Fluvastatin Prevents Renal Dysfunction and Vascular NO Deficit in Apolipoprotein E–Deficient Mice

Marianne Gervais, Sandrine Pons, Antonino Nicoletti, Claudine Cosson, Jean-François Giudicelli, Christine Richer,

Objective—The objective of this study was to investigate the effects of fluvastatin on atherosclerosis, systemic and regional hemodynamics, and vascular reactivity in apolipoprotein E–deficient (ApoE−/−) mice.

Methods and Results—Hemodynamics (fluospheres) and vasomotor responses of thoracic aorta and carotid artery were evaluated in male wild-type (WT) and untreated (ApoE−/− Control) or fluvastatin-treated (50 mg/kg per day for 20 weeks) ApoE−/− mice, all fed a Western-type diet. Plasma cholesterol and aortic root atherosclerotic lesions (ALs) were greater in ApoE−/− Control mice (19±1 mmol/L and 63 0176±87 785 μm², respectively) than in WT mice (2±1 mmol/L and 1±1 μm², respectively, P<0.01). Fluvastatin significantly decreased plasma cholesterol (−53%) but failed to limit ALs. Renal blood flow was significantly reduced in ApoE−/− Control versus WT (−25%, P<0.05) mice. This reduction was prevented by fluvastatin. Aortic and carotid endothelium-dependent relaxations to acetylcholine were not altered in ApoE−/− Control versus WT mice. In carotid arteries from WT mice, these responses were abolished after nitro-L-arginine (L-NA), whereas those from ApoE−/− Control were only partially inhibited after L-NA but fully abolished after L-NA+diclofenac. Thus, in carotid arteries from ApoE−/− mice, vasodilating prostanoiids compensate the deficit in NO availability. Fluvastatin prevented this carotid NO deficit.


Key Words: apolipoprotein E–deficient mice ■ hypercholesterolemia ■ endothelial function ■ renal perfusion ■ statin

Hypercholesterolemia and atherosclerosis are presently associated with abnormal vascular contractile and relaxant properties.1–5 Abnormal vascular reactivity combined with vascular structural alterations might be responsible for local hemodynamic changes that, when pronounced, might lead to the ischemic manifestations of atherosclerosis, such as myocardial infarction,5 stroke,5 or renal failure.8

Among the different therapeutic strategies, 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, which block biosynthesis of cholesterol, have been shown to slow the progression of atherosclerosis and decrease the risk of cardiovascular events in humans.9,10 Growing evidence suggests that some statins, in addition to their well-documented lipid-lowering property, might exert direct beneficial effects on the cellular events leading to plaque formation and rupture.11,12 These include possible effects on endothelial function,11 smooth muscle cell proliferation,1,14 and inflammatory process.15

Apolipoprotein E–deficient (ApoE−/−) mice spontaneously develop hypercholesterolemia and atherosclerotic lesions similar to those found in humans.16,17 In that respect, ApoE−/− mice are considered a pertinent model of atherosclerosis18 that offers new opportunities to study the impact of hypercholesterolemia on hemodynamics and vascular function. The effects of statins on hypercholesterolemia and atherosclerosis, as well as their influence on systemic and regional hemodynamics and vascular reactivity, have not yet been studied in this model.

Therefore, the goal of our study was to determine the effects of a hypolipidemic dose of fluvastatin on atherosclerosis progression, systemic and regional hemodynamics, and vascular function (thoracic aorta and carotid) in 30-week-old ApoE−/− mice. Our findings indicate that fluvastatin fails to decrease atherosclerotic lesions in ApoE−/− mice despite its prominent cholesterol-lowering properties and its protective effects on endothelial function and renal perfusion.

Methods

Ten-week-old male C57BL/6J mice (WT) and homozygous apolipoprotein E–deficient mice (ApoE−/−) obtained from Iffa Credo, L’Arbresle, France, were used and fed for 20 weeks on a Western-type diet containing 20% butter fat, 0.15% cholesterol, and 17% protein (UAR, Epinay-sur-Orge, France). ApoE−/− mice were ran-
domized into 2 different groups, one untreated group (ApoE−/− Control) and one treated group receiving fluvastatin 50 mg·kg−1·d−1 (ApoE−/− Fluva) incorporated in the high-fat diet from 10 to 30 weeks of age. Subset experiments were also performed in 10-week-old male C57BL/6J mice fed for 20 weeks with the Western-type diet and either untreated (WT) or treated with fluvastatin, 50 mg·kg−1·d−1 (WT Fluva) from 10 to 30 weeks of age.

Mice were housed in an air-conditioned room with a 12:12 hours light:dark cycle and had free access to tap water. All experiments complied with the European regulations on care and use of laboratory animals.

**In Vivo Hemodynamic Study**

In a first set of experiments, at the end of the study, 30-week-old WT (n=14), ApoE−/− Control (n=14), and ApoE−/− Fluva (n=14) mice were anesthetized with pentobarbital (60 mg/kg IP). The right carotid and the left femoral arteries were cannulated. The right carotid cannula was advanced into the left ventricle (LV). Blood pressure (systolic, diastolic, and mean), heart rate, LV pressure, and pressure (systolic, diastolic, and mean), heart rate, LV pressure, and cardiac output and coronary and renal blood flows were determined according to the reference blood sample technique by the use of the fluorescent microsphere method adapted to mice.19 At the end of the study, mice were killed by exsanguination, the vasculature was perfused with saline, and the thoracic aorta was processed for quantification of atherosclerotic lesions. In a subset experiment, the same hemodynamic assessments were performed at 30 weeks of age in WT (n=8) and WT Fluva (n=11) mice.

**Ex Vivo Vascular Study**

In a second set of experiments, at the end of the study, 30-week-old mice from the 3 experimental groups were anesthetized with pentobarbital (60 mg/kg, IP), and the carotid artery was isolated, quickly removed, and transferred into a 37°C Krebs’ solution containing (in mmol/L) NaCl 118.3, KCl 4.7, CaCl2 2.5, NaHCO3 25, MgSO4 1.2, KH2PO4 1.2, EDTA 0.02, and glucose 11.1. Blood was withdrawn from the right ventricle and collected in tubes containing EDTA for biochemical analysis. After exsanguination and whole-body perfusion with heparinized Krebs’ solution, the heart with the aortic root and the thoracic aorta were then removed and immediately placed in Krebs’ buffer. The aortic root was dissected for additional quantification of atherosclerotic lesions. The thoracic aorta was cleaned from loose connective tissue in the adventitia. Carotid and aortic segments (2 mm) were mounted on a small vessel isometric myograph (J-P Trading, model 400) filled with oxygenated (95% O2 /5% CO2, pH 7.4) Krebs isometric myograph (J-P Trading, model 400) filled with oxygenated (95% O2 /5% CO2, pH 7.4) Krebs solution (J-P Trading, model 400) filled with oxygenated (95% O2 /5% CO2, pH 7.4) Krebs solution (J-P Trading, model 400) filled with oxygenated (95% O2 /5% CO2, pH 7.4) Krebs solution (J-P Trading, model 400) filled with oxygenated (95% O2 /5% CO2, pH 7.4) Krebs solution (J-P Trading, model 400) filled with oxygenated (95% O2 /5% CO2, pH 7.4) Krebs solution, and either untreated (WT) or treated with fluvastatin, 50 mg·kg−1·d−1 (WT Fluva) from 10 to 30 weeks of age.

Plasma total cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol levels (mmol/L) were quantified with a Hitachi 747 analyser (Roche). HDL cholesterol content was determined after selective precipitation of ApoB-containing lipoproteins with phosphotungstic acid.

### Assessment of Atherosclerosis

#### Aortic Root

The aortic root was frozen in OCT embedding medium (Cryomatrix, Shandan) for serial cryosectioning covering 0.8 mm of the root and processed as previously described.20 Atherosclerotic lesions were expressed as the cross-section area of the lesion (μm²).

#### Thoracic Aorta

The thoracic aorta between the distal end of the aortic arch and the diaphragm was carefully dissected, immersed in 10% formaldehyde, dehydrated (graded ethanol solutions), and embedded longitudinally in paraffin. Serial longitudinal sections (3 μm thick) were performed for each vessel, taking into account the whole artery, and 8 histological slices per artery were stained with H&E and safron and analyzed. Histological lesions were identified (fatty streaks, extra-cellular lipids, and atherosclerotic plaques) and quantified.

### Statistical Analysis

Hemodynamic, vascular functional, and structural parameters and biological measurements were expressed as mean±SEM and compared using a one-way ANOVA followed by intergroup comparisons using a Student’s t test. Concentration-response curves were computed using ANOVA for multiple measurements. Atherosclerotic lesions of thoracic aorta were expressed as median±interquartile space, and median values were compared using a nonparametric Mann-Whitney analysis. P<0.05 was considered statistically significant.

### Results

#### Lipid Analysis

Untreated ApoE−/− Control mice developed severe hypercholesterolemia and hypertriglyceridemia and had a significantly lower HDL cholesterol level (−70%) compared with the age-matched WT mice (Table 1). Compared with ApoE−/− Control mice, chronic fluvastatin treatment significantly decreased plasma total cholesterol (−53%) and HDL cholesterol levels (−61%) and normalized triglycerides (Table 1).

<table>
<thead>
<tr>
<th>TABLE 1. Lipid Analysis</th>
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<tr>
<td>Total cholesterol, mmol/L</td>
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<tr>
<td>HDL cholesterol, mmol/L</td>
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<tr>
<td>Total triglycerides, mmol/L</td>
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</table>

Values are mean±SEM (n=14 per group).*P<0.05 vs WT mice; †P<0.05 vs ApoE−/− Control mice.
Atherosclerotic Lesions
After 20 weeks of a Western-type diet, no atherosclerotic lesions were detectable in the aortic root as well as in thoracic aorta from WT mice, whereas ApoE<sup>−/−</sup> Control mice developed severe atherosclerotic lesions that were not significantly opposed by chronic fluvastatin treatment, neither in the aortic root (706 ± 22 135 versus 630 ± 37 855 µm<sup>2</sup> in ApoE<sup>−/−</sup> Fluva and ApoE<sup>−/−</sup> Control, respectively, n = 15 per group, NS) nor in the thoracic aorta (total number of lesions, 5 [4 to 9] versus 8 [6 to 11] in ApoE<sup>−/−</sup> Fluva and ApoE<sup>−/−</sup> Control, respectively, n = 14 per group, P = 0.10).

In Vivo Measurements
Body weight of ApoE<sup>−/−</sup> Control mice was similar to that of age-matched WT mice but was significantly decreased by chronic fluvastatin treatment (−20%, P < 0.05). Heart weight was not significantly different between WT, ApoE<sup>−/−</sup> Control, and ApoE<sup>−/−</sup> Fluva groups (Table 2).

There were no significant differences in mean arterial pressure, heart rate, dP/dt<sub>max</sub>, cardiac output, total peripheral resistance, and coronary blood flow and resistance among WT, ApoE<sup>−/−</sup> Control, and ApoE<sup>−/−</sup> Fluva groups (Table 2). In contrast, renal blood flow was significantly decreased and corresponding resistance significantly increased in ApoE<sup>−/−</sup> Control mice compared with WT mice, and chronic fluvastatin treatment significantly prevented this renal injury (Figure 1).

In the subset experiment, fluvastatin had no effect in WT mice (Table 2). Values are mean ± SEM (n = 14 per group).

### Table 2. In Vivo Measurements

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>ApoE&lt;sup&gt;−/−&lt;/sup&gt; Control</th>
<th>ApoE&lt;sup&gt;−/−&lt;/sup&gt; Fluva</th>
</tr>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>40 ± 1</td>
<td>41 ± 1</td>
<td>33 ± 1††</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>0.16 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>80 ± 3</td>
<td>81 ± 3</td>
<td>79 ± 3</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>433 ± 17</td>
<td>427 ± 21</td>
<td>432 ± 21</td>
</tr>
<tr>
<td>dP/dt&lt;sub&gt;max&lt;/sub&gt;, mm Hg/min</td>
<td>9076 ± 413</td>
<td>8848 ± 365</td>
<td>8758 ± 401</td>
</tr>
<tr>
<td>CO, mL/min</td>
<td>21.0 ± 1</td>
<td>20.0 ± 1.0</td>
<td>20.3 ± 1.0</td>
</tr>
<tr>
<td>TPR, mm Hg · min · mL&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>3.9 ± 0.3</td>
<td>4.1 ± 0.3</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>Coronary flow, mL · min&lt;sup&gt;−1&lt;/sup&gt; · g&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>5.3 ± 0.3</td>
<td>4.8 ± 0.3</td>
<td>5.7 ± 0.5</td>
</tr>
<tr>
<td>Coronary resistance, mm Hg · min · g · mL&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>15.9 ± 1.3</td>
<td>17.4 ± 1.0</td>
<td>14.9 ± 1.1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 14 per group).

ApoE<sup>−/−</sup> Control mice were not significantly different. Chronic fluvastatin treatment significantly prevented aortic and carotid contractile responses to KCl and phenylephrine in ApoE<sup>−/−</sup> mice (Table 3, Figures 2A and 2B). In contrast, fluvastatin treatment did not enhance aortic and carotid contractions induced in WT mice by KCl (aorta, 1.77 ± 0.05 versus 1.68 ± 0.06 mN/mm; carotid artery, 0.66 ± 0.03 versus 0.71 ± 0.02 mN/mm in WT Fluva and WT, respectively, both NS) and phenylephrine (subset experiment, Figures 2C and 2D).

Acetylcholine-Mediated Relaxant Responses
Under control conditions, acetylcholine-induced concentration-dependent relaxations of carotid arteries were similar in ApoE<sup>−/−</sup> Control and WT mice (Figure 3B). In thoracic aorta, however, these responses were slightly, although not significantly, reduced in ApoE<sup>−/−</sup> Control, with

### Table 3. Ex Vivo Vascular Parameters

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>ApoE&lt;sup&gt;−/−&lt;/sup&gt; Control</th>
<th>ApoE&lt;sup&gt;−/−&lt;/sup&gt; Fluva</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoracic aorta</td>
<td>Normalized internal diameter, µm</td>
<td>1220 ± 19</td>
<td>1218 ± 15</td>
</tr>
<tr>
<td>KCl, mM/mM</td>
<td>1.81 ± 0.06</td>
<td>1.83 ± 0.07</td>
<td>1.93 ± 0.07</td>
</tr>
<tr>
<td>Phenylephrine (E&lt;sub&gt;max&lt;/sub&gt;, mN/mm)</td>
<td>1.95 ± 0.16</td>
<td>1.87 ± 0.16</td>
<td>2.61 ± 0.20†</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>E&lt;sub&gt;max&lt;/sub&gt;, % relaxation</td>
<td>82 ± 2</td>
<td>69 ± 3*</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>Normalized internal diameter, µm</td>
<td>525 ± 6</td>
<td>541 ± 7</td>
</tr>
<tr>
<td>KCl, mM/mM</td>
<td>0.67 ± 0.03</td>
<td>0.59 ± 0.03</td>
<td>0.70 ± 0.03†</td>
</tr>
<tr>
<td>Phenylephrine (E&lt;sub&gt;max&lt;/sub&gt;, mN/mm)</td>
<td>1.47 ± 0.05</td>
<td>1.41 ± 0.06</td>
<td>1.75 ± 0.06†</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>E&lt;sub&gt;max&lt;/sub&gt;, % relaxation</td>
<td>89 ± 1</td>
<td>86 ± 4</td>
</tr>
<tr>
<td>E&lt;sub&gt;Cb&lt;/sub&gt;, 10&lt;sup&gt;−8&lt;/sup&gt; mol/L</td>
<td>2.8 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td>3.2 ± 0.5</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 10–14 per group).

P < 0.05 vs WT mice; †P < 0.05 vs ApoE<sup>−/−</sup> Control mice.
E\textsubscript{max} and EC\textsubscript{50} values slightly smaller (66\%/H11\%/H10\%/H06 3 versus 76\%/H11\%/H10\%/H3% relaxation, NS) and greater (20.6\%/H11\%/H10\%/H5.9 versus 12.0\%/H11\%/H10\%/H1.6 10\%/H11\%/H8 mol/L, NS), respectively, than in WT mice (Figure 3A). In ApoE\textsuperscript{−/−}/H11\%/HControl mice, fluvastatin treatment did not affect endothelium-dependent relaxations induced by acetylcholine either in the aorta (Figure 3A) or in the carotid artery (Figure 3B).

Regarding the underlying mechanisms of endothelium-dependent relaxations, our results show that aortic relaxant responses to acetylcholine were completely abolished by a pretreatment with the NO-synthase inhibitor L-NA in WT mice as well as in ApoE\textsuperscript{−/−}/HControl mice (Figure 3C). In contrast, in the carotid artery, L-NA abolished acetylcholine-mediated relaxations in WT mice but only partially opposed those obtained in ApoE\textsuperscript{−/−}/HControl mice (maximal relaxation to Ach 10\%/H5 mol/L: 1\%/H4% in the presence of L-NA–induced inhibition of acetylcholine-mediated relaxations of thoracic aortas was unaffected by fluvastatin in ApoE\textsuperscript{−/−}/HControl mice (Figure 3C). But, fluvastatin restored NO-mediated relaxations of carotid arteries from ApoE\textsuperscript{−/−}/HControl mice (maximal relaxation to Ach 10\%/H5 mol/L in the presence of L-NA: 3\%/H1% versus 5\%/H2% in ApoE\textsuperscript{−/−}/HFluva and WT mice, respectively, NS) (Figure 3D).

### Sodium Nitroprusside–Mediated Relaxant Responses

In thoracic aortas, maximal endothelium-independent relaxations to nitroprusside were significantly decreased in ApoE\textsuperscript{−/−}/HControl mice and in ApoE\textsuperscript{−/−}/Hmice treated with 1\%/H4% in the presence of L-NA+diclofenac) (Figure 3E). L-NA–induced inhibition of acetylcholine-mediated relaxations of thoracic aortas was unaffected by fluvastatin in ApoE\textsuperscript{−/−}/HControl mice (Figure 3C). But, fluvastatin restored NO-mediated relaxations of carotid arteries from ApoE\textsuperscript{−/−}/HControl mice (maximal relaxation to Ach 10\%/H5 mol/L in the presence of L-NA: 3\%/H1% versus 5\%/H2% in ApoE\textsuperscript{−/−}/HFluva and WT mice, respectively, NS) (Figure 3D).
fluvastatin compared with WT mice. However, thoracic aorta EC_{50} values for nitroprusside were not significantly different between the 3 experimental groups, indicating that the sensitivity of aortic smooth muscle cells to exogenous NO was maintained in control and treated ApoE^{-/-} mice (Table 3). In carotid arteries, no differences in terms of maximal responses and EC_{50} values were observed between the 3 experimental groups (Table 3).

Discussion
In the present work, we evaluated in ApoE^{-/-} mice the effects of the HMG CoA reductase inhibitor, fluvastatin, administered at a dose that significantly reduced hypercholesterolemia, on hemodynamics and vascular NO availability.

Chronic fluvastatin treatment decreased total plasma cholesterol levels and normalized triglycerides in ApoE^{-/-} mice. However, despite its strong cholesterol-reducing properties, fluvastatin failed to limit atherosclerosis lesion expansion in ApoE^{-/-} mice. Such a paradoxical relationship between cholesterol levels and lesion size has already been reported after probucol treatment in ApoE^{-/-} mice.22,23 In our study, the most likely explanation for this discrepancy is that, despite the reduction in plasma cholesterol concentrations, chronic fluvastatin treatment also resulted in a concomitant decrease in HDL cholesterol. This result is of particular importance because improvement of HDL cholesterol transport has been shown to oppose atherosclerosis and its deleterious consequences in ApoE^{-/-} mice.3,44 The effect of fluvastatin on HDL cholesterol levels was quite unexpected in our study because, in humans, hypolipidemic dosages of statins, which lead to a reduction in low-density lipoprotein cholesterol, increase the HDL cholesterol levels by 5% to 10%, thus yielding an antiatherogenic lipoprotein profile.26 A decrease in the activity of the cholesterol ester transfer protein (CETP), which is the key enzyme of HDL catabolism, is thought to be the principal mechanism responsible for the increase of HDL cholesterol associated with statin treatment.27 However, a recent clinical trial has shown that statins were ineffective at reducing coronary atherosclerosis in patients with low levels of plasma CETP,28 thus suggesting that when CETP expression level is low, statins are ineffective in modulating HDL concentrations. Based on these observations, the lack of any beneficial effect of statins on HDL levels, and thus on atherosclerosis progression in our study, is not surprising, because CETP expression in mice is constitutively low.

Presently, the cardiovascular phenotype of ApoE^{-/-} mice has not been well characterized. Blood pressure has been reported to be either unchanged19,29,30 or increased.31 Atherosclerosis in ApoE^{-/-} mice has also been shown to induce an increase in aortic stiffness assessed by pulse wave velocity, resulting in decreased vascular elasticity and compliance.32 Moreover, aortic, mitral, and pulse wave velocities have been reported to be markedly increased in this model, suggesting abnormal cardiovascular adaptations.30 In our study, 30-week-old ApoE^{-/-} mice did not display any alteration of blood pressure, heart rate, cardiac output, or total peripheral resistance despite severe atherosclerotic lesions. ApoE^{-/-} mice did not develop any cardiac hypertrophy and did not exhibit any significant alteration of myocardial tissue perfusion. This result confirms those obtained in isolated perfused hearts of 30-week-old ApoE^{-/-} mice.3 Of interest is our finding that renal flow was significantly reduced in ApoE^{-/-} mice, which is the functional evidence of the previously reported histological renal lesions in the same model.31,33 Atherosclerotic renal disease involving renal artery narrowing and renal ischemia has previously been described in humans.34 Renal structural alterations as well as a decrease in NO vascular availability attributable to hypercholesterolemia or atherosclerosis development are thought to contribute to progressive renal damage.8,35 HMG CoA reductase inhibitors have been shown to improve the prognosis of various experimental renal diseases without primary atherosclerosis.36,37 This improvement, independent from plasma cholesterol reduction, has been reported to be associated with an increase in renal blood flow, followed by an increase of the glomerular filtration rate.38,39 We show for the first time that, in the context of atherosclerotic nephropathy, fluvastatin confers renal protection by limiting the abnormal rise in renal vascular resistance. This beneficial effect of fluvastatin is truly a preventive one in this specific pathological state, because no reduction in renal vascular resistance was elicited by the drug under normal conditions (WT mice).

The consequences of hypercholesterolemia on vascular contractile responses are still controversial, depending on the experimental model used (species, age, diet),2,40–42 the vascular preparation,31,43 and the contracting agent.5,42,43 In agreement with previous studies,3,44 we also found that the contractile responses to KCl and phenylephrine were not altered in the aortas and carotid arteries taken from ApoE^{-/-} mice. In addition, our data show that chronic fluvastatin treatment significantly enhances the contractile vascular responses to KCl and phenylephrine of carotid and aorta from ApoE^{-/-} mice but not from WT mice. Several explanations for this phenomenon could be advocated that would, for an unknown reason, occur only in an atherosclerotic context, including (1) an increase in intracellular calcium availability, linked to the statin-induced decrease in intracellular mevalonate concentration12,45, (2) a sensitization of the vascular smooth muscle cells to calcium; and (3) a reduced release of endothelium-derived relaxing factors or an increased release of endothelium-derived contracting factors.

An important finding of our study is that despite the major rise in plasma cholesterol and the presence of severe atherosclerotic lesions, no aortic or carotid endothelial dysfunction was observed in 30-week-old ApoE^{-/-} mice. A slight reduction in the aortic responses to acetylcholine was evidenced, however, which, because of the simultaneously observed decreased responses to SNP, suggests a weaker effect of NO on aortic smooth muscle cells rather than a local endothelial dysfunction. This lack of endothelial dysfunction in both aorta and carotid artery, also previously reported in ApoE^{-/-} mice at 20 weeks either on a normal46 or on a 0.2% cholesterol diet,44 is in disagreement with previous studies3,4,44 showing that aortic and carotid endothelium-dependent relaxations were impaired in 30- to 35-week-old ApoE^{-/-} mice. These discrepancies could be explained by the various degrees of lesion extension achieved in the different
studies. Indeed, in one study, a much more severe atherogenic diet containing 1.25% cholesterol and 0.5% cholate (versus 0.15% cholesterol in our diet) was used. In the other study, Western-type diet was started at a very early stage of ApoE−/− mice growth (4 weeks versus 8 weeks in our study). Unfortunately, atherosclerotic lesion extension was not quantified in these studies. Our results thus indicate that despite hypercholesterolemia or the presence of lesions, endothelium maintained its ability to promote relaxation.

Pertaining to the underlying mechanisms of acetylcholine-induced relaxations, aortic endothelium-dependent responses were fully abolished in our study in the presence of a NO-synthase inhibitor both in the wild-type and in the ApoE−/− mice, thus confirming the previous report of Liuba et al. Under basal conditions, endothelium-dependent carotid relaxations to acetylcholine were similar in atherosclerotic ApoE−/− and wild-type mice, but the mechanisms underlying these relaxations differed completely in the two strains. Indeed, although carotid relaxations to acetylcholine were mediated exclusively through NO in wild-type mice, a deficit in NO availability was evidenced in the carotid artery from ApoE−/− mice. Thus, the lack of effect of hypercholesterolemia on the acetylcholine-mediated relaxations of carotid arteries from ApoE−/− mice under basal conditions results from the involvement of an alternative pathway, namely an endogenous cyclooxygenase-dependent vasodilator, which compensates for the deficit in NO availability. Of interest, chronic fluvastatin treatment prevented the alteration of the NO-dependent relaxation of carotid arteries in ApoE−/− mice. Thus, that fluvastatin had no effect on lesion development but improved NO availability indicates that this latter effect was direct. Indeed, HMG-CoA reductase inhibitors are able to increase endothelial NO synthase expression or to stimulate its activity by limiting its intracellular interaction with calveolin or by decreasing its inactivation by free radicals.

In conclusion, our findings demonstrate that in ApoE−/− mice, the HMG CoA reductase inhibitor fluvastatin, administered at a cholesterol-lowering dose, exerted protective effects on renal hemodynamics and on vascular NO availability. These beneficial effects developed despite the lack of effects of fluvastatin on atherosclerotic lesion extension.

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References


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