Native \(\beta\)-Adrenergic Support for Left Ventricular Dysfunction in Experimental Mitral Regurgitation Normalizes Indexes of Pump and Contractile Function

Masayoshi Nagatsu, MD; Michael R. Zile, MD; Hiroyuki Tsutsui, MD; Phillip G. Schmid, MD; Gilberto DeFreyte, BS; George Cooper IV, MD; Blase A. Carabello, MD

Background. It is generally accepted that the adrenergic nervous system provides inotropic support for the failing heart. However, the magnitude of this support has never been studied extensively. The present study was performed to test the hypothesis that the adrenergic nervous system is capable of maintaining indexes of pump and contractile function in the normal range despite significant innate myocardial depression.

Methods and Results. We used our model of experimental canine mitral regurgitation, which produces left ventricular dysfunction after 3 months of volume overload. We studied indexes of contractile function on and off \(\beta\)-blockade at baseline and again on and off \(\beta\)-blockade 3 months after chronic mitral regurgitation had induced significant contractile dysfunction. At baseline, acute \(\beta\)-blockade caused insignificant reductions in the mass-corrected slope of the end-ejection stress-volume relation (EESVR), the end-systolic stiffness constant, and the ejection fraction–end-systolic stress and the mean velocity of circumferential fiber shortening (VCF)–end-systolic stress relations. After 3 months of chronic mitral regurgitation, all indexes of contractile function were normal in the unblocked state except for the VCF-stress relation, which was mildly reduced. However, after acute \(\beta\)-blockade after 3 months of chronic mitral regurgitation, the EESVR fell to 303±27 versus 443±24 during acute \(\beta\)-blockade before mitral regurgitation was created \((P<.05)\), and the end-systolic stiffness constant was reduced to 2.54±0.15 versus 3.27±0.11 \((P<.05)\). Only after \(\beta\)-blockade was the ejection fraction–stress relation significantly reduced for dogs with chronic mitral regurgitation. The VCF-stress relation became markedly more abnormal. The viscosity-velocity relation of myocytes isolated from the ventricles of the dogs with mitral regurgitation confirmed that substantial innate contractile depression was present.

Conclusions. After 3 months of chronic mitral regurgitation, the adrenergic nervous system was able to maintain most indexes of contractile function in the normal range despite significant depression in innate contractile function. Thus, in the absence of \(\beta\)-blockade, significant innate contractile depression may be obscured by adrenergic support. (Circulation. 1994;89:818-826.)

Key Words. • contractility • mitral valve • regurgitation • receptors, adrenergic, beta

It is generally accepted that the adrenergic nervous system provides inotropic support for the failing heart. Since acute \(\beta\)-blockade may precipitate congestive heart failure in previously compensated patients with ventricular dysfunction, native adrenergic support must be substantial enough to be clinically relevant. Yet, remarkably, the magnitude of native adrenergic support for the failing heart has not been studied extensively. Without this knowledge, it is possible that clinical and experimental studies of contractile function performed in a variety of diseases in the absence of \(\beta\)-blockade detected only subjects with the most severe contractile depression—those whom adrenergic support failed to normalize indexes of pump and contractile function. Although this group is surely an important one to study, it may not provide an accurate overview of the extent of contractile abnormalities in a given disease.

In this study, we tested the hypothesis that native \(\beta\)-adrenergic stimulation was capable of maintaining indexes of pump and contractile function in or near the normal range despite the presence of a significant reduction in native contractile state. To test this hypothesis, we examined indexes of pump and contractile function off and on \(\beta\)-blockade in normal dogs at baseline and again after 3 months of severe mitral regurgitation had induced myocardial dysfunction. Since all in vivo indexes of contractile function have limitations in application and interpretation, we also examined the contractile function of myocytes isolated from the regurgitant ventricles to corroborate the in vivo estimation of contractility.

Methods

Study Design

Indexes of pump and contractile function were studied at cardiac catheterization performed under light anesthesia in six normal dogs at baseline. Data from these animals have not
indexes of contractile function

four indexes of contractile function were examined in this study: the slope of the end-ejection stress-volume relation corrected for left ventricular mass (eesvr), the end-systolic stiffness constant (k), the ejection fraction-end-systolic stress relation, and the mean velocity of circumferential fiber shortening (vcf)-end-systolic stress relation. each has its own limitations and applications. the slope of the end-ejection stress-volume relation is relatively load independent and has correlated well with isochronally derived maximum elastance (emax). however, this slope is dependent on left ventricular size and decreases when size increases regardless of contractile state. thus, we corrected it as we have in the past for left ventricular mass. the end-systolic stiffness constant is size independent and has correlated well with an independent indicator of contractile state, the viscosity-velocity relation of isolated myocytes. whereas the above indexes have been applied primarily in the experimental laboratory, the ejection fraction-stress and vcf-stress relations have been used clinically to assess contractile function. thus, we felt it appropriate to test them in this study. eesvr and k were derived from pressure and volume data obtained by the inferior vena cava balloon technique. the ejection fraction-stress and vcf-stress relations were obtained with the data from the standard ventriculogram.

isolated myocyte function

myocytes were isolated and function was measured as previously described by investigators blinded to the in vivo results. briefly, 1 week after the in vivo evaluation of left ventricular function at 3 months of chronic mitral regurgitation, the animals were deeply anesthetized. the pericardium was excised, and the hearts were rapidly removed and placed in a cold calcium-free buffer. a wedge of left ventricle supplied by the circumflex artery was isolated, and the artery was cannulated and perfused with collagenase, allowing the disaggregation of viable myocytes. laser diffraction was then used to analyze the extent and velocity of sarcomere shortening. these studies were performed only on myocytes that suffered no significant damage from the isolation process according to criteria previously described. by variation of the viscosity of perfusate with inert methyl cellulose, the afterload on contracting sarcomeres was varied. the viscosity-velocity relation constructed in this manner is analogous to the force-velocity relation of classic cardiac mechanics.

determination of catecholamine concentrations

ten milliliters of blood was collected and centrifuged at 10 000 rpm at 4°c for 5 minutes, and 2 ml of plasma was saved and stored at -70°c until assayed. plasma norepinephrine was analyzed by radioenzymatic assay with a commercial kit for determination of catecholamines (cat-a kit, amersham corp, arlington heights, ill). norepinephrine was converted to its meta-[3h]-methoxy derivative, i.e., catechol-o-methyl transferase, in the presence of 3h methionine, extracted, and separated by thin-layer chromatography. the derivative [3h]norotane was further converted to [3h]vanillin by periodate oxidation, and 3h radioactivity was determined by liquid scintillation counting.

calculations

left ventricular volumes were calculated by the area-length method. this method has previously been validated as providing an accurate measurement of volume and mass in dogs with mitral regurgitation studied in our laboratory. the regurgitant fraction (rf) was calculated from the equation

\[
RF = \frac{(SV_A - SV_f)}{SV_A}
\]
where $SV_f$ is angiographic stroke volume (end-diastolic volume—end-systolic volume) and $SV_p$ is forward stroke volume (thermodilution cardiac output divided by heart rate).

Wall stress was calculated by Mirsky's formula. $^{26}$ The EESVR was calculated by linear regression. In this study, as in previous studies, end-ejection volume rather than true end-systolic volume was used because of its clear definition, $^{9-13}$ because little volume is expelled into the left atrium after closure of the aortic valve in this model, and because this method has correlated well with the more rigorous isochronally determined maximum elastance. $^{10}$ Although end ejection and end systole may become uncoupled in mitral regurgitation, $^{30}$ this problem is largely obviated by matching dicrotic notch pressure with end-ejection volume. $^{31}$ The slope of the EESVR was then corrected for the development of hypertrophy by multiplying it by the mass present at the time of study. The VCF was calculated from the equation

$$\text{VCF} = \frac{\text{EDD} - \text{ESD}}{\text{EDD} \times \text{ET}}$$

where EDD is end-diastolic dimension, ESD is end-systolic dimension, and ET is ejection time. Ejection time was calculated as the total time the left ventricle is ejecting either into the left atrium or aorta, taken from the time of angiographic appearance of contrast into the left atrium to time of aortic valve closure. This definition accounts for the occurrence in mitral regurgitation of substantial stenosis after aortic valve opening. The end-systolic stiffness constant ($k$) was determined by fitting end-systolic stress and end-systolic wall thickness data to the curvilinear equation $^{14}$

$$\sigma = C e^{ln(1/H)}$$

where $\sigma$ is systolic wall stress, $C$ is a constant, $k$ is the end-systolic stiffness constant, and $ln(1/H)$ is the natural logarithm of the reciprocal of wall thickness. A detailed discussion of the mathematical derivation of this index is given in the "Appendix."

**Statistics**

Dispersion from the mean is noted as ±SEM. Comparisons made regarding given parameters over the course of the study represented multiple repeated comparisons. Therefore, we tested for statistical differences using two-way ANOVA followed by a Newman-Keuls test to locate differences when they were found. Correlation coefficients for EESVR and $k$ relations exceeded .99 in all cases.

**Results**

Regurgitant fraction was $0.60 ± 0.02$ after the creation of mitral regurgitation, and it remained at this value at the 3-month study. Fig 1 demonstrates that serum catecholamines were elevated after 3 months of chronic mitral regurgitation, confirming the potential for increased adrenergic support. At baseline, $\beta$-blockade reduced heart rate from $77 ± 2$ to $69 ± 2$ beats per minute ($P < .05$). After 3 months of mitral regurgitation, unblocked heart rate was higher than at baseline: $85 ± 3$ beats per minute ($P < .05$). $\beta$-Blockade during chronic mitral regurgitation reduced heart rate to $82 ± 2$ beats per minute, a nonsignificant difference. Fig 2 demonstrates the effects of acute $\beta$-blockade on pulmonary capillary wedge pressure at baseline and after 3 months of chronic mitral regurgitation. $\beta$-Blockade at baseline caused pulmonary capillary wedge pressure to increase acutely but modestly, still within the normal range for our laboratory. After 3 months of mitral regurgitation, in the absence of $\beta$-blockade, wedge pressure was greater than it had been in the unblocked state at baseline, but it was still within the normal range. However, after acute $\beta$-blockade was instituted after 3 months of chronic mitral regurgitation, wedge pressure increased substantially and now was more than double the baseline value. As shown in Fig 3, cardiac output declined significantly after the institution of $\beta$-blockade in the baseline state. Cardiac output was significantly reduced after 3 months of chronic mitral regurgitation in the unblocked state but was reduced even more dramatically after the institution of $\beta$-blockade. Ejection fraction is demonstrated in Fig 4. It decreased significantly after the institution of acute $\beta$-blockade in the
baseline state. Three months later, ejection fraction in the unblocked state was similar to baseline. After β-blockade was instituted after 3 months of chronic mitral regurgitation, ejection fraction fell substantially to a level similar to that at baseline during β-blockade. However, this occurred when the favorable loading conditions of mitral regurgitation should have produced an ejection fraction higher than baseline. Fig 5 demonstrates two indexes of contractile function. Both fell after the institution of acute β-blockade at baseline, but the reduction was not statistically significant. After 3 months of chronic mitral regurgitation in the unblocked state, these indexes were similar to baseline. However, after the institution of acute β-blockade, both indexes fell and were significantly less than they had been in either the blocked or unblocked state at baseline. Fig 6 demonstrates the normal ejection fraction–stress relation obtained from an independent cadre of nine normal animals in the unblocked state. Only after the institution of β-blockade in dogs with chronic mitral regurgitation was contractile dysfunction manifested when the relation fell down and to the left of normal, indicating reduced ejection performance for any given level of afterload.

The VCF-stress relation with its 95% confidence interval developed from the nine normal dogs is also shown in Fig 6. The dogs with mitral regurgitation fall just outside the confidence limits in the unblocked state. The departure from normal was more striking after β-blockade.

Fig 7 shows the velocity-viscosity relation for cells taken from five normal dogs compared with cells from three dogs with chronic mitral regurgitation examined in the in vivo studies. The contractile function of the cells from the mitral regurgitant ventricles was depressed, confirming that innate contractile dysfunction was present. Further, the amount of cell dysfunction correlated well with the amount of ventricular dysfunction.

**Discussion**

An important finding of this study was that native β-adrenergic stimulation could maintain pump function and indexes of contractile function in the normal or nearly normal range despite the presence of significant innate contractile dysfunction. Although it is obvious that β-adrenergic stimulation helps maintain ventricular function, ours is the first study that estimates the magnitude of this support. By assessing innate contractile dysfunction when β-stimulation was blocked, we developed a reference point for comparison of various indexes measured when adrenergic stimulation was not blocked. Wedge pressure, cardiac output, ejection fraction, and indexes of contractile function were normal or nearly normal in the unblocked state 3 months after severe mitral regurgitation had caused contractile dysfunction. Only when β-blockade was instituted were pump and contractile dysfunction revealed. The one exception to this observation was the VCF-stress relation, which was mildly abnormal in the mitral regurgitation dogs in the absence of β-blockade.

It is likely that the overload of mitral regurgitation induces progressively worsening innate contractile function, which can be normalized by progressively using adrenergic reserve. Only when adrenergic reserve is eventually exhausted does contractile dysfunction become obvious in the unblocked state. Thus, it is likely that our previous use of β-blockade in all our studies of mitral regurgitation helped us to detect the dysfunction we found. The implication of this study is that examinations of contractile function that detect a contractile abnormality with the β-adrenergic system intact may select a group of patients with such severe dysfunction that the contractile abnormality is evident despite adrenergic stimulation. Such studies examine a
clinically important group of patients with severe disease but may be relatively insensitive to milder degrees of contractile dysfunction that was masked by adrenergic support. Although the study was not designed to compare the sensitivity of various indexes of contractile function, the study does suggest that the VCF-stress relation is sensitive, since it was the only index that demonstrated contractile dysfunction in the unblocked state.

Reliability of In Vivo Indexes of Contractile Function

Our case regarding the ability of the β-adrenergic nervous system to support contractile function rests largely on our ability to measure contractile function. The ideal index of contractile function would be sensitive to changes in inotropic state, insensitive to changes in loading, and independent of left ventricular size. The slope of the end-systolic afterload-volume relation used
in this study is relatively load independent and sensitive to inotropic changes but varies indirectly with cardiac size. Thus, when a volume overload causes an increase in left ventricular size, there is an expected decrease in the slope of this relation whether or not contractile function decreases. To overcome this problem, we multiplied the slope times the mass present at the time the slope was derived. Thus, as eccentric hypertrophy develops, the expected decrease in slope should be offset by correction with the higher mass. Despite this correction, this index demonstrated contractile dysfunction after 3 months of mitral regurgitation in the β-blocked state.

End-systolic stiffness relates strain, which is dimensionless, to stress. Because strain is dimensionless, this index of contractile function is independent of changes in cardiac size or mass that occur as hypertrophy develops. We have previously shown stiffness to be independent of loading and sensitive to changes in inotropic state. This index also showed depression in contractile function in the dogs with chronic mitral regurgitation when β-blockade was instituted.

The intent of the ejection fraction–stress and VCF-stress relation is to correct afterload-dependent indexes for afterload, resulting in a relation that reflects contractility. Both demonstrated reduced contractile function when β-blockade was instituted.

To further substantiate our assessment of contractile function, in this study as in the past, we examined an independent indicator of innate contractile function, the contractility of myocytes isolated from the affected ventricles. These studies were obtained by investigators blinded to the in vivo results. The reduced viscosity-velocity curves of the myocytes isolated from the mitral regurgitation ventricles are analogous to a reduced force-velocity relation of classic cardiac mechanics. Importantly, the myocytes are studied in a bath free of catecholamines after thorough washing to remove residual catecholamines that were present in vivo. Thus, cell function was studied in the absence of β-receptor stimulation. Both the cell indexes of contractile function in the absence of catecholamines and the in vivo indexes of contractile function in the β-blocked state were concordant and demonstrated contractile dysfunction. Other support for the presence of contractile dysfunction was the high wedge pressure that occurred in the β-blocked state and the reduced ejection fraction in chronic mitral regurgitation during β-blockade despite the presence of the favorable loading conditions that mitral regurgitation produces.

The contractile dysfunction we found on institution of β-blockade after 3 months of chronic mitral regurgitation was not merely statistically significant but also clinically relevant. β-Blockade in chronic mitral regurgitation caused a 75% increase in wedge pressure (10 to 17.5 mm Hg), a 25% decrease in cardiac output, and a substantial fall in all contractility indexes.

### Acute Versus Chronic β-Blockade

This study used acute β-blockade to remove the effects of the β-adrenergic nervous system, suddenly unmasking its level of support. These effects must not be confused with the gradual institution of chronic β-blockade, which may have beneficial effects in congestive heart failure.

In conclusion, we have found that activation of the adrenergic nervous system provides considerable support for the failing heart. Although this was suspected in the past, its impact has been substantiated here. Importantly, many indexes of contractile function appear normal in the natural state. Only after β-blockade is instituted is underlying contractile dysfunction revealed. We must caution that these data were obtained in an experimental model of mitral regurgitation, and applicability to other species and diseases is uncertain. However, it is likely that clinical studies and experimental studies that find contractile dysfunction in the natural state probably have detected those subjects whose contractile dysfunction is so severe that contractility cannot be normalized by the β-adrenergic nervous system. Such studies may underestimate the prevalence of innate contractile dysfunction in various disease states.

---

**Figure 7.** Graphs showing the sarcomere velocity/viscosity relation for normal dogs and for those with mitral regurgitation (left). The contractility of cells from the dogs with mitral regurgitation (MR) was significantly depressed (left) and correlated well with an in vivo index of contractile function (k) obtained during β-blockade (right).
Appendix*

Derivation of the Constant of End-Systolic Stiffness

Strain is defined as the deformation of a material caused by application of a force. It is usually expressed in an unstrained dimension, ln(1/H), or area, ln(A/Ao), and is a dimensionless property. We make the assumption that the myocardium is incompressible. As such, it has a constant volume equal to its area (A) times its thickness (H). Thus, changes in area are reflected by changes in thickness. As noted above, areal strain is defined as ln(A/Ao). Assuming that the myocardium is incompressible, ln(A/Ao) can be substituted for ln[(1/H)/(1/Ho)] or ln(Ho/H). Myocardial stiffness is described mathematically as the change in stress (dσ) divided by the change in strain (de), i.e., dσ/de. By altering stress (σ), one alters strain, thereby deriving the relation of σ to dσ/de (myocardial stiffness), which should reflect contractile state. Since the slope of the σ-to-dσ/de relation is defined by increments in σ and dσ/de, extrapolation to zero strain to define the slope is unnecessary. This allows our second key assumption, that ln(Ho/H) can be substituted by ln(1/H) as the expression of strain in our stress/strain relation. The stress/ strain relation is curvilinear and is expressed as σ=CKεm(1/H), where σ is stress, C is a constant, and k is the exponential constant. The constant k reflects myocardial stiffness, kSM, and thus reflects inotropic state.

Theoretical Background: Calculation of Regional Work

The σ-to-ln(1/H) relation is based on previous studies calculating regional work of the ventricle.41-45

The mechanical work done by a region of interest of the ventricular wall (regional work, RW) is the area under the tension/area curve given by the equation

\[ RW = -\int T \, dA \]

where T is the isotropic wall tension, A is the area of a regional midwall layer of the ventricle, and the integral is taken over a cardiac cycle. Tension is equal to pressure (P) times radius (r), thus tension times area is equal to \( P \times r^2 \). Since the wall thickness has the same units as volume, P×r^2 is the more familiar expression of stroke work. The accuracy of RW calculated by this method was validated by Goto et al. in the excised cross-circulated heart using a volume servopump system.

In an ellipsoid model of the left ventricle, tension (T) calculated at the equator is defined as

\[ T_e = \frac{rP}{2} \]

where Te and Tθ are the circumferential and meridional components, respectively, of wall tension at the equator. A relation between Te and Tθ is given by Laplace’s law,

\[ P = \frac{Te}{r} + \frac{Tθ}{R} \]

where r and R are the minor and major radii of curvature of the endocardial surface and P is ventricular pressure. The equilibrium of the forces at the equator in the direction of the long axis yields

\[ T_e = \frac{rP}{2} \]

From Equations 3 and 4

\[ T_e = \frac{1}{2} \left( 1 - \frac{r}{2R} \right) P \]

Substituting for r and R in Equation 5,

\[ T_e = \frac{bP}{2} \]

Therefore, T is expressed as

\[ T_e = \frac{1}{2} \left( 1 - \frac{b^2}{2a^2} \right) P \]

Since T is isotropic in the plane perpendicular to the radius, it takes the same value for every direction perpendicular to a radius through a point in the ventricular wall.

Normalization of Regional Work to a Unit Volume of Myocardium

Since larger areas of interest of the myocardium can produce more work than smaller areas of interest, it is necessary to normalize regional work to a unit volume of myocardium to compare areas of interest.42,43

Given an imaginary section of myocardium that has a volume \( V_m \), \( V_m \equiv \text{area of (A) and wall thickness (H)} \), measured along a straight line (r) perpendicular to the epicardial surface and passing through a selected point O of the section:

\[ V_m = A \times H \]

Since the myocardium is incompressible, \( V_m \) is constant. If we wish to examine regional work (RW) per unit volume (\( V_m \)) of myocardium (RWM), we divide RW by \( V_m \) (\( RW/V_m \)), which equals RW/AH. Thus, RWM=RW/AH. Recall Equation 1, RW=-\int T dA. Thus,

\[ RWM = -\int T \, dA/AH = -\int (T/H) \, dA/A = -\int (T/H) \, dlnA \]

Tension (T) divided by thickness (H) is T/H=wall stress (σ). Thus,

\[ RWM = -\int \sigma \, dlnA \]

This equation describes the area surrounded by the σ-lnA loop. The formula for calculating stress (σ) is derived by dividing tension (Equation 8) by thickness:

\[ \sigma = \frac{P \times b}{2 \left( \frac{3 b^2}{2a^2} \right) / H} \times 1332 \text{ dynes/cm}^2 \]

Meaning of lnA and Definition of Area Strain

The change in lnA (dlnA), or dA/A, in the σ-lnA relation expresses a relative change in area (“incremental area strain”). Total area strain (εA) is given by the following equation:

\[ \varepsilon_A = \int_{A_0}^{A} dA/A = \ln A - \ln A_0 = \ln (A/A_0) \]

where \( A_0 \) is the area corresponding to a state of zero stress. Definition of unstressed area (\( A_0 \)) is required to obtain the complete stress/strain relation extrapolated to the x axis. However, \( A_0 \) is not required in the analysis of the stiffness (dσ/de)–stress (σ) relation. As noted above, myocardial stiffness is defined as the change in stress (dσ) divided by the change in strain (de) or dσ/de. This relation only examines incremental changes in stress and strain to define its slope. Thus, extrapolation to \( A_0 \) is unnecessary. Therefore, stiffness

*Condensed from Reference 15.
Use of Reciprocal Wall Thickness (1/H) Instead of A

Unfortunately, changes in a regional area of interest of the myocardium are difficult to measure. Conversely, wall thickness and changes in wall thickness are easy to measure by conventional echocardiographic or cinemangiographic methods. Thus, substitution of thickness (H) for area (A), if possible, would increase the applicability of the method. The following explains how wall thickness (H) can be substituted for area in strain analysis. The myocardium is incompressible. Thus, the volume of the region of interest (Vw) is constant, even though A and H vary throughout the cardiac cycle. From Equation 9, \( A = Vw/H \), then

\[
\ln A = \ln(Vw/H) = \ln Vw + \ln(1/H)
\]

Since \( Vw \) is constant, d(lnA) = d(ln(1/H)). Thus, one can substitute ln(1/H) for lnA. ln(1/H) represents strain in the stress/stretch relation.

Acknowledgments

This research was supported in part by the National Institutes of Health grant HL-38185 (B.A.C.) and Veterans Administration Merit Reviews (B.A.C., M.R.Z.).

References


Native beta-adrenergic support for left ventricular dysfunction in experimental mitral regurgitation normalizes indexes of pump and contractile function.

M Nagatsu, M R Zile, H Tsutsui, P G Schmid, G DeFreyte, G Cooper, 4th and B A Carabello

_Circulation_. 1994;89:818-826
doi: 10.1161/01.CIR.89.2.818

_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/89/2/818

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/