Hypertension and Endothelial Dysfunction in Apolipoprotein E Knockout Mice

Renhui Yang, Lyn Powell-Braxton, Annie Ko Ogawaara, Noel Dybdal, Stuart Bunting, Osamu Ohneda, Hongkui Jin

Abstract—Mice lacking ApoE (Apoe−/−) develop initially hypercholesterolemia and latterly atherosclerosis. This study examined hemodynamics and endothelial function in 6-week-old Apoe−/− mice with hypercholesterolemia only, 7.5-month-old Apoe−/− mice with both hypercholesterolemia and atherosclerosis, and age matched controls. One day after implantation of catheters into the carotid artery, arterial pressure was measured in conscious, unrestrained mice. Compared with the respective controls, there was a significant increase in arterial pressure and the ratio of left ventricular weight to body weight in 7.5-month-old Apoe−/− mice but not in 6-week-old Apoe−/− mice. Histopathological analysis demonstrated significant renal artery disease in the form of extensive atheromatous plaques only in 7.5-month-old Apoe−/− mice, whereas no atherosclerotic lesions were found in 6-week-old Apoe−/− mice. For evaluation of endothelial function, a laser Doppler perfusion imager with a computer-controlled optical scanner was used to measure cutaneous blood perfusion on the dorsal side of one hind paw before and after topical application of mustard oil, which is known to induce nitric oxide–mediated vasodilation. The mustard oil treatment elicited a substantial increase in blood perfusion (P<0.01), which was similar between 6-week-old Apoe−/− mice and controls but significantly blunted in 7.5-month-old Apoe−/− mice versus control mice, suggesting nitric oxide–mediated vasodilation is diminished in 7.5-month-old Apoe−/− mice but not in 6-week-old Apoe−/− mice. In contrast, the increase in blood perfusion induced by topical administration of cilostazol, which induces vasodilation via cyclic adenosine monophosphate, was not different between 7.5-month-old Apoe−/− mice and controls. Thus hypertension and endothelial dysfunction observed in 7.5-month-old Apoe−/− mice may be due mainly to atherosclerosis. (Arterioscler Thromb Vasc Biol. 1999;19:2762-2768.)

Key Words: hypertension ■ endothelial dysfunction ■ Apoe−/− mice ■ nitric oxide ■ atherosclerosis

ApoE, a 34-kD glycoprotein, is a major component of plasma lipoproteins that serves as a ligand for receptor-mediated clearance of several classes of lipoproteins from plasma, including chylomicrons, VLDL, and lipoprotein remnants.1 Apoe−/− mice develop marked hypercholesterolemia and spontaneous atherosclerosis on a normal chow diet.2–9 Histopathological studies in Apoe−/− mice6,9 have revealed lesions of all phases of atherosclerosis through the aorta and its principal branches. Foam cell lesions develop as early as 8 weeks of age and progress to advanced lesions after 15 weeks.6 Sites of predilection include the aortic root, the lesser curvature of the aortic arch, aortic branches such as the carotid artery, superior mesenteric artery, renal artery, aortic bifurcation, and the pulmonary artery.6 It is likely that the extensive arterial disease may result in hemodynamic changes. For example, as atherosclerosis is the most common cause of renovascular hypertension in humans,10 renal arterial lesions in Apoe−/− mice may lead to systemic hypertension. Similarly, abundant evidence indicates that atherogenic lipoproteins and/or atherosclerosis induce endothelial dysfunction in numerous animal species and humans.11–19 Endothelial dysfunction, as evidenced by reduced endothelial nitric oxide–mediated vasorelaxation, would also influence vascular tone and hemodynamics. Furthermore, an increase in arterial pressure and impairment in endothelial function not only are interrelated but may themselves accelerate the progression of atherosclerosis. Therefore, we set out to determine whether there are changes in arterial pressure and endothelial function in Apoe−/− mice and whether the changes are due to atherosclerosis and/or hypercholesterolemia.

The present study was designed to examine arterial pressure using chronically indwelling catheters in conscious, unrestrained Apoe−/− mice at 6 weeks of age with hypercholesterolemia only and at 7.5 months of age with both hypercholesterolemia and atherosclerosis and age-matched control mice, and to determine endothelial function in vivo using a laser Doppler perfusion imager. Our results demonstrate that 7.5-month-old, not 6-week-old, Apoe−/− mice de-
velop hypertension and endothelial dysfunction. The data support the hypothesis that hypertension and endothelial dysfunction observed in Apoe\(^{-/-}\) mice may be mainly attributed to atherosclerosis.

**Methods**

**Animal Model**

Male Apoe\(^{-/-}\) mice on a >98% C57BL/6 background were originally licensed from Jan Breslow, Columbia University, New York, NY and received at approximately 4 weeks or 7 months of age. Male C57BL/6JINA mice as wild-type controls were obtained at 4 weeks of age from Charles River Breeding Laboratories (Wilmington, MA) and at 7 months of age from National Institute of Aging (Bethesda, MA). Animals were acclimated to the facility for at least 2 weeks before beginning the experiments. They were fed a normal chow diet (catalog number 5010, Purina, Gray Summit, Missouri) and water ad libitum, and housed in a light and temperature controlled room. The experimental procedures, which were approved by Genentech's Institutional Animal Care and Use Committee, conform to the guiding principles of the American Physiological Society.

**Measurement of Arterial Pressure and Heart Rate in the Awake State**

Six-week-old Apoe\(^{-/-}\) mice (n = 6) and C57BL/6JINA controls (n = 6) as well as 7.5-month-old Apoe\(^{-/-}\) mice (n = 12) and C57BL/6JINA controls (n = 10) were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) given intraperitoneally. Catheters (PE-10 fused with PE 50) filled with heparin-saline (50 U/mL) were implanted into the right carotid artery for measurement of mean arterial pressure (MAP), systemic arterial pressure (SAP), diastolic arterial pressure (DAP), and heart rate (HR). Severe atherosclerosis of the carotid artery in three 7.5-month-old Apoe\(^{-/-}\) mice prevented successful catheter implantation. Catheters were exteriorized and fixed at the back of the neck with the aid of a stainless steel wire tunneled subcutaneously and fixed with fast-polymerizing dental cement. All animals were housed individually after surgery.

One day after surgery, the arterial catheter was connected to a Model CP-10 pressure transducer (Century Technology) coupled to a polygraph (Model 7, Grass Instruments). MAP, SAP, DAP, and HR were measured and recorded simultaneously in conscious, unrestrained mice. The animals were then anesthetized with ketamine/xylazine as described above. Blood was collected by cardiac puncture for measurement of circulating lipids.

**Assessments of Cutaneous Blood Perfusion by a Laser Doppler Perfusion Imager**

To evaluate endothelial function, blood perfusion in the skin on the dorsal side of 1 hind paw was measured by a laser Doppler perfusion imager with a computer-controlled optical scanner (PIM 1.0, Lisca Inc) before and after topical application of 5% mustard oil, which is known to induce vasodilation mediated by nitric oxide. After anesthesia with ketamine/xylazine, a scanner head with He-Ne laser (Lisca Inc) was placed above the dorsal side of 1 hind paw. The paw was scanned by a laser beam moving in a rectangular pattern over an area of approximately 1 cm². The beam penetrates to a depth of approximately 0.2 mm. During the scan, moving blood cells shift the frequency of incident light according to the Doppler principle. With this noninvasive method, no physical contact is necessary between the scanning device and the tissue, which reduces extraneous influences on the perfusion to a minimum. A photodiode collects the backscattered light, and the original light intensity variations are transformed into voltage variations in the range of 0 to 10 volts. A perfusion output value of 0 volts was calibrated to 0% perfusion, 10 volts was calibrated to 100%. A color-coded image representing blood flow distribution was computer generated. Numerical perfusion values used to generate the color-coded image can be used for data analysis. The average of 2 independent measurements was performed for each experiment. The average of 2 independent measurements was used to generate the color-coded image of cutaneous blood perfusion or flow in 6-week-old Apoe\(^{-/-}\) mice (n = 16) and controls (n = 15) and in 7.5-month-old Apoe\(^{-/-}\) mice (n = 10) and controls (n = 11).

A pilot study demonstrated that topical administration of 5% mustard oil (5% in sesame oil, Chem Service, Inc), applied to the dorsal side of the hind paw using a cotton bud, rapidly increased blood perfusion in the skin of C57BL/6JINA mice, reaching a peak at 1 minute and remaining at that plateau for 5 minutes thereafter. Consequently, for this study perfusion was measured 1, 2, and 3 minutes after mustard oil application with the average of the 3 measurements taken as the posttreatment level. The effect of mustard oil on the examined in 6-week-old Apoe\(^{-/-}\) mice (n = 11) and controls (n = 8) and in 7.5-month-old Apoe\(^{-/-}\) mice (n = 10) and controls (n = 11). Application of sesame oil vehicle did not significantly alter cutaneous blood perfusion.

Because mustard oil-induced increased blood perfusion was significantly blunted in 7.5-month-old Apoe\(^{-/-}\) mice versus age-matched C57BL/6JINA animals, cilostazol (1% in 90% [dimethyl sulfoxide] DMSO) (Otsuka Pharmaceutical Co) was topically applied to a separate group of Apoe\(^{-/-}\) mice (n = 8) and controls at this age (n = 8). Cilostazol has been shown to induce vasodilation via increasing cyclic adenosine monophosphate (cAMP) rather than nitric oxide release. A preliminary study showed that cutaneous blood perfusion in response to cilostazol was maximally increased at 15 to 20 minutes in mice. The procedure was the same as before except 2 measurements were performed 15 minutes after topical administration of 1% cilostazol and averaged. Ninety percent DMSO alone had no significant effect on cutaneous blood perfusion.

**Pathological Studies on Renal Arteries**

The abdominal aorta, bilateral renal arteries, and kidneys from Apoe\(^{-/-}\) mice and controls at both 6 weeks and 7.5 months of age (n = 6 in each group) were removed, dissected, and fixed in neutral buffered formalin. After fixation, the kidneys were bisected, and 17 controls animals at 7.5 months were weighed, euthanized, and had hearts immediately removed, weighed, dissected, and had the catheters and atria individually weighed postmortem. All tissue measurements are wet weights.

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**Determination of Left Ventricular Weight**

Nine Apoe\(^{-/-}\) and 10 control mice at 6 weeks of age and 16 Apoe\(^{-/-}\) and 17 controls animals at 7.5 months were weighed, euthanized, and had hearts immediately removed, weighed, dissected, and had the catheters and atria individually weighed postmortem. All tissue measurements are wet weights.

**Statistical Analysis**

Results are expressed as mean±SEM. Two-way ANOVA was performed to assess differences in blood perfusion (%) in response to mustard oil versus vehicle or to cilostazol versus vehicle in Apoe\(^{-/-}\) versus control mice. Significant differences were then subjected to posthoc analyses using the Newman-Keuls method. Other parameters between Apoe\(^{-/-}\) mice and C57BL/6JINA rats at the same age were compared by an unpaired Student’s t test. P<0.05 was considered to be statistically significant.

**Results**

**Hypertension and Left Ventricular Hypertrophy Only in 7.5-Month-Old Apoe\(^{-/-}\) Mice**

SAP, DAP, and MAP were not different between conscious, unrestrained Apoe\(^{-/-}\) mice and controls at 6 weeks and 7.5 months of age (Figure 1). However, there was a significant increase in the arterial pressures (P<0.05) in 7.5-month-old Apoe\(^{-/-}\) mice compared with age-matched control mice (Figure 2). SAP and MAP in Apoe\(^{-/-}\) mice at 7.5 months (145.3±7.4 and 126.1±6.3 mm Hg) increased by 22 and 16 mm Hg (18% and
15%) relative to age-matched control animals. No significant difference in HR was observed between 2 groups at both 6 weeks and 7.5 months of age (the Table).

Body weight (BW), total ventricular weight, and left ventricular weight were significantly increased ($P$, 0.01) in Apoe$^{-/-}$ mice compared with age-matched control animals at 6 weeks or 7.5 months (the Table). The ratio of left ventricular weight to BW was significantly increased ($P$, 0.05) only in 7.5-month-old Apoe$^{-/-}$ mice compared with age-matched controls, whereas there was no difference in this ratio between Apoe$^{-/-}$ mice and controls at 6 weeks (the Table). The ratio of total ventricular weight to BW was not significantly different between 2 groups at either age (the Table).

**Impaired Endothelial Function Only in 7.5-Month-Old Apoe$^{-/-}$ Mice**

The basal level of cutaneous blood perfusion of the dorsal side of the hind paw measured by laser Doppler perfusion imaging was not significantly different between Apoe$^{-/-}$ mice and control animals at either 6 weeks or 7.5 months (the Table). Topical administration of 5% mustard oil increased blood perfusion in the skin significantly ($P$<0.05) in both Apoe$^{-/-}$ and control animals at both ages (Figure 3 and Figure 4, top panel). However, the increase in cutaneous blood perfusion induced by mustard oil was significantly blunted ($P$<0.05) only in 7.5-month-old Apoe$^{-/-}$ mice (Figure 4, top panel) but not in 6-week-old Apoe$^{-/-}$ mice (Figure 3) compared with age-matched controls. Administration of sesame oil vehicle had no effect on cutaneous blood perfusion. Topical administration of 1% cilostazol (in 90% DMSO vehicle) elicited a significant increase in cutaneous blood perfusion ($P$<0.01), which was similar in 7.5-month-old Apoe$^{-/-}$ compared with control animals (Figure 4, bottom panel). DMSO vehicle alone had no effect on cutaneous blood perfusion in either group.

**Hypercholesterolemia and Atherosclerosis**

Consistent with previous reports, plasma cholesterol levels were markedly elevated in both 6-week-old and 7.5-month-old Apoe$^{-/-}$ mice compared with controls (the Table). There was extensive atherosclerosis in the aorta and its main branches of 7.5-month-old Apoe$^{-/-}$ mice, whereas no artery disease was found in 6-week-old Apoe$^{-/-}$ mice.

Histopathological analysis on renal arteries revealed that five of the six 7.5-month-old Apoe$^{-/-}$ mice had significant...
renal artery disease in the form of extensive atherosclerotic plaque (Figure 5). The bilateral renal arteries contained mature atheromatous plaque extending into the vascular lumen. In contrast, the renal arteries were normal in all 6-week-old Apoe–/– mice and control mice.

**Discussion**

The present study is the first to demonstrate that 7.5-month-old Apoe–/– mice develop increased arterial pressure and impaired endothelial function in addition to hypercholesterolemia and atherosclerosis. MAP in these 7.5-month-old Apoe–/– mice was increased by 16 mm Hg (15%) as compared with wild-type control animals. Also, probably as a consequence of hypertension, Apoe–/– animals at this age exhibited mild left ventricular hypertrophy as evidenced by an increase in the ratio of left ventricular weight to BW. In contrast, 6-week-old Apoe–/– mice, which had hypercholesterolemia only without atherosclerosis, did not exhibit the abnormality in arterial pressure, endothelial function, and the left ventricle. The data indicate that hypertension and endothelial dysfunction observed in Apoe–/– mice are primarily attributed to atherosclerosis.

Consistent with prior reports on elevated lipids and arterial plaque distribution in Apoe–/– mice, this study demonstrated severe hypercholesterolemia in both 6-week-old and 7.5-month-old Apoe–/– mice and extensive atherosclerosis only in 7.5-month-old Apoe–/– mice. Renovascular hypertension is the most common secondary form of hypertension in humans. Approximately two-thirds (70%) of cases of renovascular hypertension are caused by atherosclerotic disease affecting the renal artery, and atherosclerosis accounts for 70% of all renal artery lesions. In the present study, histopathological analysis on bilateral renal arteries showed significant renal artery disease in the form of extensive atherosclerotic plaque only in 7.5-month-old Apoe–/– animals. The mature atheroma of the renal arteries extended into the vascular lumen. The thickened vascular wall and extensive atheromatous plaques may well result in a functional narrowing of the renal vessels producing renovascular hypertension in the Apoe–/– animals at this age.

Hypertension in ApoE-deficient animals could in turn speed the process of atherosclerosis. Animal experiments have clearly demonstrated that lipid-induced atherogenesis can be accelerated or retarded by manipulating arterial pressure. Hypertension not only accelerates but also

| Body Weight, Ventricular Weight, Heart Rate, Basal Cutaneous Blood Perfusion, and Plasma Cholesterol in Apoe–/– Mice and Controls |
|---|---|
| **Age** | **Apoe–/–** | **Control** |
| **BW (g)** | 6 weeks | 27.91±0.40 (9)** | 20.76±0.33 (10) |
| | 7.5 months | 36.03±0.81 (17)** | 32.07±0.54 (18) |
| **VW (g)** | 6 weeks | 0.113±0.003 (9)** | 0.085±0.003 (10) |
| | 7.5 months | 0.150±0.005 (17)** | 0.131±0.002 (18) |
| **LVW (g)** | 6 weeks | 0.084±0.004 (9)** | 0.062±0.003 (10) |
| | 7.5 months | 0.119±0.005 (17)** | 0.099±0.002 (18) |
| **VW/BW (g/kg)** | 6 weeks | 4.003±0.067 (9) | 4.070±0.131 (10) |
| | 7.5 months | 4.156±0.116 (17) | 4.089±0.038 (18) |
| **LVW/BW (g/kg)** | 6 weeks | 2.953±0.100 (9) | 2.988±0.138 (10) |
| | 7.5 months | 3.302±0.095 (17)* | 3.088±0.047 (18) |
| **HR (bpm)** | 6 weeks | 583.3±34.8 (6) | 575.7±31.6 (6) |
| | 7.5 months | 605.0±28.9 (9) | 615.5±34.4 (10) |
| **CBP (v)** | 6 weeks | 0.818±0.041 (16) | 0.907±0.032 (15) |
| | 7.5 weeks | 0.782±0.044 (26) | 0.749±0.052 (23) |
| **Plasma cholesterol (mg/dl)** | 6 weeks | 452.3±15.1 (8)** | 159.1±11.4 (7) |
| | 7.5 months | 550.3±58.9 (12)** | 117.2±8.3 (10) |

Data expressed as mean±SEM, the number in parenthesis is animal numbers. BW indicates body weight; VW, ventricular weight; LVW, left ventricular weight; HR, heart rate; CBP, basal levels of average cutaneous blood perfusion on the dorsal side of the hindpaw. *P<0.05 and **P<0.01 compared to the control group at the same age.
Exacerbates atherosclerosis in the hyperlipidemic animals. Clinical studies have shown that hypertension is associated with increased intima-media thickness and more frequent plaques in extracoronary arteries, more frequent calcifications in coronary arteries, increased wall rigidity in the aorta and peripheral arteries, and impaired endothelium-dependent vasodilation and abnormal blood rheology, which are capable of promoting thrombosis in the background of atherosclerosis.

Our finding that 7.5-month-old, but not 6-week-old, APOE−/− mice exhibited increased arterial pressure is basically consistent with an earlier report demonstrating only a modest increase in blood pressure measured by a tail-cuff method in either restrained or anesthetized mice at 9 weeks of age compared with the controls, which was not statistically significant. It is likely that the young APOE−/− mice in the earlier study are too young to develop extensive atherosclerosis and would not have renal artery disease. Previous studies on APOE−/− mice have demonstrated that the initial appearance of atherosclerotic lesions is from 3 to 4 months of age and confined to the aortic sinus at this point. Hypercholesterolemia and/or atherosclerosis may adversely affect endothelial function. To determine which one is more important to cause endothelial dysfunction, Bonthu et al examined endothelium-dependent relaxation of vascular rings in vitro in the following 2 genetic models of hypercholesterolemia: APOE−/− mice and combined APOE−/−/LDL receptor (Ldlr−/−) double-knockout mice at 19 weeks of age. In the APOE−/− mice, endothelium-dependent relaxation of proximal and distal segments of thoracic aortas, which had minimal or no atherosclerotic lesions, was normal. In the APOE−/−/Ldlr−/− mice, however, endothelium-dependent relaxation was impaired in those proximal segments of aortas that contained atherosclerotic lesions but not in distal segments that had minimal or no atherosclerotic lesions. These results suggest that endothelium-dependent relaxation of vascular rings was not impaired by hypercholesterolemia per se, rather that atherosclerosis may be necessary for inducing deficits in endothelium-dependent relaxation in vitro in these genetic mouse models. The APOE−/− animals used in the in vitro study were only 19 weeks old, an age at which the atherosclerosis development is very modest. Consistent with the in vitro findings, our in vivo study also showed that endothelial...
function was impaired in Apoe−/− animals approximately 30 weeks old, in which we find extensive atherosclerosis covering >5% of the aorta surface3–17 but not in 6-week-old Apoe−/− mice, which only exhibited hypercholesterolemia with no atherosclerosis.

Nitric oxide has been shown to be an important mediator of the vasodilator response to topical administration of mustard oil in the animal hind paw.20,21 L-NAME, an inhibitor of nitric oxide, substantially and specifically inhibits the mustard oil-induced cutaneous vasodilation.20,21 Laser Doppler perfusion imaging has been used to measure cutaneous blood perfusion or flow for experimental and clinical applications.24–28 The present study using the laser Doppler system demonstrated that topical application of mustard oil caused a significant increase in cutaneous blood perfusion in the hind paw of both Apoe−/− mice and controls. The increment, however, was significantly blunted in 7.5-month-old, not 6-week-old, Apoe−/− mice, further suggesting that nitric oxide-mediated vasodilation is diminished in the aging Apoe−/− animals. Consistent with this hypothesis, a parallel experiment using cilostazol, a selective cAMP phosphodiesterase inhibitor that has been shown to increase blood flow in the skin after topical administration,25 showed no difference in the blood flow response between the 2 groups at 7.5 months of age. Cilostazol blocks cAMP hydrolysis, elevates vascular smooth muscle cell cAMP levels, and produces vasodilation in vitro and in vivo26,27 with no effect on nitric oxide production.28 These results observed in Apoe−/− mice showed a deficit of nitric oxide-mediated vasodilation associated with extensive atherosclerosis and elevated blood pressure, which is consistent with endothelial dysfunction. The data also strongly suggest that aging Apoe−/− animals may be a good model for further studies on nitric oxide release and function.

The present study demonstrates that the Apoe−/− mice with atherosclerosis exhibit endothelial dysfunction in the cutaneous vessels. This is consistent with recent findings that in animals and patients with atherosclerosis, there is an abnormal endothelium-dependent dilation in the coronary microvasculature despite the absence of atherosclerotic lesions in these vessels.39–41 There is strong evidence in vivo that endothelial dysfunction occurs in the coronary and peripheral circulation in both conduit and resistance vessels during various stages of atherosclerosis, suggesting that endothelial dysfunction in atherosclerosis is a systemic process, not necessarily confined to vessels that develop atherosclerosis.42–44 The cause of endothelial dysfunction observed in Apoe−/− mice with atherosclerosis in the present study remains to be defined. The mechanisms underlying abnormal endothelium-dependent vascular relaxation in atherosclerosis may include decreased or abnormal production and/or release of endothelium-derived relaxing factor, destruction of endothelium-derived relaxing factor, and the concomitant release of constricting factors.39,41 A recent clinical study suggests that in patients with atherosclerosis, production of endothelium-derived cyclooxygenase-dependent constricting factors contributes to the abnormal acetylcloine-mediated dilation of the peripheral artery, which has no atherosclerotic lesions.45

In addition to renal artery stenosis induced by atherosclerosis, endothelial dysfunction may also contribute to renovascular hypertension in Apoe−/− animals. Nitric oxide is produced in renal arteries, macula densa, glomeruli, and tubules by different nitric oxide-synthases, where it is involved in physiological regulation of renal blood flow, renal autoregulation, tubuloglomerular feedback, renin release, pressure natriuresis, and tubular function.46

In summary, ApoE-deficient animals exhibited hypertension and endothelial dysfunction when extensive atherosclerosis had developed. Our study provides further evidence that chronic atherosclerosis in the mouse models of the disease results in pathophysiological changes reminiscent of those found in the advanced human disease state. However, there are 2 limitations in the present study. First, the experiments were performed at only 2 time points, although the 2 time points represented the hypercholesterolemia and hypercholesterolemia plus atherosclerosis stages, respectively in Apoe−/− mice. Second, the present study does not provide data on correlation between the severity of atherosclerosis and blood pressure or endothelial dysfunction in Apoe−/− mice. Further studies are necessary to fully elucidate the mechanisms of hypertension and endothelial dysfunction in Apoe−/− mice and other models of hyperlipidemic atherosclerosis.

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References


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