

## Review article

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### MOUSE MODELS OF EXPERIMENTAL ATHEROSCLEROSIS

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Since 1992 the mouse has become an excellent model for experimental atherosclerosis research. Until 1992, the diet - induced atherosclerosis mouse model has been used effectively, but the lesions tended to be small and were limited to early fatty-streak stage. This model was also criticized because of the toxicity and inflammatory responses due to the diet. In 1992 the first line of gene targeted animal models, namely apolipoprotein E - knockout mice was developed. Of the genetically engineered models, the apoE - deficient model is the only one that develops extensive atherosclerotic lesions on a chow diet. It is also the model in which the lesions have been characterized most thoroughly. The lesions develop into fibrous plaques; however, there is no evidence that plaque rupture occurs in this model. The LDL receptor - deficient model has elevated LDL levels, but no lesions, or only very small lesions, form on the chow diet, however, robust lesions do form on the western-type diet. The creation of apoE - knockout mice has changed the face of atherosclerosis research.

*Key words: atherosclerosis, animal models, apoE-knockout mouse, LDL receptor - knockout mouse.*

#### *ANIMAL MODELS OF ATHEROSCLEROSIS*

Atherosclerotic cardiovascular disease, the major cause of death in Western society, results from complex interactions among multiple genetic and environmental factors (1-3). Numerous animal species have been used to study the pathogenesis and potential treatment of the lesions of atherosclerosis. The first evidence of experimental atherosclerosis came into view as early as in 1908 when Ignatowski (4) reported thickening of the intima with formation of large clear cells in the aorta of rabbits fed with a diet rich in animal proteins (meat, milk, eggs). The most useful animal models have thus far been restricted to

relatively large animals, such as nonhuman primates, swine, and rabbits. Hamsters and pigeons have been used occasionally but present problems peculiar to their species. Rats and dogs are not good models for atherosclerosis because they do not develop spontaneous lesions and require heavy modifications of diet to produce vascular lesion. Despite the fact that rabbits do not develop spontaneous atherosclerosis, they are useful because they are highly responsive to cholesterol manipulation and develop lesions in a fairly short time (5). The lesions are much more fatty and macrophage-rich (inflammatory) than the human lesions and plasma cholesterol levels are extraordinarily high (very dissimilar to humans). Pigs and monkeys are better suited to model human atherosclerotic lesions. However, nowadays monkeys are not widely used due to obvious species - specific concerns (risk of extinction) and cost. The pig is a very good model - when fed with cholesterol, they reach plasma levels and atherosclerotic lesions that are quite similar to those seen in humans. Problems with the pig model are costs, the difficulties involved in maintaining the colonies and in their handling. What has been traditionally lacking was a small, genetically reproducible, murine model of atherosclerosis. Such a model could help to overcome the many problems and deficiencies of larger animals and, in particular, would permit studies of possible therapies that require relatively large numbers of animals.

Until 1992, the majority of atherosclerotic research focused on mechanisms in rabbits, with a lesser number of studies in pigs and nonhuman primates. These large animal models have provided invaluable insight. The use of pig models of the disease initially revealed that monocyte infiltration was one of the primary cellular events in the atherogenic process (6). Studies in monkeys and rabbits have been pivotal in defining the cellular events in the initiation and development of lesions (7, 8). In recent years, there has been an explosion in the number of in vivo studies that is largely attributable to the use of mouse models to study atherogenic mechanisms.

#### *MOUSE AS A MODEL OF ATHEROSCLEROSIS*

Mice are highly resistant to atherosclerosis. The only exception in mice is the C57BL/6 strain. When fed a very high cholesterol diet containing cholic acid, however, the vascular lesions in the C57BL/6 differ from the human condition in the histologic nature and location and are possibly attributed to a chronic inflammatory state rather than a genetic predisposition.

The earliest mouse model of atherosclerosis was the diet - induced model that was first characterized during the 1960s in Wissler's laboratory. Special diet contained 30% fat, 5% cholesterol, and 2% cholic acid led to atherosclerosis in C57BL/6 mice. However, this was a very toxic diet on which the mice lost weight and often got sick with morbid respiratory infections. Paigen *et al.* modified this diet by blending it one part to three parts with a 10% fat diet to yield what is

called the "Paigen diet" which consists of 15% fat, 1.25% cholesterol, and 0.5% cholic acid (9).

Although there were many uses of this model, there were also many disadvantages. The lesions are very small in mice at 4 to 5 months of age, in order of 200 to 1 000 square microns in the aortic root. The lesions are largely confined to the aortic root, and they usually do not develop beyond the early foam-cell, fatty-streak stage. The diet is also unphysiological with regard to its extremely high cholesterol content, 1.25%, and the presence of cholic acid. In addition, Lusis *et al.* have shown that this diet is in itself inflammatory, as leads to the induction of hepatic NF- $\kappa$ B activation and the expression of acute phase reactants, such as serum amyloid A (10).

Paigen *et al.* colleagues also developed assays that are widely used to quantify atherosclerosis in the mouse model. The most standard assay is the measurement of the cross-sectional lesion area in the aortic root (11). In this assay, freshly perfused and isolated hearts are fixed in formalin, embedded in gelatin, frozen, and cut into thin sections at anatomically defined sites in the aortic sinus and valve region. These sections are stained for lipids, and the lesion area is measured microscopically.

Although this model has been widely employed and is of significant use in the study of atherosclerosis, the pathology of the lesions are not ideally suited as a model for human atherosclerosis. This shortcoming led many investigators to downplay the role of the mouse as a good model of atherosclerosis. Lesion formation in the diet - induced model is largely limited to the aortic root after feeding the Paigen - diet for periods of 14 weeks to 9 months. The lesions are quite small, only several hundred to a few thousand square micrometers, and they consist almost entirely of macrophage foam cells with little evidence for smooth muscle cell involvement. Thus, this model is largely limited to the fatty streak stage and does not progress to resemble human intermediate lesions.

For many years the mouse was not used as an experimental model for atherosclerosis research because of the beliefs that mice could not survive on high - fat atherogenic diets, that lesions were not reproducible, that most mice did not get lesions, and that lesion pathology did not resemble atherosclerosis in humans. However, the use of lower - fat diets solved the survival problem; the use of inbred strains rather than random - bred mice solved the reproducibility problem; the use of susceptible strains resulted in most mice getting lesions; and longer experimental times showed that lesions with fibrous caps were produced.

The following is a list of questions that can be used to judge the usefulness of animal models of atherosclerosis: 1) What is the nature of the experimental lesions and their similarity to human lesions; 2) is the plasma lipoprotein profile and metabolism similar to metabolism in humans; 3) what is the time frame necessary for lesions to form, and how long does it take to breed the animals for the studies; 4) what is the cost of acquiring and maintaining the animals; 5) what is the ability to perform *in vivo* manipulations and imaging; and 6) what is the

ability of the model to take advantage of classical and molecular genetic approaches ?

The mouse as a model meets many of these criteria, but first it is important to acknowledge many important differences between mice and humans. The average lifespan of a mouse is about 2 years, compared to about 75 years in humans. Mice weigh much less, about 30 grams for the adult. The lipid profile in the mouse is very different from that in humans, who carry about 75% of their plasma cholesterol on LDL. Mice carry most of their cholesterol on high-density lipoprotein (HDL), which we know in humans is protective against atherosclerosis. Thus, mice fed their normal low-fat chow diet do not get atherosclerosis, while it is a common disease in humans. One difference, which is an advantage of all animal models, is the ability to control the environment and diet in mouse studies, which is impossible for long-term human studies. Human genetic studies are limited in range to various types of association studies. With mice, on the other hand, many additional kinds of genetic experiments are possible, including breeding and genetic engineering.

There are many advantages of using mice for experimental atherosclerosis research, including their relative ease and thriftiness to acquire and maintain. Their generation time is short, at about 9 weeks, 3 weeks for gestation and about 6 weeks until sexual maturity. It is easy to breed very large cohorts for experimental studies, and mice can develop atherosclerosis in a very short timeframe, as discussed below. Classical genetics in the mouse is very well established and is aided immensely by the availability of hundreds of inbred strains. Moreover, in 2002, The Mouse Genome Sequencing Consortium published the culmination of international efforts - a high quality sequence and analysis of the genome of the C57BL/6J mouse strain (12). With the coming of age of molecular genetics, it is now possible to add exogenous transgenes into mice, which can also be done in many other species. However, uniquely in mice, it is also possible to knock out or replace endogenous genes; this is one of the main advantages of working in the mouse model.

The major disadvantage of the mouse model is their small size, which makes it difficult but not impossible to perform surgical manipulations and in vivo imaging. But there have been recent advances in these techniques that have overcome many of the size limitations, such as the ability to perform imaging of abdominal atherosclerotic lesions in living mice, cardiac catheterization to determine cardiovascular function in free-ranging mice, and surgical ligation of coronary arteries giving rise to myocardial ischemia.

#### *APOLIPOPROTEIN E*

Among the factors that have been identified to date are changes in the genes involved in lipid metabolism, including the gene encoding apolipoprotein E (apoE). ApoE is a glycoprotein with a molecular size of approximately 34 kD that

is synthesized in the liver, brain, and other tissues in both humans and mice; it is a structural component of all lipoprotein particles other than low density lipoprotein (LDL). One of its most important functions is to serve as a high affinity ligand for the apoB and apoE(LDL) receptor and for the chylomicron-remnant receptor, thereby allowing the specific uptake of apoE-containing particles by the liver (13). Human apoE is polymorphic and consists of three major isoproteins (apoE-2, apoE-3, and apoE-4) of which apoE-3 is the most common. A frequent genetic variant of human apoE, apoE-2, differs from the most common form, apoE-3, by having cysteine at position 158 in place of arginine. This amino acid substitution in the LDL receptor binding region reduces the binding ability of apoE-2 to less than 2% relative to that of apoE-3. Homozygosity for the gene ApoE2 is associated with type III hyperlipoproteinemia, which is characterized by increased plasma triglyceride and cholesterol levels, yellow lipid-laden xanthomatous skin nodules, and the early development of atherosclerosis.

In addition to its important role in lipoprotein metabolism, it is likely that apolipoprotein E has other vital functions. For example, recent studies have suggested that apoE produced in the brain have a role in preventing the development of Alzheimer's disease (14).

#### *APO E - KNOCKOUT MICE - A BREAKTHROUGH*

It has been a longstanding goal of many investigators around the world to create better mouse models for lipoprotein disorders and atherosclerosis and to identify genes that may modify atherogenesis and lesion progression. In 1992 apoE - deficient mice were generated by inactivating the *ApoE* gene by targeting (15). They inactivated the apoE gene in mouse embryonic stem (ES) cells by homologous recombination. Two targeting plasmids were used, pJPB63 and pNMC109, both containing a neomycin-resistance gene that replaced a part of the apoE gene and disrupted its structure. ES cell colonies targeted after electroporation with plasmids were identified by the polymerase chain reaction (PCR) followed by genomic Southern analysis. Chimeric mice were generated by blastocyst injection with targeted lines. They gave strong chimeras, which transmitted the disrupted *apoE* gene to their progeny. Mice homozygous for the disrupted gene were produced from the heterozygotes. The facts that homozygous animals have been born at the expected frequency and that they appeared to be healthy were important. They demonstrated that lack of apoE was compatible with normal development, and they also provided another tool for studies of the phenotypic consequences of apoE deficiency. At the same time another group created also apoE - deficient mice (16). Mice homozygous or heterozygous for the disrupted *ApoE* gene appeared healthy. No difference in their body weights compared to normal mice was observed. However, significant phenotypic differences between normal animals and the homozygous mutants were observed

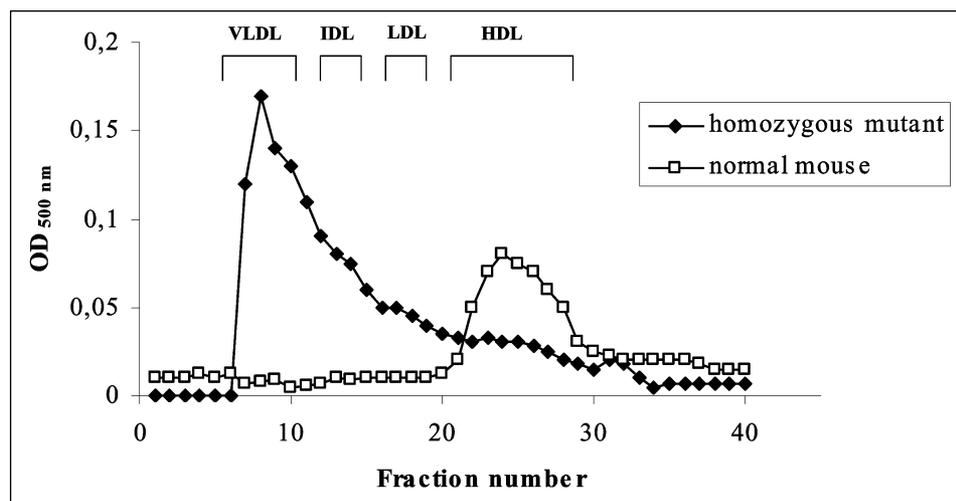
in their lipid and lipoprotein profiles (*table 1*). The apoE-knockout mice had markedly increased total plasma cholesterol levels, which were five times those of normal litter mates. These levels were unaffected by the age or sex of the animals. Although the total plasma cholesterol levels were greatly elevated in the mutants, the high density lipoprotein (HDL) cholesterol levels were only 45% the normal level. The triglyceride levels were 68% higher than those of normal animals. (These apoE-deficient mice have had a dramatic shift in plasma lipoproteins from HDL, the major lipoprotein in control mice, to cholesterol-enriched remnants of chylomicrons and VLDL - *Fig.1.*)

Mice naturally have high levels of HDL and low levels of LDL, in contrast to humans who are high in LDL and low in HDL. In addition, mice apparently lack the cholesteryl ester transfer protein, an enzyme that transfers cholesterol ester from HDL to VLDL and LDL. Despite these differences, apoE-deficient mice have phenotypes remarkably similar to those of apoE-deficient humans.

A chronological analysis of atherosclerosis in the apoE-deficient mouse has shown that the sequential events involved in lesion formation in this model are

*Table 1.* Plasma cholesterol and triglyceride levels in apoE-deficient mice on a chow diet.

Animals	Total cholesterol in mg/dl $\pm$ SD	HDL cholesterol In mg/dl $\pm$ SD	Triglyceride In mg/dl $\pm$ SD
Normal	86 $\pm$ 20	73 $\pm$ 28	73 $\pm$ 36
Heterozygous	88 $\pm$ 22	75 $\pm$ 18	102 $\pm$ 40
Homozygous	434 $\pm$ 129	33 $\pm$ 15	123 $\pm$ 51

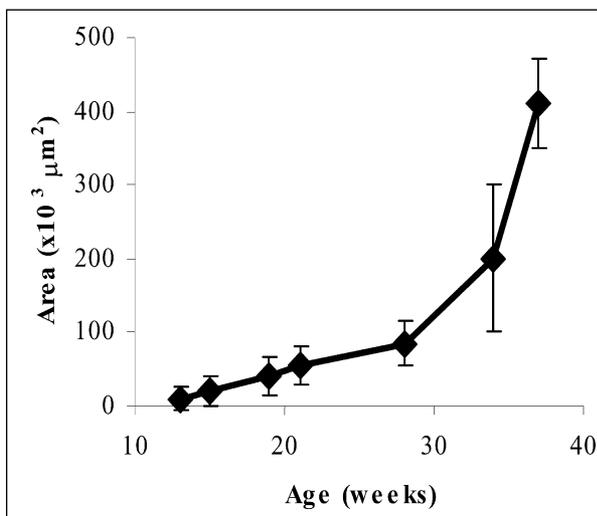


*Figure 1.* Cholesterol concentrations in lipoprotein fractions of normal and apoE-deficient mouse after fast protein liquid chromatography (FPLC).

strikingly similar to those in well - established larger animal models of atherosclerosis and in humans (17). Animals as young as 5-6 weeks of age have monocytic adhesions to the endothelial surface of the aorta that can be appreciated readily with electron microscopy (EM). EM also has demonstrated transendothelial migration of blood monocytes in similarly aged mice. By 6-10 weeks of age, most apoE - deficient mice have developed fatty - streak lesions comprised primarily of foam cells with migrating smooth muscle cells. These fatty - streak lesions rapidly progress to advanced lesions, which are heterogeneous but are typically comprised of a necrotic core surrounded by proliferating smooth muscle cells and varying amounts of extracellular matrix, including collagen and elastin (*Fig. 2*). These lesions have well - formed fibrous caps made up of smooth muscle cells and extracellular matrix that often have groups of foam cells at their shoulders. It is not uncommon for the inflammatory lesion to erode deep into the medial wall of the aorta, and some of these animals develop aortic aneurysms. Many of the lesions found in older mice develop calcified foci (18).

Other characteristics of the lesions in the apoE - deficient mouse, such as indications of oxidative change, merit attention as well (19). The atherosclerotic lesions in this mouse contain oxidation - specific epitopes. In young lesions these epitopes are predominantly localized in macrophage - rich areas, whereas in advanced lesions they are localized in necrotic regions. In addition, high titers of antibodies against the oxidized epitopes are present in the plasma of the apoE - deficient mice.

The complexity of lesions in the apoE - deficient mouse, together with the benefits of using the mouse as a model of human disease, makes it a desirable



*Figure 2.* Line plot displaying morphometric summary of vascular lesions. All measurements were performed on sections from aortic sinus.

system in which to study both environmental and genetic determinants of atherosclerosis. Initial studies examined the effects of grossly different diets on susceptibility to atherosclerosis in this animal. These studies confirmed the validity of this mouse as a model of human atherosclerotic disease and laid the groundwork for future dietary studies.

Hayek *et al.* developed a more physiological than Paigen diet - "western-type" diet for mouse studies, which is similar in composition to an average American diet of several years ago, consisting of 21% fat by weight, 0.15% cholesterol, and no cholic acid. When fed this diet, wild-type mice have a two-fold elevation in plasma cholesterol, while apoE-deficient mice have over a three-fold elevation, to about 2 000 mg/dl, again, mostly in bVLDL, but there is also an increase in LDL (16). The post-prandial clearance of intestinally derived lipoproteins is dramatically impaired in apoE - deficient mice. The apoE - deficient mouse responds appropriately to a human - like western - type diet (17). On this diet, lesion formation is greatly accelerated and lesion size is increased. In 10-week-old animals fed this diet for only 5 weeks, lesions are 3-4 times the size of those observed in mice fed a low - fat diet. In addition, monocytic adhesions and advanced lesions develop at a significantly earlier age. The results of this dietary

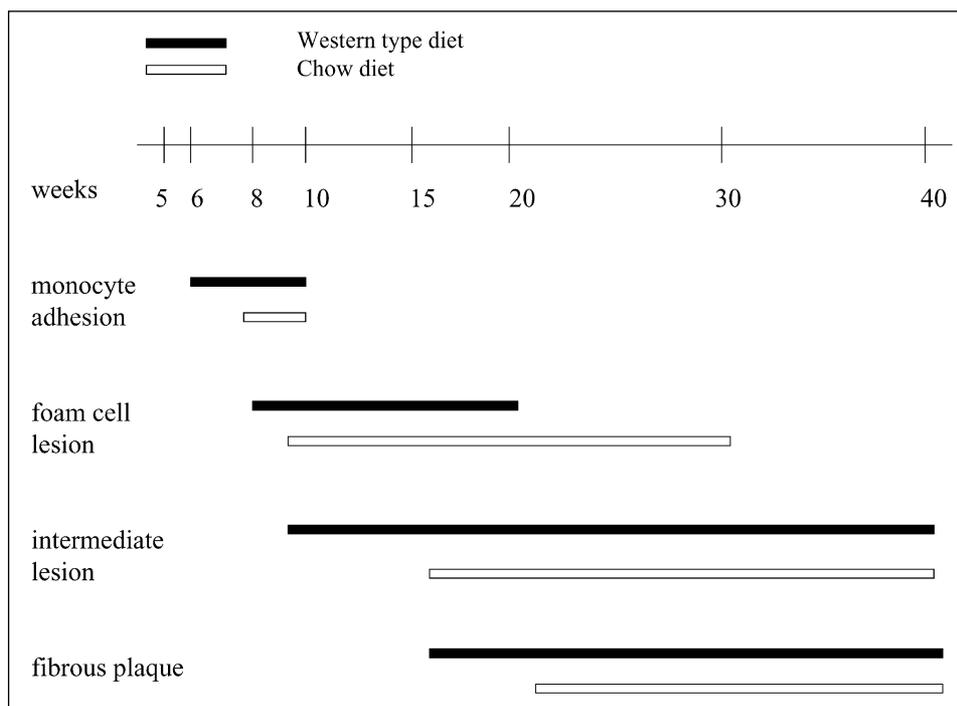


Figure 3. Diagram showing how lesion formation in chow - fed mice is delayed in comparison with mice fed the Western - type diet.

challenge demonstrate that the mouse model responds in an appropriate manner, i.e. increased fat leads to increased plasma cholesterol, which in turn leads to increased atherosclerosis (*Fig. 3*). Moreover, the data suggest that in addition to its histological similarity to humans, the mouse model exhibits a response to environmental cues resembling that of humans.

Lesions in the apoE-deficient mouse, as in humans, tend to develop at vascular branch points and progress from foam cell stage to the fibroproliferative stage with well-defined fibrous caps and necrotic lipid cores, although plaque rupture has not been observed in apoE - deficient mice or in any other mouse model. Progression of lesions appears to occur at a faster rate than in humans atherosclerosis; the rapidity of lesion progression can be advantageous in many experimental situations.

The genetic background has a major effect on atherosclerosis susceptibility in strains of apoE - deficient mice. For example, lesions from 16-week chow diet C57BL/6 apoE-KO were relatively larger than from FVB apoE-KO mice and in contrast to FVB mice there was evidence of early development of fibrous caps in these mice. In older mice, fibrous plaques from C57BL/6 apoE-KO mice were larger in size and had larger necrotic cores compared with FVB apoE-KO mice.

Comparing humans and apoE - deficient mice, lesion progression and cell types are similar, as is the presence of oxidized lipoproteins. The major difference of this mouse model, as is the presence of oxidized lipoproteins. The major difference of this mouse model, as is the case for most of the other models of experimental atherosclerosis, is that plaque rupture is not observed, whereas plaque rupture is fairly common in humans and can lead to heart attacks. One potential reason for the lack of plaque rupture in mice is that the diameter of the aorta is less than 1 mm, which is even smaller than the diameter of the major coronary arteries in humans. As the vessel diameter decreases, the surface tension increases exponentially; thus, in the mouse there may be so much surface tension that plaque rupture would not be likely to occur.

ApoE knockout mice are considered to be one of the most relevant models for atherosclerosis since they are hypercholesterolemic and develop spontaneous arterial lesions (17). Heterozygous apoE-deficient mice do not exhibit elevated plasma cholesterol levels on the chow or Western-type diet, suggesting that when mice are fed a physiological diet, a 50% decrease in apoE is not sufficient to influence fasting plasma lipids (20).

The apoE - deficient mouse contained the entire spectrum of lesions observed during atherogenesis and was the first mouse model to develop lesions similar to those of humans. This model provided opportunity to study the pathogenesis and therapy of atherosclerosis in a small, genetically defined animal.

In 1995 Kashyap *et al.* (21) described the successful correction of apoE deficiency in apoE - deficient mice by using an alternative approach involving systemic delivery to mouse liver of recombinant adenovirus vectors expressing human apoE. Thus, the single genetic lesion causing apoE absence and severe

hypercholesterolemia is sufficient to convert the mouse from a species that is highly resistant to one that is highly susceptible to atherosclerosis (22).

Mutant forms of human apoE, such as apoE3 - Leiden (23), cause a dominantly inherited form of type III hyperlipoproteinemia, and have been used for the production of transgenic mouse lines. The apo E3-Leiden transgenic mice produced increased levels of cholesterol and triglycerides in the VLDL-plus-LDL-sized fractions of plasma, and displayed an increased responsiveness to diet-induced hypercholesterolemia (24, 25).

The method of measure atherosclerosis by using the aortic root atherosclerosis assay was originally developed by Paigen *et al.* (11). The aortic root cross-sectioning assay is widely used in murine studies of atherosclerosis, allows for coincident inspection of lesion histology, and is amenable in studies using large numbers of mice. Alternative measures of atherosclerosis, such as the *en face* method, correlate with aortic root measurements. However, these methods are less amenable for studies using large numbers of mice and do not allow for inspection of lesion histology.

#### *LDL RECEPTOR DEFICIENT MICE*

Gene targeting in embryonic stem cells has recently been used to create LDL receptor - knockout (LDLR-KO) mice, a model of familial hypercholesterolemia. LDL receptor - deficient mice was made in 1993 by Ishibashi *et al.* (26). These mice have a more modest lipoprotein abnormality than the apoE - deficient mice, with increases in LDL and VLDL cholesterol leading to a total plasma cholesterol of about 250 mg/dl on a chow diet. On this diet, and at that level of plasma cholesterol, LDL receptor - deficient mice do not get atherosclerosis. However, this is a very diet-responsive model. After these mice are fed the Paigen diet, their plasma cholesterol levels soar to about 1 500 mg/dl, and large atherosclerotic lesions form (27). It has also been shown that feeding the less toxic western-type diet also leads to the development of large lesions, with plasma cholesterol levels of about 400 mg/dl. The lesion pathology in this model is not as well characterized as in the apoE - deficient model, but it does appear similar in that the lesions can progress beyond the foam - cell fatty-streak stage to the fibro-proliferative intermediate stage.

#### *OTHER MOUSE MODELS*

Overexpression of human apoA-I in apoE - deficient mice increased HDL cholesterol levels twofold and substantially decreased fatty streak and advanced fibroproliferative lesion formation (28, 29). By 4 months of age, all but 3-5% of apoE - deficient mice have had detectable fatty streaks that vary considerably in size; some are barely detectable, whereas others occlude as much as 8% of the aortic lumen. In apoE - deficient mice that overexpress human apoA-I, more than 50% of animals have no lesions by 4 months of age, and the animals that do develop atherosclerosis have lesions that are barely detectable. By 8 months of

age, apoE - deficient mice have lesions that are highly organized and that occlude on average 25% of the aortic lumen. Those apoE - deficient mice that overexpress human apoA-I have mainly immature fatty - streak lesion that occlude on average only 5% of the aortic lumen. Collectively, these data suggest that overexpression of apoA-I can diminish lesion size and slow the initiation of fatty streak formation.

The second additional mouse model is the human apoB transgenic mice created independently in Young's and Rubin's laboratories (30, 31). These mice synthesize human apoB only in their livers, as the intestinal enhancer is not present in these transgenic constructions. The human apoB transgene leads to the production of both the full-length apoB-100 protein and, via RNA editing, the truncated apoB-48 protein. These mice have mildly increased LDL and total cholesterol levels, with a lipoprotein profile showing a distinct LDL peak, in contrast to wild-type mice, which have only a distinct HDL peak. The apoB transgenic mouse is also a very diet-responsive model. Upon feeding the Paigen diet, the cholesterol goes up to about 300 mg/dl. Atherosclerosis is not observed when the mice are on fed a chow diet, but it develops in response to feeding either the Paigen diet or the western-type diet (32, 33).

More recently, apoE and LDL - receptor (LDLr) double - knockout (apoE/LDLr-DKO) mice have been created (27), representing a new mouse model that develops severe hyperlipidaemia and atherosclerosis (34). It has been reported that, even on a regular chow diet, the progression of atherosclerosis is usually more marked in apoE/LDLr-DKO mice than in mice deficient for apoE alone (35). Thus, the apoE/LDLr-DKO mouse is a suitable model in which to study the anti-atherosclerotic effect of compounds without having to feed the animals an atherogenic diet.

To study the contribution of endothelial nitric oxide synthase (eNOS) to lesion formation Kuhlencordt *et al.* (36) created apoE / eNOS double - knockout mice. It has occurred that chronic deficiency of eNOS increases atherosclerosis in apoE-KO mouse model. Furthermore, in the absence of eNOS, peripheral coronary disease, chronic myocardial ischemia, heart failure, and an array of vascular complications develop that have not been observed in apoE-KO animals.

Recently, Veniant *et al.* (37) managed to even up the cholesterol levels in chow-fed apoE-KO mice and LDLR-KO mice. They did so by making both mouse models homozygous for the apolipoprotein B-100 allele, which ameliorates the hypercholesterolemia in the setting of apoE deficiency but worsens it in the setting of LDLR deficiency. Moreover, the LDLR-KO Apob<sup>100/100</sup> mice developed extensive atherosclerosis even on a chow diet. So far this model seems to be the best as concerns the development of atherosclerosis in mice.

#### *THE EXPERIMENTAL USE OF GENE TARGETED MICE*

The apoE - deficient mouse model of atherosclerosis can then be used to: 1) identify atherosclerosis susceptibility modifying genes, by the candidate-gene

and gene-mapping methods; 2) identify the role of various cell types in atherogenesis; 3) identify environmental factors affecting atherogenesis; and 4) assess therapies that might block atherogenesis or lesion progression.

ApoE - deficient mice have also been used to look for environmental and drug effects on atherosclerosis and to test novel therapies. One of the first observations was paradoxical effects of probucol on atherogenesis in both apo E - KO (38) and LDL receptor deficient (39) mice. Probucol with strong antioxidant and cholesterol - lowering effects increased atherogenesis in apoE - KO mice by 3 folds (38). Several other compounds reduced the extent and severity of atherosclerotic lesions without affecting plasma cholesterol levels in apoE - KO mice. For example, administration of antioxidant N,N'-diphenyl 1,4 - phenylenediamine (DPPD) to apoE - KO mice resulted in a significant decrease in atherosclerosis without reducing plasma cholesterol levels (40). A marked reduction in atherosclerosis by dietary vitamin E was accompanied by no change in plasma cholesterol levels in apoE - KO mice (41). Likewise, antiatherogenic effects of the angiotensin - converting enzyme inhibitors (42-44) or the angiotensin-II receptor antagonist (45) in apoE - KO mice were independent of plasma cholesterol lowering effects.

### CONCLUSION

Gene - targeted mouse models has changed the face of atherosclerotic research (46) and helped in creation of the new theory of atherosclerosis - as an inflammatory disease (47-49).

**ABBREVIATIONS:** ApoE, apolipoprotein E; apoE-KO, apoE-knockout; LDLR-KO, LDL receptor- knockout.

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