (11)†</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>27.9 ± 0.8 (12)</td>
<td>24.1 ± 0.9 (13)</td>
<td>28.5 ± 0.7 (11)</td>
</tr>
<tr>
<td>HW/BW, mg/g</td>
<td>5.00 ± 0.16 (12)</td>
<td>11.81 ± 0.55 (13)*</td>
<td>11.5 ± 0.48 (11)</td>
</tr>
<tr>
<td>Cell length, μm</td>
<td>158 ± 3 (55)</td>
<td>192 ± 4 (55)*</td>
<td>206 ± 5 (55)*</td>
</tr>
<tr>
<td>Cell width, μm²</td>
<td>24.7 ± 0.7 (55)</td>
<td>39.2 ± 1.0 (55)*</td>
<td>38.5 ± 1.3 (55)*</td>
</tr>
<tr>
<td>Cell area, μm²2</td>
<td>3934 ± 155 (55)</td>
<td>7528 ± 237 (55)</td>
<td>7873 ± 268 (55)</td>
</tr>
</tbody>
</table>

All values are means ± SEM. Numbers in parentheses indicate sample size. *P<0.05 compared to sham, †P<0.05 compared to sham and 4 weeks.
lation state, we analyzed Cx43 expression and localization by immunoblotting and immunohistochemical staining with a panel of antibodies with defined specificities for total or phosphorylated forms of Cx43. As shown in Figure 1B, during the first 5 days after imposition of TAC, there was a transient increase in the overall abundance of Cx43 within total cardiac lysates; however, this was followed by a progressive decline, such that by 4 weeks after pressure overload, Cx43 content had fallen to 49±3.4% of control levels (n=4 each group; P<0.05). To more specifically examine the effects of TAC on Cx43 within the gap junctions, we performed Western blots on Triton X-100 insoluble fractions, which enrich for junctional membrane proteins. Both N-terminal and C-terminal pan-Cx43 antibodies demonstrated a progressive decline in junctional Cx43 abundance following TAC, diminishing to 60±2.7% of control levels 4 weeks after TAC. Moreover, phospho-specific antibodies demonstrated a substantial diminution in both pS365, as well as pS325/328/330 forms of Cx43 within a week after imposition of TAC.

These results were confirmed and extended by immunohistochemical studies, which revealed substantial loss of Cx43 gap junctional plaques within 1 week after TAC (Figure 1C). Concordant with the Western blot studies, immunostaining with antibodies specific for pS365-Cx43 and pS325/328/330-Cx43 had diminished significantly from 0.55±0.02 to 0.47±0.02 μm, and by 8 weeks after imposition of the hemodynamic overload, Cx43 had fallen to 0.42±0.03 μm. To gain some insight into the extent of residual cell–cell coupling that existed in hypertrophied hearts, we assessed the effects of the relatively selective gap junction uncoupler αGA on impulse propagation in an additional cohort of hearts studied 4 weeks after imposition of TAC. Treatment of hypertrophied hearts with αGA depressed CV further, to 0.25±0.01 m/sec. These data suggest the presence of some degree of residual gap junction function, despite the extensive GJR that occurs in response to pressure overload.

Inasmuch as cardiac hypertrophy in many animal models is associated with action potential prolongation, we also measured ventricular effective refractory periods. VERPs in hearts from 4 week TAC animals were significantly prolonged compared to sham operated controls (57.4±3.6 ms (n=9) versus 42.7±2.7 ms (n=9); P=0.005), suggesting electric remodeling of repolarizing currents and consistent with previous reports.25

We next assessed whether hypertrophied hearts were more susceptible to the induction of ventricular arrhythmias. Somewhat surprisingly, none of the 9 hearts isolated from 4 week

### Table 2. Echocardiographic Analysis of TAC Mice

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Two Weeks</th>
<th>Four Weeks</th>
<th>Eight Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham (21)</td>
<td>TAC (29)</td>
<td>Sham (21)</td>
<td>TAC (25)</td>
</tr>
<tr>
<td>LVAW, mm</td>
<td>0.92±0.03</td>
<td>0.90±0.02</td>
<td>0.89±0.04</td>
<td>1.22±0.05*</td>
</tr>
<tr>
<td>LVED, mm</td>
<td>3.86±0.06</td>
<td>3.82±0.06</td>
<td>3.84±0.07</td>
<td>3.86±0.16</td>
</tr>
<tr>
<td>FS, %</td>
<td>39.85±0.89</td>
<td>40.26±0.96</td>
<td>40.68±0.91</td>
<td>38.66±1.74</td>
</tr>
</tbody>
</table>

All values are means±SEM. LVAW, LV anterior wall; LVPW, LV posterior wall; LVID, LV internal dimension; d, diastole; s, systole; FS, fractional shortening. *P<0.05 compared to age-matched sham. Numbers in parentheses indicate sample size.

### Impulse Propagation in Hypertrophied Hearts

We next determined whether the changes in Cx43 expression were accompanied by alterations in impulse propagation, using optical mapping techniques. We observed progressive slowing of transverse conduction velocity (CVmin) following the imposition of TAC, as illustrated in Figure 1D and summarized in Table 3. As early as 1 week after TAC, CVmin had diminished significantly from 0.55±0.02 to 0.47±0.02 m/sec, and by 8 weeks after imposition of the hemodynamic overload, CVmin had fallen to 0.42±0.03 m/sec. To gain some insight into the extent of residual cell–cell coupling that existed in hypertrophied hearts, we assessed the effects of the relatively selective gap junction uncoupler αGA on impulse propagation in an additional cohort of hearts studied 4 weeks after imposition of TAC. Treatment of hypertrophied hearts with αGA depressed CV further, to 0.25±0.01 m/sec. These data suggest the presence of some degree of residual gap junction function, despite the extensive GJR that occurs in response to pressure overload.

As illustrated in Figure 1D and Table 3, the imposition of TAC, as well as pS365-Cx43 and pS325/328/330-Cx43

Figure 2. Aberrant phosphorylation of Cx43 in TAC mice. Sections were prepared at the indicated time points and stained with antibodies recognizing all forms of Cx43 (Total Cx43), S365-phosphoCx43 (pS365), or S325/328/330-phosphoCx43 (pS325). Diminution of both phosphorylated forms of Cx43 is evident as early as 1 week post-TAC. Scale bar=50 μm.
TAC mice developed ventricular arrhythmias in response to programmed electric stimulation.

**Inhibition of Pathological GJR With Spironolactone**

Aldosterone antagonists have been shown to reduce sudden cardiac death in clinical trials of myopathic patients. We therefore explored whether treatment with spironolactone might influence the extent of pathological GJR observed in response to pressure overload. Accordingly, we examined additional cohorts of mice subjected to either sham operation or TAC, with half of each group then randomized to receive either a normal chow diet or chow supplemented with spironolactone (50 mg/kg per day) for a period of 4 weeks. Interestingly, there was no effect of TAC or spironolactone on Cx43 mRNA expression (Figure 3A). Moreover, spironolactone did not diminish the overall hypertrophic response, although there was a demonstrable reduction in the extent of cardiac fibrosis (Table 4). However, compared to TAC mice receiving a normal chow diet, those treated with spironolactone showed a significant increase in the abundance of slower mobility, hyperphosphorylated forms of Cx43 (Figure 3B and Table 4), as well as a marked increase in typical gap junctional plaques (Figure 3C). The normalization of gap junctional appearance was also observed using antibodies specific for pS365-Cx43 and pS325/328/330-Cx43 (Figure 4). These molecular changes indicative of reverse remodeling were associated with a significant improvement in conduction velocity, as shown in Figure 3D and summarized in Table 4.

**Reversal of Pathological GJR With Spironolactone**

The previous experiment established that treatment with spironolactone could blunt the development of pathological GJR. We next addressed the clinically more relevant question of whether mineralocorticoid receptor antagonism could reverse the severity of GJR in hearts with established pathology. Therefore, an additional cohort of mice was subjected to TAC. Two weeks later, at which time substantial hypertrophy

Table 3. Conduction Parameters in TAC Mice

<table>
<thead>
<tr>
<th>Time Course Study</th>
<th>GA Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sham</strong></td>
<td><strong>Four Weeks Without GA</strong></td>
</tr>
<tr>
<td><strong>Sham</strong></td>
<td><strong>Four Weeks Without GA</strong></td>
</tr>
<tr>
<td>CV&lt;sub&gt;min&lt;/sub&gt;, m/sec</td>
<td>0.55±0.02 (9)</td>
</tr>
<tr>
<td>CV&lt;sub&gt;max&lt;/sub&gt;, m/sec</td>
<td>0.84±0.04 (9)</td>
</tr>
<tr>
<td>AR</td>
<td>1.54±0.07 (9)</td>
</tr>
<tr>
<td>VERP, ms</td>
<td>42.7±2.7 (9)</td>
</tr>
</tbody>
</table>

*All values are means±SEM. *P<0.05 compared to sham, †P<0.05 compared to 4 weeks without GA. Numbers in parentheses indicate sample size.

Figure 3. Inhibition of GJR with spironolactone. Groups of mice were subjected to either sham operation (S) or TAC (T) and fed either normal chow (−SPI) or chow supplemented with spironolactone (+SPI). Analyses were performed 4 weeks after surgery. A, Northern blot analysis demonstrates no changes in Cx43 mRNA abundance. B, Western blot analysis with a panCx43 antibody demonstrates increased slow-mobility Cx43 in both S+SPI and T+SPI hearts. C, Representative immunofluorescent staining demonstrates loss of Cx43 gap junction plaques in TAC−SPI hearts but substantial improvement in mice treated with spironolactone (TAC+SPI). D, Representative optical maps from each of the 4 groups. E, Representative immunofluorescent staining in reversal experiment demonstrates improvement after 2 weeks of treatment with spironolactone. F, Representative optical maps from reversal experiment.
was evident by echocardiography, half of the banded mice were randomly chosen to receive either a normal chow diet or chow supplemented with spironolactone (50 mg/kg per day) for an additional 2 week period. As previously observed, spironolactone treatment did not influence the extent of hypertrophy as determined by heart weight/body weight measurements (Table 4). Unlike the prevention study, in which spironolactone was given for 4 weeks, this shorter course of treatment was not associated with significant amelioration of the fibrotic response (Table 4). Nonetheless, as before, spironolactone treatment was associated with a significant increase in the slower mobility, hyperphosphorylated forms of Cx43 (Table 4), a marked increase in typical gap junction plaques (Figure 3E), as well as a significant improvement in epicardial conduction velocity (Figure 3F and Table 4).

**Discussion**

Impulse propagation reflects a complex interplay between structural factors and active and passive membrane properties. In the setting of pathological stressors, structural and electric remodeling occurs, leading to the formation of a substrate that may be susceptible to the initiation and maintenance of ventricular tachyarrhythmias. In this study, we evaluated the effects of pressure overload, a commonly encountered pathological stimulus, on GJR and impulse propagation. The major findings in this study include the following: (1) pressure overload hypertrophy is associated with early and progressive reduction in junctional Cx43; (2) dephosphorylation of Cx43 at serines 365 and 325/328/330 occur contemporaneously with this loss of junctional Cx43; (3) impulse propagation slows significantly during progressive hypertrophy; (4) mineralocorticoid receptor blockade blunts the development of GJR associated with pathological cardiac hypertrophy, both at the molecular and functional levels; and (5) mineralocorticoid receptor blockade potently reverses established GJR in hypertrophied hearts.

Despite evidence of substantial GJR following TAC, the hypertrophied hearts were not especially sensitive to the induction of ventricular arrhythmias. This behavior contrasts with several genetic models of uncoupling in which Cx43 expression is more profoundly reduced. At the extreme end of the spectrum are the Cx43 conditional knockout mice, in which spontaneous arrhythmias are fully penetrant; less fulminant are the heterozygous I130T ODDD mutant mice, with Cx43 levels ~10% of normal and easily inducible but without spontaneous arrhythmias. The TAC mice display an even milder phenotype, and despite evidence of substantial GJR, the incremental slowing of CV after infusion with GA suggests the presence of residual gap junction function. In addition, it is conceivable that the choice of strain used in this study reduced the arrhythmic phenotype; C57BL/6 mice are relatively resistant to induction of ventricular tachycardia.

Our data are consistent with previous studies demonstrating aberrant posttranslational phosphorylation of Cx43 in response to pathological stimuli, including models of nonischemic heart failure in the rabbit and pacing-induced heart failure in the dog. With respect to the molecular mecha-
nisms of GJR, through the use of phospho-specific antibodies, we identified at least 2 regions, serine 365 and a triplet of serines at 325, 328, and 330, within the Cx43 carboxyl terminus that are dephosphorylated as early as 1 week after imposition of hemodynamic overload. Phosphorylation at serine 365 causes a shift in Cx43 mobility to the P1 form and is thought to play a gatekeeper role preventing downregulation via protein kinase C phosphorylation at serine 368. At least one of the triplet of serines at residues 325/328/330 is phosphorylated via casein kinase I, and these sites appear to play an integral role in gap junction assembly and formation of the P2 isoform. Recent data demonstrate that phosphorylation at these sites is markedly reduced in response to cardiac ischemia. Taken together with the present data, we propose that casein kinase I–dependent phosphorylation of Cx43 may not only play a key role in normal Cx43 trafficking and membrane targeting but in the pathological remodeling of gap junctions as well. Indeed, we have recently generated site-specific Cx43 mutant knock-in mice to directly test this hypothesis.

Aldosterone antagonism has been shown in 2 major clinical trials to diminish mortality in patients with heart failure. The mechanisms responsible for this salutary effect are uncertain but appear to reflect, at least in part, a diminution in sudden arrhythmic death. Given this observation and the association of GJR with arrhythmogenesis, we tested whether spironolactone might influence Cx43 expression and impulse propagation in diseased myocardium. Indeed, in addition to the anticipated modest antifibrotic effects of concurrent aldosterone receptor blockade, the initiation of spironolactone treatment at the time of aortic banding significantly blunted the molecular and functional manifestations of GJR.

In the clinical setting, patients typically present with demonstrable heart disease. Therefore, we also examined the effects of spironolactone therapy on mice with established hypertrophy. Importantly, mice in the reversal study also demonstrated a significant enhancement of CV but without measurable diminution in the extent of fibrosis. These data argue against fibrosis playing a substantial role in the enhanced propagation. CV is also determined by inward sodium currents. However, there is evidence indicating that aldosterone enhances $I_{Na}$ in adult mouse myocytes, suggesting that mineralocorticoid blockade with spironolactone is not likely to improve CV through an increase in sodium current density. Nonetheless, additional studies will be required to ascertain the relative importance of each of these contributing factors.

Interestingly, spironolactone enhanced Cx43 phosphorylation not only in TAC mice but also appeared to have some effect in sham-treated controls. These data suggest a potential role for mineralocorticoid-dependent regulation of cell–cell coupling even at baseline, not only when neurohumoral axes are activated by pathological stressors. Whether there is a signaling pathway that includes the upstream mineralocorticoid receptor and the downstream target casein kinase I will require additional study. Additionally, it is conceivable that regulation of phosphatase activity may also play a role in the GJR process, as recently suggested by Ai and Pogwizd.

In summary, our data demonstrate that hemodynamic overload leads to substantial GJR and that spironolactone potently blunts the development of this pathological remodeling, as well as reverses, established GJR. Posttranslational phosphorylation of Cx43 appears to play an integral role in the regulation of gap junction formation as well as pathological remodeling. We suggest that modulation of gap junction regulation may represent a rational antiarrhythmic therapeutic target.

**Sources of Funding**

This study was supported by NIH grants HL64757, HL82727, and HL81336 (to G.I.F.) and GM55632 (to P.D.L.); a Glorney-Raisbeck fellowship (to N.S.); a Sarnoff Cardiovascular Research fellowship (to F.M.V.); and a Howard Hughes Medical Institute medical student fellowship (to N.S.).

**Disclosures**

None.

**References**


Gap Junction Remodeling and Spironolactone-Dependent Reverse Remodeling in the Hypertrophied Heart
Jiaxiang Qu, Frank M. Volpicelli, Luis I. Garcia, Nefthi Sandeep, Jie Zhang, Lucrecia Márquez-Rosado, Paul D. Lampe and Glenn I. Fishman

Circ Res. 2009;104:365-371; originally published online December 18, 2008;
doi: 10.1161/CIRCRESAHA.108.184044
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 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/104/3/365