This Review is part of a thematic series on the **Atherosclerosis in Diabetes: Dyslipidemia Versus Hyperglycemia**, which includes the following articles:

- Do Glucose and Lipids Exert Independent Effects on Atherosclerotic Lesion Initiation or Progression to Advanced Plaques?
- Recipes for Creating Animal Models of Diabetic Cardiovascular Disease
- The Macrophage at the Crossroads of Insulin Resistance and Atherosclerosis
- Lipids, Glucose, and Oxidative Reactions in Diabetes and Atherosclerosis

**Karin E. Bornfeldt, Guest Editor**

## Recipes for Creating Animal Models of Diabetic Cardiovascular Disease


**Abstract**—For more than 50 years, investigators have unsuccessfully tried to recreate in experimental animals the cardiovascular complications of diabetes seen in humans. In particular, accelerated atherosclerosis and dilated cardiomyopathy, the major causes of mortality in patients with diabetes, have been conspicuously absent in many mouse models of the disease. Under the auspices of the NIH, the Animal Models of Diabetic Complications Consortium has worked to address this issue. This effort has focused on the development of mouse models because of the high level of genomic information available and the many well-developed genetic manipulations that may be performed in mice. Importantly, the consortium has also worked to standardize many methods to assess metabolic and cardiovascular end points for measurement of the diabetic state and its macrovascular complications. Finally, for maximum benefits from these animal models in the study of atherosclerosis and of other diabetic complications, the consortium has created a system for sharing both the animal models and the accumulated phenotypic data with the greater scientific community. *(Circ Res. 2007;100:1415-1427.)*

**Key Words:** atherosclerosis ■ diabetes mellitus ■ diabetic cardiomyopathy ■ transgenic mice

Despite the vast clinical experience linking diabetes, obesity and the metabolic syndrome to vascular disease, little is understood about the mechanisms connecting hyperglycemia/insulin deficiency to atherosclerosis,¹ and virtually nothing is known regarding the underlying basis for differential susceptibility to vascular injury in patients with diabetes. One major impediment to progress in this area is the lack of appropriate animal models. Models in which vascular disease and heart dysfunction are clearly accelerated by diabetes would allow investiga-

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tors to define abnormal signaling pathways and test new experimental modalities. A related difficulty is the lack of standardized measures for phenotypes associated with diabetes and its complications.

To provide an experimental foundation for investigating these issues, the Animal Models of Diabetic Complications Consortium (AMDCC), under the auspices of the NIH, has established a consortium for the creation of animal models of diabetes-induced cardiovascular disease. The goals of this consortium are to generate animal models that will be useful for study of disease pathogenesis, prevention, and treatment and to test the role of candidate genes or chromosomal regions that emerge from genetic studies of complications (particularly accelerated cardiovascular diseases) in humans with diabetes. The long-term goal of the consortium is to derive, characterize, validate, and use the models for various aspects of basic, developmental, or translational research including testing, prevention, early detection, therapy, and diagnostic imaging strategies. Members of the AMDCC chose to study mice, as most animal models of diabetes are based on the laboratory mouse, in which multiple spontaneous and engineered genetic modifications have been demonstrated to produce phenotypes associated with diabetes. Mice are also attractive to the investigators because single or multiple genes can be manipulated in a genetically homogeneous background and because, like people, different strains of mice have differential susceptibilities to obesity, diabetes, and their complications. Moreover, genetic analysis is powerful in the mouse because of the availability of unique resources including complete genomic sequences for a number of strains and detailed coexpression networks for many genes and pathways. Finally, the tools and technologies for investigating pathophysiology of disease in the mouse are rapidly evolving.

The mission of the AMDCC is not only to create models for diabetic complications but also provide the models, the data relevant to their characterization, and the protocols used for their generation and analysis to the scientific community. The AMDCC web portal (http://www.amdcc.org) was designed to disseminate this data and information. Specifically, the web portal provides access to all of the strains being characterized, the experimental data being generated, the standardized protocols, analytical tools, visualization tools, bioinformatics software, and organizational information. Metabolic data and experimental readouts (such as the amount of atherosclerosis, complexity of lesions, gene expression levels, and heart function) are provided for individual animals. This provides the research community with a comprehensive resource to explore, analyze, and download the data generated from the animal models being developed and evaluated by the AMDCC. Specifically for diabetic cardiovascular complications, the validation criteria can be found at http://www.amdcc.org/cardiovascular_validation.pdf, and the protocols can be found at http://www.amdcc.org/shared/protocols.aspx.

Atherosclerosis

Investigators have studied atherosclerosis using animal models of diabetes for more than 50 years. In one of the earliest studies, Duff discovered that the cholesterol-fed rabbit had reduced atherosclerosis when treated with alloxan, an islet cell toxin that creates insulin-deficient diabetes. Moreover, in contrast to recent observations in humans with diabetes, insulin treatment in these animals was associated with increased aortic atherosclerosis. It was reasoned that the lipoprotein particles in the diabetic rabbit were too large to penetrate into the artery wall and were converted back to smaller, more atherogenic particles by the insulin treatment. Thus, despite the potentially deleterious effects of hyperglycemia and insulin deficiency, these experiments and others in nonhyperlipidemic animals clearly showed that atherosclerosis requires the presence of “atherogenic” lipoproteins. In many ways, the diabetic rabbit also illustrates the basic biology of atherosclerosis that was defined only within the past decade: smaller cholesterol-containing apolipoprotein (apo)B particles are more atherogenic, small LDL > large LDL > VLDL. In contrast, local lipolysis of VLDL might be toxic to the vessel by creating increased local concentrations of fatty acids and lysolipids, which might increase arterial permeability, and expression of adhesion molecules, inflammatory factors, and blood coagulation proteins and reduce endothelial mediated vascular dilation.

More recent experimental studies have focused on the mouse model. As in rabbits, rats, dogs, nonhuman primates, and probably humans, atherosclerosis in mice requires the development of atherogenic lipoprotein profiles. Although many species will develop such lipoprotein profiles with diet manipulation alone, the mouse has such an efficient lipoprotein clearance system that only with genetic modification can it develop an atherogenic profile, or at least a profile that allows vascular disease within an experimentally acceptable period of time. Two laboratories accomplished deletion of apoE, the protein involved in receptor clearance of remnant lipoproteins created from VLDL and chylomicrons; this allowed for the creation of the first “heart attack” mouse, i.e., a mouse that develops reproducible and quantifiable atherosclerosis. It should be noted that this mouse does not really develop a heart attack and that the creation of animals that develop a thrombosis superimposed on a ruptured vascular lesion is still a work in progress. Two other genetic alterations in the mouse, deletion of the LDL receptor (Ldlr−/−) and transgenic expression of human apoB proteins, also lead to hyperlipidemia and atherosclerosis. The 3 genetic models differ in their lipoprotein profiles with larger, more remnant lipoproteins in the apoE knockout (apoE−/−) and smaller, LDL-size particles in the Ldlr−/− model. Nonetheless, systems were put in place to superimpose diabetes in these mice, as well as in mice with other added genetic manipulations. Although diabetes accelerates vascular disease in some of these models, in most, it has been difficult to differentiate pathological effects attributable to alterations in lipoproteins from those of diabetes, hyperglycemia, and/or insulin deficiency.

There are marked differences in atherosclerosis development between mouse strains. Although the diabetes/metabolic disease community often studies disease in FVB mice that tend to develop more insulin resistance and diabetes, atherosclerosis development in this strain is not the most robust.
For this reason, the vascular biology community tends to breed their genetic mutations onto the more atherosclerosis-prone C57BL/6 strain. Other strains that are less commonly used such as DBA/2 are also atherosclerosis prone and might be useful. Even within C57BL/6 mice, there is a sexual dimorphism; female mice are more susceptible to atherosclerosis, which is not the case in humans.

Working Definition of Mouse Model of Diabetic Vascular Disease
Mouse models can be used to identify potential candidate genes for genetic analysis in humans, whereas genes conferring disease that are already identified in humans can be studied in mice. Moreover, animal models provide a critical platform with which to investigate the potential of novel therapeutic strategies. The AMDCC program has proposed that a mouse model of diabetic vascular disease should exhibit accelerated atherosclerosis, peripheral vascular disease, or microvascular disease in the setting of one of the following (Table 1): (1) insulin resistance, (2) dysmetabolic syndrome, (3) impaired glucose tolerance, (4) type 2 diabetes mellitus, or (5) type 1 diabetes mellitus. Acceleration of atherosclerosis is defined as an increase in the extent of lesions (number of lesions per unit of time) or progression of lesion histology (necrotic, lipid cores with fibrous caps surrounded by a proteoglycan matrix, or more unstable plaques). Diabetic lesions may also be characterized by increased inflammation (cells or cytokines), increased expression of certain genes (osteopontin or plasminogen activator inhibitor-1), and increased calcification compared with lesions from nondiabetic mice. Increased intralesional thrombosis and signs of more plaque rupture may be more common with diabetes. An important point is that lesion acceleration (initiation or progression) must not be solely attributable to elevated circulating cholesterol levels.

How Can Diabetes Be Created and Quantified in the Mouse?
There are several methods for creating diabetes/insulin action deficiency in the mouse (Table 2). Chemical toxins have several advantages: they require less mouse breeding, are standardized, and the diabetes can be created during different stages of lesion development. Genetic models avoid the use of potential confounding effects of toxins. Diet manipulation can create obesity as well as diabetes but with less profound hyperglycemia.

Islet Cell Ablation
One approach has been through the use of streptozotocin (STZ) to deplete β cells in the pancreatic islets. Various investigators have used protocols ranging from a single high dose of STZ (150 to 200 mg/kg) to multiple low doses of STZ (40 to 50 mg/kg per day for 5 days). Female mice are less sensitive to this islet cell toxin, and, therefore, most STZ-induced diabetic mouse studies have used males. The AMDCC adopted the low-dose strategy, in part, because this strategy minimizes nonspecific toxic effects of high-dose STZ and also provides a robust and consistent hyperglycemic response. The rationale for this approach is discussed in detail in another AMDCC publication.

Another method to induce islet cell destruction in the mouse is to generate transgenic mice expressing a viral antigen in the β cell using the rat insulin promoter. When these mice are infected with the lymphocytic choriomeningitis mouse virus, the animals develop an immune insulitis and islet cell destruction.

Genetic Models of Diabetes
Alteration of numerous individual genes involved in carbohydrate or lipid metabolism can lead to altered insulin sensitivity, body fat, and plasma lipids in mice. As an example, insulin resistance is induced by a number of genetic interventions specifically targeting the insulin-signaling pathway in muscle, adipose tissue, and the liver. Other genes in other pathways associated with triglyceride and fatty acid metabolism also alter tissue insulin sensitivity. Loss of
adipose tissue\textsuperscript{24} and adipose tissue overexpression of the transcription factor sterol regulatory element binding protein-1c\textsuperscript{25} are such examples.

Limiting islet β-cell development has produced type 1–like diabetes in mice. A haplo-deficiency of the transcription pancreatic duodenal homeobox-1 produces mild diabetes in C57BL/6 mice.\textsuperscript{26} The diabetes is more severe in the FVB strain. Transgenic mice that overexpress a calmodulin mini-gene regulated by the rat insulin II promoter develop islet cell destruction and insulin-deficient diabetes. These mice, termed OVE 26 diabetic mice, have been reported to develop diabetic complications such as cardiomyopathy.\textsuperscript{27,28} Transgenic mice with β-cell–restricted deletion of insulin or insulin-like growth factor-1 receptors,\textsuperscript{29} or insulin receptor gene regulated by the rat insulin II promoter develop islet cell–restricted deletion of insulin or insulin-like growth factor-1 receptors,\textsuperscript{29} or insulin receptor substrate-2\textsuperscript{30} develop β-cell failure and diabetes.

The Akita mouse model represents a spontaneous mutation that leads to severe hyperglycemia, hypoinsulinemia, and polydipsia beginning at 3 to 4 weeks of age.\textsuperscript{31,32} The strain was originally identified in Japan and has since been deposited in The Jackson Laboratories, where it has been back-crossed >13 generations to the C57BL/6 strain. The molecular basis for hyperglycemia in this model is a base pair substitution C96 years in the insulin-2 (ins2) gene, which affects proinsulin folding in the endoplasmic reticulum.\textsuperscript{33,34} As a consequence β cells develop protein aggregates that precipitate an endoplasmic reticulum stress response and, ultimately, severe β-cell dysfunction with islet cells that are unable to release insulin. The diabetic phenotype is most striking in male mice, which have persistent hyperglycemia to \( \approx 400 \text{ mg/dL} \) (\( >20 \text{ mmol/L} \)) throughout life. Moreover, the model has proven to replicate many of the complications of diabetes such as retinopathy,\textsuperscript{35} neuropathy,\textsuperscript{36} and nephropathy\textsuperscript{37,38} and is associated with evidence of increased oxidative stress and premature senescence.\textsuperscript{39} Thus, this mouse represents an excellent model of type 1 diabetes that is free of potential confounding effects of STZ administration and has been adopted by the AMDCC as an important platform with which to study chronic complications of type 1 diabetes.

Insulin-resistant diabetes, akin to type 2 diabetes in humans, occurs with the obesity associated with defective leptin actions. The ob/ob mouse has leptin deficiency; the db/db mouse has a defect in the leptin receptor. Both mice have obesity, insulin resistance, and some degree of diabetes. When crossed onto mice with atherosclerotic backgrounds (Ldlr\textsuperscript{-/-} or apoE\textsuperscript{-/-}), ob/ob, and db/db mice have greater atherosclerosis; however, they are also more hyperlipidemic.\textsuperscript{40} It is not easy to discern the complications of diabetes from those of dyslipidemia.

ApoA-II is the second most abundant protein on HDL, accounting for approximately 20% of the total HDL protein. ApoA-II–knockout mice exhibit increased insulin sensitivity,\textsuperscript{41} whereas transgenic mice expressing the mouse apoA-II transgene are insulin resistant and become obese.\textsuperscript{42} Transgenic expression of mouse apoA-II results in elevated HDL cholesterol levels,\textsuperscript{43} whereas expression of the human apoA-II transgene results in variable changes in HDL levels.\textsuperscript{44} Mice expressing the transgene for either human or mouse apoA-II exhibit increased susceptibility to atherosclerosis.\textsuperscript{45,46} Mouse apoA-II transgenic mice also have elevated levels of triglycerides, and plasma lipoproteins promote accumulation of lipid hydroperoxides,\textsuperscript{47,48} consistent with the accelerated atherogenesis.

What Are the Advantages of Different Diets for Production of Insulin Resistance and Atherosclerosis?

Several different diets have been used to study atherosclerosis in mouse models: some create obesity and insulin resistance, and others lead to hypercholesterolemia without alterations in insulin actions and plasma glucose, whereas the toxicity of a cholic acid diet leads to the most rapid lesion development and leads to small lesions in mice that do not have genetic alterations in lipoprotein metabolism. Laboratory strains of mice are quite resistant to atherosclerosis and to obtain lesions, an extreme diet containing 20% fat, 1.25% cholesterol, and 0.5% cholic acid (all given as percentage weight), called the Paigen diet, is required.\textsuperscript{49} Cholic acid, part of the Paigen diet, both alters plasma lipids and promotes inflammation. This diet is sometimes used to promote atherosclerosis in mice with a heterozygous deletion of the LDL receptor.\textsuperscript{49} Induced mutant mice that are genetically more susceptible to atherosclerosis require less extreme diets to develop lesions. In the apoE\textsuperscript{-/-} model, lesions form when mice are fed a chow diet that typically contains 5% fat, 0.02% cholesterol, and no cholic acid. Lesion progression can be considerably accelerated by a western-type diet containing 20% fat (milk or lard fats), 0.15% cholesterol, and no cholic acid. In the Ldlr\textsuperscript{-/-} model, lesions form very slowly on a chow (ie, low-fat) diet but will form more quickly on a western-type diet. It is also possible to produce lesions in the Ldlr\textsuperscript{-/-} model with a chow diet to which high levels of cholesterol have been added.\textsuperscript{50} Ldlr\textsuperscript{-/-} mice will also develop lesions on a semisynthetic diet (modified AIN76a) that contains 5% fat, and up to 0.15% to 0.5% cholesterol. In genetically altered animal models, non-HDL cholesterol levels of \( \approx 300 \text{ mg/dL} \) (\( >7.5 \text{ mmol/L} \)) need to be achieved in order for the animals to develop significant lesions.

Feeding of chow and low-fat semisynthetic diets does not produce excessive weight gain or insulin resistance in mouse models. In contrast, feeding high-fat diets (ie, 20% fat) induces excessive weight gain, insulin resistance, and hyperglycemia; the hyperglycemia is primarily seen in males. This effect is seen more in the Ldlr\textsuperscript{-/-} model than in the apoE\textsuperscript{-/-} model.\textsuperscript{51} The effects of insulin resistance/diabetes on atherosclerosis versus the effects of hypercholesterolemia on atherosclerosis have been evaluated in experiments in which Ldlr\textsuperscript{-/-} mice are fed low- and high-fat diets.\textsuperscript{52} It should be noted that young and old mice may have different responses to diets leading to differences in gene expression, metabolism, and atherosclerosis.

How Are Atherosclerosis Lesion Size and Complexity Best Assessed?

To create consistency among the scientific community, mouse models should be characterized with respect to atherosclerosis development and quantification of metabolic parameters related to diabetes should be set. There must be consistency and standardization in the phenotyping method-
ology. The en face method shows the extent of lesions in the aorta and is especially useful for more advanced disease, when the extent of disease within the root appears to be limited. In contrast, less advanced lesions can be quantified by measuring cross-sectional lesion area at the aortic root or brachiocephalic artery; in addition, more detailed analysis of the cells and composition of the lesion can be obtained. Although lesions are larger at the aortic root than in other locations, some investigators have put forward the observation that the brachiocephalic artery is more susceptible to advanced lesions, histologically characterized by lesion erosion, and signs of rupture.53 Sections obtained from frozen tissues that do not undergo alcohol fixation are used for oil red O staining, which identifies lesional esterified lipids, primarily cholesteryl ester. In paraffin-fixed tissues, lesion size and more histological detail including staining for matrix (collagen and proteoglycans) can be quantified. Detailed methodologies are described at http://www.amdcc.org. Although oil red staining is a standardized method, paraffin sections have the advantage of showing more detailed lesion complexity (see below). Unlike microvascular complications of diabetes that have a characteristic pathology, vascular lesions from humans with diabetes are similar to those from humans without diabetes. Although some pathologists have suggested that more vascular rupture54 and greater areas of necrotic cores55 occur in the arteries of humans with diabetes, this is a quantitative and not easily visible difference between arteries of diabetic and nondiabetic patients. Thus, lesion complexity as well as lesion extent are important parameters to be assessed in diabetic vessels. The progression of human atherosclerotic lesions is well described.56 Features such as fibrous cap and necrotic zones as well as the prevalence of specific cell types such as smooth muscle cells and macrophages, and, in some cases, quantification of symptomatic deposits of collagen, elastin, or fibrinogen (see for example57) can be detected by staining with specific antibodies or stains. An effective analysis requires summarizing data from multiple tissue sections and often uses manual tracing of each section image followed by image analysis software to help determine relative areas for lesion features and to measure relative stain intensities for key lesion constituents, for example.58 It is also possible that there are changes in gene expression on a per-cell basis in plaques from diabetic and nondiabetic arteries. For this type of analysis, laser capture microdissection has been applied in vascular biology laboratories,59 but, again, this is a fairly labor-intensive approach.

Is Atherosclerotic Lesion Regression Altered With Diabetes?

It is generally accepted that atherosclerotic vascular disease occurs in a staccato fashion with periods of progression and regression. Given that humans with diabetes are at a higher risk for coronary artery disease, in part because it is presumed that their disease progression is advanced, an important question is whether aggressive management of the dyslipidemia commonly found in diabetic patients will result in sustained plaque regression. If sustained regression were impaired in diabetes, just as in the progression studies discussed elsewhere in this article, an important question is whether the hyperglycemia per se is the impediment.

Although not yet applicable to the general investigative community, a recently developed mouse model has been used to focus on regression of established atherosclerosis.60,61 Basically, a donor mouse with atherosclerosis is used to provide an aortic arch segment with plaques that is anastomosed end to side to a recipient abdominal aorta of mouse. A donor may contain a lesion of any stage and the recipients can be chosen as appropriate for the hypothesis being tested. For example, this model has been used to test whether the normalization of the naturally low level of HDL in the apoE−/− mouse could remodel advanced plaques by transplanting aortic arch segments from apoE−/− mice into apoE−/− mice transgenic for human apoA-I.62 Similarly, the rapid normalization of the lipid profile of the apoE−/− (achieved by transplanting arteries from apoE−/− to wild-type mice) was shown to induce foam cell emigration from plaques in a process in which properties of dendritic cells were acquired.61 Because of the cumbersome and low-throughput nature of the transplant procedure, however, to be truly useful to the research community, it would be desirable to have a nonsurgical model of regression. Two such recent models include the "Reversa" mouse63 and the hypomorphic apoE mouse.64 The former is based on the Ldlr−/− platform but has the disadvantage of having multiple alleles that have been manipulated, making breeding with mice with other genotypes difficult. The latter model is apoE−/−-based and is genetically simpler. Still other possibilities are the use of adenovirus-mediated expression of apoE in apoE−/− mice65 and the use of inducible systems to turn on the expression of either the Ldlr or apoE in the corresponding knockout mice.

Present Models and Those in Development

Investigators both in and out of the AMDCC program have developed models in which vascular lesions are altered with diabetes, and many of these are reviewed elsewhere.18 Insulinopenic diabetes alone appears to alter aortic root lesion size in some models, but more extensive or accelerated disease from diabetes has been more difficult to show. In this regard, in some reports, loss of insulin and/or insulin receptors may alter lesion morphology without altering total lesion size.22,66 This phenomenon is reviewed in other articles in this series. In contrast, another report suggests that insulin actions on macrophages might be inflammatory and increase atherosclerosis lesion size.67

Many models in which diabetes is superimposed onto a hyperlipidemic background have failed to unequivocally dissociate the effects of hyperglycemia from those of hyperlipidemia. Although they are disappointing, these experiments are consistent with the idea that conventional risk factors in humans, such as dyslipidemia and hypertension, that are found with increased frequency with diabetes, especially type 2 diabetes, and not hyperglycemia are the primary causes for accelerated macrovascular disease. Studies in Ldlr−/− mice,68 HDL-deficient mice,69 apoB transgenic mice, including animals with cholesterol ester transfer protein addition,70,71 as well as some studies in apoE−/− mice,72 have failed to show more severe atherosclerosis with diabetes.

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Two approaches have recently been taken and appear to lead to increased diabetes-related vascular pathology. In one, mice overexpressing aldose reductase, the enzyme that converts glucose to sorbitol and is widely viewed to mediate a pathway leading to glucose toxicity, were bred onto the Ldpr<sup>−/−</sup> background. With STZ-induced diabetes, these mice developed more atherosclerosis. A second approach was via inhibition of a pathway for antioxidant defense. A dietary supplement with α-lipoic acid effectively prevented diabetes-induced increased atherosclerosis in apoE<sup>−/−</sup> mice and reduced oxidative stress markers and plasma levels of cholesterol and glucose. As a result of this finding, mice with hemizygous deletion of the gene for lipoic acid synthetase have been created, and the effects of reduced production of endogenous lipoic acid in these mice is being studied. Other methods are being developed to increase reactive oxygen species by reduction of antioxidant defenses such as reducing superoxide dismutases, ascorbic acid synthesis, endothelial nitric oxide production, heme oxygenase, or enzymes in the glutathione pathways. Similarly, methods are being developed to overexpress antioxidant defense enzymes, including superoxide dismutases and catalase, which can then be applied to relieving diabetic cellular stress.

**Future Directions**

Because cholesterol has such an overwhelming impact on lesion development in mouse models, the challenge continues to be the development of a mouse in which hyperglycemia or its products, hyperinsulinemia or insulin resistance, or dyslipidemia with high triglycerides and low HDL, can clearly be defined as a substantial contributor to lesion formation. This problem may necessitate using diets that lower cholesterol to approximately 300 mg/dL, with careful regulation of circulating serum cholesterol levels in the hyperglycemic or insulin-resistant mouse to levels identical to those of wild-type control mouse. This approach will likely reduce lesion extent and complexity, so that it may be difficult to definitively evaluate therapeutic strategies, but it will be useful to define pathophysiologic mechanisms. Alternately, it may be necessary to alter multiple genes in a single model to develop complex lesions, as has been shown in the creation of a mouse that has atherosclerosis and thrombosis. It should be noted that vascular events, strokes, and myocardial infarctions are extremely rare in mice, and models that have reduced plaque stability and/or thrombosis are needed. Clearly multiple factors contribute to vascular injury in humans with the metabolic syndrome or diabetes, so it is difficult to identify which of the multiple potential contributors play a substantive role. Current research efforts seek to determine whether the failure to develop human-like diabetic macrovascular disease in the mice arises from the short-term nature of rodent experiments, the superimposition of severe atherogenic dyslipidemia, the use of relatively young animals, and/or genetic differences in glucose and oxidative responses that prevent the ready development of human-like diabetic macrovascular disease in the mice. All of these factors are being explored.

**Diabetic Cardiomyopathy**

Diabetes also has direct effects on the structure and function of the heart, and diabetic cardiomyopathy is now being increasingly recognized. Diabetes alone increases the risk of cardiac failure, even after controlling for underlying coronary artery disease and hypertension. Moreover, the hearts of diabetic humans exhibit increased vulnerability in the presence of stressors such as ischemia and cardiac hypertrophy. Many clinical studies have demonstrated various functional defects in the hearts of diabetic patients, ranging from subtle defects in diastolic function to overt systolic dysfunction or impaired myocardial reserve in response to inotropic stimuli or exercise. Studies that have examined tissue samples (autopsy or cardiac biopsy) have found interstitial and replacement fibrosis consistent with myocyte loss.

Many animal studies have reproduced these clinical findings and have also demonstrated that the earliest defects that characterize the myocardium in diabetes are abnormalities in myocardial substrate metabolism (increased fatty acid oxidation and decreased glucose uptake and oxidation) and energetics (decreased mitochondrial function), followed by alterations in myocardial calcium handling. The net impact of these changes is contractile dysfunction. Studies in humans with either type 2 diabetes or obesity and insulin resistance have also demonstrated significant increases in myocardial fatty acid uptake and oxidation as well as reduced concentrations of high-energy phosphates in individuals without coronary artery disease or heart failure. Recent studies have focused on the contribution of impaired insulin signaling or insulin action to the pathogenesis of contractile dysfunction in the diabetic heart. Specifically, there is growing evidence that obesity and type 2 diabetes are associated with myocardial insulin resistance. Impaired myocardial insulin signaling may contribute to the metabolic abnormalities that exist in the diabetic heart, such as reduced glucose utilization, increased fatty acid utilization, and mitochondrial dysfunction. Moreover, impaired insulin signaling in the myocardium clearly increases the susceptibility of the heart to injury in the face of hemodynamic stressors such as ischemia and hypertension.

Another emerging mechanism is the potential role of oxidative stress. Increased markers of oxidative stress have been described in the hearts of diabetic mice. Overexpression of catalase, metallothionein, or manganese superoxide dismutases in the hearts of mouse models of type 1 diabetes reverses contractile dysfunction and mitochondrial abnormalities. In patients with the metabolic syndrome, there is local activation of the intracardiac renin–angiotensin system, which is associated with increased myocyte necrosis and apoptosis, leading ultimately to fibrosis. In more advanced stages, there is increased fibrosis, which is associated with activation of protein kinase C-β and increased expression of profibrotic cytokines such as connective tissue growth factor and transforming growth factor-β. Many of these changes have been demonstrated in animal studies as well as in human autopsy or cardiac biopsy studies. In aggregate, these changes further impair cardiac function. The speed of these changes is accelerated in the presence of ischemia.
TABLE 3. Characteristics of Diabetic Cardiomyopathy

(1) Altered myocardial insulin signaling
(2) Increased oxidative stress
(3) Altered substrate flux, more fatty acid, and less glucose oxidation
(4) Overexpression of profibrotic signaling molecules

Diabetic Cardiomyopathy in Mice

Given the multifactorial basis for cardiac dysfunction in diabetes, it will obviously be challenging to model all of these pathophysiological variables in the same heart (Table 3). However, a variety of models exist that reproduce various aspects of the syndrome. Aspects of diabetic cardiomyopathy that have been modeled in the mouse include (1) altered myocardial insulin signaling, (2) oxidative stress, (3) altered substrate flux, and (4) overexpression of profibrotic signaling molecules. In addition, studies in models of type 1 and type 2 diabetes have highlighted important similarities in the cardiac phenotypes that are associated with insulin resistance and obesity and those with insulin deficiency. Some of these models are summarized in Table 4.

An important goal in the establishment of mouse models of diabetic cardiomyopathy is the development of uniform criteria for the definition of the cardiomyopathic phenotype as applicable to the mouse, thereby providing a framework for the comparison of existing models and new models that will be developed in the future. The AMDCC has proposed criteria for diabetic cardiomyopathy in the mouse. Minimal and validation criteria were proposed.

Minimal Criteria for Mouse Models of Diabetic Cardiomyopathy

In the context of hyperglycemia or insulin resistance, a model should exhibit (1) evidence of LV dysfunction such as

TABLE 4. Models of Mouse Diabetic Cardiomyopathy

<table>
<thead>
<tr>
<th>Cardiomyopathy Mouse Model</th>
<th>Type of Diabetes</th>
<th>Relevant Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defective insulin actions or signaling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STZ</td>
<td>Type 1</td>
<td>Cardiac dysfunction, abnormal substrate metabolism decreased cardiac efficiency, mitochondrial dysfunction, increased fibrosis</td>
</tr>
<tr>
<td>OVE26</td>
<td>Type 1</td>
<td>Decreased cardiac function, fibrosis, mitochondrial dysfunction</td>
</tr>
<tr>
<td>Ob/ob</td>
<td>Type 2</td>
<td>LHV, cardiac dysfunction, lipotoxicity, abnormal substrate metabolism, decreased cardiac efficiency, mitochondrial dysfunction, impaired insulin signaling, altered gene expression, oxidative stress</td>
</tr>
<tr>
<td>Db/db</td>
<td>Type 2</td>
<td>Cardiac dysfunction, lipotoxicity, abnormal substrate metabolism, decreased cardiac efficiency, mitochondrial dysfunction, impaired insulin signaling, altered gene expression, oxidative stress</td>
</tr>
<tr>
<td>High-fat feeding</td>
<td>Type 2</td>
<td>Cardiac dysfunction, abnormal metabolism, impaired insulin signaling, decreased cardiac efficiency</td>
</tr>
<tr>
<td>CIRKO mouse (cardiomyocyte insulin receptor KO)</td>
<td>Type 2</td>
<td>Cardiac dysfunction, altered substrate metabolism, increased injury and dysfunction following LV hypertrophy</td>
</tr>
<tr>
<td>IRS-1 KO mouse; dominant negative P3K transgenic</td>
<td>Type 2</td>
<td>LVH; cardiac dysfunction and increased fibrosis following pressure overload hypertrophy</td>
</tr>
<tr>
<td>Cardiomyocyte PDK1 KO</td>
<td>Type 2</td>
<td>Dilated cardiomyopathy, mitochondrial dysfunction</td>
</tr>
<tr>
<td>Cardiomyocyte GLUT4 KO</td>
<td>Type 2</td>
<td>Cardiac hypertrophy, decreased cardiac glucose utilization, decreased recovery from ischemia</td>
</tr>
<tr>
<td>GLUT4 heterozygous KO</td>
<td></td>
<td>Cardiac hypertrophy, cardiac fibrosis, hypertension</td>
</tr>
</tbody>
</table>

Lipotoxicity

| MHC-PPARcg                  |                  | Cardiac hypertrophy, abnormal cardiac metabolism, lipotoxicity, cardiac dysfunction, cardiac fibrosis |
| MHC–acyl CoA synthase       |                  | Cardiac hypertrophy, abnormal cardiac metabolism, lipotoxicity, cardiac dysfunction, cardiac fibrosis |
| MHC-FATP                    |                  | Cardiac hypertrophy, abnormal cardiac metabolism, lipotoxicity, cardiac dysfunction |
| MHC-LPL9r                   |                  | Cardiac hypertrophy, abnormal cardiac metabolism, lipotoxicity, cardiac dysfunction, cardiac fibrosis |
| Adipose TG lipase knockout  |                  | Massive increase in cardiac triglyceride, leading to mechanical dysfunction |

Fibrosis

| MHC-PKCI-β                  |                  | Models the increase in PKCβ2 that occurs in diabetes, although expression levels are much higher; increased fibrosis, expression of CTGF, and cardiac dysfunction |

There are numerous models of enhanced cardiac fibrosis in the literature. Of relevance to diabetes are models with increased expression or activation of the renin–angiotensin signaling pathways. CoA indicates coenzyme A; FATP, fatty acid transport protein; IRS, insulin receptor substrate; PDK, pyruvate dehydrogenase kinase; P3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; TG, triglyceride.
Mitochondrial dysfunction has also been shown to be associated with mitochondrial proliferation.27,28,103,107–109 Reduced mitochondrial oxidative phosphorylation capacity107,110–112 suggests that mitochondrial dysfunction could contribute to the pathophysiology of lipotoxicity that develops in these models by exacerbating the mismatch between cellular fatty acid uptake and mitochondrial fatty acid oxidation.86 Impaired myocardial insulin signaling also develops in the hearts of animal models of type 2 diabetes.95,97–99,113 Taken together, the observations strongly implicate abnormal myocardial insulin signaling, mitochondrial dysfunction, lipotoxicity, and oxidative stress as critical early changes in the evolution of the diabetic cardiomyopathy.

Validation Criteria for Mouse Models of Diabetic Cardiomyopathy

Validation criteria for mouse models of diabetic cardiomyopathy were proposed to further characterize animals that meet the minimal criteria for diabetic cardiomyopathy. These criteria were based on known abnormalities that have been described in various rodent models of diabetes. The criteria include (1) invasive assessment of LV function in vivo to confirm systolic and diastolic dysfunction; (2) evidence of LV dysfunction in isolated perfused hearts; (3) evidence of abnormal cardiac metabolism and mitochondrial dysfunction; (4) altered gene expression, eg, increased expression of β-myosin heavy chain (MHC), decreased expression of α-MHC, and decreased expression of glucose transporters (GLUT4 and GLUT1); and (5) impaired response to stress such as pressure overload hypertrophy and myocardial ischemia. Going forward, it is likely that additional validation criteria could be added, such as evidence of oxidative stress, mitochondrial dysfunction, changes in myocardial calcium handling, activation of profibrotic signaling pathways, and evidence of microangiopathy.

Mouse Models Already Created That Fulfill the Definition of Diabetic Cardiomyopathy

Obesity-Insulin Resistance Models

Attempts were made to determine the very early effects of obesity and insulin resistance, which are clear antecedents of type 2 diabetes, on myocardial metabolism and function. Second, efforts were made to determine the mechanisms that were responsible for and to model the abnormal substrate utilization that develops in the diabetic myocardium. The evolution of cardiac dysfunction in 2 mouse models of obesity and type 2 diabetes, the ob/ob (leptin null) on the C57BL/6 background and the db/db mouse (leptin receptor null) on the C57BLKS/J6 (Kaliss) background, were intensively characterized.90,95 In both models, it was shown that impaired myocardial substrate utilization characterized by decreased rates of glucose utilization (particularly reduced rates of glucose oxidation) and increased rates of fatty acid oxidation develop at a stage when these animals are insulin-resistant and obese but before the onset of hyperglycemia. Once hyperglycemia develops, myocardial fatty acid utilization increases further, and this is the result, in part, of increased fatty acid mediated transcriptional signaling via peroxisome proliferator-activated receptor (PPAR)α. The hearts of these animals exhibit increased rates of myocardial oxygen consumption, decreased myocardial efficiency, increased rates of fatty acid oxidation, and mitochondria uncoupling.107 In light of recent evidence implicating mitochondrial superoxide generation as an activator of mitochondrial uncoupling, it is now hypothesized that oxidative stress contributes to mitochondrial uncoupling in the diabetic heart.

Models of Defective Insulin Signaling and/or Altering Glucose Uptake

Various mouse models with altered myocardial insulin signaling have been generated (Table 4). The most extensively validated model by the consortium has been mice with cardiacmyocyte deletion of insulin receptors (CIRKO). These animals develop age-related LV dysfunction. They have reduced rates of glucose utilization114 and develop mitochondrial dysfunction and evidence of oxidative stress.115 In contrast to other models of type 1 and type 2 diabetes, in this model, rates of fatty acid oxidation are not increased, which might reflect the fact that the delivery of fatty acid substrates to the heart are normal in these mice. An interesting characteristic of these mice, of relevance to diabetes, is that these animals develop increased fibrosis and reduced LV function in the face of hemodynamic stressors such as pressure overload hypertrophy or chronic catecholamine stimulation.101,116 This is characterized by increased expression of the profibrotic cytokine connective tissue growth factor, decreased vascular endothelial growth factor expression and decreased capillary density. Similar findings of impaired angiogenesis have been reported in mice with muscle and cardiomyocyte deletion of insulin receptors.117 These mice have importantly underscored the pleiotropic effects of impaired insulin action in the myocardium in contributing to the pathogenesis of changes in cardiovascular structure and function that mimic diabetic cardiomyopathy.

Other loss-of-function mutants with reduced expression of other components of the insulin-signaling pathway have also been generated and some have been characterized within the consortium. The phenotypes of these models range from early dilated cardiomyopathy in mice with cardiac and skeletal muscle loss of 3′-phosphoinositide-dependent protein kinase 1,118 to relatively minor phenotypes at rest in mice with inactivation of phosphatidylinositol 3-kinase or Akt isoforms but with increased myocardial injury in response to various stresses such as hypertension or ischemia.119–121 Given that a reduction in myocardial glucose utilization represents an early defect in the hearts of diabetics, mice with impaired myocardial glucose uptake have also been of interest. Thus mice with loss of the glucose transporter GLUT4 also mimic some aspects of diabetic cardiomyopathy. For example, heterozygous germline deletion of GLUT4 results in cardiac hypertrophy and increased interstitial fibrosis.122 Mice with cardiomyocyte-restricted knockout of GLUT4 also develop
cardiac hypertrophy, but without any increase in fibrosis. However, they do exhibit decreased contractile recovery following ischemia and reperfusion.123,124

**Models of Lipotoxicity**
Lipid accumulation leading to tissue dysfunction can arise from increased uptake or decreased lipid disposal via resecretion or oxidation. Using appropriate genetic manipulations, all 3 mechanisms have been demonstrated to produce lipid loading of the heart leading to cardiomyopathy.

**Increased Uptake**
Cardiomyocytes, like most cells, acquire fatty acids either from albumin-associated free fatty acids or from esterified lipids circulating as a component of lipoproteins. Fatty acid uptake is either via transporters such as CD36125 or through a nonreceptor process termed “flip-flop” by which the molecules solubilize within the plasma membrane.126 Lipoprotein-derived fatty acids can be released from triglyceride by lipoprotein lipase (LpL) or entire lipoprotein particles can enter cells via receptors.127 Lipotoxic cardiomyopathies have been created by myocyte overexpression of LpL combined with a knockout of PPARα128 and by transgenic expression of LpL attached to cardiomyocytes.129

Although investigators of cardiac energetics have focused on the roles of PPAR transcription factors in regulation of fatty acid oxidation, these factors also modulate lipid uptake by the heart. Overexpression of PPARs in cardiomyocytes using the MHC promoter induces genes associated with greater oxidation of lipids and increases fatty acid oxidation.130 Despite this, MHC-PPARα transgenic mice develop dilated cardiomyopathy associated with increased content of myocardial lipids. High-fat diets and STZ-induced diabetes exacerbate this cardiac dysfunction.131 Thus lipid uptake, which might increase caused by greater expression of CD36 and LpL, must exceed lipid oxidation and resecretion.132 Recently, MHC-PPARα transgenic mice were crossed with CD36 knockout mice.133 This corrected cardiomyopathy, presumably because defective lipid uptake occurred, leading to reduced myocardial lipid accumulation. Transgenic expression of PPARγ also leads to lipotoxic dilated cardiomyopathy associated with increased lipid uptake.134 In contrast, PPARδ overexpression induces lipid oxidation genes in the heart but does not cause lipotoxic cardiomyopathy.135

Further evidence that lipid accumulation is sufficient to cause cardiomyopathy is derived from several other models. Overexpression of fatty acid transport protein136 and fatty acyl–coenzyme A synthase,137 2 proteins that trap lipids within the cardiomyocyte, also leads to dilated, lipotoxic cardiomyopathy.

**Reduced Lipid Disposal**
To supply its continuous need for energy, the heart may transiently store and then hydrolyze and oxidize triglycerides. Adipose tissue triglyceride lipase is required for the first step in triglyceride lipolysis, and hormone sensitive lipase removes the second fatty acid from glycerol. A mechanically dysfunctional triglyceride “loaded” heart was created by knockout of adipose tissue triglyceride lipase.138 Defective cardiac fatty acid oxidation associated with deletion of PPARδ leads to cardiac lipid accumulation.139

**Future Directions**
Going forward, the challenge for the field will be to develop mouse models that examine other pathophysiological components of the diabetic cardiomyopathy. These include (1) developing additional models of mitochondrial dysfunction and oxidative stress; (2) examining the potential contribution of impaired microvascular function, particularly in the context of ischemia and cardiac hypertrophy; (3) examining cross-talk between impaired insulin signaling or action in the vasculature and cardiomyocyte; (4) examining the role of glucose and lipid toxicity; and (5) examining the role of increased expression of inflammatory or profibrotic cytokines in the cardiomyocytes and other cell types such as macrophages and fibroblasts.

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Recipes for Creating Animal Models of Diabetic Cardiovascular Disease

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