A Model of Psychosocial Hypertension Showing Reversibility and Progression of Cardiovascular Complications

By James P. Henry, Patricia M. Stephens, and George A. Santisteban

ABSTRACT

The sequence of pathophysiological changes that can result from the stimulating effects of a sustained disturbance of the social environment was studied in ten colonies of socially deprived mice. Sixteen formerly isolated males were placed with 16 normal females in population cages consisting of seven intercommunicating boxes. Six of these socially disturbed 32-member colonies were terminated after periods of interaction ranging from 2 days to 9 months. The remaining four were terminated a month or more after the males had been returned to individual isolation. Indirect blood pressure measurements, body and heart weights, and sections of hearts and aortas were studied in the males. Following the shorter exposures, blood pressure reverted to normal in a few days. Exposures of 6 months or more were associated with unchanged body weights and sustained increases in heart weight and blood pressure readings. In addition, there was a significant development of aortic arteriosclerosis and myocardial fibrosis. These changes persisted despite prolonged return to isolation.

KEY WORDS

myocardial fibrosis  systolic hypertension  arteriosclerosis  population cage  mice of the CBA strain

A number of laboratory models are now available that permit analysis of the effects of environmental behavioral influences on the cardiovascular system. Long-term continuous recordings of blood pressure in rhesus monkeys (1), squirrel monkeys (2), and baboons (3) have shown that sustained elevations of systolic blood pressure follow chronic exposure to aversive behavioral conditioning procedures. In a related method, we have used complex population cages stocked with CBA mice. Either both sexes or just the males are socially deprived from infancy to maturity. Males and females are then placed together in a design that induces sustained competition for territory in vigorous social interaction (4). Our hypothesis has been that the repeated confrontations serve as an intermittent functional trigger mechanism that leads to repeated arousal of the sympathetic adrenal medullary and the pituitary adrenocortical neuroendocrine response patterns (5). Use of this technique has established that the CBA strain, which is not spontaneously hypertensive, does develop sustained elevations of systolic arterial blood pressure in response to aversive behavioral conditioning (4). Our observations have been recently confirmed by Alexander (6); she used a similar cage system and a colony of normal Wistar rats. Furthermore, collaborative studies have shown that 6 months of psychosocial hypertension in CBA mice is associated with an increase in the catecholamine-synthesizing enzymes of the adrenal medulla (7), chronic interstitial nephritis, myocardial fibrosis, and arteriosclerotic degeneration of the aorta and the coronary vessels (8). The present study followed the time course of the development of the cardiovascular changes. As in clinical hypertension, the initial stages were reversible, but after a mouse had spent a significant percent of its life-span in the population cage, the measured blood pressures remained elevated and the cardiovascular complications did not regress despite a reduction in the social stimulus.

Methods

The strain of CBA Agouti mice used in these experiments has been bred in our laboratories for 18 years. The details of husbandry have been described previously (4, 9). The mice were fed a standard, normal commercial diet with a NaCl content of 1% (Purina Lab Chow). Each breeder box was stocked with a group of one male and three females which remained unchanged. Sibling mating was avoided, and runts were discarded. The young remained with their parents until they were weaned at 21-28 days. Boxes were changed weekly, using 2 inches of white pine shavings. After weaning and sexing, no more than eight mice of each
sex were maintained in the standard plastic "shoebox" 23 × 11 × 11-cm caging. All were bred, raised, and maintained in the same 15 × 30-foot air-conditioned windowless room with a 12-hour day-night cycle.

**BOXES OF MIXED MALES**

Boxes of mixed males were used in a preliminary study to determine how long it takes the blood pressure to return to normal levels after various brief periods of social interaction. The groups were made up by taking one mouse from each of a series of separate boxes of siblings. Since these males were all strangers to each other, they fought persistently although not as intensely as formerly isolated mice. Eight mice were placed in each box, and they were allowed to interact for 1, 2, 7, and 16 days. Then, instead of being returned to their original boxes, they were placed in confined isolation in glass jars and observed to see the rate at which the blood pressure returned to normal.

**POPULATION CAGES**

To stimulate social interaction, the males that were to become members of the socially interacting colonies were raised for 3.5 months in confined isolation in ½-liter glass jars after they were weaned at 12-14 days (4). Elsewhere we have shown that such formerly isolated mice are hyperaggressive and fail to establish a stable social hierarchy (10, 11). In these studies, the intensity of social disorder was reduced by using normal sibling females of the same age as the males. These females were socialized as a result of living together in the same boxes.

The special population cages that provide social stimulus and inhibit territory formation were the same as those previously described (4). They consisted of a system of six standard boxes with narrow 3.2-cm interconnecting tubes surrounding a seventh hexagonal central feeding and watering cage (Fig. 1).

A group of 16 formerly isolated males and 16 normally socialized females together constituted one experimental colony. Thus, crowding was avoided and the population density was less than five in each of the seven cages to which the mice had access. As depicted in Figure 1, the mice tended to aggregate so that some cages contained as many as 15 animals and other cages were empty.

**CONTROLS**

Since the various colonies were of different ages at termination, data from a number of appropriate control groups which were part of a previous study (8) have been included (Fig. 2). These controls were all normal boxed sibling males. Their ages were: C, 4 months (n = 21), C, 6-8 months (n = 17), and C, 10-15 months (n = 34). These previously studied groups provided standard data for body and heart weights, myocardial degeneration, and arteriosclerotic changes. In addition, 15 mice were held in isolation for a total of 9.5 months, i.e., for 6 months after the initial 3.5-month period experienced by all of the experimental males. This group, C, constituted a direct control for the data from the 6-month-old colonies. It also provided supporting data for body and heart weights and histological observations found for groups, C, C, and C. Finally, this group constituted a control for the blood pressure measurements. The other blood pressure control was the mean of the initial values observed in the members of the ten colonies just prior to their placement in the population cages.

**EXPERIMENTAL DESIGN**

As Figure 2 indicates in symbolic form, following weaning at 2 weeks, the males were isolated until adulthood in ½-liter jars for 3.5 months. Sixteen males were then placed in each of the population cages with an equal number of normal sibling females of the same age that had been raised in boxes. Following social interaction, which lasted for the periods indicated in Figure 2, the colonies were either terminated or disbanded and the males returned to isolation. Three colonies were exposed to the social stimulus for 2, 8, and 21 days and three more for 2, 6, and 9 months, respectively. To determine whether the observed changes persisted after return to a low stimulus condition, the males of two colonies, 2 + 1 and 5 + 1 months, were returned to isolation in the jars for 1 month. Those males from the two 9-month colonies were split into two groups; half of the mice were returned to isolation in the glass jars and the other half were isolated individually in the original standard cages in which they had ample living space. The times at which the various colonies were started were staggered so that autopsies could be done simultaneously and tissues needed by a collaborating laboratory could be obtained.

**BODY WEIGHT AND BLOOD PRESSURE**

All of the males were weighed once a month when their blood pressure was measured in the conscious.
HENRY, STEPHENS, SANTISTEBAN

state by a method of tail plethysmography described in previous publications (4, 9). The method followed the same detailed precautions for obtaining reliable results, the necessity for which has recently been reiterated by Pfeffer et al. (12) and Buñag (13). All of the males in any particular colony had their blood pressures measured in a single session. They were removed from the population cage and placed in standard caging in a well-ventilated warming box set at 36-38°C for 20-30 minutes before measurement. Blood pressure measurements were not made while a mouse was adapting to the restraining tube. The adaptation occurred more rapidly after a number of trials. Repeated measurements were taken until the blood pressure approached a low asymptote, and the five lowest figures consistently obtained were averaged.

AUTOPSY AND HISTOLOGY

The mice were killed by decapitation, and their abdominal and thoracic aortas and hearts were excised. The atria and vessels were then separated from the ventricles, which were weighed after expression of any

<table>
<thead>
<tr>
<th>C1</th>
<th>2 DAYS</th>
<th>8 DAYS</th>
<th>21 DAYS</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MONTHS

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 TERMINATION

SIBLING MALES IN STANDARD 23 x 11 x 11 CM CAGES
NURSING MOTHER WITH LITTER
MALE ISOLATED IN GLASS JAR (J)
SOCIA LLY STIMULATED MALE ISOLATED IN STANDARD CAGE (B)
POPULATION CAGE: STANDARD CAGES INTERCONNECTED BY 3.2 CM I.D. TUBING

FIGURE 2

Illustration of the overall experimental design. The symbols and legends below the main diagram indicate various animal husbandry procedures that were employed during this study. Groups C1, C2, and C3 represent control groups of boxed siblings terminated at 4, 8, and 10-15 months, respectively. Group C4 remained isolated in jars for 6 months after the initial 3.5-month period. The 2-, 8-, and 21-day groups represent colonies composed of 16 normal sibling females and 16 males that had been isolated for 3.5 months before they were placed in the population cage for the foregoing brief periods. The 2- and 2+1-month and the 6- and 5+1-month groups represent colonies made up like those just described. The +1 indicates that the males were returned to isolation in jars for 1 month before the termination of the experiment. Three colonies were exposed to the population cage for 9 months. Half of the 9+1-month and 9+2-month groups were reisolated in jars (J) and half in standard cages (B).
blood onto a saline-soaked paper towel. Simultaneous autopsies of the ten colonies were carried out by a team of three. A micro torsion balance which read to 0.2 mg was used for weighing. The excised hearts were sectioned through their greater diameter and through the apex. The aortas were sampled at random without concern for any particular area in the thorax or abdomen. All tissues were fixed in 10% buffered Formalin, embedded in paraffin, and sectioned at 5-6 μ. The sections of the heart and aorta were stained with periodic acid Schiff. Previous work has shown that it is realistic to discriminate the lesions into five grades from 0 to 3+ (8). Repeated evaluations of random samples has shown that the probability that any particular slide will be re-scored in the next higher or the next lower grade is high, but it is rare for it to be two or more grades away. The correlation coefficient of the score-rescore testing between three larger scoring groups into which the five grades were combined, i.e., 0 to +, + to 2+, and 3+ and above, approximated 0.7 (P < 0.01) for the aorta and 0.9 (P < 0.01) for the cardiac fibrosis (8). As in our previous study, the slides were coded and randomly mixed to ensure that grading was blind. In the normal aorta, the elastic fibers form five or six distinct wavy layers, and the long axes of the smooth muscle cells are circumferentially arranged. With persistence of elevated blood pressure, there is an increased deposition of substance within the lamellas. Such cases are scored at 1+ with a progression to 2+ as the elastic rings eventually fragment and the smooth muscle cells enlarge and vacuolate forming a palisading arrangement.

In the heart muscle of the controls, there was little fibrous tissue, but in the cases scoring 1+ there were scattered strands of fibrous tissue in the myocardium with deposits around the small vessels. In the 2+ cases scattered patches of fibrosis appeared throughout the myocardium; when they became confluent and extensive, the heart was scored as 3+.

Results

Body Weight

There were no significant differences between the body weights of the groups of boxed siblings that served as controls and those of the colony males of the appropriate age group (Fig. 3). The 10-month-old C0 control group of isolated mice also stayed within the same weight range.

Blood Pressure

The four diagrams in Figure 4 show the rate at which the blood pressure fell in the four groups of eight formerly socialized males. These mice were mixed in separate standard boxes for various brief periods and then returned to confined isolation in jars. Since these males came from different groups, they were strangers and they fought vigorously with a resultant rise in blood pressure. In the groups that experienced 1, 2, and 7 days of exposure to social interaction, blood pressure returned to normal after approximately 4 days of postexposure isolation. But after 16 days of social interaction, the return to base line took 9-10 days. These preliminary results indicated that the total period of exposure to the stimulation associated with high blood pressure would have to be longer than 2 weeks to exceed the initial reversible phase.

Figure 5 presents the mean blood pressure of all ten colonies in the open columns; the striped columns represent the two control values. The initial blood pressure is the arithmetic mean of the initial blood pressures of all colony members just before they went into their population cages, and the second control pressure, C0, is the last of the monthly blood pressure measurements of the control group of 15 mice that were isolated for a total of 9.5 months (see Fig. 2). The blood pressures were taken at monthly intervals throughout the experiment, but Figure 5 only illustrates the final readings for the various groups. The final reading was used because it represents the state of affairs with regard to social interaction at the time of autopsy when the heart and adrenal weights and the medullary enzyme levels were also determined.

The blood pressure rose progressively from the 2-day through the 8-21-day colonies. At this point,
the social interaction was at a peak as dominance-subordinate conflicts were being resolved (10), and the blood pressure had reached approximately 150 mm Hg. The 2-, 6-, and 9-month colonies had pressures approximately within this range, i.e., 150-165 mm Hg, at each of their monthly measurements. There was some return toward base line when mice from the 2-month group were placed in isolation for 1 month. This decrement was less for the colony exposed to 5 months stimulation, and the 9-month groups showed still less return to the base-line values. For all of these older colonies, the difference between these blood pressures and that of the control C4 group was highly significant (P < 0.001).

Figure 6 presents the details of the monthly blood pressure observations that were made on the three groups of mice exposed to 9 months of social interaction. The responses are presented in full because they are typical of those for all of the groups. The mean monthly blood pressure fluctuated from 140 to 165 mm Hg but appeared to become less variable after the sixth month.

The symbol "J" at 9 + 1 months and 9 + 2 months represents the blood pressure level attained by the 16 males from the two colonies that were returned to confined isolation in glass jars (Fig. 2); blood pressure in these mice stabilized at 150 mm Hg. The pressures of the remaining 16 mice that were isolated in the standard cages are indicated by the symbol "B" (Fig. 2). It is possible that the extra freedom of movement to which the formerly isolated mice had become accustomed in the population cage was associated with the observed further drop in blood pressure. Even so, the three sets of measurements of these two groups of eight mice individually isolated in cages remained at approximately 140 mm Hg, a value significantly in excess of the resting control level (P < 0.001).

HEART WEIGHT

A sustained blood pressure elevation and arteriosclerotic changes were closely associated with the observed increase in ventricular weight; Figure 7 shows the changes in this parameter. The first group of mice fell short of the mean for the older mice, because the animals had not quite attained full growth when they were exposed to the 2 days of social interaction; hence, their hearts were small at autopsy. However, the 8- and 21-day weights exceeded that of the C4, 4-month-old control group. The 2-month and 2 + 1-month groups showed a small but significant elevation over the corresponding 6-8-month-old controls (C). Although the
Average systolic blood pressure at termination. The abscissa is the same as that in Figure 3. The open histograms represent the last of the series of monthly readings of systolic blood pressure in the males in each of the colonies under study. The striped column labeled initial blood pressure represents the mean initial blood pressure of all of the groups, and the striped column marked C represents the blood pressure of the control group which was isolated after weaning and 10 months old when blood pressure was measured just prior to termination of the experiment (see Fig. 2). The significance of the differences between the appropriately aged controls and the various experimental groups was determined by paired Student’s t-tests. The asterisks denote significant differences from the control values in the various groups: ** = $P < 0.01$ and *** = $P < 0.001$.

Blood pressure was already sustained at the 150 ± 20-mm Hg level after the third week, a large increase in heart weight did not appear until the sixth month. Moreover, it vanished after a month of return to isolation in spite of sustained hypertension. At the ninth month, however, the changes in heart weight appeared to be fixed. There was a highly significant difference in heart weights between this group and the appropriate 10-15-month-old controls (C) ($P < 0.001$).

**Aortic Arteriosclerosis**

Figure 8 shows the changes in the aortas. They were similar to although less severe than those we have previously described (8). The mean for the 2-, 8-, and 21-day mice ranged between ± and +. By 6 months there was a significant disturbance of the normal structure. The + to 2+ value implies that there was, on the average, some fragmentation of the elastic rings with an increase in collagen and elastin within the lamellas enlarging them. On occasion, enlargement and radial orientation of the smooth muscle cells with respect to the lumen were found. The level of the arteriosclerotic lesions in the aortas of the two groups exposed to 9 months of social stimulation and returned to isolation remained significantly elevated ($P < 0.01$) and $P < 0.001$, respectively. There were only slight changes (±) for the C1, C2, and C3 boxed sibling controls and for the C4 group in isolation in jars.

**Myocardial Degeneration and Fibrosis**

Figure 9 records the combined incidence of myocardial degeneration and fibrosis. Significant changes first became apparent at a minimal score of + in the 6- and 9-month groups. The 5-month group that was returned to isolation showed control levels of involvement. By contrast, there was actually an increase in the 9-month group that was returned to isolation for 2 months. The significantly elevated ($P < 0.001$) mean score of 2+ in this colony indicates a considerable investment of fibrous tissue between the muscle bundles and around the small vessels, together with small scattered patches of fibrous tissue throughout the myocardium. The boxed sibling controls (C1–C3) and the isolated controls (C4) showed no significant pathological changes.

**Discussion**

The results of this study confirm and extend the observations of previous investigators who have studied the effects of social interaction on the cardiovascular system in various species of animals (10-13). The effects of social interaction on cardiovascular function are complex and involve a variety of factors, including the type of social interaction, the duration of the interaction, and the individual and group characteristics of the animals involved. The results of this study suggest that social interaction has a significant impact on cardiovascular function, and that the effects of social interaction on blood pressure may be mediated through changes in the cardiovascular system itself.
Discussion

BODY WEIGHT

The absence of a significant difference in body weight between the socially stimulated mice and appropriately aged groups of control boxed siblings indicates that despite the vigorous interaction there is no serious metabolic deficiency in the members of the various colonies. This finding was confirmed by the fact that despite the sustained fighting only five mice died as a result of social interaction and the full complement of males was available in half of the colonies at the termination date. The data also indicate that differences in heart weight between the control groups in the standard boxes and the groups in the population cages are not related to a difference in body weight.

BLOOD PRESSURE

Our previously published observations have established that the blood pressure of unanesthetized boxed sibling males and of isolated males as measured by the cuff technique remains a consistent 125 ± 12 mm Hg (4, 10). On the other hand, our original observations (4) of a sustained elevation of the average systolic blood pressure of populations of mice that have been socially interacting have now been repeatedly confirmed (6, 7, 10, 11, 14). Furthermore, the present work, which shows that the blood pressure of mice that have been exposed for prolonged periods remains elevated despite prolonged reisolation, repeats earlier findings (4).

Finally, the original elevation in the blood pressure in the population cage is not an expression of increased muscular activity. Daniel (15) used activity wheels to train mice; he demonstrated that the blood pressure of two groups of mice that ran an average of 8 and 20 miles a week, respectively, for 5 weeks did not change, i.e., control 112 mm Hg, experimental 107 and 116 ± 8 mm Hg, respectively.

The significance of environmental stimulation in inducing increased blood pressure has recently been discussed in connection with the spontaneously hypertensive rat. Lais et al. (16) have shown that young spontaneously hypertensive rats raised from birth in a quiet dark room fail to develop an arterial blood pressure as high as that found in rats raised in normal light. This effect may be related to the fact that the young respond with exaggerated activation to normal environmental stimuli. Lundgren (17), reporting the work of Folkow's group, suggests that in the spontaneously hypertensive rat the neurogenic factor may be an intermittent functional trigger mechanism which progressively leads to resetting of baroreceptor controls and to structural changes in precapillary resistance vessels.

Finally, the original elevation in the blood pressure in the population cage is not an expression of increased muscular activity. Daniel (15) used activity wheels to train mice; he demonstrated that the blood pressure of two groups of mice that ran an average of 8 and 20 miles a week, respectively, for 5 weeks did not change, i.e., control 112 mm Hg, experimental 107 and 116 ± 8 mm Hg, respectively.

The significance of environmental stimulation in inducing increased blood pressure has recently been discussed in connection with the spontaneously hypertensive rat. Lais et al. (16) have shown that young spontaneously hypertensive rats raised from birth in a quiet dark room fail to develop an arterial blood pressure as high as that found in rats raised in normal light. This effect may be related to the fact that the young respond with exaggerated activation to normal environmental stimuli. Lundgren (17), reporting the work of Folkow's group, suggests that in the spontaneously hypertensive rat the neurogenic factor may be an intermittent functional trigger mechanism which progressively leads to resetting of baroreceptor controls and to structural changes in precapillary resistance vessels.

Finally, the original elevation in the blood pressure in the population cage is not an expression of increased muscular activity. Daniel (15) used activity wheels to train mice; he demonstrated that the blood pressure of two groups of mice that ran an average of 8 and 20 miles a week, respectively, for 5 weeks did not change, i.e., control 112 mm Hg, experimental 107 and 116 ± 8 mm Hg, respectively.
to an increase in the adrenal medullary catecholamine-synthesizing enzymes (7); if the changes are the same as those in the spontaneously hypertensive rat, the wall-to-lumen ratio of the precapillary vessels increases, leading to raised resistance even at normal levels of smooth muscle activity (17). At first such changes will subside when the stimulus is withdrawn, but when the hypertension has been sustained for a long time, as Wolinsky has shown (18-20), the percent of mucopolysaccharides and smooth muscle noncollagenous proteins that accumulate in the media diminishes in favor of fibrous proteins, elastin, and collagen. These wall elements show far less regression than smooth muscle when the stimulus is removed. Such changes of a degenerative nature would account for the sustained elevation seen in those mice of our colonies that were exposed to 6 and 9 months of social stimulation.

**HEART WEIGHT**

In the 2-, 8-, and 21-day colonies, the observed increase in heart weight was presumably associated with a moderate cardiac muscle hypertrophy in response to an increase in cardiac output as well as blood pressure. The highly significant heart weight increase in the 6-month colony ($P < 0.001$) and the three 9-month groups ($P < 0.001$) was not a response to further blood pressure elevation, since this parameter remained stable after the first month (Fig. 5). However, collagen invasion of the hearts and fibrosis of the ventricles may well have contributed to the increased heart weights seen in the aging colonies. Lundgren (21) points out that following release of renal artery obstruction the time course and the extent of regression of left ventricular hypertrophy and parameters indicating vascular medial hypertrophy are nearly identical, suggesting a close similarity in structural adaptation. The normotensive control of 10-15-month-old boxed siblings, C3, showed no increase in heart weight despite their age. Indeed, the C3 group of confined inactive isolated mice had slightly lower heart weights than did the more active standard boxed mice.

**MYOCARDIAL DEGENERATION AND FIBROSIS**

The myocardial damage score subsided in the group that was isolated for 1 month following 5 months in the colony. This fall paralleled the return of heart weight to normal. In the 9-month colonies, both heart weight and fibrosis scores stayed elevated despite the return to isolation. As in our previous studies (8), these changes significantly exceeded those found in control boxed siblings of the same age ($P < 0.001$).

In his recent discussion of clinical hypertension Kaplan (22) has outlined the time course of the stages in the progression of the typical case. In the first three decades, it is labile and reversible; this is the stage of pre- and early-hypertension. By age 30-50 years, although still asymptomatic, established hypertension has begun; the blood pressure is elevated whenever it is measured and arteriosclerosis is in progress. In the 40-60-year age group, when more than half of the expected life-span has passed, the incidence of life-threatening complications of the heart, aorta, brain, and kidneys has risen sharply (15). The equivalent half-life-span of a CBA mouse is approximately 12-15 months, the age of colonies that had been exposed to the longer periods of social interaction and were developing complications. The raised arterial blood pressure with the increased heart size and the progressive...
arteriosclerosis and myocardial fibrosis create an interlocking picture which suggests that murine psychosocial hypertension has aspects that model the human condition.

References

15. Daniel TN: Method for preparing coronary arterial corrosion casts of the mouse heart. M.S. Thesis (Physiology), University of Southern California, Los Angeles, June, 1970
A model of psychosocial hypertension showing reversibility and progression of cardiovascular complications.
J P Henry, P M Stephens and G A Santisteban

*Circ Res.* 1975;36:156-164
doi: 10.1161/01.RES.36.1.156

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1975 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/36/1/156

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/