Mouse Models for Atherosclerosis and Pharmaceutical Modifiers

Susanne Zadelaar, Robert Kleemann, Lars Verschuren, Jitske de Vries-Van der Weij, José van der Hoorn, Hans M. Princen, Teake Kooistra

Abstract—Atherosclerosis is a multifactorial highly-complex disease with numerous etiologies that work synergistically to promote lesion development. The ability to develop preventive and ameliorative treatments will depend on animal models that mimic the human subject metabolically and pathophysiologically and will develop lesions comparable to those in humans. The mouse is the most useful, economic, and valid model for studying atherosclerosis and exploring effective therapeutic approaches. Among the most widely used mouse models for atherosclerosis are apolipoprotein E-deficient (ApoE−/−) and LDL receptor-deficient (LDLr−/−) mice. An up-and-coming model is the ApoE*3Leiden (E3L) transgenic mouse. Here, we review studies that have explored how and to what extent these mice respond to compounds directed at treatment of the risk factors hypercholesterolemia, hypertriglyceridemia, hypertension, and inflammation. An important outcome of this survey is that the different models used may differ markedly from one another in their response to a specific experimental manipulation. The choice of a model is therefore of critical importance and should take into account the risk factor to be studied and the working spectrum of the compounds tested. (Arterioscler Thromb Vasc Biol. 2007;27:1706-1721.)

Key Words: mouse models | atherosclerosis | pharmaceutical drugs | statins | ACE inhibitors | AT1 receptor antagonists | PPAR | LXR

Despite significant advances in treatment and in understanding of its biology, coronary atherosclerosis remains the leading cause of morbidity and mortality of men and women in industrialized societies. Hypercholesterolemia, particularly of low-density lipoprotein (LDL) cholesterol and very low-density lipoprotein (VLDL) cholesterol, is a well-established risk factor for the incidence of atherosclerosis and its pathologic complications. For the past 20 years, the statin class of cholesterol-lowering drugs has been the mainstay for treatment of hypercholesterolemia, hypertriglyceridemia, hypertension, and inflammation. An important outcome of this survey is that the different models used may differ markedly from one another in their response to a specific experimental manipulation. The choice of a model is therefore of critical importance and should take into account the risk factor to be studied and the working spectrum of the compounds tested. (Arterioscler Thromb Vasc Biol. 2007;27:1706-1721.)

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Definition of atherogenic mechanisms in humans is hindered by the complexity and chronicity of the disease process. Another complication is the inability to sequentially characterize lesions in an individual patient, despite rapid progress in noninvasive detection modalities.

Therefore, there has been a reliance on animal models for the disease to dissect the pathogenetic steps and causalities. Mouse models in particular have proved useful to study atherosclerotic lesion development, and a number of recent reviews have extensively discussed the various mouse models available (for example, and references therein). Transgenic and knockout mouse models for atherosclerosis have also been instrumental in evaluating existing and finding and testing new atherosclerotic drugs. Here, we review studies on the response of those mice on an atherogenic diet to drugs directed at treatment of the risk factors hypercholesterolemia, hypertriglyceridemia, hypertension, and inflammatory status. Also mouse studies were reviewed in which questions were addressed about role and causality of the various risk factors in a therapeutically relevant manner by genetically, immunologically, nutritionally, or pharmacologically modifying key components of the atherosclerotic process. An important outcome of this survey is that the different

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models used may differ strikingly from one another in their response to a specific experimental manipulation. The choice of a model is therefore of critical importance and should take into account the risk factor to be studied and the working spectrum of the compounds tested (viz. lipid-lowering, hypotensive, antiinflammatory, or combinations thereof).

After providing a brief overview of relevant mouse models for atherosclerosis allowing assessment of the above risk factors, we have explored the extent to which current anti-atherosclerotic drugs reduce atherogenesis in each of the models. The pharmaceutical modifiers tested, experimental settings used, and outcomes of these studies are summarized in Table 1.

**Mouse Models for Atherosclerosis**

Wild-type mice are quite resistant to atherosclerosis as a result of high levels of antiatherosclerotic HDL and low levels of proatherogenic LDL and VLDL. All of the current mouse models for atherosclerosis are therefore based on perturbations of lipoprotein metabolism through dietary or genetic manipulations.

Among the most widely used mouse models are apolipoprotein E–deficient mice (ApoE<sup>−/−</sup> mice), in which targeted deletion of the apoE gene leads to severe hypercholesterolemia and spontaneous atherosclerosis, and LDL receptor–deficient mice (LDLr<sup>−/−</sup> mice), in which atherosclerosis develops especially when fed a lipid-rich diet. A newly emerging model is the ApoE<sup>−/−</sup>Leiden (E3L) transgenic mouse, in which a mutated form of the human apoE3 gene has been introduced; E3L mice have a hyperlipidemic phenotype, develop atherosclerosis on being fed cholesterol, and are more sensitive to lipid-lowering drugs than ApoE<sup>−/−</sup> and LDLr<sup>−/−</sup> mice.

Some relevant characteristics of the above models are summarized below.

**Apolipoprotein E–Deficient (ApoE<sup>−/−</sup>) Mice**

ApoE is synthesized in the liver and in macrophages and has a number of important antiatherogenic functions. As a constituent of plasma lipoproteins it serves as a ligand for the cell-surface lipoprotein receptors such as LDL-receptor (LDLr) and LDLr-related proteins (LRPs), thereby promoting the uptake of atherogenic particles from the circulation. Consequently, homozygous deletion of the apoE gene in mice results in a pronounced increase in the plasma levels of LDL and VLDL attributable to the failure of LDLr- and LRP-mediated clearance of these lipoproteins. The most obvious phenotype of ApoE<sup>−/−</sup> mice is the spontaneous development of atherosclerotic lesions, even on a standard chow diet which is low in its fat content (<40 g/kg) and does not contain cholesterol. Lesions of ApoE<sup>−/−</sup> mice resemble their human counterparts and develop over time from initial fatty streaks to complex lesions. This process can be strongly accelerated by a high-fat, high-cholesterol (HFC) diet. Because of the rapid development of atherosclerosis, the ApoE<sup>−/−</sup> model has been used widely, despite considerable limitations. A major drawback of the complete absence of apoE protein is that the model is dominated by high levels of plasma cholesterol. For instance, on a chow diet plasma cholesterol concentrations are about 8 mmol/L, compared with 2 mmol/L for the parent C57Bl/6 mouse, and can become >70 mmol/L on a HFC diet. Another shortcoming is that most plasma cholesterol is confined to VLDL and not to LDL particles as in humans. Furthermore, there is mounting evidence that apoE protein has additional antiatherogenic properties besides regulating the clearance of lipoproteins. For example, it is thought that apoE exerts antiatherosclerotic effects by its antioxidant, antiproliferative (smooth muscle cells, lymphocytes), antiinflammatory, antiplatelet, and NO-generating properties. Also, apoE can modulate immune activation: it inhibits T-cell proliferation and is essential for normal innate immune function. These immunomodulatory effects of apoE are of relevance in atherosclerosis, which besides lipoprotein accumulation is characterized by immune/inflammatory activation. Indeed, reconstruction of macrophage-specific expression of apoE reduces atherosclerosis in ApoE<sup>−/−</sup> mice, whereas reconstitution of C57Bl/6 mice with macrophages from ApoE<sup>−/−</sup> mice increases atherosclerosis (see Tenger and Zhou, and references therein). In addition to this, apoE is a strong acceptor of cellular cholesterol and as such involved in foam cell formation as well as reverse cholesterol transport. Because of the complete absence of apoE in ApoE<sup>−/−</sup> mice the study of the above processes and the effects of drugs thereupon is restricted in this model.

**LDL Receptor–Deficient (LDLr<sup>−/−</sup>) Mice**

In humans, mutations in the gene for the LDLr cause familial hypercholesterolemia. Mice lacking the gene for LDLr display a modestly elevated plasma cholesterol level when maintained on a regular chow diet (about 5 mmol/L versus 2 mmol/L in wild-type animals), and they develop atherosclerosis only slowly. On HFC diet feeding, LDLr<sup>−/−</sup> mice show strongly elevated plasma cholesterol (>25 mmol/L) and rapid development of atherosclerosis. The plasma lipoprotein profile of LDLr<sup>−/−</sup> mice resembles that of humans, with the cholesterol being confined mainly to the LDL fraction. Interestingly, LDLr<sup>−/−</sup> mice coupled with an ApoB-editing deficiency (LDLr<sup>−/−</sup>/ApoBEC<sup>−/−</sup> mice) or combined with human ApoB100 transgenic mice (LDLr<sup>−/−</sup>/ApoB<sup>−/−</sup>) show a large increase in plasma LDL cholesterol and develop atherosclerosis on a low-fat diet. The morphology of the lesions in LDLr<sup>−/−</sup> mice is comparable to that in ApoE<sup>−/−</sup> mice, with the plaques developing in a time-dependent manner, starting from the proximal aorta. In all, the LDLr<sup>−/−</sup> mouse represents a more moderate model than the ApoE<sup>−/−</sup> mouse, mainly because of the milder degree of hyperlipidemia.

**ApoE<sup>−/−</sup>Leiden (E3L) Transgenic Mice**

The ApoE<sup>−/−</sup>Leiden mutation is a rare dominant-negative mutation in the human APOE3 gene. It is characterized by a tandem duplication of codons 120 to 126 and associated with familial dysbetalipoproteinemia in humans. ApoE<sup>−/−</sup>Leiden transgenic (E3L) mice have been generated by introducing a human APOE<sup>−/−</sup>Leiden gene construct into C57Bl/6 mice. Besides the APOE<sup>−/−</sup>Leiden gene, this construct consists of the APOC1 gene and a promoter element that regulates the expression of APOE and APOC1 genes (see and refer-
<table>
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<tr>
<th>Group</th>
<th>Dose (Mg/kg bw/d)</th>
<th>Time (weeks)</th>
<th>Diet</th>
<th>Mouse Model</th>
<th>Sex</th>
<th>Effect on Cholesterol</th>
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<td>12</td>
<td>?</td>
<td>E−/−</td>
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<td>29</td>
<td>?</td>
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<td>8</td>
<td>?</td>
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<td>←</td>
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<td>?</td>
<td>E/^−^</td>
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<td>←</td>
<td>↓</td>
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<td>5</td>
<td>8/16</td>
<td>?</td>
<td>E/^−^</td>
<td>δ</td>
<td>←</td>
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<td>20</td>
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<td>E/^−^</td>
<td>δ</td>
<td>←</td>
<td>↓</td>
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<td>E/^−^</td>
<td>δ</td>
<td>←</td>
<td>↓</td>
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**AT1 receptor antagonists**

| Olmesartan    | 1/3               | 10           | 1.25% chol       | E/^−^        | δ   | ←                    | ↓                | 90   |
|               | 0.5/3             | 6            | 1.25% chol       | E/^−^        | δ   | ←                    | ↓                | 82   |
|               | 10                | 25           | 0.15% chol       | E/^−^        | δ   | ←                    | ↓                | 89   |
|               | 9.3               | 24           | 0.5% chol        | E3L          | γ   | ↓                    | ↓                | unpublished data, 2003 |
| Valsartan     | 0.1/1             | 10           | 1.25% chol       | E/^−^        | δ   | ←                    | ↓                | 27   |
|               | 0.5               | 6            | 1.25% chol       | E/^−^        | δ   | ?                    | ↓                | 91   |
| Candesartan   | 1                 | 12           | 1% chol          | E/^−^        | ?   | ←                    | ↓                | 42   |
|               | 0.5               | 16           | 0.15% chol       | E/^−^        | δ   | ?                    | ↓                | 92   |
| Telmisartan   | 0.3/3             | 12           | chow             | E/^−^        | δ/γ | ←                    | ↓                | 93   |
|               | 1                 | 12           | chow             | E/^−^        | δ   | (V)LDL↑ LDL↓          | ↓                | 25   |
| Irbesartan    | 50                | 12           | chow             | E/^−^        | γ   | ↑                    | ↓                | 87   |
|               | 10                | 20           | chow             | E/^−^        | δ   | ←                    | ↓                | 25.88 |
|               | 50                | 7            | 1.25% chol       | E/^−^        | δ   | ?                    | ↓                | 74   |
| Losartan      | 5                 | 10           | chow             | E/^−^        | ?   | ?                    | ↓                | 78   |
|               | 25                | 12           | chow             | E/^−^        | ?   | ←                    | ↓                | 84   |
|               | 20/30             | 12           | chow             | E/^−^        | γ   | ←                    | ←                | 146  |
|               | 100 mg/L          | 12           | chow             | E/^−^        | γ   | ←                    | ↓                | 85   |
| Losartan + angiotensin II | 5/25 | 12 | chow | E/^−^ | δ/γ | ← | ↓ | 86 |

**PPARα agonist/1/fibricates**

| Gemfibrozil | 100 | 20 | chow | E/^−^ | ? | LDL ↓ LDL↑ | HDL↑ LDL↓ not quant. | 109 |
| Ciprofibrate | 0.05%/w/w | 13 | chow | E/^−^ | δ/γ | ↑ | ↑ | ↑ | 103 |
|               | 100 | 21 | 0.4% chol | LDLR^-^ | γ | ↑ | ↑ | ↑ | not quant. | 104 |
| GW7647 | 2.5 | 14 | 1.25% chol | LDLR^-^ | δ | ← | ↓ | 111 |
| Fenofibrate | 100 | 8 | 0.2% chol | E/^−^ | δ | ← | ← | 108 |
|               | 100 | 8 | 0.2% chol | h apoA-I E/^−^ | δ | ← | ↓ | 108 |
|               | 200 | 14 | 0.2% chol | E/^−^ | δ | ↑ | ← | 106 |
|               | 0.1% w/w | 14 | 0.2% chol | E/^−^ | δ | ↑ | ← | 107 |
|               | 10 | 11 | chow | E/^−^ | γ | (V)LDL↑ LDL↓ | ← | 105 |
|               | 0.05%/w/w | 13 | chow | E/^−^ | δ/γ | ↑ | 103 |
|               | 100 | 10 | 0.2% chol | h apoE2Ki | δ | (V)LDL↑ LDL↓ | ↓ | 114 |
|               | 100 | 24 | 1.25% chol | LDLR^-^ | δ | ↓ | ↓ | 112 |
|               | 30 | 18 | 0.5% chol | E3L | γ | ↓ | ↓ | 113 |
| Bezafibrate | 0.05%/w/w | 13 | chow | E/^−^ | δ/γ | ↑ | 103 |
| WY14643 | 0.02%/w/w | 13 | chow | E/^−^ | δ/γ | ↑ | 103 |

Continued
ences therein). Although E3L mice still express endogenous apoE protein, the clearance of apoE-containing lipoproteins is impaired, albeit less dramatically than in ApoE-/- mice. The introduction of the APOC1 gene may further increase plasma lipid levels by diminished lipolysis and VLDL uptake through both the LDLr and LRP.

E3L mice show significant elevations of plasma cholesterol and triglycerides on a regular chow diet and are, in contrast...
to wild-type mice, highly responsive to fat-, sugar-, and cholesterol-containing diets, resulting in strongly elevated plasma cholesterol and triglyceride levels, with a prominent increase in VLDL- and LDL-sized lipoprotein fractions. Plasma lipid levels can easily be adjusted to a desired concentration by titrating the amount of cholesterol and sugar in the diet. As compared with ApoE−/− and LDLr−/− mice, E3L mice represent a moderate mouse model for hyperlipidemia (cholesterol levels on chow are about 2 mmol/L and do not exceed 25 mmol/L on a HFC diet). In addition, the plasma cholesterol and triglyceride levels respond strongly to changes in hepatic VLDL production. Therefore, drugs and diets influencing the chylomicron and VLDL production show parallel effects on plasma cholesterol and triglyceride levels. In this respect, E3L mice are more sensitive than ApoE−/− and LDLr−/− mice and respond to hypolipidemic compounds with cholesterol-lowering.

E3L mice develop atherosclerotic lesions with all the characteristics of human vascular pathology, varying from fatty streak to mild, moderate, and severe plaques. Atherosclerosis development starts at the aortic root and progresses along the entire arterial tree in a time-dependent fashion.

E3L mice crossbred with human cholesteryl ester transfer protein (CETP)-expressing mice display an elevated basal cholesterol level and an even more human-like lipoprotein profile. CETP expression in E3L mice shifts the distribution of cholesterol from HDL toward VLDL/LDL, and strongly (7-fold) increased atherosclerosis development.

### 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibitors (Statins)

Statins are widely prescribed cholesterol-lowering drugs with proven efficacy in humans that act by inhibiting 3-hydroxy-3-methylglutaryl (HMG) Coenzyme A (CoA) reductase, a rate-limiting enzyme of cholesterol biosynthesis. Clinically relevant statins include atorvastatin, cerivastatin, fluvastatin, lovastatin, pravastatin, pitavastatin, rosuvastatin, and simvastatin. It is important to note that the plasma cholesterol-lowering effect of statins results mainly from an enhanced hepatic uptake of LDL via upregulation of LDLr expression and to a lesser extent from a reduced endogenous cholesterol biosynthesis.

With the advent of hyperlipidemic mouse models suitable for the study of atherosclerosis, it is now possible to reexamine and compare the effect of clinically established statins with respect to their antiatherosclerotic efficacy. It is thought that the mouse models reviewed here may afford a more focused examination of the action of statins and provide, for new compounds, better prediction of the human response.

However, as summarized in Table 1, statins that are efficacious in lowering cholesterol in man do not necessarily do so in the established ApoE−/− and LDLr−/− models, this being in contrast to E3L mice which respond well to statins.

Treatment of ApoE−/− mice with pravastatin, fluvastatin, cerivastatin, pitavastatin, or simvastatin did not lower plasma cholesterol25–32 or even increased plasma cholesterol.33–37 Cholesterol-lowering effects of statins in ApoE−/− mice were only then observed when the cholesterol concentration in the experimental diet was relatively low (0.15% w/w)38–40 or when more powerful third generation statins (atorvastatin; rosuvastatin) were used.41–43

Despite absence of a hypcholesterolemic effect, cerivastatin,31 pitavastatin,31 fluvastatin,27,31 pravastatin,26 and simvastatin32 reduced aortic lipid deposition in statin-treated ApoE−/− mice. However, when atherosclerotic plaques were analyzed in more detail, controversial observations were made. Some studies report antiatherogenic effects such as decreased intraplaque hemorrhage and calcification46 and increased fibrous cap thickness.46 A study with fluvastatin in ApoE−/− documents absence of an effect on lesion size and lesion number despite pronounced total cholesterol-lowering and an increase in HDL cholesterol.38

The LDLr−/− model (or the combined ApoE−/−/LDLr−/− model) is much less used to evaluate the antiatherogenic properties of statins. In the few papers published the effects of statins on plasma cholesterol levels and atherosclerosis in LDLr−/− mice appeared to be variable. Low-dose pravastatin did not significantly decrease atherosclerosis in LDLr−/− mice,44,46 despite lowering of plasma cholesterol45 (no plasma cholesterol data were reported by Kwak et al44). In another LDLr−/− study atorvastatin was found to lower plasma cholesterol, but no lesion scores were reported.39 Simvastatin caused a reduction in atherosclerosis in LDLr−/− mice, beit with or without46 the lowering of plasma cholesterol.

In contrast to the ApoE−/− and LDLr−/− models, the effects of statins in the E3L model are more uniform and human-like, and results reported so far all consistently show hypolipidemic and antiatherosclerotic effects. Statins tested in E3L mice include atorvastatin,47–50,143 lovastatin,51 pravastatin (unpublished data, 2003), and rosuvastatin.52,53 The E3L model has also been used to evaluate antiatherosclerotic effects of statins independent of cholesterol-lowering (“pleiotropic effects” of statins).47,50,52 Comparison of a statin-treated group versus a cholesterol-matched control group revealed that statins can reduce atherosclerosis beyond and independent of the reduction achieved by cholesterol-lowering alone. Pleiotropic (including antiinflammatory) statin effects in the vasculature and the liver may at least partly explain these additional beneficial effect of statins (see also54).

### Other Cholesterol-Lowering Drugs

In recent years, there has been a significant effort in developing new (classes of) cholesterol-lowering drugs that can be used alone or in combination with statins. These drugs are in particular directed toward preventing cholesterol absorption and promoting cholesterol secretion (1 and references therein). Among the few examples published (Table 1) are studies on ezetimibe and inhibitors of Acyl-CoA:cholesterol acyltransferase (ACAT). Ezetimibe selectively inhibits intestinal uptake and absorption of dietary and biliary cholesterol in small intestinal enterocytes. The drug inhibited cholesterol absorption by 90% and reduced atherosclerosis by 97% in ApoE−/− mice fed a Western diet containing 0.15% w/w cholesterol.55,56 Similar results were obtained in ApoE−/−/Leiden mice (unpublished data, 2007) and by Basso et al in LDLr−/− mice.57 The reductions in cholesterol in ApoE−/− mice occurred in the VLDL and LDL fractions, whereas HDL
cholesterol levels were increased by ezetimibe treatment. These findings correspond with clinical observations. Co-administration of a statin and ezetimibe synergistically decreased LDL and raised HDL levels, compared with a single-drug treatment in human adults.58

Removal of cholesterol from lipid-laden macrophages (“foam cells”) can lead to regression of atherosclerosis or stabilization of the plaque. There is a dynamic balance between the amount of free cholesterol and cholesteryl esters (for storage) within the cell, which is regulated by two enzymes located in the endoplasmic reticulum: Acyl-CoA:cholesterol acyltransferase-1 (ACAT1) and neutral cholesterol ester hydrolases (nCEH). By inhibiting cholesterol esterification, free cholesterol can exit from foam cells, return to the liver via HDL, and be secreted in the bile. An ACAT inhibitor (F-1394) decreased plasma cholesterol and atherosclerosis in ApoE−/− mice.59 Although F-1394 did not reduce plasma cholesterol in ApoE−/−/LDLr−/− double knockout mice, it did also reduce atherosclerosis.60 In E3L mice, the ACAT inhibitor avasimibe reduced cholesterol and atherosclerosis, the latter even more than can be explained by its cholesterol-lowering effect alone.61 Studies on combinations of ACAT inhibitors and statins in mice have not been reported yet. Of note, an additional beneficial effect of avasimibe above simvastatin was observed in a rabbit atherosclerosis model,62 and a human study reports that avasimibe above simvastatin was observed in a rabbit atherosclerosis model.62,63

More recent findings indicate that manipulating ACAT2 expression, which is primarily found in the liver and small intestine, may be an interesting alternative. ACAT2−/− mice backcrossed to ApoE−/− mice had fewer lesions, compared with wild-type controls.64

Hypotensive Drugs (ACE Inhibitors and AT1 Receptor Antagonists)

Epidemiological investigations clearly point out that hypertension is associated with exaggerated atherosclerosis and that elevated blood pressure (BP) is highly predictive for future atherosclerosis-associated cardiovascular events.65 Hypertension also increases the rate of atherosclerotic plaque development in models for atherosclerosis.66–68 Hypertension can occur in two forms, at elevated or at normal plasma angiotsin II (Ang II) levels. Ang II, which is generated from angiotensin I by the angiotensin-converting enzyme (ACE), is the principal effector of the renin-angiotensin system (RAS) and modulates BP (Figure). Using hypertensive Ang II−/− mice with either elevated plasma Ang II levels or normal plasma Ang II levels, Mazzolai et al showed that both forms of hypertension led to a similar increase in lesion extension compared with normotensive mice, but the atherosclerotic plaques of the hypertensive animals with high Ang II were more advanced and less stable.69 In addition, these mice also showed enhanced systemic and vascular inflammation. In agreement with these observations, infusion of Ang II in ApoE−/− mice indeed enhances vascular inflammation, increases atherosclerotic lesion size, and promotes unstable plaque phenotype.69 Daugherty et al reported the presence of pronounced abdominal aortic aneurysms in ApoE−/− mice infused with Ang II.70 Weiss et al also found that Ang II-induced hypertension accelerates the initiation and progression of atherosclerosis in ApoE−/− mice.71 Conversely, when hypertension was induced to a similar level by administering norepinephrine (noradrenaline), they found only a modest increase in atherosclerosis. In two other studies Ang II administration to ApoE−/− mice stimulated atherosclerotic lesion formation in the absence of any significant increase in BP or plasma cholesterol levels.70,72

Most of the known effects of Ang II, including its proinflammatory effects, are related to Ang II type 1 (AT1) receptor activation (Figure). Genetic disruption of the AT1A receptor in LDLr−/− or ApoE−/− mice leads to inhibition of atherosclerotic lesion formation, irrespective of BP or plasma cholesterol levels.73,74

Inhibition of Ang II action on the arterial wall by blocking its production with ACE inhibitors or by blocking binding to its receptors on cells with AT1 receptor antagonists was shown to attenuate atherosclerosis (Table 1): Hayek and colleagues reported that the ACE inhibitor captopril suppressed the development and progression of atherosclerosis in ApoE−/− mice.75,76 Most ACE inhibitors tested in ApoE−/− mice showed similar reductions in atherosclerosis.25,76–81 Low dose enalapril and temocapril in ApoE−/− mice and fosinopril in LDLr−/− mice did not have this atherosclerosis reducing capacity.76,82,83

Keidar et al and others78,84–86 found that the AT1 receptor antagonist losartan significantly inhibited the development of atherosclerotic lesions in the ApoE−/− model. Similar results in ApoE−/− mice were reported for the AT1 receptor antagonists irbesartan,25,74,87,88 olmesartan,82,89,90 valsartan,27,91 candesartan,42,92 and telmisartan.25,93 Moreover, olmesartan also showed this effect in E3L mice (unpublished data, 2003). Several studies have shown that these antiatherosclerotic effects of ACE inhibitors and AT1 receptor antagonists could be dissociated from their BP-lowering ability: The ACE inhibitors fosinopril and ramipril, and the AT1 receptor antagonist losartan diminished atherosclerosis in ApoE−/− mice independently of lowering BP.78,80 Similar results in ApoE−/− mice were found with nonhypotensive doses of the ACE inhibitor temocapril and the AT1 receptor blocker olmesartan.82
Treatment of ApoE\(^{-/}\) mice with either the nonspecific antihypertensive drug hydralazine or AT\(_1\) receptor blocker irbesartan reduced systolic BP to the same level; however, only irbesartan treatment reduced atherosclerosis and improved endothelial function.\(^{74}\) Suganuma found that ApoE\(^{-/}\) mice that underwent uninephrectomy (UNx) to induce mild renal dysfunction showed higher BP and a dramatic increase in the extent and number of atherosclerotic lesions.\(^{85}\) The AT\(_1\) receptor antagonist losartan but not hydralazine strongly decreased the UNx-induced acceleration in atherosclerosis, despite equivalent reduction in BP.

A mediator of Ang II effects in the vessel wall after AT\(_1\) receptor activation is the superoxide-producing NAD(P)H oxidase (see Tsuda et al.\(^{82}\) and references therein). In ApoE\(^{-/}\) mice lacking the gene for p47 NADPH oxidase subunit the atherosclerotic burden is markedly decreased, underlining the relevance of oxidative stress and NAD(P)H oxidase for the atherosclerotic process.\(^{94}\)

**Combination Therapy With Hypotensive Drugs**

Because statins and AT\(_1\) receptor blockers attenuate atherosclerosis through different mechanisms, combined use of the two types of drugs might therefore be expected to produce a greater antiatherosclerotic effect compared with either drug alone (supplemental Table I, available online at http://atvb.ahajournals.org).\(^{95}\) Indeed, simultaneous administration of AT\(_1\) receptor blocker/statin combinations, notably candesartan/losartan, valsartan/bisindolylmaleimide, or olmesartan, to high-cholesterol-fed ApoE\(^{-/}\) mice reduced atherosclerosis to a greater extent than each drug alone.\(^{27,42}\) However, Grothusen et al reported that a combined treatment of ApoE\(^{-/}\) mice with RAS-blockade (ramipril or telmisartan) and atorvastatin may have additive effects on systemic cardiovascular risk markers even in the absence of lipid reduction, but additional effects on atherosclerotic progression and stability were not observed in this model.\(^{28}\) In E3L mice, combination therapy with olmesartan and pravastatin did additively reduce atherosclerosis, resulting in fewer lesions, which were less severe and more stable (unpublished data, 2003).

**Nuclear Hormone Receptors**

The nuclear hormone receptor superfamily of ligand-activated transcription factors regulates gene expression in such diverse processes as metabolism, development, and reproduction. The family has 48 members in humans, and includes, for example, retinoid, steroid, and thyroid hormone receptors.

The subfamilies known as peroxisome proliferator-activated receptor (PPAR) and liver-X-receptor (LXR) have emerged as dominant regulators of processes that influence cardiovascular risk, namely various aspects of lipid and glucose metabolism, insulin sensitivity, as well as inflammation.\(^{96–101}\)

Interestingly, PPARs and LXRs not only show these effects at the systemic level but also regulate lipid homeostasis and inflammation in macrophages, endothelial cells, and smooth muscle cells within the vessel wall. Drugs that specifically activate these receptors may therefore retard the development of atherosclerosis at several levels.\(^{96}\)

**PPARs**

There are three distinct PPAR subtypes, PPAR\(_\alpha\), PPAR\(_{\beta/\delta}\) (hereafter referred to as PPAR\(_\delta\)), and PPAR\(_\gamma\). Although there is overlap in natural ligands (fatty acids, eicosanoids) that are capable of activating the 3 PPARs, each receptor subtype has a tissue-specific expression pattern and exhibits overlapping but distinct biological activities.\(^{1,96}\)

While PPAR\(_\alpha\) is expressed in metabolically active tissues, including the liver, heart, kidney, and skeletal muscle, PPAR\(_\delta\) is expressed more ubiquitously, and PPAR\(_\gamma\) is expressed predominantly in adipose tissue, but is also found in skeletal muscle, liver, and colon. The expression of PPARs in cells of the artery wall may be of importance for some of the effects of PPAR agonists on atherosclerosis. The clinical relevance of PPAR-regulated processes is underscored by the successful use of fibrates (PPAR\(_\alpha\) agonists) and thiazolidinediones (TZD; PPAR\(_\gamma\) agonists) to treat hyperlipidemia and type-2 diabetes, respectively (Table I).

**PPAR\(_\alpha\)**

Studies on the role of PPAR\(_\alpha\) in mouse models for atherosclerosis have yielded complex results. Tordjman et al surprisingly observed that PPAR\(_\alpha^{-/-}\)/ApoE\(^{-/}\) mice had fewer atherosclerotic lesions than control ApoE\(^{-/-}\) mice, suggesting a proatherogenic role of PPAR\(_\alpha\).\(^{102}\) The PPAR\(_\alpha^{-/-}\) mice were found to be less insulin resistant and to have a lower blood pressure compared with controls, potentially at least partially explaining the unexpected outcome. In another study, Fu et al found that treatment of ApoE\(^{-/}\) mice with a PPAR\(_\alpha\) agonist, ciprofibrate, aggravated hyperlipidemia, and increased atherosclerosis.\(^{103}\) The same group also reported enhanced plasma cholesterol levels and atherosclerosis development in LDLr\(^{-/-}\) mice treated with ciprofibrate.\(^{104}\)

Several groups found no effect on atherosclerosis in ApoE\(^{-/-}\) mice treated with fenofibrate, even when plasma cholesterol levels were increased in some of these studies.\(^{105–107}\) Notably, Duez et al showed that fenofibrate reduced the lesion surface area of ApoE\(^{-/-}\) mice carrying a fenofibrate-inducible human Apo-Al transgene.\(^{108}\)

Also other laboratories demonstrated that PPAR\(_\alpha\) and its agonists can be antiatherogenic. Calkin et al demonstrated that gemfibrozil decreases atherosclerosis in ApoE\(^{-/-}\) mice in association with a reduction in LDL cholesterol.\(^{109,110}\) Li et al showed that activation of PPAR\(_\alpha\) by a highly specific and potent agonist (GW7647) inhibited atherosclerosis in hyperlipidemic LDLr\(^{-/-}\) mice, without significantly altering the diet-induced hyperlipidemia.\(^{111}\) Similarly, Srivastava et al recently reported that fenofibrate reduces atherosclerosis in LDLr\(^{-/-}\) mice in conjunction with a decrease in plasma cholesterol. Our group, using E3L mice, found that fenofibrate reduces atherosclerosis more than can be explained by the cholesterol-lowering effect of fenofibrate per se.\(^{112}\) Impaired recruitment of monocytes/macrophages, reduced vascular and systemic inflammation, and stimulation of cholesterol efflux may all contribute to the additional beneficial effect of fenofibrate.

Hennuyer et al similarly reported that fenofibrate treatment significantly improved lipoprotein metabolism toward a less atherogenic phenotype and delayed the development of ath-
erosclerosis in a dyslipidemic nondiabetic murine model, human ApoE2 knock-in mice (E2KI mice).114

The discrepancies observed between the mouse atherosclerosis findings might be attributable to the animal models used, the type of agonist, the diet, and the duration of the experimental treatment.

**PPARγ**

Studies on PPARγ are in general agreement that activation of this receptor is beneficial for reducing atherosclerosis,105,115–118 despite unaffected104,119–122 or even increased plasma cholesterol.105,122 Li et al were the first to describe the inhibitory effects of two structurally distinct agonists of PPARγ on the progression of atherosclerosis.113 In these studies, LDLr−/− mice were challenged with a Western diet for 10 weeks in the presence or absence of PPARγ agonists. Treatment with rosiglitazone or the tyrosine-derived insulin sensitizer GW7845 led to a marked reduction (60% to 80%) in lesion area in male mice. In another study, using female LDLr−/− mice, PPARγ agonists surprisingly failed to prevent atherosclerosis or to correct hyperinsulinemia induced by a high-fat diet.115 Because TZDs are effective in ameliorating insulin resistance in female humans, it is unclear why these compounds have failed in female mice.

The marked reduction in lesion size observed by Li et al115 could result from the direct actions of TZDs on cells of the arterial wall or from improvements in systemic metabolic parameters. To address this possibility, Collins et al treated male LDLr−/− mice maintained on a high-fat or high-fructose diet with troglitazone for 3 months.117 Although both diets promoted atherosclerosis, mice fed on the high-fat diet developed insulin resistance and diabetes, whereas those fed the high-fructose diet remained normoglycemic. Notably, although troglitazone therapy reduced en face aortic lesion area in both dietary groups, improvements in insulin sensitivity were observed only in the mice maintained on a high-fat diet. These findings suggest that the antiatherogenic actions of TZDs can be independent of their beneficial effects on insulin resistance and could arise from direct actions on vascular cells. Others have demonstrated similar antiatherosclerotic effects of troglitazone in ApoE−/− mice.118

The above treatment studies provide compelling evidence in support of the antiatherogenic actions of TZDs in vivo; however, they do not clarify the contribution of macrophage-specific PPARγ expression to disease progression. This issue has been addressed by studies in which bone marrow from either wild-type or highly chimeric PPARγ−/− mice were transplanted into LDLr−/− mice.116 Reconstituted mice were subsequently challenged with a Western diet for 8 weeks to induce a moderate degree of atherosclerosis. Remarkably, transplantation of PPARγ−/− bone marrow into LDLr−/− mice led to a marked increase in atherosclerotic lesion area, suggesting that PPARγ and its transcriptional targets have atheroprotective functions in plaque macrophages. In accordance with this view, troglitazone-treated mice display small lesions, which contained proportionally fewer macrophages, suggesting that troglitazone strongly inhibited macrophage accumulation.117

**Dual PPARα/γ Agonists**

With PPARα and PPARγ agonists displaying distinct antiatherogenic and anti diabetic effects, investigators are currently looking into the development of compounds with dual activity ("coagonists"), ie, ligands that activate both PPARα and PPARγ. This new class of combined PPARα/γ agonists should provide a new therapeutic approach via complementary metabolic actions and should integrate the actions of fibrates (regulating lipoprotein metabolism and antiinflammatory) and TZDs (regulating insulin resistance and blood glucose levels, and antiinflammatory), thereby addressing several of the risk factors for cardiovascular disease. Claudel et al demonstrated that a PPARα/γ coagonist, GW2331, decreased atherosclerosis by 32% in ApoE−/− mice.105 Zuckerman et al provided a mechanistic explanation by showing that PPARα/γ coagonist LY465608 inhibits macrophage activation using peritoneal macrophages from ApoE−/− mice.123 Another PPARα/γ coagonist, tesaglitazar, reduced atherosclerosis by 92% in E3L mice.124 In line with the observations of Zuckerman, tesaglitazar exerted antiatherosclerotic effects beyond plasma cholesterol-lowering, including antiinflammatory, NFκB-reducing vascular effects. Chira et al,125 using female LDLr−/− mice, also found that tesaglitazar reduced atherosclerosis via lipid-independent mechanisms, probably at least in part by direct actions on the vessels. At variance with the above findings, a recent study in ApoE−/− mice using the non-TZD PPARα/γ coagonist 3q showed increased atherosclerosis, despite decreased plasma cholesterol, possibly as a result of a concomitant decrease in HDL and an increase in aortic expression of genes (vascular cell adhesion molecule (VCAM)-1, MCP-1, CD36, P-Selectin) associated with plaque development.110

**PPARδ**

The role of PPARδ has long been enigmatic, but recent studies have identified it as a regulator of lipid metabolism and energy expenditure.97 Four studies have evaluated the consequences of PPARδ activation on development of atherosclerosis in mice, but the impact of PPARδ on disease show apparent discrepancies. Transplantation of PPARδ-null bone marrow progenitor cells into LDLr−/− mice resulted in less atherosclerosis than in LDLr−/− mice receiving wild-type progenitor cells, suggesting that PPARδ is proatherogenic.126 Li et al found that the PPARδ agonist GW7842 did not alter the progression of atherosclerosis in hyperlipidemic LDLr−/− mice, compared with untreated mice, despite a decrease in inflammatory cytokine expression in atherosclerotic lesions.111

Whereas activation of PPARδ proved to be ineffective under hyperlipidemic conditions, a recent study using the same mouse model but under more moderate hypercholesterolemic conditions reports a reduction of atherosclerosis with the PPARδ agonist GW0742X.127 The authors demonstrate that antiinflammatory effects of GW0742X in aorta and adipose tissue may contribute to the antiatherogenic effect observed. A significant reduction of plasma cholesterol and triglyceride levels and a strong decrease of atherosclerosis with PPARδ agonists were also observed in E3L mice in our
laboratory (unpublished data, 2007). In all, these studies with PPARγ agonists show that these agonists can exert atheroprotective effects in cases of mild or moderate levels of hypercholesterolemia, but that they may not be as effective as the PPARα- and γ-selective agonists.28 The currently available evidence from mouse animal models supports the concept that PPARα and PPARγ not only act to control lipid and glucose at a systemic level but also have important actions in cells that determine the development and clinical course of atherosclerosis.

LXRs
Two LXR isoforms have been described so far, LXRα and LXRβ. LXRβ has a ubiquitous tissue distribution, whereas LXRα predominates in liver, adipose tissue, and intestinal tissue, as well as macrophages. Natural LXRs ligands include intermediates and end products of sterol metabolism, and both isoforms appear to respond to the same natural and synthetic ligands. The ability of LXRs to control genes involved in cholesterol efflux in macrophages, hepatic bile acid synthesis, and intestinal cholesterol absorption, to limit inflammation, and to improve glucose tolerance makes them attractive targets for the development of drugs for treatment of cardiovascular, metabolic, or inflammatory diseases. It is thought that LXR agonists can effectively mediate cholesterol efflux and prevent foam cell formation through upregulation of ABCA1 cholesterol transporter and apoE. Another direct effect of LXR agonists on vessel wall are antiinflammatory effects in macrophages which are thought to slow down lesion progression.

However, LXRs also regulate genes participating in lipogenesis and induce hypertriglyceridemia in mice.129,130 These findings raise the question of whether the activation of LXRs promotes or inhibits atherosclerosis.

Both gain-of-function and loss-of-function studies indicate that activation of the LXR pathway is antiatherogenic. Treatment of ApoE<sup>−/−</sup> and LDLr<sup>−/−</sup> mice (Table 1) with a synthetic LXR agonist (GW3965) decreases atherosclerosis by more than 50%.129 Furthermore, GW3965 was shown to exert direct effects on vascular gene expression, increasing expression of the ATP-binding cassette subfamily genes ABCA1 and ABCG1 (involved in cholesterol efflux from macrophages) in the aortas of mice. Comparable results were obtained by Terasaka et al who observed that T-0901317, a synthetic LXR agonist, significantly inhibited the development of atherosclerotic lesions in LDLr<sup>−/−</sup> mice achieved by bone marrow transplantation, evidence was provided that these atheroprotective effects of T-0901317 were dependent on LXR activity in macrophages.132 In addition to their effects on cholesterol metabolism, activation of the LXRs by synthetic agonists has an inhibitory effect on inflammatory gene expression in macrophages by antagonizing NF-κB signaling,97 pointing to a second potentially antiatherogenic mechanism of these receptors. Studies performed in our laboratory also showed a strong antiatherosclerotic effect and confirmed the quenching of NF-κB activity with T-0901317 in E3L mice (unpublished data, 2007). To date, the relative contribution of enhanced cholesterol efflux and repression of inflammation to the beneficial activity of the LXR activators is unclear and constitutes an important topic for future research.

Unfortunately, a major side effect of LXR agonists remains the increase of VLDL and severe lipogenesis observed in rodents. A recent study demonstrates that separate activation of LXRα and LXRβ yields distinctive lipid outcomes in vivo.133 Most importantly, the results lend support to the idea that LXRβ-selective agonists may raise HDL-cholesterol and stimulate macrophage cholesterol efflux without causing liver triglyceride accumulation.

Summary and Concluding Remarks
The ideal animal model of atherosclerosis will mimic the human subject metabolically and pathophysiologically and will develop lesions comparable to those found in humans. Given the complex multifactorial character of atherogenesis, no one species will be suitable for all studies. Mouse models have proved useful to study atherosclerotic lesion development and exploring effective therapeutic approaches. However, differences in anatomy, lipid metabolism, and gene expression complicate translation of experimental results obtained in mice to humans. First, unlike in humans, the primary circulating lipoprotein in mice is HDL, which makes wild-type mice very resistant to the development of atherosclerosis.17

Also, whereas human liver produces only apoB100, mice produce both apoB100 and its truncated form, apoB48.15 To make lipoprotein profiles more human-like and to overcome resistance to atherosclerosis, knockout (eg, ApoE<sup>−/−</sup>, LDLr<sup>−/−</sup>, LDLr<sup>−/−</sup>/ApoBEC<sup>−/−</sup>) and transgenic (eg, E3L, LDLr<sup>−/−</sup>/ApoB<sup>−/−</sup>) mouse models have been generated. Differences in the severity of hypercholesterolemia, the location of atherosclerotic plaques (aorta in mice versus coronary arteries in humans), the course of the disease (fulminate in mice versus indolent in humans), and the absence of end-stage ischemic lesions, as well as the fact that murine atherosclerosis is not associated with occlusive coronary
artery disease, myocardial infarction, cardiac dysfunction, and premature death, which are the hallmarks of human coronary heart disease, might hinder the translation of the findings from mice to humans. Second, some genes that regulate glucose, fatty acid, and cholesterol metabolism are expressed differentially across species. For example, two adipokines, adiponectin and resistin, are expressed by both adipocytes and macrophages in humans, but only by adipocytes in mice. Similarly, PPARα, the target of hypolipidemic fibric acids, has been found to play an essential role in regulating cholesterol efflux from human, but not mouse, macrophages. Also, CETP, a plasma glycoprotein that facilitates transfer of cholesteryl esters from HDL to apo-B-containing lipoproteins such as VLDL and LDL, is present in humans but not in mice. E3L mice have recently been crossbred with human CETP expressing mice and display the expected shift in distribution of cholesterol from HDL toward LDL/LDL, as previously also observed in ApoB/hCETP transgenic mice and in a transgenic hCETP rat line.

Treatment of E3L/CETP mice with fenofibrate, atorvastatin and niacin (unpublished data, 2007) also resulted in decreased plasma cholesterol and triglyceride levels and elevated LDL similarly as observed in humans.

Last, transcription factors that control gene expression in one species might not be crucial regulators in another. This difference is exemplified by the regulation of proteins which the target of hypolipidemic fibric acids, has been found to play an essential role in regulating cholesterol efflux from human, but not mouse, macrophages. Also, CETP, a plasma glycoprotein that facilitates transfer of cholesteryl esters from HDL to apo-B-containing lipoproteins such as VLDL and LDL, is present in humans but not in mice. E3L mice have recently been crossbred with human CETP expressing mice and display the expected shift in distribution of cholesterol from HDL toward LDL/LDL, as previously also observed in ApoB/hCETP transgenic mice and in a transgenic hCETP rat line.

Treatment of E3L/CETP mice with fenofibrate, atorvastatin and niacin (unpublished data, 2007) also resulted in decreased plasma cholesterol and triglyceride levels and elevated LDL similarly as observed in humans.

| TABLE 2. Summary of Effects of Pharmaceutical Modifiers in the 3 Mouse Models Reviewed |
|---------------------------------|-----------------|-----------------|-----------------|
| Statis                           | chol            | athero          | chol            | athero          | chol            | athero          |
| ACE inhibitors                   | ↔               | va              | ↔               | va              | ↔               | va              |
| AT-R antagonists                 | ↔               | ↓               | ↔               | ↓               | ↔               | ↓               |
| PPAR agonists                    |                 |                 |                 |                 |                 |                 |
| PPARα                            | ↑               | ↔               | va              | ↓               | ↓               | ↓               |
| PPARγ                            | ↔               | ↓               | va              | ↓               | nd              | nd              |
| PPARδ                            | nd              | nd              | ↔               | va              | ↓               | ↓               |
| PPARα/γ                          | va              | ↓               | ↔               | ↓               | ↓               | ↓               |
| LXR agonists                     |                 |                 |                 |                 |                 |                 |
| LXRα,β                           | ↓               | ↓               | ↓               | ↓               | ↑               | ↑               |
| Miscellaneous                    |                 |                 |                 |                 |                 |                 |
| Ezetimibe                         | ↓               | ↓               | ↓               | ↓               | ↓               | ↓               |
| ACAT-inhibitors                  | ↓               | ↓               | nd              | nd              | ↓               | ↓               |
| va indicates variable; nd, not determined. |
lipoproteins; the E3L model comes out better to the other models and appears more useful in predicting effects in humans. In addition to the E3L model there are other knock-in mouse models, such as ApoE2, ApoE3, ApoE4 knock-in (ki) mice,14,16 that could potentially also be responsive to lipid-lowering drugs. Indeed E2ki mice were shown to respond to the PPARα activator fenofibrate, but the PPARγ activators rosiglitazone and pioglitazone did not affect plasma cholesterol or atherosclerosis in this model.11,14 In general, these new knock-in models have not been thoroughly investigated yet and await further testing and validation.

Importantly, apoe not only affects the clearance of lipoproteins, but has other, notably antiinflammatory, immunomodulatory, and antiatherogenic properties.9,13 Because of the complete absence of apoe the study of drug effects in the ApoE−/− model is restricted and may not necessarily mimic or be similar in humans. This and the insensitivity of ApoE−/− and LDLr−/− mice to many hypolipidemic compounds is the more important because the current search for improving existing therapies and finding and testing new antiatherosclerotic compounds are predominantly centered around the pharmaceutical targets dealt with in this review, viz. hypercholesterolemia, hypertriglyceridemia, and inflammation. Important areas for investigation and new avenues for treatment include combination therapy (for example, statins with ACE inhibitors, PPARs [single or co-agonists], CETP inhibitors), design of more potent and selective PPARα activators, development of synthetic LXR agonists that overcome the undesired effects of the currently available synthetic LXR agonists on plasma lipids, elevation of HDL cholesterol, or slowing down and dampening (chronic) systemic and vascular inflammation.

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Disclosures

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References


70. Auerbach B, Sliskovic DR. The combined effect of inhibiting both ACAT and Ca(2+) channel blockade on atherosclerosis in apoE-deficient mice. Arterioscler Thromb Vasc Biol. 2004;24:534–539.


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