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# Rodent models of diabetic cardiomyopathy

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Diabetic cardiomyopathy increases the risk of heart failure in individuals with diabetes, independently of co-existing coronary artery disease and hypertension. The underlying mechanisms for this cardiac complication are incompletely understood. Research on rodent models of type 1 and type 2 diabetes, and the use of genetic engineering techniques in mice, have greatly advanced our understanding of the molecular mechanisms responsible for human diabetic cardiomyopathy. The adaptation of experimental techniques for the investigation of cardiac physiology in mice now allows comprehensive characterization of these models. The focus of the present review will be to discuss selected rodent models that have proven to be useful in studying the underlying mechanisms of human diabetic cardiomyopathy, and to provide an overview of the characteristics of these models for the growing number of investigators who seek to understand the pathology of diabetes-related heart disease.



## Introduction

Cardiovascular complications are the leading cause of diabetes-related morbidity and mortality (Garcia et al., 1974). Although increased coronary atherosclerosis is the major cause of cardiac complications in diabetic patients, an increased risk for the development of heart failure remains that is independent of coronary artery disease and hypertension. More than 30 years ago, Rubler et al. described four diabetic patients suffering from heart failure who had normal coronary arteries and no other obvious etiologies for heart failure (Rubler et al., 1972). Other studies have shown that the increased risk for developing heart failure persists in diabetic patients after adjusting for age, blood pressure, weight, cholesterol and coronary artery disease (Kannel and McGee, 1979; Ho et al., 1993). This has led to the use of the term 'diabetic cardiomyopathy', which has been defined as ventricular dysfunction occurring in diabetic patients in the absence of coronary artery disease and hypertension (Regan et al., 1977; Fein, 1990). The term now includes diabetic individuals with diastolic dysfunction, the prevalence of which may be as high as 60% in well-controlled type 2 diabetic patients (Nicolino et al., 1995; Di Bonito et al., 1996; Poirier et al., 2001; Schannwell et al., 2002; Bell, 2003; Di Bonito et al., 2005).

Although diabetic cardiomyopathy is increasingly recognized, the underlying mechanisms are still incompletely understood. Most knowledge of the disease mechanisms has been gained from studies in animal models of obesity, insulin resistance or diabetes, supported by studies in genetically modified animals that mimic discrete pathophysiological mechanisms that are observed commonly in diabetic hearts. The focus of the present review will be to discuss selected rodent models that have proven to be useful in studying the underlying mechanisms of human diabetic cardiomyopathy.

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## Rodent models as a tool to study diabetic cardiomyopathy

Rodents are useful model organisms with which to study the underlying mechanisms of diabetic cardiomyopathy. They are relatively resistant to the development of atherosclerosis, unless specific atherogenic gene manipulations are introduced (Ishibashi et al., 1994; Coleman et al., 2006). Thus, the effects of obesity, insulin resistance and diabetes on the heart, which are independent of coronary artery disease, can be studied. In recent years, mouse models have evolved as the preferred rodent model in cardiac research. Mice are easy to maintain in the laboratory, have short breeding cycles and, genetically, are related closely to humans. The mouse and human genomes are approximately the same size, contain almost the same number of genes (99%) and show extensive synteny (conserved gene order) (Waterston et al., 2002). The major advantage of mice compared with rats is the ease of generating gain-of-function or loss-of-function mutants, which is facilitated by the availability of the complete genome sequence for a number of mouse strains. Specific gene deletion or gene overexpression strategies can be used to rescue or exaggerate diabetic cardiomyopathy (Belke et al., 2000; Semeniuk et al., 2002), or to mimic specific traits of diabetic cardiomyopathy (Finck et al., 2002). In addition, many different mouse strains with genetically homogeneous backgrounds are available, which, like humans, have varying susceptibility to diabetes-induced changes in cardiac structure and function. Although the Zucker diabetic fatty rat represents a useful model for obesity and type 2 diabetes, the difficulty of genetic manipulation in rats has limited the experimental possibilities in this animal model, although novel gene engineering techniques for rats are now available. In the past, the rat was the preferred model for cardiac physiologic measurements owing to its larger size when compared with the mouse. However, numerous techniques have now been adapted (miniaturized) and validated so that cardiac function can be assessed reliably in the mouse in vivo and ex vivo, including isolated heart perfusions, hemodynamic measurements by cardiac

catheterization, cardiac magnetic resonance imaging (MRI) and echocardiography, which has even been extended to view the hearts of murine fetuses as early as embryonic day 10 (James et al., 1998; Severson, 2004). In addition, novel techniques with increased sensitivity for the detection of contractile abnormalities have been developed, such as high temporal resolution cardiac cine-MRI, which has demonstrated the presence of diastolic dysfunction in diabetic *db/db* mouse hearts (Yue et al., 2007; Stuckey et al., 2008).

The utility of rodents as a model organism for understanding human diabetic cardiomyopathy could be questioned. For example, the length of the cardiac cycle in mice is a tenth of that in humans, and differences also exist in the expression of ion channel and contractile protein isoforms. Thus, murine cardiac physiology may not exactly reproduce traits of human cardiac pathophysiology (James et al., 1998). However, rodent models have many traits in common with human diabetic cardiomyopathy. For example, rodent models of obesity, insulin resistance and type 2 diabetes have identified left ventricular hypertrophy, diastolic dysfunction, increased cardiac fatty acid uptake and utilization, decreased cardiac efficiency, impaired mitochondrial energetics, increased myocardial lipid storage, and impaired $Ca^{2+}$ handling (An and Rodrigues, 2006; Boudina and Abel, 2007; Bugger and Abel, 2008), which mirror similar observations that have been made in humans with type 1 diabetes, or with type 2 diabetes and obesity. Individuals with obesity, type 1 diabetes or type 2 diabetes have been shown to develop diastolic dysfunction and cardiac hypertrophy (Carugo et al., 2001; Poirier et al., 2001; Szczepaniak et al., 2003; McGavock et al., 2007). Using in vivo positron emission tomography (PET) technology, Peterson and colleagues demonstrated that cardiac fatty acid uptake and utilization are increased in obese and insulin-resistant women, and that cardiac efficiency correlates negatively with body mass index (BMI) (Peterson et al., 2004). They also demonstrated that the hearts of individuals with type 1 diabetes exhibited increased myocardial oxygen consumption ($MVO_2$) and impaired insulin-mediated glucose uptake. The increase in fatty acid utilization was proportional to serum fatty acid levels, which increased in proportion to the severity of hyperglycemia (Peterson et al., 2008). $^{31}P$ magnetic resonance spectroscopy has revealed decreased creatine phosphate (PCr)/ATP ratios in the hearts of subjects with type 1 and type 2 diabetes; the decrease is proportional to the degree of diastolic dysfunction, suggesting a role for impaired cardiac mitochondrial energetics (Metzler et al., 2002; Diamant et al., 2003; Scheuermann-Freestone et al., 2003). Using magnetic resonance spectroscopy, Szczepaniak et al. showed that myocardial triglyceride storage correlates with BMI and is correlated inversely with regional systolic function (Szczepaniak et al., 2003). Increased myocardial triglycerides have been shown to exist also in type 2 diabetes (McGavock et al., 2007). These findings were confirmed by Sharma et al., who demonstrated a fivefold to sixfold increase in intramyocardial lipid levels in obese, or type 2 diabetic, patients suffering from non-ischemic heart failure that was associated with a transcriptional profile similar to that of the Zucker diabetic fatty rat (Sharma et al., 2004). Finally, myofilament function is depressed in skinned fibers as a result of decreased $Ca^{2+}$ sensitivity, suggesting that $Ca^{2+}$ handling may also be impaired in human diabetic cardiomyopathy (Regan et al., 1977; Jweied et al., 2005). Thus, experimental findings in rodent models are likely to have utility in identifying underlying mechanisms of human diabetic cardiomyopathy. A summary of common abnormalities in human and rodent type 2 diabetic cardiomyopathy is presented in Table 1. An overview of the molecular mechanisms that are proposed to contribute to the development of diabetic cardiomyopathy is illustrated in Fig. 1. For a more comprehensive and detailed discussion of the basic mechanisms and pathology of diabetic cardiomyopathy, the reader is referred to previously published reviews on this topic (Hayat et al., 2004; An and Rodrigues, 2006; Boudina and Abel, 2007; Bugger and Abel, 2008; Asghar et al., 2009).

## Rodent models of diabetic cardiomyopathy

The cardiac phenotypes in models of type 1 and type 2 diabetes show significant overlap. Both models are characterized by increased fatty acid utilization, decreased glucose utilization, impaired calcium handling, compromised mitochondrial energetics, and increased connective tissue content in the heart. Thus, models of type 1 and type 2 diabetes have been used interchangeably to understand pathophysiological mechanisms of diabetic cardiomyopathy. However, recent studies have revealed important differences between models of type 1 and type 2 diabetes. Mitochondrial reactive oxygen species (ROS) production is increased in the hearts of type 2 diabetic models, whereas type 1 diabetic models show no increase or even reduced production of ROS that originate from mitochondria (Boudina et al., 2007; Bugger et al., 2008; Herlein et al., 2009). Fatty acid-induced mitochondrial uncoupling is another trait of type 2 diabetic hearts that does not seem to be present in type 1 diabetic models (Boudina et al., 2007; Bugger et al., 2008). Thus, in some circumstances, pathophysiological mechanisms for cardiomyopathy may differ between type 1 and type 2 diabetes.

The interpretation of experimental findings should also take into account the etiology of obesity and diabetes in a given model. Models can differ in the severity of obesity and diabetes, and may display distinct susceptibility to cardiomyopathy depending on the genetic background of the rodent strain. In some animal models, confounding effects owing to toxic drug treatment, or specific effects of an underlying genetic mutation that leads to obesity and type 2 diabetes, should be taken into account. We will present selected animal models, divided by the type of diabetes, that have been used to study diabetic cardiomyopathy. The pathogenesis of diabetes will be described, the cardiac abnormalities will be

**Table 1. Summary of common cardiac abnormalities in obese and type 2 diabetic patients, and in animal models of obesity and type 2 diabetes**

| | Obese/diabetic patients | *ob/ob* | *db/db* | ZDF |
|---|---|---|---|---|
| Diastolic function | ↓ | ↓ | ↓ | ↓ |
| LV mass | ↑ | ↑ | ↑ | ↑ |
| Cardiac efficiency | ↓ | ↓ | ↓ | = |
| Fatty acid oxidation | ↑ | ↑ | ↑ | ↑ |
| Lipid content | ↑ | ↑ | ↑ | ↑ |
| Mitochondrial energetics | ↓ | ↓ | ↓ | |
| $Ca^{2+}$ handling | ↓ | ↓ | ↓ | = |

LV, left ventricular; ZDF, Zucker diabetic fatty.
See text for references.

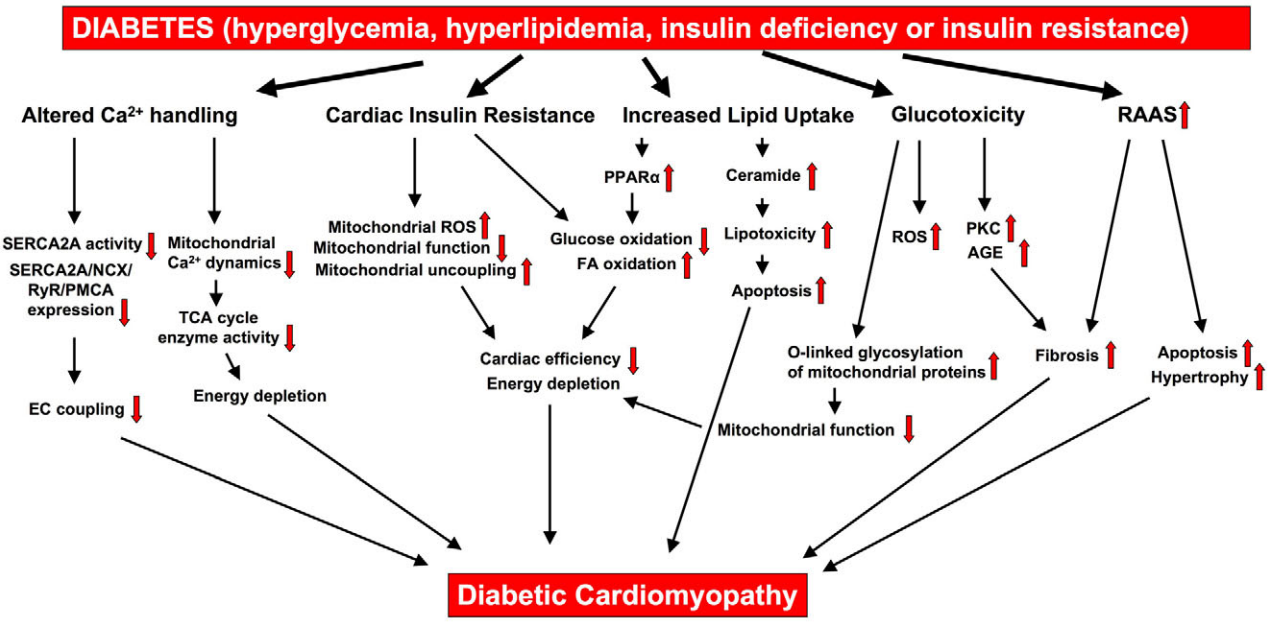


**Fig. 1. Schematic of mechanisms that may impair myocardial function in diabetes.** Five major mechanisms and their downstream consequences are summarized: impaired calcium cycling, myocardial insulin resistance, increased lipid uptake, glucotoxicity and activation of the renin-angiotensin-aldosterone system (RAAS). The mechanisms linking diabetes with these pathways are incompletely understood and it is not known whether these pathways share a common pathophysiology. Moreover, it is likely that these mechanisms are interrelated and may exacerbate each other. SERCA2A, sarcoendoplasmic reticulum $Ca^{2+}$-ATPase 2A; NCX, $Na^{2+}/Ca^{2+}$ exchanger; PMCA, plasma membrane $Ca^{2+}$-ATPase; RyR, ryanodine receptor; EC coupling, excitation-contraction coupling; TCA cycle, tricarboxylic acid cycle; PPARα, peroxisome proliferator-activated receptor α; ROS, reactive oxygen species; PKC, protein kinase C; AGE, advanced glycation end products.

summarized, and the advantages and pitfalls of each respective model will be discussed. Finally, we will discuss genetically engineered models that have been generated to mimic specific diabetes-associated cardiac alterations. A comparison of cardiac abnormalities between the type 1 and type 2 diabetic models that are reviewed is shown in Table 2. Models not reviewed in this article are listed in Table 3.

## Models of type 1 diabetes

### The streptozotocin (STZ) model

The most frequently used model of type 1 diabetes is the streptozotocin (STZ) model. STZ is a glucosamine-nitrosourea antibiotic that is similar structurally to glucose and is taken up preferentially by the GLUT2 glucose transporter in insulin-producing pancreatic β-cells (Schnedl et al., 1994). Intraperitoneal treatment with STZ results in β-cell toxicity and necrosis, leading ultimately to insulin deficiency (Bonnevie-Nielsen et al., 1981). Both high-dose regimens with a single dose of STZ (up to 200 mg/kg) and low-dose regimens with consecutive injections of low doses of STZ have been applied to animals to cause diabetes. Since STZ is known to cause extrapancreatic genotoxic effects, the Animal Models of Diabetic Complications Consortium (AMDCC) recommends the low-dose protocol with five consecutive injections of 50 mg/kg STZ (www.amdcc.org). Using this protocol, rodents develop hyperglycemia within 7 to 14 days after the first injection. STZ-treated mice show increased serum fatty acid, triglyceride and cholesterol levels, whereas insulin levels progressively decrease with the duration of diabetes (Islas-Andrade et al., 2000).

Most studies in STZ-diabetic mice report systolic and diastolic dysfunction that increases in severity in proportion to the duration of diabetes. Echocardiographic analyses have shown decreased rates of circumferential shortening and fractional shortening (Nielsen et al., 2002; Suarez et al., 2008). Reduced left ventricular (LV) systolic pressure and diminished ±dP/dt (rate of pressure rise or fall during systole and diastole, respectively) have been demonstrated using LV catheterization (Kajstura et al., 2001; Van Linthout et al., 2008). Diastolic dysfunction has been suggested by increased LV diastolic pressure, measured by catheterization, and by abnormal patterns of mitral inflow and pulmonary venous flow using Doppler echocardiography (Kajstura et al., 2001; Lacombe et al., 2007). In vitro, peak LV pressure and ±dP/dt are reduced in Langendorff-perfused hearts (Trost et al., 2002; Suarez et al., 2004; Suarez et al., 2008).

Studies of cardiac metabolism reveal increased fatty acid oxidation (FAO), and increased expression of the genes encoding peroxisome proliferator-activated receptor α (PPARα) and FAO proteins, whereas, glucose oxidation and pyruvate dehydrogenase activity are reduced (Flarsheim et al., 1996; Chatham and Forder, 1997; Depre et al., 2000; Finck et al., 2002; How et al., 2006). Consistent with these observations, proteomic studies demonstrated an increased abundance of FAO proteins within mitochondria as early as 1 week after the onset of diabetes (Turko et al., 2003). Mitochondrial respiratory function declines progressively with various substrates that have been tested, including α-ketoglutarate, pyruvate and succinate (Flarsheim et al., 1996; Lashin et al., 2006). Creatine kinase activity is decreased in

**Table 2. Cardiac abnormalities in type 1 and type 2 diabetic rodent models**

| | STZ | OVE26 | Akita | *ob/ob* | *db/db* | ZDF |
|---|---|---|---|---|---|---|
| Cardiac size | = | | ↓/= | ↑ | ↑ | ↑ |
| Cardiac function | ↓ | ↓ | ↓ | ↑/↓ | ↓ | ↓ |
| Cardiac efficiency | ↓ | | = | ↓ | ↓ | = |
| Mitochondrial energetics | ↓ | ↓ | ↓ | ↓ | ↓ | |
| Lipid storage | ↑ | | | ↑ | ↑ | ↑ |
| Fatty acid oxidation | ↑ | | ↑ | ↑ | ↑ | ↑ |
| Glucose oxidation | ↓ | | ↓ | ↓ | ↓ | ↓ |
| $Ca^{2+}$ handling | ↓ | ↓ | ↓ | ↓ | ↓ | = |
| Oxidative stress | ↑ | ↑ | | ↑ | ↑ | |

STZ, streptozotocin.
See text for references.

STZ hearts, possibly as a consequence of reduced mRNA expression of the enzyme (Popovich et al., 1989). Studies investigating oxidative stress in STZ-diabetic hearts revealed increased cellular ROS levels, enhanced superoxide production, increased NADPH oxidase expression (subunit p47) and decreased GSSG/GSH ratios (ratio of oxidized to reduced glutathione) (Ghosh et al., 2004; Ghosh et al., 2005; Ceylan-Isik et al., 2006; Lashin et al., 2006; Wold et al., 2006; Singh et al., 2008). The mitochondrial origin of increased superoxide remains controversial because direct measurements of mitochondrial superoxide production showed no increase in STZ hearts (Herlein et al., 2009).

STZ hearts also display perturbations in intracellular $Ca^{2+}$ handling, including reduced expression and activity of sarcoendoplasmic reticulum $Ca^{2+}$-ATPase 2a (SERCA2a), reduced $Na^{+}$-$Ca^{2+}$ exchanger expression, impaired sarcoplasmic reticulum calcium release and reuptake, and compromised mitochondrial $Ca^{2+}$ cycling (Lopaschuk et al., 1983; Flarsheim et al., 1996; Hattori et al., 2000; Choi et al., 2002; Zhao et al., 2006; Suarez et al., 2008). Furthermore, several studies have demonstrated increased connective tissue content in STZ-diabetic hearts, which can be attenuated by treatment of mice with the aldosterone antagonist spironolactone, suggesting that increased aldosterone action may contribute to cardiac fibrosis (Miric et al., 2001; Westermann et al., 2007; Singh et al., 2008; Ueno et al., 2008; Van Linthout et al., 2008). Cardiac angiotensin II receptor density and synthesis is increased in STZ hearts, and increased superoxide production, apoptosis and fibrosis can be inhibited, at least partially, by treatment with angiotensin receptor blockers or angiotensin-converting enzyme (ACE) inhibitors (Brown et al., 1997; Singh et al., 2008).

The most important advantages of the STZ model are that diabetes can be induced easily in mice and rats, and that the model permits the evaluation of diabetes on the heart in varying genetic background strains. Diabetes can easily be superimposed in genetically altered mice, which allows the creative design of sophisticated mechanistic studies, without prolonged waiting periods, as would be necessary if mutant mouse strains were crossed with genetic models of diabetes. Diabetes can also be induced at different ages, which allows the effects of diabetes on the heart to be investigated at various stages in the life cycle of the organism.

An important limitation of the STZ model is the potential for extrapancreatic genotoxic effects (Bolzan and Bianchi, 2002). For example, changes in hepatic gene expression, including downregulation of genes related to glucose and lipid metabolism, occur as early as 48 hours following STZ treatment and before elevation of systemic glucose levels, suggesting that STZ has direct effects on gene expression that are unrelated to hyperglycemia (Kume et al., 2005). In the heart, STZ may directly impair cardiac contractile function through a p38 MAP kinase-dependent oxidative stress mechanism (Wold and Ren, 2004). In addition, the severity of diabetes can vary in the STZ model with some animals developing ketosis, whereas others do not. In this circumstance, mitochondrial dysfunction developed only in the presence of ketosis, despite equivalent degrees of hyperglycemia (Lashin and Romani, 2004).

**Table 3. Additional models of type 1 and type 2 diabetes with cardiomyopathy**

| Type of diabetes | |
|---|---|
| **Type 1 diabetes** | **References** |
| NOD mouse | Pacher et al., 2002 |
| Alloxan | Fein et al., 1985; Zola et al., 1988 |
| BB rat | Rodrigues and McNeill, 1990; Broderick and Hutchison, 2004; Broderick and Poirier, 2005 |
| **Type 2 diabetes** | |
| Goto-Kakizaki rat | Desrois et al., 2004a; Desrois et al., 2004b |
| KK $A^{y}$ mouse | Ye et al., 2004 |

NOD, non-obese diabetic.

## The OVE26 mouse model

The OVE26 mouse (on the FVB background) was generated by Epstein et al. in 1989 (Epstein et al., 1989). Overexpression of the $Ca^{2+}$-binding protein calmodulin in pancreatic β-cells led to insulin-deficient diabetes within the first week of life owing to pancreatic β-cell damage, although the exact mechanism of cell damage remains to be elucidated (Epstein et al., 1989). OVE26 mice develop increased serum triglyceride levels, have reduced insulin levels, and survive for 1 to 2 years without insulin administration (Epstein et al., 1989; Liang et al., 2002).

In the OVE26 mouse, cardiac contractility has been studied mainly in isolated cardiomyocyte preparations. Several studies have demonstrated impaired peak shortening, prolonged time to peak

shortening, prolonged time to 90% re-lengthening, and reduced maximal velocities of shortening and re-lengthening (Duan et al., 2003; Ye et al., 2003; Zhang et al., 2003). By contrast, no significant reduction of contractile force was observed in Langendorff perfusions of OVE26 diabetic hearts (Liang et al., 2002).

Ultrastructural analyses of OVE26 hearts show areas with swollen mitchondria, mottled matrices and broken mitochondrial membranes, accompanied by impairment in pyruvate-supported mitochondrial state 3 respiration (Shen et al., 2004; Shen et al., 2006). In addition, mitochondrial content is increased in OVE26 hearts and analysis of the cardiac proteome revealed the induction of several mitochondrial proteins, suggesting increased mitochondrial biogenesis in these hearts (Shen et al., 2004; Shen et al., 2006). Several studies also indicate that oxidative stress occurs in OVE26 hearts. In these hearts, GSH levels are reduced, catalase expression is induced and malondialdehyde levels are increased; furthermore, the incubation of isolated cardiomyocytes in high glucose medium increases cellular ROS levels, which potentially results from increased mitochondrial superoxide generation (Ye et al., 2003; Shen et al., 2004; Ye et al., 2004; Shen et al., 2006). Importantly, overexpression of metallothionein, catalase or manganese superoxide dismutase (MnSOD) at least partially reverses some of the cardiac abnormalities in OVE26 mice, including mitochondrial ultrastructural abnormalities, mitochondrial dysfunction and impaired contractility (Liang et al., 2002; Ye et al., 2003; Ye et al., 2004; Shen et al., 2006). Impairment in the intracellular $Ca^{2+}$ handling of OVE26 hearts has been reported as increased resting $Ca^{2+}$ levels, attenuated $Ca^{2+}$-induced $Ca^{2+}$ release, delayed recovery of the intracellular $Ca^{2+}$ transient, and reduced expression of SERCA2a and the $Na^{+}$-$Ca^{2+}$ exchanger (Ye et al., 2003; Ye et al., 2004; Kralik et al., 2005). With respect to myocardial substrate oxidation, it has only been shown that total glucose transporter-4 (Glut4) levels and insulin-stimulated Akt phosphorylation are not reduced in OVE26 mice; substrate oxidation rates have yet to be reported (Duan et al., 2003).

Compared with the STZ model, the findings in the OVE26 mouse are not confounded by potential extrapancreatic drug toxicity. In addition, OVE26 mice survive for more than 1 year, thus allowing the long-term effects of diabetes to be investigated on the heart, whereas the survival of STZ diabetic rodents is limited. However, OVE26 mice develop diabetes in the first week postpartum, that is, at a very early stage in postnatal development, which may influence cardiac development and lead to myocardial adaptations that might not necessarily recapitulate the consequences of the type 1 diabetes that develops during adulthood.

### The heterozygous *Ins2*$^{+/-}$ Akita diabetic mouse

A more recently discovered model of type 1 diabetes is the Akita diabetic mouse (Yoshioka et al., 1997). This mouse develops diabetes as a consequence of a single base pair substitution in the *Ins2* gene, resulting in impaired folding of proinsulin, which leads to protein aggregate-induced endoplasmic reticulum stress in pancreatic islets and eventual β-cell failure (Yoshioka et al., 1997; Ron, 2002). Akita mice on the C57BL/6 background consistently develop hyperglycemia, by as early as 5 to 6 weeks of age, which is associated with increased serum fatty acid and triglyceride levels (Bugger et al., 2008). Since hyperglycemia is less pronounced in female Akita mice, male mice are usually studied. Akita mice die between 40 and 50 weeks of age.

Because of the recent discovery of the Akita mouse, relatively few studies are available that describe the cardiac phenotype of this mouse model. Although Lu et al. reported an almost 50% reduction in fractional shortening, estimated by echocardiography, long-term studies from our laboratory could not confirm significant contractile dysfunction in Akita mice in vivo (Lu et al., 2007; Bugger et al., 2008). Using isolated working heart perfusions, we identified only subtle impairment in LV-developed pressure, whereas the inotropic response to isoproterenol treatment or insulin was impaired significantly, suggesting that basal cardiac contractility is only mildly affected in Akita mice, whereas cardiac reserve appears to be impaired (Bugger et al., 2008).

FAO rates are increased in Akita hearts, whereas glucose oxidation rates are reduced (Bugger et al., 2008). In addition, mitochondrial function is compromised; the expression of genes encoding for subunits of mitochondrial oxidative phosphorylation (OXPHOS) complexes is reduced; OXPHOS and TCA cycle proteins are reduced; and the density of mitochondrial cristae is severely decreased, despite an increase in mitochondrial content (Bugger et al., 2008; Bugger et al., 2009). A recent proteomics study from our laboratory suggests that reduced signaling through the peroxisome proliferator-activated receptor gamma co-activator 1 (PGC-1) transcriptional regulatory cascade may contribute to reduced TCA cycle and OXPHOS subunit content, thereby leading to cardiac mitochondrial dysfunction in Akita mice (Bugger et al., 2009). Akita mice show no signs of cardiac mitochondrial uncoupling or impairment in cardiac efficiency, as observed in type 2 diabetic models (Boudina et al., 2007; Bugger et al., 2008). Mitochondrial superoxide production and total cellular ROS levels are not increased in Akita hearts, suggesting that mitochondrial oxidative stress might not be present in mouse hearts in this model (Bugger et al., 2008). Akita hearts have decreased L-type $Ca^{2+}$ current density, which may, at least in part, be the result of reduced expression of L-type $Ca^{2+}$ channels on the cardiomyocyte surface (Lu et al., 2007).

In contrast to the OVE26 mouse, the onset of diabetes occurs in Akita mice at 5 to 6 weeks of age, which is more similar to humans who develop type 1 diabetes at between 15 and 25 years of age. Similar to OVE26 mice, no confounding drug effects have to be taken into account. Besides cardiomyopathy, the Akita model also replicates other typical complications of diabetes, such as retinopathy, neuropathy and nephropathy. Although originally backcrossed into the C57 background, the Akita mouse is now also available on the FVB background at Jackson Laboratories.

## Models of type 2 diabetes

### The *ob/ob* mouse

*ob/ob* mice develop diabetes as a consequence of recessive mutations in the obesity (*ob*, also known as *Lep*) gene. In 1994, Friedman's group identified the gene product of the obesity gene as the adipocytokine leptin (Zhang et al., 1994). The *ob* gene is mutated in both available strains of *ob/ob* mice. In *ob/ob*$^{2J}$ mice, no mature *ob* RNA is synthesized, whereas, in *ob/ob*$^{1J}$ mice, a truncated protein is synthesized that is then degraded within the adipocyte (Zhang et al., 1994; Moon and Friedman, 1997). Thus, in both models, obesity and diabetes result from leptin deficiency

owing to long-term failure of appetite suppression in the hypothalamus (Friedman and Halaas, 1998). By as early as 4 weeks of age, *ob/ob* mice on the C57BL/6 background are moderately obese, display hyperinsulinemia and have impaired glucose tolerance, but are not yet diabetic (Buchanan et al., 2005). By 15 weeks, these mice are severely obese and develop type 2 diabetes. Serum fatty acid and triglyceride levels are increased in some studies, which may depend on the nutritional state (peak fed, post-absorptive or fasted) and the age investigated (Mazumder et al., 2004; Buchanan et al., 2005). Besides hyperphagia, *ob/ob* mice are characterized by decreased body temperature, markedly increased body fat content, decreased energy expenditure and activity, and infertility (Coleman, 1978). *ob/ob* mice die at approximately 14 months of age (Barouch et al., 2006).

The contractile phenotype of *ob/ob* mouse hearts is subtle. *ob/ob* mice develop cardiac hypertrophy with only mild or no impairment in systolic function, as measured by echocardiography (Barouch et al., 2003). However, *ob/ob* mice appear to have diastolic dysfunction, as evidenced by reduced ratios of early to late (E/A) transmitral flow velocities in Doppler flow analysis (Christoffersen et al., 2003). Using cardiac catheterization, contractile function is normal or increased in vivo (Buchanan et al., 2005). In addition, in isolated working heart perfusions, contractile function is not impaired or only mildly impaired (Barouch et al., 2003; Mazumder et al., 2004; Buchanan et al., 2005). By contrast, myocardial oxygen consumption is increased in *ob/ob* mice, resulting in decreased cardiac efficiency, which may contribute to impaired cardiac reserve in *ob/ob* hearts (Christoffersen et al., 2003; Mazumder et al., 2004; Boudina et al., 2005; Buchanan et al., 2005). In isolated *ob/ob* cardiomyocytes, peak shortening and the maximal velocities of shortening and re-lengthening are depressed (Li et al., 2006).

The rates of FAO and myocardial triglyceride storage are increased in *ob/ob* mice, whereas the rates of glucose oxidation are decreased, and cardiac insulin resistance develops (Lee et al., 2001; Christoffersen et al., 2003; Mazumder et al., 2004; Buchanan et al., 2005). The mitochondrial respiratory capacity is reduced in *ob/ob* hearts with various substrates, and mitochondrial ATP synthesis is uncoupled from oxygen consumption when hearts are exposed to high concentrations of fatty acids (Boudina et al., 2005). *ob/ob* cardiomyocytes have an increased malondialdehyde content, reduced GSH/GSSG ratios, increased protein carbonyl formation, and increased levels of the p47 and gp91 subunits of NADPH oxidase, suggesting that oxidative stress occurs in *ob/ob* hearts (Li et al., 2006). *ob/ob* cardiomyocytes have elevated intracellular resting $Ca^{2+}$ concentrations, prolonged intracellular $Ca^{2+}$ decay, diminished responsiveness to extracellular $Ca^{2+}$, and decreased SERCA2a activity (Li et al., 2006). $Ca^{2+}$ transients are smaller and slower, and sarcoplasmic reticulum (SR) $Ca^{2+}$ reuptake is impaired (Fauconnier et al., 2005; Van den Bergh et al., 2008). Apoptotic cell death and caspase 3 activity are also increased in *ob/ob* hearts (Barouch et al., 2006; Van den Bergh et al., 2008).

*ob/ob* mice recapitulate the metabolic phenotype of humans with insulin resistance and obesity, and the cardiac phenotype of *ob/ob* mice shares many traits with the hearts of humans with obesity and type 2 diabetes (Table 1). This model allows the evaluation of the early effects of obesity and insulin resistance on cardiac function, and the effects of additional hyperglycemia at older ages. It is important to acknowledge that leptin deficiency may confound the results owing to potential specific effects that leptin may exert on cardiac function. Leptin has been proposed to have pro- or anti-hypertrophic effects, to regulate heart rate, and to exert cardioprotective effects following ischemia-reperfusion (Carlyle et al., 2002; Barouch et al., 2003; Rajapurohitam et al., 2003; Smith et al., 2006). In humans, the metabolic syndrome is characterized by hyperleptinemia and leptin resistance. Thus, impaired leptin action in *ob/ob* mice could mimic specific effects of leptin resistance in human obesity. However, it is unclear whether peripheral organs, including the heart, are indeed resistant to the action of leptin, and the potential contribution of impaired cardiac leptin action to abnormal cardiac function in *ob/ob* mice remains to be elucidated. Leptin signaling has significant effects on immune cells, and defects in innate and adaptive immunity have been described in *ob/ob* mice (Sheena and Meade, 1978; Meade et al., 1979; La Cava and Matarese, 2004; Matarese et al., 2005; Otero et al., 2006). For example, cardiac injury induced by viral myocarditis is more pronounced in *ob/ob* mice than in their lean controls, probably owing to a defective T-cell response (Kanda et al., 2004). However, with the exception of steatosis, we have not observed significant pathological changes in *ob/ob* mouse hearts.

## The *db/db* mouse

The *db/db* mutations, which arose initially on the C57BL/Ks background, are another model of obesity and type 2 diabetes that develop because of the lack of hypothalamic leptin action (Coleman, 1978). In contrast to *ob/ob* mice, leptin action is impaired in *db/db* mice because of a leptin receptor (Ob-R) defect. Owing to abnormal splicing, the insertion of a premature stop codon into the *db* transcript leads to the long form of the leptin receptor (Ob-Rb) being replaced with the short-form isoform (Ob-Ra) (Chen et al., 1996; Lee et al., 1996). Since Ob-Rb is responsible for leptin action in the hypothalamus to regulate appetite, body weight and energy expenditure, the lack of Ob-Rb receptors leads to increased obesity despite increased serum leptin levels in these mice. Although glucose tolerance is normal in 4-week-old *db/db* mice, this model develops severe type 2 diabetes by 8 weeks of age and is equivalently obese to *ob/ob* mice (Buchanan et al., 2005). *db/db* mice have early hyperinsulinemia and, in most studies, serum fatty acid and triglyceride levels are increased (Aasum et al., 2003; Buchanan et al., 2005; Hafstad et al., 2006). *db/db* mice on the C57BL/6 background are similar phenotypically (in terms of body weight and glucose homeostasis) to *ob/ob* mice.

Contractile disturbances are more pronounced in *db/db* (C57BL/Ks) mice when compared with *ob/ob* mice, which probably reflects the earlier onset and greater severity of hyperglycemia. *db/db* mice develop cardiac hypertrophy as evidenced by increased LV mass and wall thickness in cardiac MRI assessments (Yue et al., 2007). Using echocardiography, reduced fractional shortening and a reduction in the velocity of circumferential shortening have been demonstrated (Semeniuk et al., 2002; Carley et al., 2004; Pereira et al., 2006). Cardiac output, LV-developed pressure and cardiac power are all reduced in isolated, working *db/db* hearts, whereas LV end diastolic pressure is increased (Belke et al., 2000; Aasum et al., 2003; Carley et al., 2004; Hafstad et al., 2006; Hafstad et al., 2007). Similar contractile deficits are observed in Langendorff-perfused *db/db* hearts, in which ±dP/dt, peak systolic pressure, rate pressure product and developed pressure are all reduced (Belke et al., 2004;

Boudina et al., 2007). In addition, diastolic function is impaired when assessed by echocardiography or MRI (Semeniuk et al., 2002; Stuckey et al., 2008). Furthermore, cardiac efficiency is decreased in *db/db* mice, probably owing to fatty acid-induced mitochondrial uncoupling (Buchanan et al., 2005; How et al., 2006; Boudina et al., 2007).

FAO and myocardial lipid storage are increased in *db/db* mice, whereas glucose uptake and oxidation are decreased, accompanied by cardiac insulin resistance (Belke et al., 2000; Lee et al., 2001; Aasum et al., 2003; Carley et al., 2004; Buchanan et al., 2005; Hafstad et al., 2006; Yue et al., 2007). In addition, mitochondrial function is impaired and mitochondrial content is increased (Boudina et al., 2007). Increased mitochondrial superoxide generation and increased malondialdehyde and 4-hydroxynonenal levels suggest that there is oxidative stress in the hearts of *db/db* mice (Boudina et al., 2007). Perturbations in cardiac $Ca^{2+}$ handling are found in these mice, as shown by decreased systolic and diastolic levels of $Ca^{2+}$, decreased rates of $Ca^{2+}$ decay and $Ca^{2+}$ leakage from the SR (Belke et al., 2004). $Ca^{2+}$ transients, L-type $Ca^{2+}$ current and SR $Ca^{2+}$ load are all reduced in *db/db* hearts (Pereira et al., 2006). Cardiac mineralocorticoid receptor blockade with eplerone normalizes reduced cardiac adiponectin expression in *db/db* mice, suggesting that an activated renin-angiotensin-aldosterone system (RAAS) may have negative effects on cardiac function in these mice (Guo et al., 2008). Sympathetic activation and reduced cardiac parasympathetic tone have been demonstrated in *db/db* mice, indicative of cardioregulatory autonomic dysfunction (Goncalves et al., 2009). As in *ob/ob* mice, the direct contribution of impaired myocardial leptin action to cardiac dysfunction is incompletely understood.

## The Zucker fatty rat and Zucker diabetic fatty (ZDF) rat

Zucker fatty rats have a homozygous missense mutation in the *Fa* (also known as *Lepr*) gene encoding the rat leptin receptor (Ob-R). The mutation occurs in a sequence of the *Fa* gene that is common to all leptin receptor isoforms, including Ob-Rb (Iida et al., 1996; Phillips et al., 1996). Obesity develops because of non-functioning leptin receptors, possibly owing to a receptor dimerization defect (Phillips et al., 1996). Zucker fatty rats are hyperphagic, obese and develop increased serum triglyceride, fatty acid and insulin levels, but are not hyperglycemic (Luiken et al., 2001; Coort et al., 2004). The phenotype of the Zucker diabetic fatty rats (ZDF rats) originated from selective breeding of Zucker fatty rats that exhibited high glucose levels, thus ZDF rats are an inbred strain generated from the outbred Zucker fatty rat. ZDF rats are obese, hyperinsulinemic, hyperglycemic, hyperleptinemic and have consistently increased serum fatty acid and triglyceride levels (Clark et al., 1983; Golfman et al., 2005; Wang et al., 2005). ZDF rats are obese and insulin resistant until 6 weeks of age but, during this period, they are euglycemic. Hyperglycemia starts to develop at around 6 weeks of age and, by the age of 10-12 weeks, stable hyperglycemia has developed and insulin levels begin to fall owing to pancreatic β-cell insufficiency.

In general, the cardiac phenotypes of the Zucker models have been investigated less thoroughly compared with *ob/ob* and *db/db* mice. Changes in substrate oxidation and abnormalities in cardiac contractility are less pronounced in Zucker fatty rats than in diabetic ZDF rats. Zucker fatty rats develop cardiac hypertrophy and interstitial fibrosis (Luiken et al., 2001; Conti et al., 2004). Reduced cardiac power has been observed in isolated, perfused Zucker fatty rat hearts, whereas another study demonstrated increased rate pressure products in this model (Vincent et al., 2001; Young et al., 2002). Cardiac efficiency appears unaltered in this model (Young et al., 2002). Carbohydrate oxidation is reduced in Zucker fatty rats, whereas oleate oxidation and FAO gene expression are not increased (Young et al., 2002). However, palmitate uptake is enhanced in isolated cardiomyocytes of Zucker fatty rats, and myocardial lipid content is increased (Luiken et al., 2001; Vincent et al., 2001; Coort et al., 2004). Increased lipid peroxide levels, increased total superoxide dismutase activity, and the induction of antioxidant enzymes suggest that there may be oxidative stress in Zucker fatty rat hearts (Vincent et al., 2001; Conti et al., 2004). No change in SERCA2a mRNA expression has been observed in Zucker fatty rats (Young et al., 2002; Golfman et al., 2005).

In ZDF rats, impaired cardiac contractility has been observed more consistently. Fractional shortening, as measured by echocardiography, is reduced in 20-week-old ZDF rats, and cardiac power, dP/dt and rate pressure product are all reduced in isolated perfused hearts of ZDF rats (Zhou et al., 2000; Sharma et al., 2004; Golfman et al., 2005; Wang et al., 2005). The hearts of ZDF rats develop hypertrophy and increased myocardial lipid storage (Zhou et al., 2000; Lee et al., 2001; Sharma et al., 2004; Golfman et al., 2005). Rates of FAO and FAO gene expression are increased in the hearts of ZDF rats, whereas carbohydrate oxidation, pyruvate dehydrogenase flux and Glut4 expression are all decreased (Chatham and Seymour, 2002; Sharma et al., 2004; Golfman et al., 2005; Wang et al., 2005). No change in SERCA2a mRNA expression has been observed in ZDF rats (Golfman et al., 2005).

The obese and diabetic Zucker rats represent useful models to investigate the effect of obesity and/or type 2 diabetes on the heart. It is important to point out that hyperglycemia does not develop in Zucker fatty rats, as opposed to *ob/ob* mice, therefore providing a unique model with which to conduct longitudinal studies on the long-term effects of obesity on the heart. The genetic background is heterogeneous, which more closely resembles the human condition. By contrast, ZDF rats are inbred so direct comparisons between obese and diabetic Zucker rats are complicated owing to differences in the genetic background. Compared with the mouse models, serum lipid levels appear to be altered more dramatically in the ZDF rat. As outlined above, the possibilities of additional genetic manipulation are limited compared with mice. Similar to *ob/ob* and *db/db* mice, a specific effect owing to impaired leptin action may contribute to the cardiac phenotype in obese and diabetic Zucker rats.

## Diet-induced obesity and diabetes

To circumvent potential problems related to altered leptin signaling, many researchers have begun to evaluate models of diet-induced obesity and diabetes. Western diets (high fat and high sucrose) lead to obesity, insulin resistance and diabetes, particularly when applied to C57BL/6 mice (Symons et al., 2009). However, the degree of hyperglycemia and insulin resistance is not as severe as that observed in leptin or leptin receptor mutant mice. After 2 weeks of a Western diet, C57BL/6 mice develop changes in myocardial substrate utilization that precede the development of obesity and severe insulin resistance. Specifically, rates of glucose oxidation and

glycolysis are reduced, and myocardial FAO and oxygen consumption are increased (Wright et al., 2009). The extent of these changes is similar to those observed in more extreme models of obesity such as *ob/ob* mice. Short-term Western diets do not impair cardiac function, which develops in C57BL/6 mice after 20 weeks (Kim et al., 2005). The onset of cardiac dysfunction following Western diets is more rapid in Wistar rats, in which high-fat feeding for 7 weeks leads to myocardial steatosis, impaired contractile function and mitochondrial degeneration. Myocardial fatty acid uptake is increased in Wistar rats fed on a Western diet owing to increased sarcolemmal CD36 (Ouwens et al., 2005; Ouwens et al., 2007). Substrate oxidation and myocardial oxygen consumption have not yet been evaluated in this model. However, taken together, these studies indicate that caloric excess might be sufficient to induce metabolic defects that are associated with diabetic cardiomyopathy. It is important to note that isocaloric high-fat diets which do not induce obesity or insulin resistance appear to improve cardiac function in rat models of heart failure and cardiac hypertrophy (Okere et al., 2005; Rennison et al., 2008; Rennison et al., 2009), implicating a deleterious role for hyperinsulinemia and impaired glucose homeostasis in the associated cardiac defects that develop following ingestion of a Western diet.

## Genetically engineered mice to evaluate potential mechanisms underlying diabetic cardiomyopathy

Genetic engineering of mice has been used to evaluate the specific role of discreet pathways in the development of cardiac dysfunction in diabetes. Some investigators designed rescue experiments in which a specific abnormality had been restored using genetic engineering (particularly transgenic overexpression), and the potential beneficiary effect of genetic engineering on cardiac function in the setting of diabetes has been investigated. Overexpression of SERCA2a in STZ-diabetic mice and overexpression of human GLUT4 in *db/db* mice are examples of strategies that have successfully normalized contractile dysfunction in the respective diabetic models (Belke et al., 2000; Semeniuk et al., 2002; Trost et al., 2002). Other investigators have generated a variety of models that reproduce a single aspect of diabetic cardiomyopathy. These models are useful to investigate more specifically the impact of particular abnormalities on cardiac function and to further elucidate the molecular mechanisms. In recent years, the concept has been put forward that metabolic abnormalities in diabetic hearts contribute to the development of impaired contractility. As a consequence, a number of models have been generated that mimic these cardiac metabolic abnormalities. In particular, increased fatty acid utilization and triglyceride storage, as well as impaired cardiac insulin signaling, have been implicated in the pathogenesis of diabetic cardiomyopathy. Some of these models will be discussed below, and other models are summarized in Table 4.

The impact of increased cardiac fatty acid utilization in the absence of diabetes-associated systemic metabolic alterations has been investigated in mice with cardiomyocyte-specific overexpression of PPARα (Finck et al., 2002). PPARα is a nuclear receptor that increases the expression of most genes involved in FA uptake, transport, and oxidation, and whose expression is increased in some models of diabetic cardiomyopathy (Desvergne and Wahli, 1999; Finck et al., 2002). Remarkably, mice overexpressing PPARα only in the heart demonstrate a phenotype that shares many similarities with diabetic cardiomyopathy, including LV hypertrophy; ventricular dysfunction; increased FAO rates and FAO gene expression; decreased glucose oxidation and GLUT4 expression; increased myocardial triglyceride storage under fasted conditions; and reduced SERCA2a expression (Finck et al., 2002). Thus, many traits of diabetic cardiomyopathy were recapitulated by overexpression of PPARα, underscoring the important role that altered myocardial substrate metabolism plays in the pathogenesis of diabetic cardiomyopathy. Because these mice are not diabetic, they represent a useful model for further elucidating the molecular mechanisms by which intrinsic alterations in cardiac metabolism may contribute to cardiac dysfunction.

**Table 4. Additional genetic models that mimic discreet pathophysiological aspects of diabetic cardiomyopathy**

| Mouse model | |
|---|---|
| **Defective insulin signaling** | **References** |
| Dominant negative PI3K | McMullen et al., 2003 |
| Heart and skeletal muscle PDK1 KO | Mora et al., 2003 |
| Cardiomyocyte GLUT4 KO | Abel et al., 1999 |
| GLUT4 heterozygous KO | Stenbit et al., 1997 |
| UCP-DTA mouse | Duncan et al., 2007 |
| **Lipotoxicity** | |
| Adipose TG lipase (ATGL) KO | Haemmerle et al., 2006 |

KO, knockout; PI3K, phosphoinositide 3-kinase; PDK1, 3-phosphoinositide-dependent protein kinase 1; UCP-DTA, uncoupling protein-diphtheria toxin A.

Myocardial lipid storage is increased in diabetic hearts, and the toxic effects of lipid overload have been implicated in the pathogenesis of diabetic cardiomyopathy. Mouse models have been generated that may be useful to investigate the underlying mechanisms by which lipotoxicity may contribute to cardiac dysfunction in diabetes. In one model, long-chain acyl-CoA synthetase 1 was overexpressed exclusively in cardiomyocytes, which increases cardiomyocyte fatty acid uptake. Depending on the degree of overexpression, these mice develop cardiac lipid accumulation and dilated cardiomyopathy, potentially as a consequence of increased apoptosis (Chiu et al., 2001). Interestingly, adenovirus-mediated hyperleptinemia prevented cardiac dysfunction and lipid overload in these mice (Lee et al., 2004). The mechanisms for this effect are not clear but could include decreased delivery of fatty acids to the heart or increased AMP-activated protein kinase (AMPK) activation in the heart leading to increased rates of fatty acid oxidation. In another model, fatty acid uptake was increased by cardiomyocyte-restricted overexpression of the sarcolemmal fatty acid transporter FATP1. In this model, rates of FAO are increased, glucose oxidation is reduced, and mice show signs of diastolic dysfunction with preserved systolic function (Chiu et al., 2005). Diastolic sarcomere length and relaxation kinetics seem to be independent of the impairment in intracellular $Ca^{2+}$ handling in these mice (Flagg et al., 2009). Yagyu et al. generated mice that express human lipoprotein lipase (LPL) with a cell-attachment glycosylphosphatidylinositol anchor ($LPL^{GPI}$) in cardiomyocytes. These mice express $LPL^{GPI}$ on the cardiomyocyte surface, resulting in cardiac lipid accumulation and the development of dilated cardiomyopathy (Yagyu et al., 2003). Thus, all of these models mimic some aspects of the cardiac phenotype that is observed in

models of diabetes and can, therefore, serve as models to further dissect the mechanisms by which increased cardiac fatty acid delivery and lipid accumulation may contribute to cardiac dysfunction in diabetes.

Diminished glucose oxidation rates in cardiomyocytes occur as early as 48 hours after the induction of diabetes by STZ treatment, which is reversed by insulin treatment (Chen et al., 1984). Insulin-stimulated cardiac glucose uptake and utilization is also impaired in type 2 diabetic hearts (Mazumder et al., 2004). To investigate the specific role of impaired cardiac insulin signaling without confounding effects from systemic metabolic perturbations, our laboratory generated mice with a cardiomyocyte-specific deletion of the insulin receptor (CIRKO mice). CIRKO mice develop contractile dysfunction (but not heart failure) that is associated with decreased glucose and fatty acid oxidation (Belke et al., 2002). CIRKO mice also develop mitochondrial dysfunction, have increased mitochondrial superoxide production, and display fatty acid-induced mitochondrial uncoupling (Boudina et al., 2009). The mitochondrial phenotype closely mirrors the impairment in mitochondrial function that is observed in *ob/ob* and *db/db* mice, suggesting that impaired cardiac insulin signaling per se contributes to the development of cardiac dysfunction in diabetic hearts. Interestingly, cardiac dysfunction does not seem to be much more pronounced in diabetic mice when compared with CIRKO mice, although a direct comparison between the contractile function of CIRKO mice and diabetic mice in the same study has not yet been reported. Certainly, the contribution of hyperglycemia to diabetes-associated cardiac dysfunction needs to be re-evaluated in the face of the increasing evidence that additional molecular mechanisms may impair cardiac contractility independently of hyperglycemia (Fig. 1). It is also worth mentioning that a general reduction in substrate oxidation rates and mitochondrial function has been demonstrated for failing hearts, suggesting that preceding or co-existing insulin resistance may, under certain conditions, contribute to the oxidative defects observed in heart failure (Hoppel et al., 1982; Ide et al., 2001; Ventura-Clapier et al., 2004; Neubauer, 2007). Indeed, epidemiological studies suggest that insulin resistance is an independent risk factor for heart failure (Swan et al., 1997; Doehner et al., 2005). Thus, CIRKO mice represent a useful model to dissect the mechanisms by which impaired insulin signaling may compromise mitochondrial function in diabetic hearts, independently of hyperglycemia and hyperlipidemia, which may even have relevance for the pathology of cardiac diseases beyond diabetic cardiomyopathy.

## Conclusions and perspective

Many studies suggest the existence of a human diabetic cardiomyopathy for which the underlying mechanisms are incompletely understood because of experimental limitations in humans. Rodent models of type 1 and type 2 diabetes share several traits with human diabetic cardiomyopathy and have greatly advanced our understanding of the underlying pathology of diabetic cardiomyopathy. Each model has certain limitations and no perfect model exists that exactly phenocopies the human condition. Genetic heterogeneity, as well as differences in lifestyle among humans, makes the generation of an appropriate model challenging. However, features identified in a variety of rodent models have subsequently been identified in human studies. Thus, despite their limitations, rodent models have proven to be valuable tools that may increase our understanding of human diabetic cardiomyopathy. Additional models of type 1 and type 2 diabetes, as well as genetically engineered mice that mimic specific abnormalities, are expected to be discovered or generated in the future. In addition, under-investigated models are expected to be characterized further. It is also likely that more sophisticated experimental strategies, including genetic engineering techniques, will allow us to more specifically evaluate mechanisms that increase the risk for the development of heart failure in diabetic humans without coronary artery disease. Moreover, we anticipate the development of new models that will test the role of potential therapeutic targets that might ameliorate diabetic cardiomyopathy. Although advances in genetic engineering might have outpaced the development of experimental techniques that allow reliable physiologic investigation of these models, investigators are now better equipped with techniques to characterize such mouse models (Severson, 2004; Yue et al., 2007). In light of the current obesity epidemic, novel therapeutic strategies are of utmost importance to reduce cardiac complications in diabetic patients, which represent a major burden for health care budgets. We are optimistic that research on animal models of type 1 and type 2 diabetes will continue to provide insights into the pathology of diabetes-related cardiac complications, from which novel therapeutic strategies may originate.

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