Diet and Murine Atherosclerosis

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Abstract—Lipid-enriched diets are often used to induce or accelerate the rate of atherosclerotic lesion development in murine models of atherosclerosis. It appears that the induction of persistent hypercholesterolemia to levels \( \geq 300 \) mg/dL is required for the development of experimental atherosclerosis in the mouse. A variety of different diets have been used that vary in the level of cholesterol, the level and type of fatty acid, and the absence or presence of cholate. Each of these components as well as the protein source has been shown to influence lipoprotein level and/or atherosclerosis, with dietary cholesterol being the major proatherogenic component. In some instances the effects of these components on the expression of hepatic genes relevant to lipid homeostasis has been observed. An appreciation of the effect of the differences in diet composition on these processes is important to compare results from different atherosclerosis studies, so the composition of the diets used should always be reported or referenced. Cholate should not be used unless its effects are being specifically investigated. (Arterioscler Thromb Vasc Biol. 2006;26:242-249.)

Key Words: atherosclerosis ■ cholate ■ cholesterol ■ diet ■ fatty acids ■ murine models

The mouse has become the pre-eminent species for the study of experimental atherosclerosis. Mice in the wild generally consume a low-fat diet and this forms the basis for the formulation of the standard mouse or rodent chow containing 4% to 6% fat (weight fat per weight of diet) and a cholesterol content \(<0.02\% \) (w/w). The current popularity of the mouse as a species for the study of experimental atherosclerosis rests on the ease with which its genome can be manipulated. Almost all of these genetic manipulations in mice used for atherosclerosis research depend on the disruption of normal lipoprotein regulation and metabolism. The mouse is naturally a high high-density lipoprotein (HDL) animal, bearing relatively low steady-state concentrations of very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL). All dietary and genetic means for promoting atherosclerosis involve a change in this balance with the apoprotein B (apoB) containing lipoproteins becoming more dominant. Most strains of mice are relatively resistant to the development of atherosclerosis even with a change in the lipoprotein profile. Among mouse strains, C57BL/6 mice are the most atherosclerosis sensitive strain and therefore the most widely used. Most of what follows in this review focuses on experiments with this murine strain, whether as wild-type mice or mice with genetic manipulations.

We have summarized the phenotypes of the various mouse models used for atherosclerosis research in Table 1. The phenotype characteristics refer to steady-state plasma cholesterol levels, dominant lipoprotein phenotype, and a gross description of the lesion distribution and phenotype. A common feature in each of these models is the accumulation of VLDL and/or LDL in the plasma. In the genetic models this is accomplished by the lack of the ligand (apoE) or the receptor (LDLR) playing major roles in the clearance of non-HDL lipoproteins. Some animal models develop quite complex and widespread atherosclerosis while being fed normal mouse chow diet. Included in this group are the apoprotein E-deficient (apoE \(^{-/-}\) ) mouse model, \(^1-4\) and more complex genetic models based on LDL receptor deficiency (LDLR \(^{-/-}\) ) in which apoB100 is the major form of apoB present in the plasma. The latter is accomplished by over expression of human apoB as a transgene \(^5\) or elimination of apoB48 expression by deletion of the apoB editing enzyme apobec-1 so that murine apoB100 is exclusively expressed. \(^6\) Plasma total cholesterol levels ranged from 500 to 800 mg/dL. In the apoE-deficient background, most of the accumulating non-HDL lipoproteins contain apoB-48. However, manipulation of the apoB gene to allow for the exclusive expression of either apoB48 or apoB100 in the apoE \(^{-/-}\) background exerted only modest effects on the atherosclerosis susceptibility, especially when mice with similar plasma cholesterol levels were compared. \(^7\) Each of these models when fed the Western-type diet (Table 2) develop approximately twice the plasma cholesterol levels, ranging from 1000 to 1600 mg/dL (Table 1), and develop atherosclerosis throughout the vascular tree more rapidly—advancing atherosclerosis by at least 6 weeks based on our experience in the apoE \(^{-/-}\) model (unpublished data).

It was generally thought that LDLR \(^{-/-}\) mice maintained on chow would not develop atherosclerosis to any significant extent. \(^8\) Many of the earlier experiments were performed with mice in a mixed genetic background. However, using inbred animals deficient in the LDLR, mice develop modest lesions
TABLE 1. Phenotype of Murine Models of Atherosclerosis

<table>
<thead>
<tr>
<th>Mouse Model</th>
<th>Diet</th>
<th>TC (mg/dL)</th>
<th>Prominent Lipoprotein Phenotype</th>
<th>Lesion Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>Chow</td>
<td>600</td>
<td>VLDL, IDL</td>
<td>Widely distributed</td>
<td>3</td>
</tr>
<tr>
<td>LDLR&lt;sup&gt;-/-&lt;/sup&gt;, apobec-1&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>Chow</td>
<td>400–650</td>
<td>LDL</td>
<td>Widely distributed, complex</td>
<td>6</td>
</tr>
<tr>
<td>ApoB tg, LDLR&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>Chow</td>
<td>800</td>
<td>LDL (modest VLDL)</td>
<td>Widely distributed, advanced</td>
<td>5</td>
</tr>
<tr>
<td>LDLR&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>Chow</td>
<td>200–300</td>
<td>LDL</td>
<td>Very modest</td>
<td>10</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>Western type</td>
<td>1300–2000</td>
<td>VLDL, IDL</td>
<td>Widely distributed, complex</td>
<td>*</td>
</tr>
<tr>
<td>LDLR&lt;sup&gt;-/-&lt;/sup&gt;, apobec-1&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>Western type</td>
<td>1600–1750</td>
<td>VLDL, LDL</td>
<td>Widely distributed, complex</td>
<td>6</td>
</tr>
<tr>
<td>LDLR&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>Western type</td>
<td>1500–2000</td>
<td>VLDL, LDL</td>
<td>Widely distributed</td>
<td>12</td>
</tr>
<tr>
<td>LDLR&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>High fat, cholate</td>
<td>1880</td>
<td>VLDL, LDL</td>
<td>Widely distributed</td>
<td>8</td>
</tr>
<tr>
<td>ApoB tg</td>
<td>Paigen diet (cholate)</td>
<td>310</td>
<td>LDL</td>
<td>Widely distributed, fatty streaks</td>
<td>14</td>
</tr>
<tr>
<td>Wild-type</td>
<td>Paigen diet (cholate)</td>
<td>140–190</td>
<td>VLDL, HDL</td>
<td>Small, proximal aorta (fatty plaques)</td>
<td>13, 20</td>
</tr>
</tbody>
</table>

*Reardon CA and Getz GS unpublished.

in the aortic root<sup>9</sup> or throughout the aorta.<sup>10</sup> Similar lesions were observed on a semisynthetic low-fat, low-cholesterol diet.<sup>11</sup> When fed the Western-type diet, all LDLR<sup>-/-</sup> mice develop widespread atherosclerosis, with the lesions becoming quite complex with increasing duration of feeding.<sup>12</sup> However, wild-type mice<sup>13</sup> and mice expressing human apoB-100 transgene<sup>14</sup> do not develop lesions unless fed a high-cholesterol, cholate-containing diet.

Wild-Type Mice

The initiation of atherogenesis in wild type rodents by dietary manipulation was first described by Robert Wissler and colleagues in the 1950s.<sup>15–17</sup> This group used bile acid or bile salt as a stimulant for the induction of lesion formation in rats. Thomas and Hartroft<sup>18</sup> also used a high-fat diet containing 30% cocoa butter, 5% cholesterol, and 2% sodium cholate to induce myocardial infarction in rats. Beverly Paigen and colleagues mixed this diet with breeding chow containing at least 11% fat in 1 part to 3 parts to generate what has become known as the Paigen diet. The final composition of the Paigen diet is 15% fat, 1.25% cholesterol, and 0.5% sodium cholate.<sup>13,19,20</sup> The fat in this diet is primarily composed of saturated fatty acids derived from either cocoa butter or butter fat.<sup>19,20</sup> Because the predom-
inant fat in the Paigen diet is saturated, 1% corn oil is often added to avoid polyunsaturated fatty acid deficiency. The addition of cholate accentuates the hypercholesterolemia induced by the cholesterol and high fat content of the diet by facilitating fat and cholesterol absorption and perhaps by inhibition of cholesterol 7α-hydroxylase, the rate-limiting enzyme of cholesterol conversion to bile acid. This latter effect likely represents the major regulatory mode of action of cholate.

The Paigen diet was used to examine the sensitivity of different strains of wild-type mice to atherosclerosis. Sustained hypercholesterolemia induces the formation of very early fatty streak lesions in the aortic root and proximal aorta in atherosclerosis susceptible mouse strains. Seldom does this diet induce more advanced lesion in wild-type mice. Among the most atherosclerosis susceptible strains are the C57BL/6 mice. Other strains of mice were more resistant to the development of atherosclerosis, with some strains not developing any lesions. There is no correlation between the degree of response of plasma lipids to the diet and the extent of atherosclerosis sensitivity in different strains of wild-type mice. In fact, some quite atherosclerosis resistant strains actually have significantly higher non-HDL cholesterol levels in response to this diet than does the C57BL/6 mouse. The variation between strains has been used to map quantitative trait loci (QTL) for lipoprotein response and atherosclerosis response. The orthologs in the mouse genome may help to map QTL in the human genome.

The C57BL/6 strain was used by Paigen and her collaborators to fine-tune the effect of the lipid components of the diet on atherosclerosis and plasma lipids by systematically varying them within narrow ranges. Dietary cholesterol between 0.5% and 1.0% did not result in statistically significant differences in plasma lipids or proximal aorta lesion size. Similarly, there was no statistical difference in these parameters on varying the cholic acid content of the diet between 0.1% and 0.5%. However, lesion formation was more consistent with diets containing 1.0% cholesterol and 0.5% cholic acid. In contrast to cholesterol and cholic acid, the nature of the fat in the diet did influence the extent of lesion development in the proximal aorta. C57BL/6 mice were fed diets containing 1% cholesterol, 0.5% cholic acid, and 1 of 7 different sources of dietary fat, each at 15%. The diets varied in the proportion of short-chain (8 to 14 carbons) and long-chain (16 to 18 carbons) saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. The development of fatty streak lesions in the proximal aorta was correlated with the percentage of saturated fatty acid in the fat source and inversely with the amount of monounsaturated fatty acid in the diet. Coconut oil, containing predominantly short-chain saturated fatty acids, was an exception, exhibiting smaller lesions than expected for a diet enriched in saturated fat. Dairy fat was the most atherogenic of the seven food fats used. The size of lesions was insensitive to variation in the amount of polyunsaturated fatty acids in these diets, which varied between 1% and 15% of total dietary fatty acids. Whereas all of the 7 diets increased the level of non-HDL lipoproteins and hepatic cholesterol ester and triglyceride content, these levels did not correlate with the size of the proximal aortic lesions.

In addition to examining the effects of varying the levels of cholesterol or cholate in the high-fat, cocoa butter diet on atherosclerosis, a recent experiment has been reported in which each of these 3 components have been selectively omitted. The presence of dietary cholesterol was necessary to elevate plasma cholesterol levels. In the absence of cholate, plasma cholesterol levels were slightly reduced, but the absence of fat had no effect on plasma cholesterol levels. However, the absence of either cholate or cholesterol stimulated plasma triglyceride levels. Also emerging from these experiments is the observation that dietary cholesterol induces genes of acute inflammation in the liver, whereas cholate induces hepatic genes particularly related to fibrosis. A close inspection of the array data suggests that some of the gene changes noted with cholesterol may be mediated in part by effects on bile acid metabolism.

The effects of high-cholesterol diets on wild-type C57BL/6 mice has also been studied using semisynthetic diets based on the AIN-76a formulation containing 4.3% fat (1.9% cocoa butter and 2.4% soy oil) supplemented or not with 0.5% cholesterol. After 1 week of feeding, the diet supplemented with cholesterol did not have an influence on plasma cholesterol levels in either males or females, while substantially increasing liver cholesterol and cholesteryl ester content. Gene array analysis on the liver of these mice identified >60 genes that were either upregulated or downregulated by dietary cholesterol. A small proportion of the cholesterol regulated genes were also identified in the study by Vergnes et al. From this, one may conclude that plasma cholesterol is not always a sensitive monitor of a change in cholesterol homeostasis. In contrast, in rabbits, cholesterol feeding regularly influences plasma cholesterol levels. The difference in response of plasma cholesterol between rabbits and mice is not attributable to a differential regulation of 7α hydroxylase, the rate-limiting enzyme in the conversion of cholesterol to bile acid.

The cholate containing diet has been widely used for atherosclerosis studies, even up to the present time. However, cholate has pleiotropic effects and its use has the potential to confound interpretation both with respect to lipoprotein homeostasis and the chronic inflammation that is at the core of athero sclerotic lesions. As a bile salt, it facilitates cholesterol absorption and with the hepatobiliary circulation exerts a feedback control on cholesterol transformation to bile acid. Thus, its presence in the diet will increase cholesterol loading and hence hypercholesterolemia. As mentioned, it also promotes the expression of genes involved in hepatic fibrosis. Furthermore, cholate is a ligand for the nuclear hormone receptor FXR, whose activity regulates the expression of multiple genes involved in lipoprotein metabolism. Among these genes are apoCII and apoCIII, whose alterations in expression could account for the fact that the presence of cholate in the diet is associated with a lower plasma triglyceride than is the case for similar diets without cholate. In LDLR−/− mice, feeding a diet containing 0.5% cholate for 5 to 7 months resulted in obvious skin xanthomata, which we have not observed in our experiments feeding a Western-type diet for 3 to 5 months. Because of these complex effects we believe that cholate should not be used in atherogenic diets unless the specific effects of cholate are being investigated.

**LDLR-Deficient Model**

Aside from the series of articles describing dietary effects in wild-type C57BL/6 mice, the most frequently used murine model for the study of diet and atherosclerosis is the LDLR−/−
mouse. Compared with wild-type mice, LDLR−/− mice maintained on chow diet exhibit elevated levels of LDL but develop vascular lesions very slowly, even in mice fully inbred into the C57BL/6 background.8,10 However, the addition of fat and cholesterol to the diet results in significant increases in the level of VLDL and VLDL remnants as well as further increases in LDL levels, and the relatively rapid development of advanced atherosclerosis over 2 to 3 months. The first study to demonstrate the usefulness of the LDLR−/− mouse for the study of atherosclerosis used a modified cholate containing diet with 7.5% cocoa butter, 1.25% cholesterol, and 0.5% cholate.8 The lipoprotein phenotype of these animals was similar to that seen in human patients with familial hypercholesterolemia (Table 1).

The ability to induce advanced atherosclerosis in LDLR−/− animals with diets has provided the opportunity for examining the effect of varying the level of cholesterol and the nature and level of the dietary fat and assessing the vascular response in more detail than in wild-type mice. Examples are listed in Table 2. The simplest extension of the studies in wild-type mice is to feed LDLR−/− mice the same diet lacking cholate [or cholic acid], ie, 15% fat and 1.25% cholesterol (for example, reference 29). This dietary formulation is fairly widely used. The source of the fat is either cocoa butter, which is free of cholesterol, or anhydrous milk fat, which adds up to 0.05% of cholesterol. This additional cholesterol is not expected to have a significant impact on a base of 1.0 to 1.25% cholesterol.

To define the role of dietary cholesterol and cholate in LDLR−/− mice, semisynthetic diets built on the AIN-76a formulation were compared.10 The diets contained either 20% fat with 0.5% cholesterol, 20% fat with 1.25% cholesterol, or the latter diet supplemented with 0.5% cholate. The fat source was primarily cocoa butter, with a small amount of soy oil added as a source of essential fatty acids. In the absence of cholate, increasing the cholesterol content of the diet from 0.5% to 1.25% had no significant effect on plasma lipids, lipoproteins, or total aortic lesion area. However, the addition of sodium cholate to the high-fat, high-cholesterol diet increased lipoproteins but only slightly increased aortic lesion area, especially in the thoracic aorta. Thus, cholate is not required for robust lesion development in the LDLR−/− mouse model. The lesions in the cholate diet group were qualitatively similar to those seen in the cholate-free, high-cholesterol diets. There was no evidence of cholate-induced hepatotoxicity in these experiments. The mice used in these experiments were not in a homogeneous C57BL/6 genetic background.

This work was extended by Teupser, Persky, and Breslow,11 who compared various levels of dietary cholesterol supplementation of a low-fat (4.3%) semisynthetic diet in LDLR−/− mice fully inbred in the C57BL/6 genetic background. Five dietary cholesterol levels were compared (0.0%, 0.02%, 0.15%, 0.30%, and 0.5%). Relative to the mice fed the diet without cholesterol, plasma cholesterol levels were not elevated in the 0.02% cholesterol diet group. Interestingly, the 3 highest cholesterol diets induced similar higher levels of plasma cholesterol, caused primarily by elevations in VLDL and LDL cholesterol. Cholesterol levels in the liver paralleled that in the plasma. Lesion size was also similar in the three highest cholesterol diet groups at both the aortic root and brachiocephalic artery after 16 weeks of diet feeding, and in all cases the lesions were significantly larger than was observed with the diets containing 0% and 0.02% cholesterol. Therefore, it appears that the greatest sensitivity of the LDLR−/− model to cholesterol is between dietary cholesterol levels of 0.02% and 0.15%. These experiments make the strong point that cholesterol, rather than the high fat content, is the major atherogenic component of the high-fat/high-cholesterol diet.

By far, the most widely used high-fat diet for atherosclerosis experiments is the so-called Western-type diet, which contains 21% fat and 0.15% cholesterol. The source of the fat is usually anhydrous milk fat, which contributes an additional 0.05% cholesterol, making the total cholesterol in the diet 0.2%. This diet was first used by Plump et al12 in their article describing the apoE−/− mouse. This diet is now used for most experiments involving the treatment of either LDLR−/− or apoE−/− mice with a high-fat, high-cholesterol diet, except in those cases in which the influence of specific dietary constituents on lipoprotein metabolism or atherosclerosis is being explored. In our experience, feeding a Western-type diet leads to a rapid increase in plasma cholesterol in LDLR−/− deficient mice, so that by 2 weeks a new equilibrium level of plasma lipid and lipoproteins is achieved. This diet also increases the titer of autoantibodies to an epitope found on oxidized LDL and this increase is correlated with the extent of atherosclerosis in the LDLR−/− mice.13 The Western-type diet with 21% fat and 0.15 to 0.20% cholesterol and a diet containing 18% milk fat and 0.25% cholesterol yield similar plasma lipid levels and atherosclerotic lesion size in the aortic root and innominate artery.12 This is consistent with results using low-fat, semisynthetic diets showing that 0.15% dietary cholesterol induces the same level of atherogenesis as does 0.5% dietary cholesterol.11 It is worth noting that the Western-type diet sold by Harlan Teklad is not precisely the same diet as that provided by Research Diets, Inc. The major differences are in the carbohydrate sources and that the Research Diets Western-type diet contains 1.0% corn oil in addition to the 21% milk fat. Thus, Research Diets Western-type diet is less likely to be accompanied by any signs of essential fatty acid deficiency.

A modified Western-type diet was compared with a high fructose diet to examine the role of insulin resistance in atherogenesis.14 The modified Western-type diet involved a reduction of milk fat to 16% and the addition of 5% lard to maintain the 21% fat composition of the diet. The fructose diet also contained 5% lard, but no milk fat. No cholesterol was added to either diet, although the presence of the animal fat will contribute some dietary cholesterol. The modified Western-type diet produced somewhat lower plasma cholesterol levels than is usually observed with the standard Western-type diet, (ie, ~900 mg/dL contrasted with a level that is approximately twice this). Interestingly, the modified Western-type diet induced more insulin resistance, but less atherosclerosis, than the fructose diet. This study indicates that high dietary saturated fat in the presence of low cholesterol levels can also promote the development of aortic atherosclerosis. Furthermore, the supervision of insulin resistance on hypercholesterolemia does not necessarily aggravate atherosclerosis.
LDLR−/− mice backcrossed to C57BL/6 mice for >10 generations have been fed isocaloric diets that contained 0.2% cholesterol with fat derived from combinations of different plant oils to generate diets enriched in saturated fatty acids, monounsaturated fatty acids, and n-6 polyunsaturated fatty acids. The total amount of fat in the diets was 18.5% except for a diet that had half of the fat replaced with carbohydrate. The plasma cholesterol and apoB containing lipoprotein responses to these diets were rank ordered as monounsaturated > saturated > saturated > polysaturated. The aortic root atherosclerosis response was highest for the carbohydrate and monounsaturated fat diets compared with the saturated fat diet. Thus, in contrast to wild-type C57BL/6 mice, a monounsaturated fat diets compared with the saturated fat diet. In these studies there was a good correlation between VLDL cholesterol and lesion size. It is not ascertained whether the predominant change in VLDL cholesterol levels in the LDLR−/− animals fed these diets is the result of changes in production or catabolic rates. In a slightly different dietary setting (18% fat and 0.25% cholesterol) and in a mixed C57BL/6 and 129 background, a significant atheroprotective effect of safflower oil (n-6 polyunsaturated fatty acid rich) contrasted with milk fat was noted in LDLR−/− mice. Whether formulating saturated fat diets with vegetable fat or animal fat will influence the lipoprotein and atherosclerosis response in LDLR−/− mice remains to be conclusively determined.

Using LDLR−/−RAG−/− mice, we have noted an interaction of the immune system and dietary fatty acid in the LDLR−/− background. Immune deficiency in LDLR−/− mice results in lower plasma lipoprotein level when fed chow or a diet containing milk fat as the source of the dietary fat (18.5% fat and 0.25% cholesterol). This was not the case when the milk fat was replaced with safflower oil (unpublished data). Difference in hepatic lipoprotein production is not responsible for the lower plasma lipids. There is evidence in the literature to suggest that there is a partial impairment of immune function in the presence of high levels of polyunsaturated fatty acid, perhaps mediated by effects on the PPAR family of nuclear hormone receptors.

There are numerous studies of murine atherosclerosis that have used the Western-type diet in the LDLR−/− model. They have not all been cited here for want of space and because few have addressed the impact of dietary components on atherosclerosis.

More Complex LDL Receptor-Deficient Models

There are 2 additional genetic models based on the LDLR−/− background that develop significant atherosclerotic lesions without the need for dietary supplementation with high fat or high cholesterol. The first of these is the animal model lacking both the LDLR and the apoB editing enzyme apobec-1. The lipoprotein phenotype in these animals on chow more closely resembles that of familial hypercholesterolemia, with LDL being the predominant lipoprotein, than is the case for LDLR−/− animals fed a high-fat diet. The average plasma cholesterol ranges from 400 to 650 mg/dL on chow and >1600 mg/dL when fed a Western-type diet. Extensive aortic atherosclerosis was noted by 28 to 32 weeks of age in animals fed chow. Despite its similar lipoprotein profile to humans, this model has not been used for dietary studies.

The second of these complex models involves the addition of a transgene for human apoB100 to the LDLR−/− animal. The mean plasma cholesterol for these animals fed chow is ~800 mg/dL. Whereas LDL is the predominant lipoprotein, the presence of the apoB100 transgene markedly increased the triglyceride content of the plasma and the LDL, and increased atherosclerosis in the whole aorta. In an LDLR−/−, apoB100-only model, which also develops atherosclerosis on a chow diet, the development of atherosclerosis can be significantly attenuated by essentially eliminating hepatic lipoprotein secretion and thus drastically lowering plasma cholesterol levels. The reduction in hepatic lipoprotein secretion was achieved by Cre-mediated removal of the hepatic microsomal triglyceride transfer protein gene. Elimination of hepatic VLDL secretion was equally atheroprotective whether animals were fed chow or a high-fat diet. Intestinal lipoprotein secretions were unaffected in this model.

The LDLR−/−, apoB100 transgenic mouse model has been used by Rudel et al to study the effect of diets with different fatty acid composition, but without any additional dietary cholesterol supplementation. The effect of these diets on lipids and atherosclerosis were contrasted with a commercial diet containing 4.6% fat primarily composed of polyunsaturated fats and 0.06% cholesterol. Palmitic acid, oleic acid-rich safflower oil, safflower oil, fish oil, and a trans fatty acid blend were used to obtain diets enriched in saturated fat, cis monounsaturated fatty acid, n-6 polyunsaturated fatty acid, n-3 polyunsaturated fatty acid or trans monounsaturated fatty acid, respectively. The total fat content of each diet was 4% and the cholesterol content was 0.005%. Total plasma cholesterol ranged from 2000 to 600 mg/dL and was rank ordered from trans monounsaturated fatty acids> saturated fatty acids> cis monounsaturated> n-6 polyunsaturated fatty acids> n-3 polyunsaturated fatty acids and commercial diet. There was a very good positive correlation between total plasma cholesterol levels and the extent of aortic lesion with the different diets. Like the LDLR−/− mice, cis monounsaturated fatty acid provided little if any atheroprotection in the LDLR−/−, apoB100 transgenic mice.

ApoE-Deficient Model

The study of murine atherosclerosis was greatly stimulated following the description and general availability of apoE−/− mice, which develop lesions while being fed a chow diet. Unlike the wild-type mice, which develop lesions only in the proximal aorta, apoE−/− mice develop lesions throughout the macrovasculature. Over time these lesions become quite complex, progressing well beyond the fatty streak. The Western-type diet accelerates the development of lesions of all stages, from the foam cell lesion to the fibrous plaque. The lesions from animals fed the Western-type diet are more lipid-rich than in those animals fed chow.

Mice heterozygous and homozygous for the apoE-null allele and wild-type mice have been compared while being
fed chow or the high-fat/cholesterol/cholate Paigen diet. On chow, heterozygous apoE-deficient mice (apoE<sup>+/−</sup>) are essentially indistinguishable from wild-type animals with respect to plasma lipids and lipoproteins and do not develop atherosclerosis in the proximal aorta. In contrast, the homozygous apoE-deficient mice (apoE<sup>−/−</sup>) were hypercholesterolemic and developed significant atherosclerosis. When fed the high-fat/cholesterol/cholate diet, the heterozygous apoE<sup>+/−</sup> mice also had plasma lipid levels comparable to wild-type mice. The remaining wild-type apoE allele was sufficient to generate plasma apoE levels equal to those of wild-type animals. Despite this, larger lesions were observed in the homozygous apoE<sup>−/−</sup> mice than wild-type animals (by ∼10-fold). Thus, the absence of one allele of apoE renders the mice more susceptible to diet-induced atherosclerosis. In contrast, the homozygous apoE<sup>−/−</sup> mice on the atherogenic diet were significantly hypercholesterolemic (>2000 mg/dL) and proximal aorta lesions were at least 50-fold larger than in the heterozygous mice. Lipid deposits were detected in the liver of all mice fed the high-fat/cholesterol/cholate diet and were also noted in other organs in the homozygous apoE-deficient mice, such as esophagus, colon, lung, and skin.

Like the LDLR<sup>−/−</sup>, apobec-1<sup>−/−</sup> model, a limited number of studies have been performed in the apoE<sup>−/−</sup> mice to explore the role of dietary components on lipoprotein metabolism and atherosclerosis. With respect to the lipid composition of the diets, the role of dietary fatty acids from different sources was examined in the study by Merkel et al. However, unlike the modest effects of these dietary fats on lipoproteins and atherosclerosis in the LDLR<sup>−/−</sup> model, no effects were observed in the apoE<sup>−/−</sup> model. However, the apoE<sup>−/−</sup> mice have been used to explore the effects of protein source. Soy protein-fed apoE<sup>−/−</sup> animals have less atherosclerosis than those for whom casein was the major protein source. These experiments used 10% olive oil as the source of fat in the diet with or without the addition of 1% cholesterol and 0.25% cholate. Whether cholesterol or cholate were present, soy protein led to lower atherosclerosis in various aortic segments, despite similar plasma cholesterol levels. Atheroprotection by soy was manifested in the LDLR<sup>−/−</sup> mice as well. Soy protein has less methionine, a precursor of homocysteine, than is the case for casein. However, plasma homocysteine levels were not significantly different in the mice fed the 2 different diets. Soy protein also has more arginine, the substrate for nitric oxide production. Additional studies involving diets in which the isoflavones were extracted with alcohol have implicated this component of soy as contributing to its atheroprotective effect in addition to the protein component. That the soy isoflavones may be functioning as phytoestrogens is suggested by the requirement for the presence of the estrogen receptor-α for the manifestation of its atheroprotective effect.

**More Complex ApoE-Deficient Models**

The elevated lower density lipoproteins (VLDL and remnants) of apoE<sup>−/−</sup> mice are characterized by a disproportionate elevation of apoB48. Interestingly, chow-fed apoE<sup>−/−</sup> mice expressing only apoB100 had modestly lower cholesterol levels in the plasma and less extensive atherosclerosis than apoE<sup>−/−</sup> mice expressing only apoB48. In all these animals there was a good correlation between total plasma cholesterol and extent of aortic atherosclerosis.

ApoE<sup>−/−</sup> mice have been compared with LDLR<sup>−/−</sup> mice and with mice deficient in both apoE and the LDLR while fed a normal chow diet or a high-cholesterol, high-fat, and cholate diet. ApoE is a ligand present on chylomicron remnants that allow them to be removed by the LDLR, the LDLR-related protein, and perhaps also heparan sulfate proteoglycans. However, it must be borne in mind that apoE may have other influences on lipoprotein and nonlipoprotein metabolic and cellular functions. For example, in its absence, cholesterol absorption is reduced by 25%.47

On a chow diet, LDLR<sup>−/−</sup>-deficient mice have approximately half the total plasma cholesterol of apoE<sup>−/−</sup> mice, whereas plasma cholesterol levels in the apoE<sup>−/−</sup>, LDLR<sup>−/−</sup> mice were comparable to the apoE<sup>−/−</sup> mice. The apoE<sup>−/−</sup>, LDLR<sup>−/−</sup> mice on chow have an elevation of both LDL and remnants. With the feeding of a 1.25% cholesterol, 7.5% cocoa butter, and 0.5% cholate diet, all mice exhibit a rapid increase in total plasma cholesterol levels so that by 2 weeks they are close to their steady state level. Intermediate-density lipoprotein (IDL)/LDL levels are similar in all 3 mice fed the high-fat/cholesterol diet, whereas chylomicron remnant/VLDL levels are similar in the apoE<sup>−/−</sup> mice and apoE<sup>−/−</sup>, LDLR<sup>−/−</sup> mice. Remnant/VLDL levels are lower in the LDLR<sup>−/−</sup> mice.

**Diet and Regression of Atherosclerosis**

All of these discussions have focused on atherosclerosis initiation and progression. However, regression of atherosclerosis is also of major clinical and therapeutic significance. Related to this, there is an increasing appreciation of the dynamic nature of the atherosclerotic plaque. One must distinguish between retardation of progression and true regression of pre-existent lesions. Many experiments have been performed on regression in humans, nonhuman primates, and rabbits. More recently, regression has been approached in mice, using the 2 models on which this review has focused.

A novel approach to regression of pre-existing atherosclerotic lesions has involved the transplantation of a segment of the aorta from apoE<sup>−/−</sup> mice into a wild-type C57BL/6 recipient mouse having normal lipid levels (<100 mg/dL). Lesions in the transplanted aorta were reduced in size by ∼50% within 3 weeks. This was accompanied by a substantial reduction in macrophage foam cells. This approach has highlighted the dynamic nature of the lipid loaded macrophages, which can rapidly migrate from the transplanted segment to the draining lymph nodes. In a second study, apoE<sup>−/−</sup> animals maintained on the Western-type diet for 6 months, and therefore exhibiting advanced lesion in the aorta, were either transferred to a chow diet or used as donors of aortas for transplantation into chow fed apoE<sup>−/−</sup> mice. The plasma cholesterol was reduced by half from ∼1,000 mg/dL to ∼400 to 500 mg/dL, after diet shift and was similar to that in the recipients of the aortic transplants. Yet there was no evidence of regression in either group of animals when analyzed 5 months later. Together these studies suggest that the presence of apoE in the plasma, low plasma lipid levels,
or both are necessary to promote regression of atherosclerotic lesions.

A second novel model that has been used to study atherosclerosis regression in mice is the hypomorphic apoE/Mx1-Cre mouse that expresses an apoE4-like variant at 2% to 5% of normal levels of apoE. These mice have normal lipid levels while maintained on a chow diet. Yet when placed on a diet containing 16% fat, 1.25% cholesterol, and 0.5% cholic acid, the plasma cholesterol increased to \(\sim 1000\) mg/dL primarily because of an accumulation of remnants. This level of plasma cholesterol is \(\sim 5\)-times the level the same diet would induce in a wild-type mouse. After 18 weeks on this diet, the animals were placed on a chow diet for an additional 16 weeks either without or with the induction of the full expression of apoE (induced mice). The induction of apoE expression was achieved by activation of the Cre-mediated excision of the neomycin resistance cassette from intron 3 of the hypomorphic apoE gene. On switching to a chow diet, plasma lipid levels were significantly reduced and the plasma lipid profiles were similar in the uninduced and induced animals, with HDL being the primary plasma lipoprotein. The percent of the aorta containing atherosclerotic lesion was reduced by 40% in the uninduced animals and by a further 30% in the induced mice. Subendothelial foam cells were reduced in the lesions in both sets of mice, whereas in the induced group there was also a substantial reduction of neutral lipid from the fibrotic core of the lesions. These results indicate the sensitivity of atherosclerosis to the level of apoE expression in the presence of low plasma cholesterol and is reminiscent of the results on progression with heterozygous apoE\(^{-/-}\) mice on a high-fat diet.\(^{50}\)

Finally, adenoviral mediated hepatic overexpression of human apoE3 in LDLR\(^{-/-}\) mice maintained on a Western-type diet and with established atherosclerotic lesions resulted in a reduction in lesion size in the aorta and aortic root, as well as a reduction in the proportion of macrophages in the lesions. This occurred without any change in plasma cholesterol levels.\(^{54}\) This study highlights the importance of apoE expression on regression of atherosclerosis in the absence of effects on plasma lipids.

**Conclusion**

The information summarized has indicated that a variety of diets containing various types and levels of fatty acids, different levels of cholesterol, and the absence or presence of sodium cholate have been used for atherogenesis studies. What does appear to be obligatory for promoting atherosclerosis is the induction of persistent hypercholesterolemia to levels \(>300\) mg/dL. Because each of these dietary components is capable of independently influencing plasma lipid levels and atherosclerosis, sometimes differentially in different animal models, the composition of the diet used in atherosclerosis studies should be referenced or described. Cholate should be avoided because of its many effects on cholesterol absorption, expression of genes involved in lipoprotein metabolism via the FXR nuclear receptor, and possibly other effects. The Western-type diet has become the diet used in the majority of murine atherosclerosis studies to induce hypercholesterolemia. The cholesterol component of this diet is probably the major proatherogenic agent. However this diet has the confounding effect of also inducing insulin resistance. The murine models discussed in this review are amenable to further more refined study of the role of dietary components, including the nonfat components, in atherogenesis. Whereas the mouse has proven to be a very valuable species for the study of atherogenesis and its influence by diet, we need to recognize that these studies may not be predictive of diet effects in humans.

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**References**


