Effect of Age on the Development of Cardiac Hypertrophy Produced by Aortic Constriction in the Rat

Shogen Isoyama, Jeanne Y. Wei, Seigo Izumo, Peter Fort, Frederick J. Schoen, and William Grossman

To test the hypothesis that the capacity to develop left ventricular (LV) hypertrophy might diminish with advancing age, we examined the hypertrophic response to ascending aortic constriction in 3 groups of adult Fischer 344 rats (9 months, 18 months, and 22 months of age). Aortic constriction was created so that aortic cross-sectional areas would be the same for the 3 groups of rats. Four weeks after imposition of aortic constriction, there was no significant difference in peak LV pressure, peak-to-peak and mean systolic pressure gradients between left ventricle and aorta, cardiac output, LV minute work, or cross-sectional area of the aortic constrictions in the 3 groups. In 9-month-old aortic-constricted rats, LV dry wt (LVDW)/body wt, LVDW/tibial length, and myocyte width increased by 23% (p<0.01), 14% (p<0.01), and 27% (p<0.01), respectively, compared with sham-operated rats. In contrast, in 18-month-old and 22-month-old aortic-constricted rats, LVDW/body wt and LVDW/tibial length were unchanged compared with sham-operated controls, and increases in myocyte width were only modest 4 weeks following constriction. RNA concentration in the myocardium 5 days after constriction increased by 21% (p<0.001) in 9-month-old rats but showed no significant rise in 18-month-old rats. These results suggest that advancing age is associated with a diminished capacity for hypertrophy in response to acute pressure overload and that a reduced ability to synthesize protein may be one of the major contributing factors to a diminished capacity for hypertrophy in advanced age. (Circulation Research 1987;61:337-345)

Cardiac hypertrophy may be regarded as an adaptive physiologic response to increased functional demands on the heart. However, when the functional demands exceed adaptive limits, heart failure ensues. It is well appreciated that when subjected to similar stress, heart failure occurs more frequently in old patients compared with younger cohorts. This higher incidence of heart failure in old patients might be associated with a diminished capacity for hypertrophy in response to given stress.

In animal studies, it has been suggested that the hypertrophic response in the adult state may be different from that in the growing phase. Several investigators have examined the effect of age on the capacity for development of cardiac hypertrophy in adult animals; however, the observations in these studies are controversial. McCafferty and Edington reported that the extent of increase in heart weight in response to exercise was greater in young adult rats than in old rats. In contrast, Fiorini et al reported that there was no age difference in the extent of myocardial hypertrophy produced by thyroid hormone administration in the mouse. In a report by Zitnik and Roth, there was no age difference in the extent or time course of hypertrophic response after administration of thyroid hormone in the rat. These controversies might be attributable in part to the different types of stimulus and/or different mechanisms of myocardial hypertrophy.

To test the hypothesis that the capacity for hypertrophic response of the myocardium might diminish with age, ascending aortic constriction was employed to increase left ventricular pressure load, and the extent of myocardial hypertrophy under the same degree of aortic constriction in young adult and old rats was examined since increased left ventricular pressure is a common trigger of heart failure in elderly patients.

Materials and Methods

We used 3 age groups of male Fisher 344 rats (Harlan Sprague-Dawley Inc., Indianapolis, Ind.) under contract with the National Institute of Aging (first group, 9 months old; second group, 18 months old; third group, 22 months old). The males of this colony show a 50% mortality at 24 months of age. Before surgery, all rats were housed for at least 3 days in the Animal Quarters of the Harvard-Thorndike Laboratory, Beth Israel Hospital, at 23 ± 1°C on a 12-hour light/dark
of rats killed at 4 or 8 weeks after aortic constriction. Therefore, in the present study, 16-gauge needles were used to create aortic constriction of the same cross-sectional area among the 3 age groups and rats were killed at 4 weeks after operation to compare the extent of hypertrophy among those groups.

**Hemodynamic Measurements and Calculations**

Body weights were measured before (initial body weight) and 4 weeks after the surgery (final body weight). At 4 weeks after the surgery, each rat was anesthetized with urethane (1.1 g/kg i.p.). Urethane was chosen as the anesthetic for hemodynamic measurements because it does not depress myocardial function and has a longer duration of action. After tracheal intubation was performed as described above, the rat was placed on a slightly warmed electric heating pad (39 ± 1°C). Under controlled ventilation with room air, the neck region was carefully opened, and the right carotid artery was approached by dividing the sternocleidomastoid, digastric, and omohyoid muscles close to the trachea and retracting the sternocleidomastoid muscle. A polyethylene cannula (Intramedic polyethylene tubing, PE-50, Becton Dickinson Co., Mountain View, Calif.) was inserted into the right carotid artery, and aortic pressure was monitored using a Statham P23 physiologic transducer. The left thorax was opened at the third or fourth intercostal space. The pericardium was carefully opened, and the heart was exposed. The descending aorta was gently dissected free from the surrounding tissues, and an electromagnetic flow probe (2.5-mm diameter, BL 6025-H55, Biotronex Laboratory, Kensington, Md.) was positioned around the ascending aorta at the distal portion of the thread (used for constriction) to measure ascending aortic flow, which represents cardiac output excluding coronary flow. Particular care was taken so that the electromagnetic flow probe did not change the aortic pressure level or pulse contour. Zero level of aortic flow was defined as the beginning of the upstroke of the tracings.

Next, the left ventricular cavity was approached from the left ventricular base with a 19-gauge needle, through which the left ventricular pressure was measured using a strain gauge physiologic transducer (model MS20-BA26AAS, Electromedics Inc., Englewood, Colo.). Zero pressure reference was taken at the midlevel of the heart. Recordings of phasic and mean pressures and flow were continuously displayed on a multichannel recorder (recorder 260, Gould Inc., Instruments Division, Cleveland, Ohio). Left ventricular end-diastolic pressure was measured by high amplification of the left ventricular pressure signal. Cardiac output was calculated as heart rate times stroke output (measured by the aortic flow probe) and was divided by final body weight to give ml/min/kg. Left ventricular minute work was calculated as heart rate times left ventricular stroke work. The cross-sectional area of the constricted ascending aorta was calculated at 1 week after aortic constriction attained 80% of the level of left ventricular hypertrophy observed in those of rats killed at 4 or 8 weeks after aortic constriction.
using Gorlin's formula. After the hemodynamic measurements were performed, each rat was killed, and its heart was removed, stripped of fat and appendages, and divided into a right ventricular free wall portion and a left ventricular-septal portion. After determining the total left ventricular wet weight, the left ventricle was cut into 2 portions. The free wall portion was fixed with 10% neutral buffered formalin and stored in this solution until histologic examination. The other portion involving the ventricular septum was weighed and dried to constant weight at 70°C (48-72 hours). The dry wt/wet wt ratio of the second portion was obtained, and total left ventricular dry weight was calculated from the total left ventricular wet weight and the dry wt/wet wt ratio of the second portion. One leg was removed above the knee joint, and the length of the tibia from the condyles to the tip of the medial malleolus was measured using a method modified from Yin et al.

**Histologic Examination**

Transmural myocardial sections were cut in a transverse plane perpendicular to the apex-to-base axis. Sections were processed conventionally for histologic examination (dehydrated in graded alcohols and embedded in paraffin), but in 6-μm sections, and stained with hematoxylin-eosin. For each section, a mean myocyte width was determined by measurement of transnuclear widths of random, longitudinally oriented myocytes in the circular midwall muscle bundles with a calibrated microscope eyepiece reticle (10 cells for each sample) on random fields at a magnification of 400×. Degree of fibrosis was semiquantitatively described for each specimen as follows: score 0, no fibrotic lesion; score 1, minimal or focal fibrotic lesions; score 2, focal fibrotic lesions and subendocardial scars; score 3, multifocal fibrotic lesions or large subendocardial scars; score 4, generalized scarring. All measurements and histologic observations were made by a single observer. All histologic data were collected without knowledge of other data.

**RNA Measurement in the Myocardium**

To determine the protein-synthesizing capacity of the myocardium, RNA content of myocardial tissue was determined using 2 additional groups: 9-month-old rats (n = 10) and 18-month-old rats (n = 10). The rats were operated on as described above, creating aortic constriction or performing a sham operation, and killed 5 days after operation. Each sample of left ventricle was saved in liquid nitrogen until biochemical analysis. The individual samples were homogenized in 4 mol guanidine thiocyanate and extracted at 65°C with a phenol/chloroform/isoamyl alcohol mixture. After centrifugation, the aqueous phase was saved, and the organic phase was reextracted. The combined aqueous phase was completely deproteinized by extraction twice with phenol/chloroform/isoamyl alcohol followed by extraction twice with chloroform/isoamyl alcohol. After ethanol precipitation and centrifugation, the sample was dissolved in 6 mol guanidine hydrochloride and reprecipitated by a half volume of ethanol to remove DNA and t-RNA. The RNA pellet was dissolved in sterile water, and its concentration was determined by measuring the absorbance at 260 nm. In all cases, the 260/280 nm absorbance ratio was greater than 2.

**Statistical Analysis**

Variables measured are expressed as mean ± SEM. The statistical significance of differences in mean values from 2 groups of sham-operated and the aortic-constricted rats were assessed by the unpaired Student's t test. The Bonferroni correction was applied for multiple comparisons to reduce the possibility of chance significance.

**Results**

**Changes in Heart Weight and Histologic Findings**

All rats weighed approximately 340 g at the start of the experiment. Body weights differed little over the 9-22-month-old age range. Final body weights increased slightly 4 weeks after initial surgery in both sham-operated and aortic-constricted 9-month-old rats but did not in either the 18- or 22-month-old rats. Tibial length tended to be slightly increased with age (4.38 ± 0.03 cm in 9-month-old rats, 4.43 ± 0.01 cm in 18-month-old rats, 4.45 ± 0.02 cm in 22-month-old rats, P<0.05) but did not show a significant difference between sham-operated and aortic-constricted rats.

Figure 1 shows the left ventricular (LV) dry weight, LV dry wt/final body wt, and LV dry wt/tibial length. In sham-operated rats, LV dry weight increased and was 7% higher in 22-month-old rats than in 9-month-old rats (p<0.1). After aortic constriction, LV dry weight in 9-month-old rats increased by 14% compared with age-matched sham-operated rats (192 ± 7 vs. 168 ± 4 mg, p<0.01). In contrast, LV dry weight in the 18- and 22-month-old rats with aortic constriction did not increase compared with sham-operated controls (173 ± 5 vs. 171 ± 6 mg, NS, in 18-month-old rats and 180 ± 5 vs. 183 ± 7 mg, NS, in 22-month-old rats with sham-operation and aortic constriction, respectively).

Since changes in body size occur with aging and surgery, it is important to examine changes in left ventricular mass relative to parameters of body size. Interestingly, the ratio of LV dry weight to final body weight increased with age in sham-operated rats (0.473 ± 0.013 mg/g in 9-month-old rats, 0.492 ± 0.006 mg/g in 18-month-old rats, and 0.539 ± 0.014 mg/g in 22-month-old rats, p<0.01). However, the ratio of LV dry weight to final body weight in 9-month-old rats increased by 23% in the aortic-constricted group (0.581 ± 0.031 mg/g) compared with age-matched sham-operated rats (p<0.01), while in 18- and 22-month-old rats, there was no difference between aortic-constricted and sham-operated rats. Since there is evidence that the ratio of LV dry weight to tibial length allows a more appropriate adjustment for body size, this ratio was calculated for all groups. Once again, there was evidence of increased left ventricular mass in aortic-constricted 9-month-old
Left ventricular (LV) dry weights, LV dry wt/final body wt, and LV dry wt/tibial length in 9-month-old (9M), 18-month-old (18M), and 22-month-old (22M) rats. Sham, sham-operated rats; AoC, aortic-constricted rats; NS, not significant; Bars, mean ± SEM.

Figure 1. Left ventricular (LV) dry weights, LV dry wt/final body wt, and LV dry wt/tibial length in 9-month-old (9M), 18-month-old (18M), and 22-month-old (22M) rats. Sham, sham-operated rats; AoC, aortic-constricted rats; NS, not significant; Bars, mean ± SEM.

Figure 1. Left ventricular (LV) dry weights, LV dry wt/final body wt, and LV dry wt/tibial length in 9-month-old (9M), 18-month-old (18M), and 22-month-old (22M) rats. Sham, sham-operated rats; AoC, aortic-constricted rats; NS, not significant; Bars, mean ± SEM.

**Hemodynamic Changes**

Hemodynamic data for each of the 3 age groups are summarized in Table 2. There was no significant difference in peak systolic left ventricular pressure, peak aortic pressure, or mean aortic pressure among the 3 age groups of rats with aortic constriction. Left ventricular end-diastolic pressure increased slightly with age and was slightly higher in the aortic-constricted rats than in the sham-operated rats in each age group; however, these changes were not statistically significant. No age group showed a significant difference in cardiac output between sham-operated and aortic-constricted rats (Table 2). Heart rate tended to decrease with age in sham-operated rats (p<0.01 in 22-month-old rats). However, there was no difference between aortic-constricted and sham-operated rats.

Peak-to-peak pressure difference and mean systolic pressure difference between left ventricle and aorta were similar among the 3 age groups of aortic-constricted rats as seen in Figure 3. Additionally, there was no significant difference in cross-sectional area of the constricted ascending aorta or in left ventricular minute work among the 3 age groups of rats with aortic constriction (Figure 3).

**RNA Concentration in the Myocardium**

As summarized in Table 3, there was no significant difference in RNA concentration in the myocardium between the 2 age groups of sham-operated rats. RNA concentration in aortic-constricted 9-month-old rats increased by 21% compared with age-matched sham-operated rats and was significantly higher than in 18-month-old rats with aortic constriction. In contrast, RNA in 18-month-old rats with aortic constriction showed only a 4% increase compared with sham-operated rats. These results correlated with changes in left ventricular dry weight and myocyte width in 9- and 18-month-old rats.

**Discussion**

Many studies have been reported concerning the myocardial response to stress in neonatal animals. It
Figure 2. Histologic sections obtained from hearts of 9-month-old (Panels a and b), 18-month-old (Panels c and d), and 22-month-old (Panels e and f) rats with sham-operation and aortic-constriction, respectively. Stained with hematoxylin and eosin, magnification 375×.
is generally accepted that a volume or pressure overload in neonates results in significant cardiac enlargement accompanied by mitotic division of both myocardial and nonmyocardial cells (hyperplasia), while compensatory cardiac enlargement in adult animals is achieved by growth of preexisting myocardial cells (hypertrophy) and nonmyocardial cell mitotic division.

However, there is little information concerning adaptation of the adult heart to increased loading with advancing age. The extent as well as time course of cardiac hypertrophy induced by thyroid hormone in rats of advanced age (22-24 months) was reported to be similar to that in young adult rats. In another report, there was a longer lag period before hypertrophy in mice of advanced age (26 months). McCafferty and Edington examined the effect of age on cardiac hypertrophy during chronic running exercise, and reported that exercise increased heart weight in rats 3-15-months old. However, the extent of increase in heart weight in response to exercise was greater in young rats than in old rats. Furthermore, initiation of exercise at an older age (approximately 20 months) decreased the heart weight compared with age-matched controls.

In our study, left ventricular hypertrophy was substantially less in rats of advanced age (18 to 22 months) than in young rats in response to a similar grade of increased afterload for 4 weeks. This diminished hypertrophic response was also found in morphometric measurement of individual myocytes. In fact, a significant increase in left ventricular weight was not observed, and an increase in myocyte width was modest in the old rats following aortic constriction.

Table 1. Histologic Analysis in the 3 Age Groups of Rats

<table>
<thead>
<tr>
<th></th>
<th>9 months</th>
<th>18 months</th>
<th>22 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>AoC</td>
<td>Sham</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Myocyte width (μm)</td>
<td>20.4 ± 0.8</td>
<td>25.9 ± 0.9*</td>
<td>29.2 ± 0.6§</td>
</tr>
<tr>
<td>Fibrosis score</td>
<td>0.6 ± 0.7</td>
<td>0.9 ± 0.9</td>
<td>1.6 ± 0.5‡</td>
</tr>
</tbody>
</table>

Sham, sham-operated rats; AoC, aortic-constricted rats; mean ± SEM.

* p < 0.01, † p < 0.05, statistical significance of differences between mean values in sham-operated and aortic-constricted rats in each age group. ‡ p < 0.05, § p < 0.01, statistical significance of differences between mean values in 9-month-old rats and in different age rats in sham-operated or aortic-constricted rats.

Table 2. Hemodynamic Data in the 3 Age Groups of Rats

<table>
<thead>
<tr>
<th></th>
<th>9 months</th>
<th>18 months</th>
<th>22 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>AoC</td>
<td>Sham</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Peak systolic LVP</td>
<td>91 ± 3</td>
<td>127 ± 13*</td>
<td>95 ± 9</td>
</tr>
<tr>
<td>End-diastolic LVP (mm Hg)</td>
<td>4.6 ± 0.8</td>
<td>5.6 ± 0.9</td>
<td>5.1 ± 1.0</td>
</tr>
<tr>
<td>Peak systolic AoP</td>
<td>89 ± 3</td>
<td>94 ± 9</td>
<td>94 ± 10</td>
</tr>
<tr>
<td>Mean AoP (mm Hg)</td>
<td>69 ± 4</td>
<td>75 ± 7</td>
<td>79 ± 11</td>
</tr>
<tr>
<td>CO (ml/min)</td>
<td>80 ± 9</td>
<td>63 ± 5</td>
<td>76 ± 7</td>
</tr>
<tr>
<td>CI (ml/min/kg)</td>
<td>223 ± 21</td>
<td>182 ± 16</td>
<td>212 ± 16</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>371 ± 17</td>
<td>361 ± 8</td>
<td>345 ± 9</td>
</tr>
</tbody>
</table>

Sham, sham-operated rats; AoC, aortic-constricted rats; LVP, left ventricular pressure; AoP, aortic pressure; CO, cardiac output; CI, cardiac index; mean ± SE. * p < 0.05, † p < 0.01, statistical significance of differences between mean values in sham-operated and aortic-constricted rats in each age group. ‡ p < 0.05, § p < 0.01, statistical significance of differences between mean values in 9-month-old rats and different age rats in sham-operated or aortic-constricted rats.
by guest on May 31, 2017 http://circres.ahajournals.org/ Downloaded from

pressure gradient between the left ventricle and aorta

Increased cardiac hypertrophy resulted. In the present study, relatively moderate degrees of aortic constriction estimated by peak left ventricular pressure or by pressure gradient between the left ventricle and aorta were employed because mortality following aortic constriction was high in old rats (22 months). Given a more intense stimulus, it is possible that an increase in left ventricular weight might be observed even in rats of advanced age (assuming the animals survive).

In sham-operated rats, left ventricular weight and myocyte width increased with age. In addition, histologic examination revealed that myocardial tissues obtained from older rats showed more fibrosis. Similar findings associated with senescence per se have been reported by McCafferty and Edington,9 Li et al,16 Yin et al,17,22 Wei et al,21 and Anversa et al.26 If the extent of hypertrophy occurring with senescence alone has reached a maximum level, it might be possible to obtain further hypertrophy by pressure overload in advanced age. However, in the reports of Florini et al10 and Zitnik and Roth,14 stimulation by thyroid hormone produced further myocardial hypertrophy, even in rats of advanced age. Therefore, in our study, the degree of hypertrophy produced by pressure overload in rats of advanced age should not have been limited by existing hypertrophy associated with senescence, although it seems likely that the hypertrophy caused by senescence alone might change the level of sensitivity to stimulation for myocytes to develop further hypertrophy.

McCafferty and Edington6 examined the effect of age on cardiac hypertrophy produced by chronic running exercise and suggested that there might be a threshold age (approximately 15 months old) beyond which the initiation of a training program no longer affects heart muscle growth. Although aortic constriction rather than exercise was employed as the stimulus to cardiac hypertrophy, our observations are consistent with their findings.

Johnson et al21 and Meerson et al28 have reported that in myocardium of old rats or old humans, RNA concentration decreased, and the rate of RNA and protein synthesis as well as degradation were diminished. In our study, young adult rats showed a significant increase in myocardial RNA concentration following aortic constriction, but old rats did not. Since the majority (>97%) of total cellular RNA in our preparation is ribosomal RNA, a failure to increase ribosomal RNA in old rats indicates that the capacity to synthesize protein is diminished. This diminished capacity for protein synthesis appears to be one of the contributing factors to the diminished hypertrophic response in rats of advanced age.

The different findings in the extent of myocardial hypertrophy in animals of advanced age produced by thyroid hormone administration10,11 or, in our study, aortic constriction may reflect differences in the mechanisms by which these stimuli provoke myocardial hypertrophy.2,4,5,8,29,30 Many factors within the myocardial cell17,31 modulate the process linking increased circulatory demand to myocardial hypertrophy and/or the process of protein synthesis and degradation. Thyroid hormone has been shown to regulate cardiovascular responsiveness to catecholamines through stimulation of β-adrenoceptors in vivo11,32 and in vitro.33 Increases in β-adrenoceptor density11 and protein synthetic rates10 after thyroid hormone treatment may be reduced in the senescent compared with the young adult heart. It has been

Table 3. RNA Concentration in the Myocardium of the 2 Age Groups of Rats

<table>
<thead>
<tr>
<th>2 Age Groups of Rats</th>
<th>9 months</th>
<th>18 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>AoC</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>963 ± 22</td>
<td>1,168 ± 31</td>
</tr>
<tr>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>980 ± 10</td>
<td>1,021 ± 16</td>
</tr>
<tr>
<td>NS</td>
<td>p&lt;0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Sham, sham-operated rats; AoC, aortic-constricted rats; NS, not significant. mean ± SEM.
demonstrated that aging has no effect on the extent of thyroid-induced cardiac hypertrophy in mice and rats. It is possible that thyroid hormone may differentially modulate cardiovascular adrenergic responsiveness and protein synthesis in young adult and senescent animals under similar conditions of pressure overload.

Stimulation of α-adrenoceptors may modulate the hypertrophic response of cardiac muscle at the cellular level. In intact animals, subhypertensive doses of norepinephrine caused myocardial hypertrophy. Furthermore, estimated by cell volume, surface area, and protein content, norepinephrine plus β-receptor blockade produced hypertrophy in cultured myocytes, which suggests that stimulation of α-receptors may trigger hypertrophy in myocardial cells. It is possible that the circulating catecholamine or myocardial catecholamine levels were higher after aortic constriction than the circulating catecholamine or myocardial catecholamine levels were higher after aortic constriction in young rats than in old rats and/or that the number or affinity of α-adrenoceptors in old rats was diminished compared with young rats in our study.

The role of baroreceptor function should also be considered in interpreting the results of our study. In our aortic-constriction preparation, the arterial baroreceptors were relatively unloaded, while the systemic hypertension of renal or subdiaphragmatic origin, these receptors would be subjected to pressure loading comparable in degree to that experienced by the cardiopulmonary receptors located in the left ventricle. Baroreceptor interactions with protein synthetic mechanisms may be important in mediating the age effect on the myocardial hypertrophic response. Further studies are needed to determine whether these factors may contribute to the diminished capacity for myocardial hypertrophic response in rats of advanced age.

Acknowledgments

The authors are grateful to Drs. Janice and Marc Pfeffer for helpful advice and Helen Shing and Sara Murray for technical assistance (histology).

References


**Key Words**

- left ventricular hypertrophy
- adult aging
- senescence
- afterload
- RNA
Effect of age on the development of cardiac hypertrophy produced by aortic constriction in the rat.
S Isoyama, J Y Wei, S Izumo, P Fort, F J Schoen and W Grossman

*Circ Res.* 1987;61:337-345
doi: 10.1161/01.RES.61.3.337

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 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/61/3/337

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation Research* is online at:
http://circres.ahajournals.org/subscriptions/