Abstract

In humans, diabetes mellitus (DM) is considered a heterogeneous metabolic disorder. Swine have been used as a model for many human conditions including type 1 (insulin-deficient) and type 2 (insulin-resistant) DM research because of their phenotypic similarities to humans including: cardiovascular anatomy and function, metabolism, lipoprotein profile, size, tendency to obesity, and omnivorous habits. There is phenotypic overlap between the two types of DM and pig models show characteristics and complications of both. Streptozotocin and alloxan have been used to create insulin deficient diabetes in pigs. One of the most unique and useful phenotypes is that these insulin-deficient pigs develop more severe coronary atherosclerosis than nondiabetic controls. It is not fully understood why patients with either type 1 or type 2 DM have increased severity and diffuseness of atherosclerosis compared with nondiabetic patients. The current human epidemic of type 2 DM and its attendant cardiovascular complications underscore the unmet need for creating a useful, readily available animal model of type 2 insulin resistant DM that also develops coronary artery atherosclerosis. The phenotypic susceptibility to coronary atherosclerosis makes pigs an attractive species to identify causative mechanisms. Suggested criteria for validation of an animal model of humanoid type 2 DM are described. The goal would be to develop a useful animal model for mechanistic studies as well as to develop and test novel therapeutics both for type 2 DM as well as its cardiovascular complications.

Key Words: animal model; atherosclerosis; cardiovascular disease; glucose intolerance; insulin resistance; minipig; obesity; swine; type 2 diabetes mellitus

Introduction

Diabetes mellitus (DM) comprises a heterogeneous group of disorders with the common characteristic of elevated blood glucose (Harris 2004). The range of normal, prediabetic and diabetic glucose values for humans is shown in Figure 1 (Diagnosis and Classification of Diabetes Mellitus 2005). These values are derived from large population studies and are consensus driven. These values are also relevant to pigs and provide a framework for establishing the diagnosis of DM in experimental studies. A discussion of some of the similarities and differences in glucose metabolism and glucose tolerance between pigs and humans is given in a review of type 1 DM in the minipig (Larsen and Rolin 2004).

Type 1 DM is due to pancreatic β-cell destruction leading to insulin deficiency and is generally characterized by notable symptoms (weight loss, polyuria, and polydipsia), abrupt onset at a young age but usually after puberty, immune-mediated loss of β-cells by anti-islet cell antibodies, and a need for exogenous insulin therapy. Type 2 DM is characterized by insulin resistance (IR) and hyperglycemia and differs from type 1 DM in that patients are generally overweight and asymptomatic in the early stages. Type 2 DM usually has a slow onset and, until recently, most often occurred in adults (Kaufman 2005). IR is defined as a decreased biological response to normal concentrations of serum insulin that over time leads to compensatory hypersulinsulinemia (Kohen-Avramoglu et al. 2003). Type 2 DM is often preceded by hyperinsulinemia and impaired glucose tolerance with 50% of patients with hyperinsulinemia progressing to develop diabetes (Kaufman 2005). Although fully manifested diabetes mellitus confers a greater risk for cardiovascular disease and other complications than isolated IR without hyperglycemia, the presence of IR is a major independent risk factor with relative risk

Abbreviations used in this article: DM, diabetes mellitus; FH, familial hypercholesterolemia; GLP, glucagon-like peptide; GLUT4, glucose transporter 4; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; IGF, insulin growth factor; INSR, insulin receptor; IR, insulin resistance; IRS, insulin receptor substrate; LDL, low-density lipoprotein; LO, lipoygenase; PLD, phospholipase-dependent; PPAR, peroxisome-proliferative-activated receptor; SMC, smooth muscle cell; SZT, streptozotocin; TG, triglyceride; TNF, tumor necrosis factor; VLDL, very low-density lipoprotein.
ratios between 2.2 and 2.7 when multifactorial linear regression analysis is utilized to assess risk (Howard 1999; Kohen-Avramoglu et al. 2003; Taskinen 1995). Thus, both IR and hyperglycemia appear to mediate the pathophysiology of type 2 DM independently.

This review focuses on different pig strains and models of diabetes mellitus with an emphasis on their relationship to insulin resistance and type 2 DM (Table 1). We include some pig models in which diabetes was induced with alloxan or streptozotocin (SZT). Although these pigs may be considered to model type 1 DM (acute onset and low insulin levels), the features often overlap with many characteristics of type 2 DM (insulin resistance, dyslipidemia, and low insulin levels in the late stages). We also focus on the cardiovascular complications because other complications such as retinopathy, neuropathy, and nephropathy have not been extensively reported in these pig models.

Legal and Ethical Issues

Animal welfare laws vary considerably around the world. The literature includes numerous articles in which the use of animals in research is discussed (e.g., Akins et al. 2005; Tuffery 1995). It should be noted that there are those who believe that animals should not be used for experimental purposes. Nevertheless, it is possible to minimize distress and have humane endpoints with well-designed studies. Research involving animals should be a balance between knowledge gained and potential harm to animals. The utilization of pigs in diabetes research, whether induced experimentally or developed through breeding for selected traits, must take into account the potential adverse effects. To study the complications associated with diabetes, mechanisms should be in place to prevent or alleviate adverse consequences of these complications. Induction and maintenance of diabetes require adequate support. With adequate monitoring, good husbandry practices, and appropriate veterinary care, it is possible to minimize adverse effects and to produce new information that will benefit both experimental animals and humans.

Background

There is a growing epidemic of type 2 DM in children and adults and the associated diabetic complications, especially cardiovascular disease (Kohen-Avramoglu et al. 2003; Zimet et al. 2001). Strategies to ameliorate IR have been hampered by a poor understanding of the genotypes predisposed to this phenotype and, specifically, the pathophysiological mechanisms that when activated in IR patients accelerate atherosclerosis and lead to other complications. Research has focused on the loss of peripheral insulin sensitivity in muscle that is associated with an abnormal increase in hepatic glucose output leading to changes in the rate of peripheral glucose disposal (Cline et al. 1999; Lofgren et al. 2000). Chronic hyperglycemia has deleterious effects on both insulin secretion and insulin activity (glucotoxicosis). Thus, hyperglycemia may be partially responsible for the defective insulin mediated glucose disposal in both type 1 and type 2 DM. In addition, it has been proposed that IR leads to β-cell dysfunction, including decreased and delayed insulin secretion.

Multiple murine models have been useful in studying the consequences of genetic manipulations that induce IR (Michael et al. 2000). The most informative among these have been structural changes in the insulin receptor (INSR) and the downstream signaling molecules insulin receptor substrate (IRS)-1 and IRS-2 (Kido et al. 2000; Michael et

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**Figure 1** Glucose tolerance categories. The diagnosis of diabetes mellitus is based on fasting and 2-hr plasma glucose values on oral glucose tolerance test (OGTT). *The diagnosis of diabetes can be based on unequivocal symptoms and a random glucose measurement of 200 mg/dL. Adapted from the American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care January 28, 2005, Suppl 1:S37-S42.
### Table 1 Characteristics of pig strains used as models of type 2 diabetes mellitus

<table>
<thead>
<tr>
<th>Strain</th>
<th>S/A</th>
<th>Diet</th>
<th>Characteristics of diabetic state—hyperglycemia, hyperinsulinemia</th>
<th>Lipid profile</th>
<th>Body weight/body composition</th>
<th>Genetic alterations and complications</th>
<th>Use</th>
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<tbody>
<tr>
<td>Yucatan (Panepinto et al. 1982; Phillips et al. 1982)</td>
<td>NA</td>
<td>NA</td>
<td>High K group K &gt;3.5 Low K group K &lt;1.5 Low K-hyperglycemic during lactation. ↓ glucose tolerance with high-fat diet</td>
<td>FFA- High K mean 863 mEq/L Low K mean 1094 mEq/dL TG- High K mean 145 mg/dL Low K 127-133 mg/dL</td>
<td>Control approx 50 kg Become obese when fed ad lib</td>
<td>Modified pancreatic receptor or postreceptor response Thickened capillary membranes in muscle sections</td>
<td>Produced by selective breeding Not currently available</td>
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<tr>
<td>Yucatan (Boullion et al. 2003; Korte et al. 2005; Mokelke et al. 2005a; Mokelke et al. 2003; Mokelke et al. 2002; Phillips et al. 1980)</td>
<td>A</td>
<td>HF</td>
<td>Fasting glucose (Dixon et al. 2002) Control 49 mg/dL HF 43 mg/dL Diabetic HF 316 mg/dL</td>
<td>Cholesterol (Dixon et al. 2002) Control 79 mg/dL HF 358 mg/dL Diabetic HF 419 mg/dL LDL cholesterol Control 35 mg/dL HF 197 mg/dL Diabetic HF 255 mg/dL HDL Control 32 mg/dL HF 127 mg/dL Diabetic HF 102 mg/dL HDL/LDL Control 1.03 HF 1.53 Diabetic HF 2.54</td>
<td>Body wt (Mokelke et al. 2005a) Control 60 kg Diabetic HF 69 kg</td>
<td>Coronary artery and myocardial dysfunction Diabetic high-fat-fed pigs had increased atherosclerotic plaques in aorta Retinal capillary basement membrane thickening</td>
<td>Model of diabetic dyslipidemia and can be used to study mechanisms, interventions, and atherosclerosis. Model for early retinal microvascular change (intervention and mechanism)</td>
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<tr>
<td>Yucatan (Sebert et al. 2005a; Sebert et al. 2005b)</td>
<td>Overfed (1.5 × cal/s)</td>
<td>Fasting glucose ↑ in overfed pigs (4.5 vs. 3.7 mmol/L) Glucose infusion rate (insulin sensitivity) Control 14.5 mg/kg/min Overfed 6.4 mg/kg/min</td>
<td>TG ↑ in overfed pigs</td>
<td>Body wt Control 51 kg Overfed 107 kg Porcine Obesity index Control 0.47 Overfed 0.97</td>
<td>Modification of gene expression in skeletal muscles</td>
<td>Model for childhood obesity and insulin sensitivity</td>
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</tbody>
</table>
Table 1 (continued) Characteristics of pig strains used as models of type 2 diabetes mellitus

<table>
<thead>
<tr>
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<th>Genetic alterations and complications</th>
<th>Use</th>
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<tbody>
<tr>
<td>Sinclair (Dixon et al. 1999; Roberts et al. 2001)</td>
<td>A</td>
<td>HF</td>
<td>Fasting glucose Control 3 mmol/L HF 4 mmol/L Diabetic HF 17 mmol/L Fructosamine Control 175 mmol/L Diabetic HF 300 mmol/L</td>
<td>TG Control 0.34 mmol/L HF 0.38 mmol/L Diabetic HF 0.99 mmol/L Cholesterol Control 1.9 mmol/L HF 7.76 mmol/L Diabetic HF 13 mmol/L LDL Control 0.98 mmol/L HF 3.54 mmol/L Diabetic HF 7.96 mmol/L</td>
<td>12-mo-old body weight not given</td>
<td>Vascular dysfunction, mild carotid Atherosclerosis</td>
<td>Model for pharmacotherapy for prevention of vascular disease associated with diabetes</td>
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<td>Gottingen (Johansen et al. 2001; Larsen and Rolin 2004; Larsen et al. 2002)</td>
<td>NA</td>
<td>HF</td>
<td>Fasting glucose ↑ Control 3.6 mM HF-HE 4.3 mM Insulin ↑ Control 23 pM HF-HE 80 pM Insulin response to IV glucose (AUC) Control 771 pM × min HF-HE 6741 pM × min</td>
<td>Cholesterol ↑ Control 1.82 mmol/L HF-HE 2.03 mmol/L TG Control 0.13 mmol/L HF-HE 0.24 mmol/L (TG ↑ with age)</td>
<td>Body wt Control 21 kg HF-HE 26.8 kg (visibly obese) Control 25 kg HF-HE 33 kg % total body fat Control 6.1% HF-HE 13.2%</td>
<td>None given</td>
<td>Obese model that shares metabolic impairments with humans and may serve as a model of insulin resistance and metabolic syndrome</td>
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<td>Göttingen (Kjems et al. 2001; Larsen et al. 2003)</td>
<td>S</td>
<td>NA</td>
<td>Fasting glucose (S 125 + NIA 67 mg/kg) Control (pre) 3.6 mM Diabetic (post) 5.0 mM Fasting insulin Control (pre) 54.3 pM Diabetic (post) 25.5 pM Impaired glucose tolerance Impaired pulsatile insulin secretion</td>
<td>NA</td>
<td>Body wt Range 14-32 kg (multiple protocols)</td>
<td>Reduced β-cell mass Hyperglucagonemia</td>
<td>Model suitable for studying pathophysiology and agents for treatment of diabetes. Varying degrees of glucose intolerance and diabetes dependent on the dose of NIA + S</td>
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<tr>
<td>Strain</td>
<td>S/A</td>
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<td>HS</td>
<td>Control 101 mg/dL</td>
<td>Cholesterol</td>
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<td>HFHS 165 mg/dL</td>
<td>HDL cholesterol</td>
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<td>Fasting insulin Control 11 mU/mL</td>
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<td>HFHS 20 mU/mL</td>
<td>HDL 41 mg/dL</td>
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<td>↓ insulin sensitivity</td>
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<td>HFHS 406 µmol/L</td>
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<td>Ossabaw (Dyson et al. 2006; Hausman et al. 1983; Meserole and Etherlon 1984; Mokelke et al. 2005b)</td>
<td>NA</td>
<td>HF</td>
<td>↓ glucose clearance</td>
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<td>Obese 88 kg</td>
<td>Obese Ossabaw become hypertensive ↑ neointima hyperplasia Glucose intolerance exacerbated with HF diet</td>
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<td>Insulin-resistant</td>
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<td>Yorkshire (Askari et al. 2002; Gernity et al. 2001; Natarajan et al. 2002; Suzuki et al. 2001)</td>
<td>S</td>
<td>HF</td>
<td>Fasting glucose Controls and HF 3.3-6.1 mmol/L (not different)</td>
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<td>10- to 12-mo-old Yorkshire pigs weigh ~175 kg</td>
<td>Dyslipidemia Severe Coronary and aortic atherosclerosis</td>
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<td>Diabetic and Diabetic HF 3-4 times that of controls</td>
<td>Cholesterol</td>
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<td>and HF ~25 mmol/L</td>
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<td>Diabetic HF ~70 mmol/L</td>
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These studies have shown that mutations altering insulin-stimulated glucose transport in muscle lead to secondary changes in fat cells that are similar to those that occur in type 2 DM. Likewise, loss of insulin sensitivity in muscle or fat also results in stimulation of hepatic glucose output. Recently, novel studies with peroxisome-proliferative-activated receptor (PPAR\(^1\)) genes and agonists have led to the discovery of new regulatory interactions between metabolism, inflammation, and atherosclerosis (Lee et al. 2003; Levi et al. 2003; Li et al. 2000; Tordjman et al. 2001). These genetically manipulated murine models have been very useful in characterizing the effects of altering the function/signaling of the INSR and PPAR pathways, but they do not recapitulate all of the essential elements of the predisposing phenotype (e.g., IR, obesity, hypertriglyceridemia, and hypercholesterolemia) that lead to impaired glucose tolerance and the development of type 2 DM. Notably, rodent models mimic some of the changes that occur in human IR, but the development of hyperlipidemia and atherosclerosis requires an additional genetic manipulation such as deletion of apolipoprotein-E or low-density lipoprotein (LDL) receptor (Hasty et al. 2001; Zhang et al. 1992). The atherosclerosis that develops in these mice has minimal proliferative changes and occurs almost exclusively in proximal ascending aorta. Although adding or subtracting various genetic traits is useful in looking at genetic interactions, this requirement for multiple genetic manipulations may complicate the use of rodents for testing the role of subtle changes that IR might exert on diabetes complications.

There is an unmet need for a useful animal model to determine the mechanisms that mediate the development of complications in IR type 2 DM (Kohen-Avramoglu et al. 2003; Zimmet et al. 2001). The rationale for choosing pigs is based on several issues. Humans and pigs have similar pharmacokinetics after subcutaneous drug administration, gastrointestinal structure and function, pancreas morphology, and overall metabolic status (Larsen and Rolin 2004). Moreover, pigs have been informative in studies of cardiovascular, renal, and ophthalmic complications associated with SZT-induced type 1 DM (Askari et al. 2002; Gerrity et al. 1979; Nichols et al. 1992; Prescott et al. 1991, 1995). Pigs fed a high-fat high-cholesterol diet develop coronary, aortic, iliac, and carotid atherosclerotic lesions, anatomical locations extremely relevant to the human condition. Most importantly, these lesions recapitulate the histopathology seen in humans: proliferative lesions consisting of smooth muscle cells, macrophages, lymphocytes, foam cells, calcification, fibrous caps, necrotic and apoptotic cells, and extracellular matrices (Brodala et al. 2005; Gerrity et al. 1995, 1992; Prescott et al. 1991, 1995). Results of testing medicines (e.g., statins) and devices (e.g., stents) in pigs have been regarded as having a high positive predictive value for subsequent translation to humans (Hasler-Rapacz et al. 1996; Johnson et al. 1999).

Table 1 (continued) Characteristics of pig strains used as models of type 2 diabetes mellitus

<table>
<thead>
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<th>Use</th>
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</thead>
<tbody>
<tr>
<td>Familial Hypercholesterolemic</td>
<td>NA</td>
<td>NA</td>
<td>Not developed</td>
<td>CHOLESTEROL 316 mg/dL</td>
<td>MIXED COMMERCIAL BREED</td>
<td>MISSENSE MUTATION (C253 (\rightarrow) T253)</td>
<td>CORONARY AND AORTICATHEROSCLEROSIS</td>
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<td>(FH) (Prescott et al. 1995; Prescott et al. 1991)</td>
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<td>HDL-C 22 mg/dL</td>
<td>BACKGROUND</td>
<td>SUBSTITUTING ARG94 (\rightarrow) CYSTHEL94 IN THE LDL RECEPTOR</td>
<td>POTENTIAL FOR CHEMICALLY INDUCED DIABETES</td>
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<td>LDL-C 275 mg/dL</td>
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<td>AND/OR INSULIN RESISTANCE</td>
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<td>LDL/HDL 8.1</td>
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<td>CHOLESTEROL 105 mg/dL</td>
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<td>TRIGLYCERIDES 29 mg/dL</td>
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</tbody>
</table>

$^{a}$NA, not applicable; $^{K}$, value; $^{S}$, streptozotocin; $^{A}$, Alloxan; $^{HF}$, high fat; $^{HS}$, high sucrose; $^{HE}$, high energy; $^{HC}$, high cholesterol; FFA, free fatty acids; IR, insulin resistance; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglyceride; AUC, area under curve; NIA, nicotinamide.
However, human diabetics have not enjoyed the same benefit of drug-eluting stents as nondiabetic patients due to accelerated disease progression (Finn et al. 2005; Flaherty and Davidson 2005; Iakovou et al. 2005). The mechanism for this accelerated disease progression is unknown but is likely due to the systemic metabolic perturbations that occur in DM. Such mechanisms may also be operative in the development of diffuse atherosclerosis in peripheral (e.g., iliac, femoral, and carotid) arteries. Thus, pigs have great potential as a relevant animal model of insulin-resistant type 2 DM to identify mechanisms that lead to the development of diabetic complications and to develop and test novel therapeutic approaches.

Yucatan Minipigs

The Yucatan pig is native to the Yucatan Peninsula of Mexico. Its early development and characterization for laboratory use took place at Colorado State University (Panepinto 1996). Selective breeding has been reported to be a successful strategy for producing pigs with impaired glucose tolerance. During the 1970s, researchers at Colorado State University used this strategy to develop two lines of Yucatan minipigs with altered glucose tolerance, “low K” with impaired tolerance and “high K” with enhanced tolerance (Phillips and Panepinto 1986; Phillips et al. 1982). The impaired glucose tolerance was due to a decrease in peripheral insulin concentration resulting from decreased insulin secretion in response to a glucose challenge. The low serum insulin levels in this line did not appear to be due to im paired synthesis and storage of insulin but were consistent with a modified pancreatic receptor or postreceptor response as suggested by the finding that these pigs had normal insulin release in response to isoproterenol challenge. These pigs also had normal numbers of islets and β-cells (Phillips and Panepinto 1986). Females from this strain rapidly became obese, and some developed insulin resistance and diabetes and exhibited both hyperinsulinemia and hyperglycemia during gestation and lactation. When these pigs were fed a high-fat diet and sucrose as the carbohydrate source, they had a marked increase in peripheral insulin concentration and prolonged elevation of plasma glucose (Phillips et al. 1981). In this study, impaired glucose tolerance or dietary changes did not affect serum lipids except for an elevation in fasting free fatty acid concentration.

Although selective breeding was shown to be feasible, several generations were required. Glucose intolerance was not observed in the F7 generation of the low-K line (Hand et al. 1987), and these pigs are not currently available. Thus, chemical induction is an attractive alternative and has been the primary means for creating diabetic pigs.

Recently a Yucatan minipig model of diabetic dyslipidemia at the University of Missouri at Columbia has been described in which alloxan (175 mg/kg intravenously) was given to induce diabetes. Feed and fluids were used to maintain positive energy balance and prevent wasting (Bouillion et al. 2003). Plasma glucose was maintained at 300 to 400 mg/dL with supplemental insulin, dyslipidemia with plasma cholesterol levels at 280 to 405 mg/dL, and triglyceride levels at 55 to 106 mg/dL. Pigs maintained normal weight (lean) or gained two times normal weight (obese). The advantage of this model is that it is not a wasting disease as can occur in other hyperglycemic models.

This diabetic swine model (alloxan and fat diet in the Yucatan strain) has been used to examine the effect of Atorvastatin on diabetes and atherogenesis by researchers at the University of Missouri (Dixon et al. 2002; Otis et al. 2003). Compared with dyslipidemic nondiabetic pigs, the diabetic swine had larger LDL that resembled intermediate-density lipoprotein (IDL), increased coronary atherosclerosis as determined by intravascular ultrasound, and increased collagen deposition in internal mammary artery. Atorvastatin decreased total and very low-density lipoprotein (VLDL) triglycerides and protected from or reduced the extent of atherosclerosis but had minimal effects on dyslipidemic profile or LDL/HDL ratio in the doses used. The authors suggest that Atorvastatin may have other antiatherogenic effects in diabetes perhaps by altering inflammation (Youssef et al. 2002).

This diabetic model is one of the few pig studies in which microvascular disease has been examined and reported. After 20 wk, microvascular retinal changes were evaluated (Hainsworth et al. 2002). The diabetic pigs had thicker retinal capillary basement membranes than nondiabetic pigs. The dyslipidemia induced by feeding a high-fat diet did not appear to contribute to the basement membrane thickening. This model can be useful in identifying mechanisms in diabetic retinopathy and evaluating early interventions.

Researchers at the University of Missouri have extended the use of this model to show enhanced contractibility in coronary arteries of diabetic pigs and that this enhanced contractility is prevented by exercise (Mokelke et al. 2005a). Diabetes was induced with alloxan (125 mg/kg intravenously), and animals were maintained at fasting blood glucose levels between 300 and 400 mg/dL and total cholesterol >300 mg/dL (high-fat, high-cholesterol, and sucrose diet) as before. Pigs that were trained to exercise on a treadmill exhibited a resting bradycardia and a lower heart rate in response to work load consistent with improved fitness. Coronary blood flow was lower in diabetic dyslipidemic pigs than in controls (nondiabetic, high-fat diet) and exercise on the treadmill prevented the decrease. Both sedentary and exercised diabetic dyslipidemic pigs had a lower response to bradykinin (endothelial-dependent relaxation) than controls (nondiabetic, high-fat diet). In prostaglandin F2α-constricted vessels, dilation was depressed in sedentary diabetic dyslipidemic animals compared with the nondiabetic control and exercised diabetic dyslipidemic pigs. Adenosine (endothelial-independent) dilation was depressed in diabetic dyslipidemic compared with control and diabetic dyslipidemic exercised pigs. In vivo microvascular
tone was greater in diabetic dyslipidemic animals, and this was prevented by exercise.

Recently, a model for childhood obesity and insulin resistance without chemical induction was created by overfeeding (1.5 times the recommended amount of calories) a Western style diet that was high in saturated fat and carbohydrates with a high glycemic index to 4-mo-old Yucatan minipigs until adulthood (Sebert et al. 2005a). The overfed pigs became obese and had a porcine obesity index (based on abdominal and neck measurements) and respective weight that were approximately twice those of controls fed normally. The overfed pigs also developed lower insulin sensitivity as measured by the euglycemic hyperinsulinemic clamp method than did control-fed pigs. Gene expression of glucose transporter 4 (GLUT 4<sup>+</sup>), INSR, and PPAR was lower in muscle of adult overfed pigs compared with normal fed pigs consistent with increased insulin resistance. In adipose tissue, GLUT4 transcription was increased, indicating possible enhanced glucose uptake or lipogenesis. In addition, leptin expression was increased. Plasma insulin growth factor 1 (IGF-1<sup>+</sup>) was increased in the younger overfed pigs compared with normally fed pigs. Overfed pigs had higher LDL-cholesterol:HDL-cholesterol ratios than pigs fed normally.

### Sinclair Minipigs

The Sinclair miniature pig was originally developed at the Hormel Institute of the University of Minnesota in the 1950s by breeding four feral strains. A Yorkshire domestic pig was later introduced into the strain. Significant characterization and development took place later at the Sinclair Comparative Medicine Research Farm at the University of Missouri (Panepinto 1996). Researchers at the University of Missouri have used alloxan (175 mg/kg intravenously)-induced diabetic Sinclair minipigs to examine the relationship of diabetes to vascular dysfunction. The induced diabetic state was characterized by a postprandial hyperglycemia (6 times that of controls) and reduced insulin response to an intravenous glucose load, which generally did not require insulin support. Vascular function in diabetic Sinclair minipigs fed an atherogenic diet was compared with that of nondiabetic pigs fed an atherogenic diet and nondiabetic pigs fed a control diet (Dixon et al. 1999). The diabetic pigs fed the high-fat diet had lipoprotein profiles similar to human diabetics, with a shift of the cholesterol into the LDL fraction. In diabetic pigs fed the high-fat diet, 75% of coronary artery segments showed contractile oscillations in response to prostaglandin F<sub>2α</sub> compared with 25% for the high-fat-fed nondiabetic pigs and 10% for non-diabetic pigs. Endothelial-dependent relaxation in response to bradykinin was nearly abolished in brachial arteries of the diabetic pigs fed the high-fat diet while remaining unchanged in the high-fat controls. Carotid artery atherosclerosis was increased in the high-fat-fed diabetic pigs compared with the other groups.

### Göttingen Minipigs

The Göttingen minipig was developed in the 1960s at the Institute of Animal Breeding and Genetics of the University of Göttingen, Germany, by crossbreeding the Vietnamese, Hormel, and German improved Landrace swine. Researchers at Novo Nordisk with others have developed and used the Göttingen minipig to study physiological aspects of diabetes and pharmacological therapies for diabetes. A summary of the Göttingen minipig as a model of diabetes was provided in a recent ILAR Journal issue (Larsen and Rolin 2004). The reference values for glucose and lipid homeostasis have been well characterized for the Göttingen minipig. Plasma glucose, leptin, fructosamine, insulin, c-peptide, triglyceride, free fatty acids, and cholesterol were measured as pigs aged and became heavier, revealing that plasma glucose, fructosamine, and triglycerides increase with age (Larsen et al. 2001). When fed a high-fat high-energy diet, the Göttingen minipig may serve as a model for the “metabolic syndrome” (Johansen et al. 2001). Female Göttingen minipigs fed a high-fat high-energy diet to induce obesity had increased body weight and fat content. Although preprandial plasma glucose and insulin concentrations were not altered, insulin response to intravenous glucose was increased (Johansen et al. 2001). Male Göttingen minipigs fed a high-fat high-energy diet also became obese (increased weight and body fat) and had increased fasting blood glucose and insulin levels compared with normally fed controls (Larsen et al. 2001). Although oral glucose tolerance was not changed, insulin response to intravenous glucose was increased while glucose clearance was unchanged.

The Göttingen minipig with reduced β-cell mass following nicotinamide (67 mg/kg intravenously to protect the β-cells partially) and SZT (125 mg/kg intravenously) or alloxan (80 mg/kg intravenously) was used to examine changes in pulsatile insulin secretion. In this strain, a decrease in β-cell mass leads to impaired pulsatile insulin secretion, reduced postprandial hepatic insulin clearance, and relative hyperglucagonemia (Kjems et al. 2001). Other studies with this strain have also demonstrated reduced insulin pulsatility. However, the authors indicate that neither β-cell mass nor the slight hyperglycemia seen in this model completely accounted for the disturbed insulin pulsatility observed in human type 2 DM (Larsen et al. 2003).

In addition to physiological studies, this model has been used to examine therapies for hyperglycemia. A glucagon-like peptide 1 (GLP1<sup>+</sup>) derivative NN2211 was evaluated for treatment in hyperglycemic minipigs (Ribel et al. 2002). GLP1 is an effective but unstable antidiabetic agent, and NN2211 is a long-acting derivative of GLP1. After treatment with NN2211, more glucose was required in the hyperglycemic clamp procedure to maintain plasma glucose 1.5 to 2.0 mmol above baseline. Insulin variations were higher, and glucagon levels were suppressed. In oral glucose tolerance testing, NN2211 reduced glucose excursion with no change in insulin. This type of study demonstrates
the potential value of hyperglycemic swine in the development of new therapies for glucose control.

**Yorkshire and Yorkshire-crossed Strains**

The Yorkshire is an old breed developed in the Yorkshire area of northern England, and it was first imported into the United States in 1893. This commercial breed is noted for its large frame, long body, and bacon-type conformation (Anderson and Kiser 1967). This swine model has been well characterized over the past 20 yr for its use in atherosclerosis research. A Yorkshire swine model of SZT-induced diabetes (50 mg/kg/day intravenously for 3 days) maintained without supplemental insulin was established at the Medical College of Georgia with others by reducing β-cell numbers to approximately 20% of normal (Askari et al. 2002; Gerrity et al. 2001; Natarajan et al. 2002; Suzuki et al. 2001). The hyperglycemia (fasting glucose levels 3-4 times that of controls) that resulted did not affect plasma cholesterol levels. These swine, like others with chemically induced diabetes, were reported to have impaired glucose tolerance to oral glucose tolerance testing. Compared with controls, coronary atherosclerosis was exacerbated, with lesions containing calcification and a higher rate of smooth muscle proliferation. Occlusive lesions were also reported in the small epicardial branches of the coronary arteries in diabetic pigs, with induction of dyslipidemia by feeding a high-fat diet. Monocyte/macrophage function that is altered by hyperlipidemia was further altered by diabetes. These diabetic dyslipidemic pigs had elevations in macrophage lipid metabolism. Monocyte acyl-CoA-cholesterol acyltransferase activity was increased with enhanced 12 lipoxygenase and H₂O₂ production.

Although this model is established by the acute destruction of β-cells that is characteristic of type 1 DM, the lack of insulin dependence, increased triglycerides, and glucose intolerance resemble late-stage type 2 DM to some extent. Because increased cardiovascular complications are seen in both type 1 and type 2 human diabetics, classification may be less important than understanding the mechanisms that mediate these increased complication rates. One of the most important aspects of using pigs to study vascular disease in diabetics is the ability to define the precise biochemical changes and mechanisms that initiate and perpetuate lesion progression, which are unique to the insulin-resistant and/or hyperglycemic state. There appears to be a lack of direct effect of glucose on smooth muscle cell (SMC) proliferation in vitro. Studies were undertaken to determine the mechanism for the increase in smooth muscle accumulation and proliferation seen in the diabetic pigs (Askari et al. 2002). Diabetes increased plasma triglycerides that contained palmitic, linoleic, stearic, oleic, and arachidonic fatty acids. IGF-1 is abundant in atherosclerotic lesions of diabetic pigs, whereas arteries from nondiabetic controls contain little. Oleate and linoleate markedly enhance the growth-promoting effects of IGF-1 through phospholipase-dependent (PLD) pathways in SMCs isolated from these diabetic dyslipidemic animals (Askari et al. 2002). The authors proposed that the diabetic accelerated atherosclerosis was caused in part by increased IGF-1-induced PLD activity and generation of diacylglycerol fueled by triglycerides containing oleate and linoleate.

A valuable aspect of the swine model is that it can be used to extend the in vitro data to the in vivo situation, and to identify molecular mechanisms. The lipoxygenase (LO) pathway is associated with oxidant stress and the pathogenesis of atherosclerosis. LO, a protein product of arachidonic metabolism, has been postulated to play a role in the accelerated atherosclerosis seen in diabetes. Immunostaining of abdominal and coronary arteries from diabetic pigs fed a high-fat diet demonstrated increased 12-LO protein compared with dyslipidemic nondiabetic pigs (Natarajan et al. 2002). Leukocyte-type 12-LO protein and 12-LO mRNA expression were also significantly greater in the arteries of the diabetic pigs. Increased oxidant stress as measured by the production of hydrogen peroxide after phorbol-myristate acetate stimulation was also observed in monocytes from peripheral blood in the dyslipidemic diabetic pigs compared with dyslipidemic-only and diabetic-only pigs, indicating a synergistic effect. This work then identified a mechanism by which oxidant stress may mediate diabetic complications.

One study (Otieno et al. 2005) related to commercial pork production and involving a Yorkshire and Berkshire intercross was designed to determine whether polymorphisms of genes implicated in human hyperglycemia and insulin resistance were associated with porcine muscle and fat measurements. Although carried out for the potential enhancement of lean pork production, results may prove useful in identifying additional characteristics of swine for modeling type 2 DM. Investigators examined the genes for human proteins MAPK8, RETN, HSD11B1, and AKT2, which are implicated in type 2 DM. MAPK8 is implicated in type 2 DM by mediating tumor necrosis factor α (TNFα)-induced insulin resistance. Disruption of MAPK8 in mice increases insulin sensitivity. RETN inhibits glucose uptake. HSD11B1 converts inactive glucose to active form, and inhibition of HSD11B1 decreases glucose concentrations in mice. AKT2 binds GLUT4 and mediates glucose binding in muscle and fat cells. In this study, polymorphisms of the MAPK8, RETN, and HSD11B1 genes were identified that were associated with fat deposition traits in pigs. Selective breeding for some of these polymorphisms may well produce better swine models of type 2 DM.

**Chinese Guizhou Minipig**

The Chinese Guizhou minipig is one of numerous strains of miniature pigs native to China, and it has proven suitable for biomedical research (Panepinto 1996). This strain has also been used to study the interaction of type 2 DM and atherosclerosis. Chinese Guizhou minipigs fed a high-fat high-sucrose diet for 6 mo produced a mild elevation in plasma
total cholesterol and triglycerides without chemical destruction of islets (Xi et al. 2004). The animals were heavier and fatter. Fasting blood glucose was elevated compared with control diet animals, and plasma fasting insulin levels, although elevated initially, were not significantly different from controls. The pigs fed a high-fat high-sucrose diet were less sensitive to insulin than the control pigs. The authors speculated that this reduced sensitivity may represent induction of insulin resistance followed by progressive decline in pancreatic β-cell function. These high-fat high-sucrose-fed pigs were glucose intolerant and had impaired acute insulin secretion in oral glucose tolerance testing. They had higher plasma total cholesterol, triglycerides, and free fatty acids. They also had increased serum TNFα, TNFβ, and free fatty acids, which are modulators of glucose metabolism. Obese humans and animals have increased TNF mRNA expression in adipose tissue. Histological analyses demonstrated adipose hypertrophy, high triglyceride staining, hepatic steatosis, and a decrease in pancreatic β-cell mass. Atherosclerosis fatty streak lesions were present in the aorta of the pigs fed a high-fat high-sucrose diet.

This Chinese Guizhou minipig fed a high-fat high-sucrose diet has also been useful in new drug testing. A synthetic lipoprotein lipase activator (N0-1886) tested in this model inhibited adipose enlargement, suppressed plasma TNFα, free fatty acids, and glucose, and increased glucose clearance and insulin response to intravenous loading. It also reduced total cholesterol and increased HDL cholesterol (Yin et al. 2004). This model should be advantageous in studying the effects of diabetes on atherosclerosis.

Ossabaw Pigs

Ossabaw pigs hold promise to overcome the lack of genetically determined swine models of type 2 DM (Buhlinger et al. 1978; Cote et al. 1982; Wangsness et al. 1980). Ossabaw pigs are believed to be the descendants of pigs brought from Spain that escaped and were isolated on the Ossabaw Islands off the coast of Georgia (Brisbin et al. 1977). Thus, this unique strain of pigs has lived in relative genetic isolation for centuries surviving on abundant food in the fall and relative starvation conditions in the winter. Such a scenario may have selected for a “thrift genotype,” which has been offered as an explanation for the high prevalence of type 2 DM in certain populations (Lee et al. 2005; Prentice et al. 2005). The Ossabaw pigs have also been reported to demonstrate insulin resistance. It has been shown that insulin binding to liver microsomes during growth decreased in Yorkshire but not in Ossabaw pigs (Meserole and Etherton 1984). The binding affinity was lower for liver microsomes than for pancreatic β-cells. These findings have suggested that the liver in Ossabaw pigs is relatively insensitive to insulin.

Plasma lipoproteins of Ossabaw pigs have been characterized in 10- to 12-mo-old animals. Ossabaw pigs have increased triglycerides, cholesterol, and VLDL. The VLDL in Ossabaw pigs are two fold larger than Yorkshire. No differences were seen in LDL. The VLDL correlated with backfat thickness, and adiposity correlated with high-density lipoprotein (HDL) (Etherton and Kris-Etherton 1980).

In contrast to contemporary commercial pigs, which are selected for leanness, Ossabaw pigs are considered obese, thus exhibiting an important risk factor for the development of type 2 DM. Notably, the backfat measures 2.64 cm in Yorkshire whereas it measures 5.97 cm in age-matched Ossabaw pigs (Hausman and Martin 1981). Dietary restrictions to 65% that of ad libitum dietary intake for 20 wk resulted in a seven fold decrease in adipose tissue, a six fold decrease in number of adipocytes, and a 50% decrease in muscle mass for Yorkshire pigs (Etherton and Kris-Etherton 1980; Etherton et al. 1982). However, in Ossabaw pigs this dietary restriction resulted in only a 2.6 % decrease in adipose mass, no change in number of adipocytes, and no loss of muscle mass. It has been reported that in Ossabaw pigs lipolytic enzyme adaptation occurs in adipose tissue but not liver, and that the enzymatic response to fasting and refeeding is more dynamic in lean pigs than Ossabaw pigs (Buhlinger et al. 1978). Ossabaw pigs are, however, not a large size. Yorkshire lean commercial pigs weigh 175 kg at 10 to 12 mo, whereas Ossabaw pigs weigh 88 kg at the same age. Yorkshire pigs are fast growing, whereas Ossabaw pigs are slow growing and have lower plasma growth hormone (Kasser et al. 1981; Wangsness et al. 1977).

Recently Ossabaw pigs have been shown to be a novel model of metabolic syndrome (obesity, insulin resistance, glucose intolerance, dyslipidemia, and hypertension) (Dyson et al. 2006). When fed a high-fat high-cholesterol diet for 20 to 40 wk, Ossabaw pigs were more obese, had higher peak glucose and insulin following glucose tolerance testing, had increased serum triglycerides, and developed higher blood pressure than Yucatan pigs fed the same diet (Dyson et al. 2005; Mokelke et al. 2005b). In addition, hyperlipidemic Ossabaw pigs that appear to model the metabolic syndrome had greater neointimal hyperplasia in stented segments of coronary arteries compared with Yucatan pigs (Zafar et al. 2005). These findings make the Ossabaw pig an exciting model for continued studies to investigate the accelerated cardiovascular complications associated with diabetes without chemical induction.

Familial Hypercholesterolemic Pigs

Pigs with familial hypercholesterolemia (FH) created by Drs. Jan and Judith Rapacz in Madison, Wisconsin, spontaneously exhibit hypercholesterolema and develop severe coronary and abdominal aortic atherosclerosis while being fed low fat pig chow (Prescott et al. 1991, 1995). The pig FH causative mutation represents a missense mutation (C253→T253), in which Arg94→Cys94 is substituted in the LDL receptor and is inherited in an autosomal fashion.
Low-Birth-Weight Pigs and Type 2 Diabetes Mellitus

In humans, low birth weight is associated with an increased risk of glucose intolerance and type 2 DM and cardiovascular disease in adult life (Poore and Fowden 2004). The thrifty genotype hypothesis proposes that suboptimal nutrition in utero causes adaptations to occur in the fetus to slow growth but these adaptations are detrimental once full nutrition is achieved. Notably, low-birth-weight piglets have abnormal cardiovascular function and poor glucose metabolism (Poore et al. 2002). Insulin sensitivity measured as a decrease in plasma glucose after intravenous injection of insulin was reduced in pigs as adults that were smaller in the neonatal period. The study suggests that postnatal growth in pigs, rather than low birth weight per se, was an important determinant of insulin sensitivity as an adult.

Criteria for Validating Models of Type 2 Insulin-resistant Diabetes Mellitus

Complications due to diabetes mellitus generally take years to develop in humans. Cardiovascular complications remain the most common cause of death among diabetics, but considerable morbidity and mortality are also associated with an increased risk of complications in renal, ophthalmological, gastrointestinal, and central and peripheral nerve tissues. Modeling these complications in animals also requires allowing sufficient time. The National Institutes of Health-sponsored website for the Animal Models of Diabetic Complications Consortium (AMDCC) has many detailed protocols listed for characterizing renal, neurological, retinal, and cardiovascular complications in rodent models of diabetes that can be extrapolated to pigs: https://www.amdcc.org/index.aspx. Carefully designed studies in validated models are needed to determine whether future mechanistic and intervention studies could be done in a shorter time frame and thus with less expense. Such studies must account for the pathophysiological changes that occur in type 2 DM to link these changes to the complications. A seven-step process to characterize an animal model of human type 2 DM is listed in Table 2. The criteria that each step addresses are discussed briefly below.

Step 1: Document Fasting Hypersinsulinemia, Insulin Sensitivity, and Serum Glucose Levels

Linking insulin resistance to a given diabetic complication requires documenting its severity during the study period. Fasting and postprandial glucose and insulin levels, insulin clamping studies, and frequently sampled insulin glucose tolerance testing have been used (Garvey et al. 2003; Goff et al. 2005). These assays must be reproducible and sensitive to variables that alter insulin sensitivity. For example, large weight gains or losses would be predicted to be accompanied by inverse changes in insulin sensitivity. To measure long-term glucose control, hemoglobin A

Step 2: Determine Lipoprotein Concentration and Particle Size

Detailed lipoprotein analyses are essential to understanding the relevance of a given animal model to the dyslipidemia present in the majority of human type 2 DM. The human IR phenotype is characterized by accumulation of small dense LDL particles as opposed to large LDL particles (Carr et al. 1999; Gardner et al. 1996; Kohen-Avramoglu et al. 2003) and by suppression of large HDL particles (Brown et al. 1990; Deeb et al. 2003; Garvey et al. 2003; Goff et al. 2005; Howard et al. 2003). Thus, not only are the magnitude of lipoprotein changes important, but the distribution and size of particles are also important in macrovascular disease.

Step 3: Obtain Total Body Fat Measurements

An estimate of total body fat will determine whether there is a correlation between total body fat and IR and whether changes in total body fat over time correlate with changes in

Table 2 Steps for validating models of type 2 insulin-resistant diabetes mellitus

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Document fasting hypersinsulinemia, insulin sensitivity, and serum glucose levels.</td>
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<tr>
<td>2</td>
<td>Determine lipoprotein concentration and particle size.</td>
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<tr>
<td>3</td>
<td>Obtain total body fat measurements.</td>
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<tr>
<td>4</td>
<td>Characterize serum markers.</td>
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<tr>
<td>5</td>
<td>Measure arterial blood pressure.</td>
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<tr>
<td>6</td>
<td>Document end-organ damage due to type 2 insulin-resistant diabetes mellitus.</td>
</tr>
<tr>
<td>7</td>
<td>Document reduction in end-organ damage due to type 2 insulin-resistant diabetes mellitus with intervention.</td>
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</tbody>
</table>
IR to determine the influence of total body fat on the degree of IR and diabetes complications. As in humans, the distribution of body fat in swine may be important in determining the risk associated with obesity, so the distribution should be analyzed (Dyson et al. 2006). Noninvasive measures of body fat allow for serial measurements throughout the course of a study. Recent studies in Yucatan swine have shown that simple measures can be predictive (Witczak et al. 2005).

Step 4: Characterize Serum Markers

Regulatory factors secreted from adipose tissue (adipokines) are the subject of increased research to determine their role in obesity and type 2 DM. C-reactive protein, interleukin-6, TNFα, leptin, plasminogen activator inhibitor-1, adiponectin, resistin, and fibrinogen are frequently elevated in human type 2 DM (Harris et al. 1999; Hunt et al. 2002). In the currently available animal models of type 2 DM, it is not yet known whether (1) changes in the levels of these markers can distinguish between insulin sensitive and IR animals, (2) there is a progressive change in inflammatory markers over time, (3) these changes correlate with the severity of complications, (4) the changes in these markers correlate with changes in fat mass and changes in insulin resistance, and importantly (5) which measurements correlate best with the changes that have been measured in affected humans.

Step 5: Measure Arterial Blood Pressure

A large percentage of human type 2 diabetics are hypertensive, therefore control of blood pressure is essential to retard or prevent complications such as renal failure, retinopathy, or accelerated atherosclerosis (Bell et al. 2005; Buch et al. 2005; Cueto-Manzano et al. 2005; McFarlane et al. 2005; Teitelbaum et al. 2005). Validated noninvasive methods for measuring blood pressure are available in several species (Krege et al. 1995; Mesangeau et al. 2000; Springett et al. 2001). The range of pig arterial blood pressure values is very close to that of humans and varies to some extent with age, gender, and strain (Chow et al. 1999; Goodrich et al. 2001). Of note, blood pressure appears to be dysregulated in pigs with SZT-induced, insulin-deficient DM (Mesangeau et al. 2000). It should be noted that most studies in humans that establish levels considered hypertensive are considerably larger than is feasible in most experimental pig herds. Thus, comparison of diabetic versus controls (i.e., concurrent nondiabetic pigs or changes with induction of DM) will help establish the impact of changes in blood pressure on the specific complication(s) being studied.

Step 6: Document End-organ Damage Due to Type 2 Insulin-resistant Diabetes Mellitus

Development of severe and diffuse coronary, aortic, and peripheral (e.g., iliac, femoral, and carotid) atherosclerosis is the most common cause of death and disability among human type 2 diabetics. Thus, measuring atherosclerosis in a given animal model must account for severity and diffuseness as well as the rate of progression. Endothelial function studies and assessment of vascular reactivity as described in studies above are also important to consider. Screening the urine for protein and performing definitive histological analysis are required to determine whether IR is associated with the development of renal disease. The histopathological characterization would include light and electron microscopic detection of renal histopathology and immunohistochemical detection of immunoglobulin and complement deposition. Characterization of changes in cardiac muscle, retinal arteries, and central and peripheral nerves depends on study design and target organs. Current animal models of diabetic microangiopathy have limitations. Although the pig has proven useful for studying macrovascular disease, there are very few references in the literature to microvascular disease in diabetic pigs. It is necessary to collect tissues, especially from long-term studies, to evaluate the microvasculature. Finally, documenting changes in expression levels of insulin-sensitive genes in liver (e.g., FOXO-1 and PEPCK) as well as fat and skeletal muscle will be very important for the design of subsequent mechanistic studies that will lower insulin resistance.

Step 7. Document Reduction in End-organ Damage Due to Type 2 Insulin-resistant Diabetes Mellitus with Intervention

To document that an animal exhibiting these criteria is a valid model of human type 2 DM and its complications requires mechanistic studies in appropriate numbers of treated and control animals showing that treatment of the DM is accompanied by prevention or reduction of the severity of a given complication.

Summary and Future Directions

Pigs have many attributes that make them suitable for creating an animal model of type 2 DM to determine the mechanisms that mediate cardiovascular complications. Important examples of attributes include the following:

- Pigs develop atherosclerosis in anatomical locations that are relevant to the human condition (Brodala et al. 2005; Hasler-Rapacz et al. 1995). These animals represent an ideal model for identifying causative mechanisms and testing new treatment strategies in these vascular beds, especially in the presence of type 2 DM.
- Pigs develop atherosclerotic lesions that recapitulate the histopathology seen in humans (Brodala et al. 2005; Gerrity et al. 1979; Nichols et al. 1992; Prescott et al. 1991, 1995).
- The strains of pigs reviewed in this article docu-
ment that pigs recapitulate many of the metabolic abnormalities present in type 2 DM.

The current limitations pigs impose are mostly due to size and expense. The Ossabaw pigs hold promise for providing animals of a more suitable size and an inherited predilection to insulin resistance. FH pigs exhibit a spontaneous dyslipidemia characteristic of that found in IR humans, obviating the need for and expense of additional dietary supplementation.

It should be emphasized that defining the precise biochemical changes that occur in type 2 DM and correlating these changes with specific complications will be essential for identifying potential mechanisms that initiate and perpetuate complications that are unique to the insulin-resistant and/or hyperglycemic state. The pig model appears to be ideal for using these mechanistic data to develop and test potential new treatment strategies. The ultimate validation of an animal model of diabetic complications will require reducing the severity of a given complication with successful treatment of the DM, including metabolic control with decreased insulin resistance or hyperglycemia, implantation of devices (e.g., stents), or other novel therapeutics.

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References


