Aortic Constriction Exacerbates Atherosclerosis and Induces Cardiac Dysfunction in Mice Lacking Apolipoprotein E

Jiao-Hui Wu, John Hagaman, Shinja Kim, Robert L. Reddick, Nobuyo Maeda

Abstract—Despite considerable evidence suggesting that hypertension contributes to the development and progression of atherosclerosis, the causative links remain unclear. We have tested the effects of chronic hypertension induced by suprarenal aortic constriction on the development of atherosclerosis in apolipoprotein E–deficient (Apoe−/−) mice. Compared with a sham operation, narrowing the aortic luminal diameter by 33% increased blood pressure proximal to the constriction by ≈15 mm Hg, but the pressures distal to the constriction were unchanged. Kidney renin mRNA and plasma renin activity were also unaffected. Compared with plaque size after the sham operation, atherosclerotic plaque size in the aortic root 8 weeks after coarctation was increased to 245% and 152% in males and females, respectively. Aortic segments at the constriction were free of atherosclerotic deposits, but segments proximal to the constriction were dilated and had atherosclerotic lesions. Thrombi were present immediately below the constriction in Apoe−/− and wild-type vessels. Surprisingly, compared with wild-type mice, the Apoe−/− mice were more susceptible to the cardiac hypertrophy and dysfunction induced by pressure overload. Thus, aortic coarctation exacerbates atherosclerosis in vessels proximal to the constriction without a concomitant increase in the renin-angiotensin system. Our study also suggests that apolipoprotein E plays an important role in modulating cardiac hypertrophy. (Arterioscler Thromb Vasc Biol. 2002;22:469-475.)

Key Words: animal models ■ hypertension ■ thrombosis ■ acidophilic macrophage pneumonia ■ echocardiography

Hypertension and atherosclerosis are 2 of the most important causes of morbidity and mortality in humans. Numerous clinical and epidemiological studies have identified systemic arterial hypertension as an independent and potent risk factor for the development of atherosclerotic disease. For example, the Framingham study found that diastolic blood pressure (BP), systolic BP, and pulse pressure are predictors of coronary heart disease risk.1,2 The extent of atherosclerosis in the aorta and coronary arteries of subjects in the Pathobiological Determinants of Atherosclerosis in Young (PDAY) study3 was greater in hypertensive compared with normotensive individuals. Similarly, an association between ultrasound-measured carotid artery intima/media thickness and systolic BP was demonstrated in the Atherosclerosis Risk in Communities (ARIC) Study.4

Animal models with combined genetic risks for atherosclerosis and hypertension have also shown that elevated BP accelerates atherogenesis. Thus, lack of endothelial NO synthase increases BP and exacerbates atherosclerosis in mice lacking apoE (Apoe−/− mice).5 Similarly, Dahl salt-sensitive hypertensive rats that overexpress the human cholesterol ester transfer protein develop severe hyperlipidemia and atherosclerosis and show decreased survival.6 Nevertheless, current data do not allow an unequivocal distinction between the physical effects on the atherogenic process of the increased systemic BP and the effects on vasculature caused by concurrent changes in circulating vasoactive mediators, and causative links between hypertension and atherosclerosis have yet to be defined.

Surgical coarctation of the aorta in combination with high dietary cholesterol has been shown to markedly increase atherosclerosis in rabbits and monkeys.7–9 In the present study, we have applied aortic coarctation to alter the BPs of Apoe−/− mice with the aim of understanding the interaction between BP changes and a genetic form of hyperlipidemia. We find that aortic coarctation in mice accelerates atherosclerosis in the aorta proximal to the constriction. Organized thrombi are found immediately distal to the constriction independent of hyperlipidemia. A surprising new finding is that compared with wild-type mice, Apoe−/− mice are significantly more susceptible to the cardiac hypertrophy induced by chronic coarctation of the aorta.

Methods

Animals

Apoe−/− mice10 were backcrossed at least 7 generations to a C57BL/6 genetic background. C57BL/6 mice were used as the wild-type control group. Care and experimental procedures were in compliance with the Principles of Laboratory and Animal Care established by the
Aortic Constriction
Mice were anesthetized with isoflurane and placed in the supine position on warm pads under an operating microscope. The abdomen was opened, and the abdominal aorta at the suprarenal level was freed of the surrounding adventitial adipose tissue by gentle dissection. The aorta between the celiac and superior mesenteric arteries was constricted by tying a 6-0 silk suture ligature against a 28-gauge needle for males and 30-gauge needle for females to yield an approximate 33% narrowing of the luminal diameter when the needle was removed. For the sham operations, 18-gauge needles and 20-gauge needles were used for males and females, respectively. Coarctation was applied when the mice were 2 months of age, and they were euthanized at 4 months of age for evaluation.

Echocardiography
Echocardiograms were obtained on conscious mice with gentle restraint at 2, 4, 6, and 8 weeks after surgery. Two-dimensional guided M-mode echocardiography was performed by using HDI 5000 echocardiograph equipment (ATL) and a 7.5-MHz transducer. Heart rate was calculated by using the period between 2 consecutive end-diastolic points from pulsed Doppler echocardiography. Left ventricular (LV) mass was calculated as described.

Intra-arterial BP Measurement
Mice were anesthetized with isoflurane, and the right carotid and right femoral artery were cannulated with flame-stretched PE 20 tubing. Catheters were connected to MLT1050 precision transducers and Power-Laboratory recording equipment (ADI Instruments). Approximately 90 minutes after the mice had recovered from the anesthesia, intracarotid and femoral artery BP s were measured simultaneously. An average of 10 measurements was taken as a mean value for each animal.

Histology and Lesion Measurements
Mice were euthanized at 4 months of age. Blood was collected from the heart into a tube containing EDTA (final concentration 5 mmol/L) for plasma lipid analysis. The heart and vascular tree were perfused by intracardiac infusion with 10 mL of 4% paraformaldehyde in PBS under physiological pressure. Heart segments that contained the aortic sinuses were serially sectioned for morphological evaluation of atherosclerotic lesions. Thoracic aortas were excised, freed of adventitial fat, and examined under microscopy. Specimens were subsequently dried and weighed, and lipids were extracted by methanol/chloroform (1:1 [vol/vol]). Serial 10-μm-thick sections of the contiguous abdominal aorta were cut every 100-μm distance from 5 mm above the suture level to 5 mm below for evaluation. Paraffin-embedded tissue sections were stained with hematoxylin and eosin (H&E), with periodic acid-Schiff stain (PAS), or with trichrome. Aortic segments were also embedded in epoxy resin, and sections were stained with toluidine blue.

Renin mRNA and Plasma Activity
Total RNA was prepared from homogenized kidney by using ABI 6700 (ABI). Renin mRNA levels were determined by real-time quantitative reverse transcription–polymerase chain reaction with ABI 7700 (ABI) with the use of 5'-ACAGTATCCCAACAGGGAGACAG-3' and 5'-GCACCTGGAGCCAGACAGGA-3' as primers and 5'-FAM-CTGCTCATGGCAATCC-Tamra-3' as the detection probe. Amplification of β-actin mRNA was used as an internal standard with 5'-CTGCTGACGGCCAGGCATTC-3' and 5'-CAAGAAGGAGGCTTGGA-AAAAGA-3' as primers and 5'-TET-CATATTGGCCAAGCGGCTTG-TCCG-Tamra-3' as the detection probe. Plasma concentration of active renin was determined by radioimmunoassay as described.

Statistical Analysis
Data were analyzed by using a JMP software package (SAS Institute Inc). Means of different groups were compared by ANOVA. Time-dependent changes were analyzed by MANOVA with repeated measures.

Results
Aortic Coarctation and Survival of Animals
Surgical coarctation (banding) of the abdominal aorta of 2-month-old Apoe−/− or wild-type mice yielded an approximately one third narrowing of the luminal diameter (see below). Aortas of the sham-operated mice had no luminal narrowing. All mice recovered from anesthesia, but approximately 20% of sham-operated mice and 40% of the banded mice died within a week after surgery (most died within 1 or 2 days). Postmortem examination of some banded mice that died after 24 hours showed thrombi at or near the banded area. No additional losses of mice were seen 1 week after banding, except in the banded Apoe−/− group. Thus, 34 of 86 banded Apoe−/− mice died during the first week, and 9 died between 6 and 8 weeks after surgery. Some of the later deaths were by aortic dissection, and some mice showed signs of heart failure. All the mice included in the present study that were alive at 8 weeks after surgery appeared healthy. There was no significant difference in weight gain between the sham-operated and banded groups (Table 1). Compared with sham-operated hearts, the hearts of male and female Apoe−/− mice were significantly enlarged after banding and showed an approximately 50% increase in the heart-to-body weight (HW/BW) ratio (P<0.0001). In contrast, the increase of the HW/BW ratio was significantly greater in Apoe−/− compared with Apoe−/− male mice (P<0.01). There was no significant difference between Apoe−/− and Apoe−/− female mice. Aortic and coronary artery sections from Apoe−/− and Apoe−/− male mice 8 weeks after banding showed large areas of fibrotic tissue and atherosclerotic lesions that did not appear in sham-operated mice (Fig. 2). The increase of the HW/BW ratio was significantly greater in Apoe−/− compared with Apoe−/− female and male mice (P<0.05).
Increased BP Proximal to the Coarctation

Aortic banding significantly increased BP proximal to the constriction (Table 2). The differences between the carotid and femoral artery BPs of individual animals averaged 25±2 mm Hg (n=52) in the banded group versus 10±1 mm Hg (n=32) in the sham-operated group (P<0.0001). The increased pressure differentials resulted mainly from an increased carotid BP in the banded mice (126±3 mm Hg in banded mice compared with 111±2 mm Hg in sham-operated mice, P<0.001). The difference in the femoral artery BP between banded and sham-operated mice was not significant (100±2 versus 101±2 mm Hg, P=0.3). There were no statistically significant differences in BPs (carotid or femoral) between the wild-type and Apoe−/− mice or between the sexes. The HW/BW ratio of individual Apoe−/− animals was positively correlated with the differential BPs between their carotid and femoral arteries (R²=0.31, P=0.0001; n=68).

Short-term aortic constriction above the renal arteries can increase renin production in the kidneys, which can sense the reduced BP distal to the constriction. However, we found no difference in renin mRNA levels in the kidneys or in plasma renin activity of banded and sham-operated mice at 8 weeks after banding (Table 2), which is consistent with the absence of significant differences between the femoral arterial BPs of the banded and the sham-operated animals.

Vessels in the Vicinity of Constrictions

Cross sections of the abdominal aorta of the banded area were examined by light microscopy (Figure 1). The position of the banding applied to the aorta was identified by the presence of the suture (Figure 1D and 1E). The banding narrowed the aortic luminal area in the Apoe−/− and wild-type mice to 39±4% (n=11) relative to that in the sham-operated mice (100±5%, n=5). The intima contained 2 or 3 layers of small cuboidal cells, most likely representing proliferation of smooth muscle cells to accommodate the increased pressure (Figure 1E and 1F). Lipids were absent in the intima.

The aorta proximal to the banding site was dilated with a luminal diameter ~30% larger than the diameter of the

**TABLE 2. Increased BPs of Apoe−/− Mice Proximal to the Aortic Constriction**

<table>
<thead>
<tr>
<th>Mice</th>
<th>Sex</th>
<th>Treatment</th>
<th>Carotid BP, mm Hg</th>
<th>Femoral BP, mm Hg</th>
<th>ΔBP, mm Hg</th>
<th>HR, bpm</th>
<th>Renin mRNA, ng/mg</th>
<th>Renin Activity, ng Ang I/mL per h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoe−/−</td>
<td>M</td>
<td>Sham</td>
<td>109±3 (15)</td>
<td>101±3 (15)</td>
<td>8.5±2 (15)</td>
<td>696±16 (14)</td>
<td>19.8±4.3 (6)</td>
<td>51±4 (6)</td>
</tr>
<tr>
<td>Apoe−/−</td>
<td>M</td>
<td>Banding</td>
<td>126±4* (24)</td>
<td>100±3 (24)</td>
<td>26.3±3.1* (24)</td>
<td>641±16† (22)</td>
<td>24.9±3.3 (10)</td>
<td>58±6 (6)</td>
</tr>
<tr>
<td>Apoe−/−</td>
<td>F</td>
<td>Sham</td>
<td>111±3 (13)</td>
<td>99±3 (13)</td>
<td>12.1±2 (13)</td>
<td>652±13 (12)</td>
<td>31.9±5.0 (7)</td>
<td>ND</td>
</tr>
<tr>
<td>Apoe−/−</td>
<td>F</td>
<td>Banding</td>
<td>127±5* (16)</td>
<td>99±4 (16)</td>
<td>27.0±4.2* (16)</td>
<td>676±10 (19)</td>
<td>31.1±4.4 (9)</td>
<td>ND</td>
</tr>
<tr>
<td>WT</td>
<td>M</td>
<td>Banding</td>
<td>106±3 (3)</td>
<td>90±4 (3)</td>
<td>16.2±1.5 (3)</td>
<td>688±21 (7)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>WT</td>
<td>F</td>
<td>Sham</td>
<td>118±6 (4)</td>
<td>108±3 (4)</td>
<td>10.2±5.5 (4)</td>
<td>647±23 (4)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>WT</td>
<td>F</td>
<td>Banding</td>
<td>126±5 (9)</td>
<td>104±3 (9)</td>
<td>21.9±6.0 (9)</td>
<td>641±19 (6)</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

BP indicates mean intra-arterial BP, ΔBP, BP differences between carotid and femoral arteries; and HR, heart rate. Values are mean±SE. The numbers of animals are in parentheses. BP was measured at 8 wk after banding. The amount of renin message is expressed as nanograms per milligram of the total cellular RNA. HR was measured by echocardiogram. BPs of WT sham males were not determined.

*P<0.01 vs sham; †P<0.01 vs sham; ‡P<0.05 vs sham.
sham-operated aorta (Figure 1A). In 10 of the 12 banded Apoe/− mice examined, the aortas immediately above the constriction contained atherosclerotic lesions of various sizes, ranging from foam cell accumulations to well-developed plaques (Figure 1B). In 4 mice, the medial layer was disrupted, and plaque material was deposited outside the vessel wall (Figure 1C). Plaques were not present further upstream (>500 μm) from the banding site. The presence of abdominal aortic lesions is rare in Apoe/− mice at 4 months of age, and none of the 6 sham-operated Apoe/− mice that were examined had plaques in this area.

Vessels immediately distal to the site of banding were slightly dilated, with the luminal diameter increased by ∼10% relative to the sham-operated vessels. In all 6 Apoe/− vessels and in the 3 wild-type vessels evaluated, there were large thrombi attached at multiple locations to the aortic wall (Figure 1G). Smooth muscle cells within the medial layers near the thrombi were elongated and contained lipids, but foamy macrophages were not present (Figure 1H). Most of the thrombi were well organized and composed of fibrous materials, but occasionally, small aggregates of platelets were present on the surface (Figure 1I), suggesting that thrombus formation at this location was an ongoing process. However, even in the hyperlipidemic environment of Apoe/−, lipids were not identified as a component of these thrombi. Thrombi in the same area were also found in the vessels of 3 of the 6 Apoe/− mice 4 weeks after the banding but in none of the vessels of 9 mice 2 weeks after banding (not shown). None of the sham-operated mice developed thrombi near the site of banding.

Cardiac Hypertrophy and Other Pathological Complications.

We used echocardiography to monitor changes in the heart induced by aortic banding. Abdominal aortic banding of Apoe/− mice induced a time-dependent increase in LV end-diastolic dimension (LVEDD; P <0.01 by MANOVA versus sham, Figure 2A) and LV mass (P <0.01, Figure 2B) and a trend toward a decrease in ejection fraction (P =0.066, Figure 2C). Hearts were hypertrophied by 4 weeks after banding and began to dilate significantly after 6 weeks. At 8 weeks, 5 of 41 banded Apoe/− mice had an ejection fraction of <30%, indicating heart failure. In contrast, neither the LV diameter nor the heart mass was increased significantly in wild-type mice after banding. Thus, the Apoe/− mice are more prone to develop cardiomegaly and heart failure in response to chronic cardiac overload induced by aortic constriction than are wild-type mice. Heart rate was not significantly influenced by sex, treatment, or genotype (Table 2).

Histological examination showed that the LVs and right ventricles were dilated in the 8-week banded Apoe/− mice. Myocardial hypertrophy and cardiac damage, as evidenced by larger myocytes with enlarged nuclei and extensive interstitial and perivascular fibrosis, were present in the hearts of the banded Apoe/− mice (Figure 3A and 3B). In addition, in 8 of 37 Apoe/− mice, the left atrium was enlarged and firm and contained large organized thrombi (Figure 3C). Foam cells were found on the left atrial wall adjacent to the thrombus and, presumably, were the consequence of a disturbed blood flow in this area (Figure 3D). Most of the mice with atrial thrombi also showed evidence of pulmonary congestion (Figure 3E). The lungs contained intra-alveolar collections of enlarged macrophages that were filled with acidophilic needle-shaped crystals (Figure 3F) that are typical of acidophilic macrophage pneumonia. Acute inflammatory cells were absent. Bronchi and alveolar spaces contained PAS staining of the proteinaceous materials (Figure 3E). Areas of fibrosis were present in some animals. Neither the sham-operated Apoe/− mice nor the banded wild-type mice had atrial thrombi or pulmonary congestion, implicating the detrimental effects of the Apoe mutation under these circumstances.

Increased Aortic Atherosclerosis Proximal to the Constriction

Lesions in the aortic arch were significantly increased in banded Apoe/− mice compared with sham-operated mice (Figure 4A through 4D). All the thoracic aortas from banded mice (6 males and 5 females) contained at least 3 large plaques in the aortic arch, whereas no aortas from the sham-operated mice (6 males and 5 females) had >1 plaque. The cholesterol ester content of the excised thoracic aortas of banded Apoe/− mice was 244±38% that of the sham-operated vessels per aorta or 166±16% per dry weight (n = 11 and 9, respectively; P <0.001; effects of the sex of the animal were not significant).

The mean size of lesions within the aortic root of banded males (86 000±8000 μm²) and females (128 000±9000 μm²) were 245% and 152% those of sham-operated males (35 000±7000 μm²) and females (84 000±8000 μm²), respectively (Figure 4E, P <0.0001 for effects of banding). No qualitative changes in the plaque morphology were notable in association with the banding (not shown). The lesion size was not correlated with either HW/BW ratio or ejection fraction.
of the heart of the individual animals, suggesting that cardiac dysfunction and atherosclerosis are independent processes in this system.

Discussion

We have used mechanical methods to increase the aortic BP in mice to study the relationships between hypertension, atherosclerosis, and Apoe genotype. Our data show that BP and atherosclerosis in the aorta proximal to the constriction were increased by banding the suprarenal abdominal aorta of Apoe−/− mice, whereas no changes occurred in the BP and atherosclerosis in the distal aorta. We also found that Apoe−/− mice are significantly more susceptible than are wild-type mice to the cardiac hypertrophy induced by chronic constriction of the aorta.

The effects of chronic aortic constriction on atherosclerosis in Apoe−/− mice confirm the effects observed in Watanabe heritable hyperlipidemic rabbits,7 in rabbits fed high cholesterol diets,8 and in cynomolgus monkeys.9 All these experiments demonstrate a marked increase in atherosclerotic lesions in response to elevated BP. The suggested explanations include the possibility that an increased intimal permeability induced by hypertension might lead to an increased rate of entry of lipoproteins into the intima16,17 or that the higher pressure could increase endothelial or medial damage, thereby triggering the initiation of plaque formation by recruiting proinflammatory leukocytes to the sites.18

Angiotensin II is a potent vasoactive mediator that has profound effects on atherosclerosis. Thus, mice that overexpress a human renin transgene and a human angiotensinogen transgene are hypertensive and develop larger lesions than do nontransgenic mice when these mice are fed a high cholesterol diet.19 Daugherty et al20 and Weiss et al21 have shown that systemic infusion of angiotensin II at supraphysiological levels of 0.7 mg/kg per day dramatically increased atherosclerosis in Apoe−/− mice in the relatively short time of 4 weeks. Although these 2 studies differ (in that Daugherty et al did not see any BP increase in their animals, whereas Weiss et al reported that the treatment increased BP by 40 mm Hg), a marked infiltration of inflammatory cells in plaques and in the adventitia was noted by both studies, which agree that this is indicative of the proatherogenic effects of angiotensin II.

One of the hypotheses explaining the mechanism by which banding of the abdominal aorta above the renal arteries induces hypertension postulates that the constriction causes a reduction in renal blood flow, which in turn activates the renin-angiotensin system.15 In support of this idea, studies have shown that circulating renin levels22 and renin mRNA in the renal afferent arterioles23 increase 3- to 4-fold after abdominal aortic constriction. However, other studies have shown that circulating renin levels return to normal within several days after the surgery22 and that chronic coarctation does not alter mean BPs distal to the coarctation.24 Our data agree with and extend the latter observations by showing that renin mRNA levels in the kidney, plasma renin activity, and the femoral BP in Apoe−/− mice 8 weeks after banding are not different from those in sham-operated mice. Thus, it is very unlikely that systemic angiotensin II levels are chronically altered in the banded animals compared with the sham-operated animals. Nevertheless, coarctation induced a sustained increase in BP and enhanced lesion development.

Although the increased atherosclerosis present in the proximal aorta and its branches is similar to that described in other animal models, the presence of organized thrombi in the portion of the aorta just distal to the coarctation has not, to our

Figure 3. Histological examination of heart (A through D) and lung (E and F) of Apoe−/− mice after 8 weeks of aortic coarctation. A and B, Interstitial (A) and perivascular (B) fibrosis in the myocardium is illustrated as blue staining of collagens (Masson’s trichrome, original magnification ×100). C and D, Organized thrombus (th) is present in left atrium (la) in panel C (original magnification ×5) and panel D (original magnification ×20). Arrow in panel D indicates foam cells on the wall of the left atrium (la), ao indicates aorta; rv, right ventricle, and ra, right atrium. Stain was Sudan IV. E, The bronchus (arrow) contains a cellular infiltrate of macrophages and mononuclear cells that are admixed with mucoid material (H&E stain, original magnification ×150). F, Large histiocytic cells (arrows) are present within alveolar spaces (H&E stain, original magnification ×500).
Cardiac dysfunction was not observed in any wild-type mice despite the fact that some of them had BP increases that were more than those seen in Apoe−/− mice with cardiac dysfunction. This suggests that a lack of apoE may exacerbate cardiac hypertrophy and subsequent dysfunction. Although little is known about the role of apoE in heart function, our earlier work has shown that apoE is highly expressed in the mouse heart, whereas its expression is low in skeletal muscles. In addition, Hartley et al have reported significantly elevated HW/BW ratios of the 13-month-old Apoe−/− mice compared with the wild-type mice. Many circulatory problems, including severe aortic atherosclerosis, can contribute to the hemodynamic changes in old Apoe−/− mice. However, our present study showed that the hearts of 2-month-old Apoe−/− mice, although normal, have a significantly larger LVEDD and a smaller ejection fraction than are seen in wild-type C57BL/6 mice (Figure 2). Furthermore, our unpublished data (J. Knowles, N. Maeda, 2001) show that the HW/BW ratios of Apoe−/− mice at 4 months of age are ~15% larger than those of the wild-type mice with a similar genetic background (6 to 8 generations of backcrossing to C57BL/6). The degrees of atherosclerosis at these ages are still minimal. Although we cannot completely eliminate the possibility that some residual contribution of strain 129 derived genes linked to the ApoE locus and that differing in the 2 strains is responsible for this phenotype, a more conservative suggestion is that apoE is important for cardiac energy metabolism and has direct effects in modulating cardiac hypertrophy. Further studies are necessary to elucidate the mechanisms whereby apoE may exert this function.

Acknowledgments

This study was supported by National Institutes of Health grants HL-62845 and HL-42630. The authors thank Dr H. Rockman for advice on transverse aortic constriction and Drs H.-S. Kim, J. Knowles, L. James, and O. Smithies for useful discussions.

References


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doi: 10.1161/hq0302.105287

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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