The BBZDR/Wor Rat Model for Investigating the Complications of Type 2 Diabetes Mellitus

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Abstract

Congenic and inbred strains of rats offer researchers invaluable insight into the etiopathogenesis of diabetes and associated complications. The inbred Bio-Breeding Zucker diabetic rat (BBZDR)/Wor rat strain is a relatively new and emerging model of type 2 diabetes. This strain was created by classical breeding methods used to introgress the defective leptin receptor gene (*Lepr^{fa}*) from insulin-resistant Zucker fatty rats into the inbred BBDR/Wor strain background. The diabetic male BBZDR/Wor rat is homozygous for the fatty mutation and shares the genetic background of the original BB strain. Although lean littermates are phenotypically normal, obese juvenile BBZDR/Wor rats are hyperlipidemic and hyperleptinemic, become insulin resistant, and ultimately develop hyperglycemia. Furthermore, the BBZDR/Wor rat is immune competent and does not develop autoimmunity. Similar to patients with clinical diabetes, the BBZDR/Wor rat develops complications associated with hyperglycemia. The BBZDR/Wor rat is a model system that fully encompasses the ability to study the complications that affect human type 2 diabetic patients. In this review, recent work that has evaluated type 2 diabetic complications in BBZDR/Wor rats is discussed, including the authors' preliminary unpublished studies on cardiovascular disease.

Key Words: BBZDR/Wor rats; cardiovascular disease; diabetes; nephropathy; neuropathy; retinopathy

Human Type 2 Diabetes Mellitus

Diabetes mellitus is a term that is used loosely to describe a group of disorders simply characterized by hyperglycemia (elevated blood sugar levels). The hyperglycemic state can result from the destruction of pancreatic insulin-producing beta cells, the inadequate action of insulin due to faulty insulin secretion and/or peripheral resistance to the action of insulin. Primarily hyperglycemia may be controlled through diet and/or exogenous insulin therapy; however, diabetics continue to experience both acute and chronic metabolic complications.

Type 2 diabetes (formerly called "adult onset" diabetes or non-insulin-dependent diabetes mellitus) is a term applied to several categories of diabetic patients, including a majority with an undefined cause. Characteristics consist of a number of health disorders, including obesity, polycystic ovary disease, and syndrome X (also known as metabolic syndrome); features are similar to those of type 2 diabetes, including dyslipidemia (elevated triglycerides and decreased high-density lipoprotein [HDL[1]] levels), hypertension, and insulin resistance (Arthur et al. 1999; Kotake and Oikawa 1999; Lender et al. 1997; Pugeat and Ducluzeau 1999; Watanabe et al. 1999). Type 2 diabetes is more prevalent than type 1 diabetes; it affects more than 6% of the total US population with more than 1.3 million new cases diagnosed each year. Overall, diabetes is the sixth leading cause of death in the United States. Disturbingly, the number of new cases affecting adolescents is increasing exponentially, and type 2 diabetes in the United States is reaching epidemic proportions (CDC 2003).

Type 2 diabetes is generally recognized as the culmination of genetic and environmental risk factors, and genetic susceptibilities comprise one of the greatest risk factors. The concordance rate among identical twins is higher in type 2 diabetics than seen in type 1 diabetics (Kaprio et al. 1992; Medici et al. 1999). Aside from the small number of patients with mutations in proinsulin, glucokinase, insulin receptor genes, or mutations in specific mitochondrial functions, the frequent genetic risk factors include insulin resistance, decreased insulin secretion, and postreceptor defects (Weir 1996). A second prevalent risk factor associated with type 2 diabetes is obesity, a major health problem affecting more than 1 billion people worldwide (Imai 2003). Modifiable behavioral risk factors associated with insulin resistance include poor diet, lack of exercise, smoking, and stress (Kelly 2000).

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[1]Abbreviations used in this article: BB, Bio-Breeding; BBDP, BB diabetic-prone; BBDR, BB diabetic-resistant; BBZDR, BB Zucker diabetic rat; BRB, blood-retinal barrier; CAD, coronary artery disease; DPN, diabetic polyneuropathy; ECM, extracellular matrix; *fa, Lepr^{fa}* fatty mutation at the leptin receptor locus; GK, Goto-Kakizaki; GLUT-2, glucose transporter type-2; HDL, high-density lipoprotein; IGT, impaired glucose tolerance; LDL, low-density lipoprotein; OLETF, Long-Evans Tokushima fatty; PPARγ, proliferator-activated receptor-gamma; PTCA, percutaneous coronary artery angioplasty; TGase, transglutaminase; TGF-β, transforming growth factor-beta; TP, testosterone propionate; TZD, thiazolidinedi one; VEGF, vascular endothelial growth factor; ZDF, Zucker diabetes fatty.

The pathogenesis of type 2 diabetes is not well understood. The two metabolic characteristics of type 2 diabetes are (1) alterations in insulin secretion and (2) inability of peripheral tissues to respond to insulin (insulin resistance). Prediabetics present with impaired glucose tolerance (IGT[1]) due to faulty insulin secretion. Over time, the demand for insulin production due to increased insulin resistance leads to excessive stress on the beta cells, which can lead to complete beta-cell failure. Therapies for type 2 diabetics are limited. Overweight individuals who present with impaired glucose tolerance are encouraged to lose weight, adhere to recommended diets, and exercise regularly, because weight loss and muscle gain delay the onset of type 2 diabetes (Pan et al. 1997). In the presence of continued hyperglycemia, patients may receive drugs that reduce the overall blood glucose level (Chiasson et al. 1994; Coniff et al. 1995; Iwamoto et al. 1996). The effectiveness of many of these glucose-lowering drugs is temporary and within 4 to 5 yr of therapy can no longer compensate for beta-cell failure (Turner et al. 1996). Thiazolidinediones (TZDs[1]) can act as adjunct therapy to increase the effectiveness of glucose-lowering drugs, but recent evidence also suggests that TZDs alone may rejuvenate beta cells (Bell 2003). Therapeutic use of TZDs leads to partial improvement (20-40%) of insulin-stimulated glucose removal in several human insulin-resistant states (reviewed in Olefsky 2000). TZDs can act through the adipocyte nuclear peroxisome proliferator-activated receptor-gamma (PPARγ) to enhance insulin sensitization, but they may also work through PPARγ-independent pathways through direct interaction with muscle and liver (Furnsinn and Waldhausl 2002; Kahn and Flier 2000; Olefsky 2000). As the disease progresses, type 2 diabetics may also require exogenous insulin therapy.

Long-term poor glycemic control in diabetic patients leads to the development of microvascular (neuropathy, retinopathy, and nephropathy) and macrovascular (coronary artery disease) complications. The results of the United Kingdom Prospective Diabetes Study, the Diabetes Control and Complications Trial, and the Epidemiology of Diabetes Interventions and Complications study have clearly shown that long-term glycemic control is important to prevent the accompanying complications of diabetes (Keen 1994; Klein 1995; Malone et al. 2001; Molyneaux et al. 1998; Peterson and Smith 1995; Service and O'Brien 2001; White et al. 2001; Zhang et al. 2001; Zinman 1998). Clinicians and patients therefore routinely monitor the control of diabetes and development of complications through blood chemistry molecular markers, including blood glucose levels and glycosylated hemoglobin (Peters et al. 1996; Weir 1996). The pathways involved in the development of complications of type 2 diabetes are similar to those in type 1 diabetic patients (nonenzymatic glycosylation, increased flux through the polyol pathway, activation of protein kinase C, and increased hexosamine pathway flux). These pathways are discussed elsewhere in this issue of *ILAR Journal* (Eiselein et al. 2004).

Animal Models for Type 2 Diabetes

Numerous induced and spontaneous rodent models have been used to model various defects of human type 2 diabetes. A comprehensive overview of these models is beyond the scope of this review. We instead briefly review several frequently used spontaneous rat models: Otsuka Long-Evans Tokushima fatty (OLETF[1]), Goto-Kakizaki (GK[1]), Zucker Diabetes Fatty (ZDF[1]), and Bio-Breeding Zucker diabetes-resistant (BBZDR[1])/Wor rats. The relevant characteristics of these animal models compared with human disease are listed in Table 1 and discussed below.

OLETF Rat

In the mid-1980s, spontaneous diabetes was detected in an outbred colony of Long-Evans rats maintained at the Tokushima Research Institute, Otsuka Pharmaceuticals (Shima et al. 1999). Subsequent selective breeding for more than 20 generations has led to the generation of a spontaneously diabetic strain of rat that displays polyuria, polydipsia, and slight obesity (Man et al. 1997). The OLETF rat develops hyperphagia and insulin resistance between 12 and 24 wk of age, and mild obesity, hyperglycemia, and hyperinsulinemia between 20 and 28 wk of age. By 40 wk of age, the diabetic rats are hypoinsulinemic and exhibit defects in insulin secretion (Kawano et al. 1991, 1992; Yamamoto et al. 1999). Obese OLETF rats are unable to control individual meal size due to the loss of cholecystokinin-A receptors (Bi and Moran 2002, 2003). Diabetic disease sequelae in the OLETF rat are similar to human disease although there is a gender bias with diabetes observed in most (86%), but not all, male rats (Kawano et al. 1991, 1992; Yamamoto et al. 1999). These rats have proven useful in studying the effects of exercise and diet on the development of type 2 diabetes, to test the efficacy of antidiabetic agents, and to study the complications of diabetes (Shima et al. 1999). However, the utility of this strain is hampered by lack of availability to the research community outside Japan.

GK Rat

The GK rat is a widely accepted model for research in type 2 diabetes. The GK rat was created by selective breeding of Wistar rats for oral glucose intolerance, and it is >35 generations inbred (Goto et al. 1988). Hyperglycemia in GK rats has been mapped to the genomic segment *Niddm1,* which encodes at least two loci responsible for high blood glucose (Fakhrai-Rad et al. 2000; Galli et al. 1999). Males and females become diabetic at weaning age, most likely due to an overall inherent lack of normal beta cell mass. Diabetes in the GK rat is characterized by fasting hyperglycemia, impaired secretion of insulin in response to glucose,

Table 1 Comparative characteristics of spontaneous type 2 diabetes in humans and in BBZDR/Wor, ZDF, GT, and OLETF rat model systems[a]

	Human[b]	BBZDR/Wor	ZDF	GK	OLETF
Age of onset (hyperglycemia)	Adult adolescence on rise	10 wk	~7 wk	~12 wk	12-24 wk
Obesity (hyperleptinemia)	+	+	+	–	+ (slight)
Insulin resistance (hyperinsulinemia)	+	+	+	+	+
Prediabetes fasting IGT	14.9%	+	+	+	+
MHC genes	HLA-DQ and DR	$RT1^{u}$	$RT1^{uv1}$	$RT1^{u}$	$RT1^{u}$
Other known diabetes-associated genes	*Obh-1, CTLA-4.* At least 2 loci, perhaps ≥16	*fa/fa*	*fa/fa*	*Niddm/kdp1*	*Obh-1, Dmo1, Olep1, Olep2*
Gender effect (%)	M = F (50%)	M = ~100% F = <3%	M = ~100%* F = ~0%; unless induced	M = F	M = 86% F = 0%
Microvascular complications					
Retinopathy	up to 80%	+	–	+	+
Neuropathy	70-80%	+	+	+	+ (sucrose fed)
Nephropathy	10-21%	+	+	+	+
Macrovascular complications					
CAD	65% cause of death	+	–	+ (microangiopathy)	+ (microangiopathy)

[a]BBZDR/Wor, Bio-Breeding Zucker diabetic rat; ZDF, Zucker diabetic fatty rat; GK, Goto-Kakizaki rat; OLETF, Otsuka Long-Evans Tokushima fatty rat; IGT, impaired glucose tolerance; CAD, coronary artery disease; MHC, major histocompatibility complex; M, male; F, female; +, present; –, not present.
[b]CDC [Centers for Disease Control and Prevention]. National Diabetes Fact Sheet: General Information and National Estimates on Diabetes in the United States, 2002. US Department of Health and Human Services. Atlanta: CDC.
*Diet induced.

and hepatic and peripheral insulin resistance. In contrast to other rodent models of type 2 diabetes, the GK rat is nonobese and therefore does not accurately mimic all of the features of human type 2 diabetes. However, late onset complications such as retinopathy, microangiopathy, neuropathy, and peripheral nephropathy have been described in the literature (Carmo et al. 2000; Murakawa et al. 2002; Riley et al. 1999; Sato et al. 2003; Yoshida et al. 1996). In an attempt to generate a more appropriate nephropathy model, this strain was recently used for the introgression of genes from the Fawn-hooded hypertensive (FHH) rat, which develops progressive renal disease in the absence of diabetes (Nobrega et al. 2004).

ZDF Rat

The ZDF is an outbred rat model that spontaneously expresses diabetes. The homozygous mutation (*fa/fa*) of the leptin hormone receptor results in the development of type 2 diabetes in male rats when they are fed a high-energy rodent diet (Purina 5008). The diabetic ZDF begins to develop hyperglycemia at ~7 wk of age, and glucose levels in the fed state typically reach 500 mg/dL by 10 to 11 wk of age. Triglyceride and cholesterol levels of obese rats are higher than those of lean rats. Very high lipid levels can be induced in the obese ZDF using high saturated fat and sucrose-containing diets. Obese male infertility hampers research in these rats, and it has been addressed by the use of testosterone propionate (TP^{1}; Hemmes and Schoch 1988). Depending on the amount and duration of TP administration, obese males increase their probability of ejaculation and sexual activity. Diabetic ZDFs do not spontaneously develop hypertension or cardiovascular disease (Clark et al. 1983).

Type 2 Bio-Breeding (BB^{1}) Rat Models

We wished to develop a model for type 2 diabetes that encompassed all of the human features and could be used to study the associated complications. We chose the BB rat strain background for development of the model. Since the mid-1970s, the BB rat has been used as an animal model for human type 1 diabetes, which comprises ~10% of all diabetes mellitus. This model has been extremely useful for studying both spontaneous diabetic-prone ($BBDP^{1}$) and induced diabetic-resistant ($BBDR^{1}$) diabetes and associated

diabetic complications. The BB rat model of type 1 diabetes and human type 1 diabetes are more comprehensively addressed in accompanying reviews in this issue (Mordes et al. 2004; Eiselein et al. 2004, respectively).

BBZDP/Wor Rat

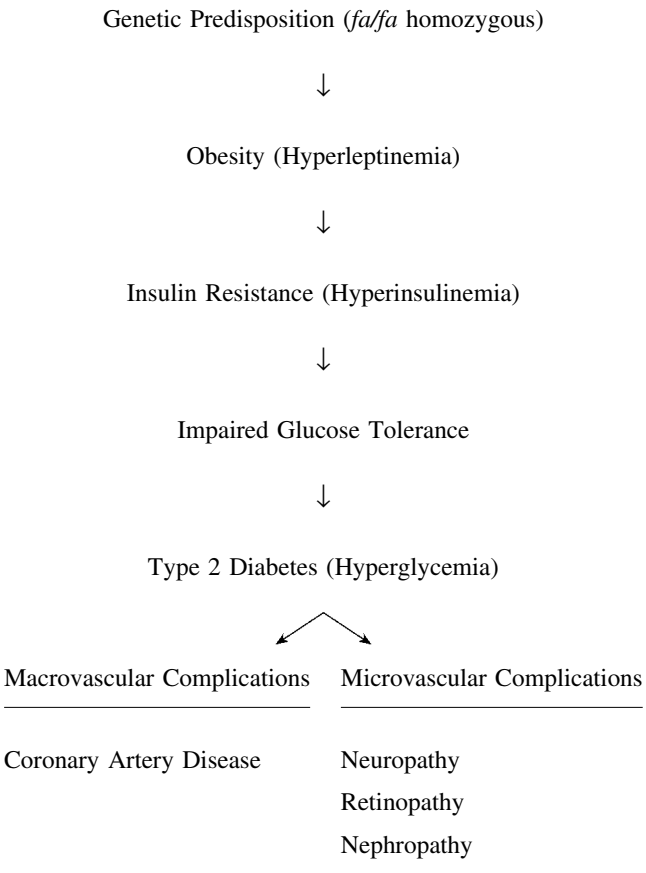
The BBZDP/Wor strain carries the *Iddm2* type 1 diabetes-associated genetic locus (reviewed by Mordes et al. 2004) in addition to encoding the *Lepr*^{fa} mutation. In this strain, diabetes is manifested by lymphopenia, obesity, hyperinsulinemia, and autoimmune diabetes (Guberski et al. 1988, 1993; Vernet et al. 1995). Islets from obese rats reveal beta-cell hyperplasia, and diabetes develops due to a combination of insulin resistance and autoimmune insulitis (Guberski et al. 1988). Although the BBZDP/Wor rat is a potentially important animal model of type 2 diabetes, interpretation of the existing data is often complicated by the presence of both type 1 and type 2 diabetes characteristics. We have previously shown that injections of BBDR/Wor splenocytes into BBZDP/Wor rats correct lymphopenia and prevent autoimmune insulitis (Guberski et al. 1988). However, nonlymphopenic obese male BBZDP/Wor rats still become diabetic, in spite of the absence of insulitus. These results suggested the ability to create a nonlymphopenic type 2 diabetic rat model from these animals.

BBZDR/Wor Rat

The BBZDR/Wor rat is an inbred rat strain (>40 generations) that was developed as an animal model for type 2 diabetes and is emerging as the most applicable model of type 2 diabetic complications. To produce the BBZDR/Wor type 2 diabetic rat, classical genetic methods were used to remove the recessive *Iddm2* gene responsible for lymphopenia and spontaneous autoimmunity and retain the *Lepr*^{fa} (*fa*^{1}) mutation by crossing BBZDP/Wor animals with the lean, nondiabetic BBDR/Wor rats. Both male and female obese BBZDR/Wor rats are infertile, and strain lines are maintained through mating of heterozygous (lean) littermates. Although obese females rarely (<3%) develop disease, the obese male BBZDR/Wor rat spontaneously develops type 2 diabetes at approximately 10 wk of age (~100%) when fed standard rat chow (Purina 5010; Ellis et al. 1998, 2000, 2002; and our unpublished data). Heterozygous lean rats of either sex do not develop glycosuria or hyperglycemia. Thus, obese females and lean littermates are generally used as age-matched controls, and except where noted otherwise, this article focuses on the research generated using the type 2 diabetic male.

Salient features of the BBZDR/Wor diabetic rat include dyslipidemia, hyperglycemia, insulin resistance, hypertension, and decreased levels of the beta cell-specific glucose transporter type-2 (GLUT-2[1]) (Ellis et al. 1998; Sima and Merry 1997; our unpublished observations). In comparison, we have observed that obese female BBZDR/Wor rats have impaired glucose tolerance, but rarely (<3%, age of onset 21 wk) develop hyperglycemia. In addition, as in human type 2 diabetics, we observed that the BBZDR/Wor male and female rats developed IGT (glycemic values that exceeded 200 mg/dL by 2 hr after dextrose injection) and insulin resistance (manuscript in preparation). We analyzed pancreatic islets isolated from lean and obese BBZDR/Wor. Islets from lean nondiabetic BBZDR/Wor rats were normal in all respects and displayed maintenance of normal islet architecture and normal levels of glucagon, insulin, and GLUT-2 (Figure 1, A and B; GLUT-2 not shown). In contrast, islets from young obese diabetic males were profoundly enlarged and demonstrated beta-cell hyperplasia and mild fibrosis, commonly seen in islets from patients with type 2 diabetes (Figure 1, C and D). An overall reduction in insulin and GLUT-2 was also observed. Progression of diabetes led to a decrease in beta-cell mass and disorganization of islet architecture seen in older diabetic obese males (Figure 1, E and F). Studies of pancreatic sections from older diabetic rats immunostained for insulin revealed focal reduction in beta-cell insulin content (Figure 1F). We also observed an overall reduction of GLUT-2 staining of beta-cell surface membranes (data not shown).

Collectively, these data show that similar to the human disease, the BBZDR/Wor type 2 rat demonstrates classic disease progression, as outlined below. Furthermore, rats with 4 mo of diabetes develop both microvascular and macrovascular complications, as described below.



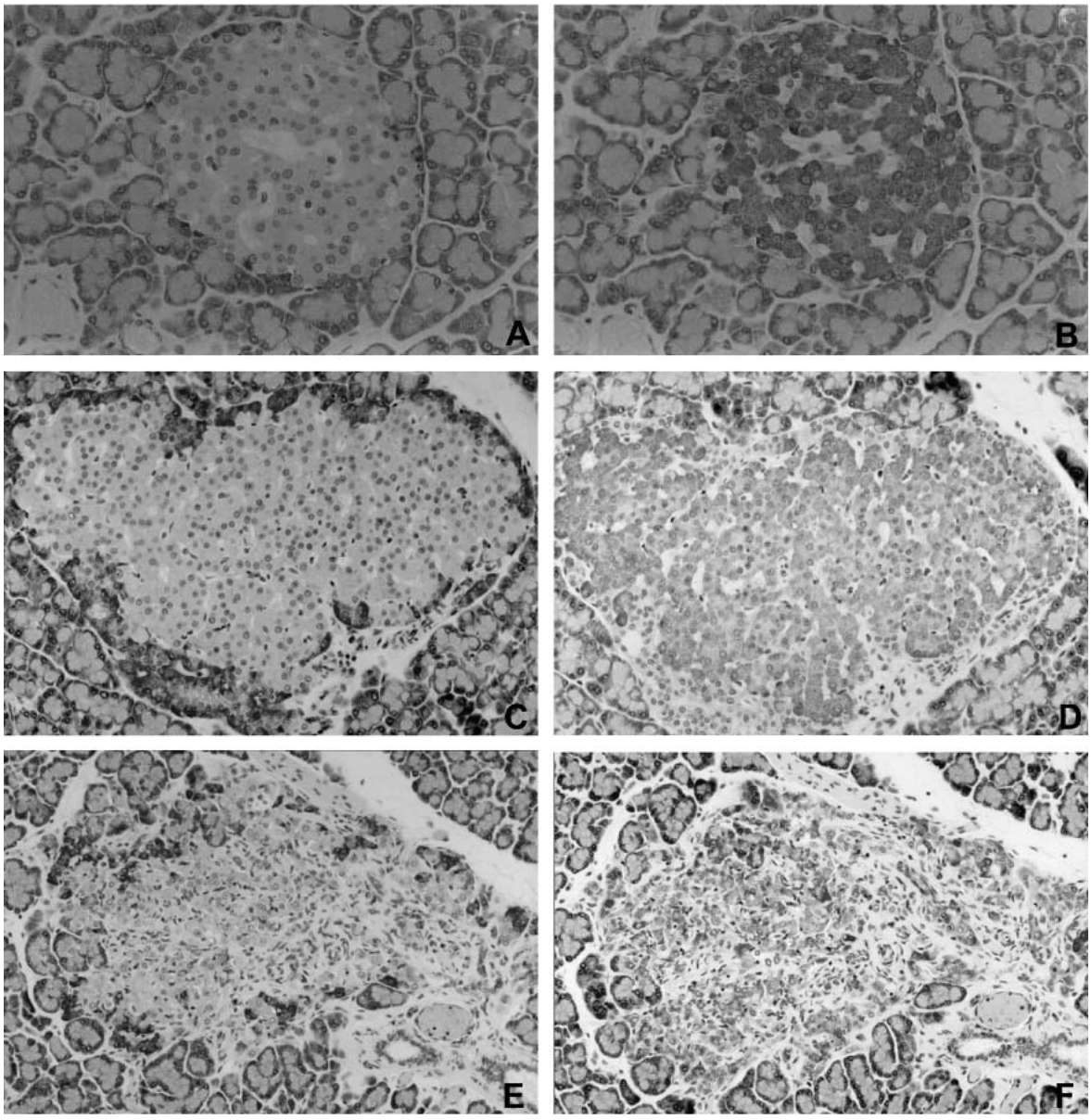


Figure 1 BBZDR/Wor pancreatic islet morphology. Pancreata from lean (A and B), obese prediabetic (C and D), and obese diabetic (E and F) BBZDR/Wor rats were isolated and processed for immunohistochemistry. Consecutive, fixed hemotoxylin and eosin-stained sections were immunostained for glucagon (A, C, E) or insulin (B, D, F). Representative images are shown.

Microvascular Complications (Neuropathy, Retinopathy, and Nephropathy)

Glucose-mediated vascular damage is directly caused by flux within four molecular pathways (reviewed by Eiselein et al. 2004). These pathways include increased polyol flux (with increases in sorbitol and fructose), increased hexosamine pathway flux, activation of various protein kinase C isoforms, and an increase in the formation of advance glycation end products. The development of therapies to control and/or correct diabetic complications is directed at enzymes that control these pathways and restore the altered flux. Although some of these therapies appear to be successful in treating an individual complication, they do not treat diabetic complications collectively. In addition to complications induced by hyperglycemia, many type 2 diabetics have the added impact of complications due to dyslipidemia (Battisti et al. 2003; Johansen and Birkeland 2003). The BBZDR/Wor rat would be an ideal model to separate these aspects of type 2 diabetes because obese females present with dyslipidemia in the absence of hyperglycemia (unpublished observations). Future work will focus on this characteristic. However, as described below, much work studying microvascular complications has already been performed in the BBZDR/Wor obese male diabetic rat.

Diabetic Neuropathy

Diabetic neuropathy is the most common diabetic complication, which affects 70 to 80% of type 2 diabetic patients (Sima and Sugimoto 1999). Diabetic neuropathy is a major complication that can present as several syndromes, which affect motor, sensory, and autonomic nerves. One of these syndromes, diabetic polyneuropathy (DPN[1]), is a heterogeneous condition with symptoms ranging from peripheral sensory deficits (loss of sensation in limbs) to autonomic neuropathy (Levitt et al. 1996; Tesfaye et al. 1996). Fulminant DPN can result in amputation of limbs and significant mortality once the autonomic nervous system is involved. Peripheral nerve dysfunction in DPN results from the additive effects of nerve damage accompanied by impaired nerve regeneration (Pierson et al. 2002; Sima, 2003; Xu and Sima, 2001; Xu et al. 2002). The disease pathogenesis is multifactorial and may involve polyol pathways, abnormalities in vasoactivity, nonenzymatic glycosylation, and possibly genetic susceptibility. Dysfunctional synthesis and slowed transport of neurofilaments in diabetic rats have been suggested as an additional contributing cause of human DPN (Xu et al. 2002).

The comparison between DPN in type 2 diabetic BBZDR/Wor and type 1 diabetic BBDP/Wor rats has highlighted several differences that also reflect differences seen in human type 1 and type 2 diabetics, as outlined below (Sima and Sugimoto 1999; Sima et al. 2000). These differences between the rat strains illustrate the advantages of studying genetically similar models with distinct disease syndromes to increase our understanding of disease pathogenesis. In both strains of diabetic rats, DPN is characterized by a progressive slowing of nerve conduction velocity, axonal atrophy, and degeneration. The slowing of nerve conduction velocities (the first sign of DPN in human and animal models) is more severe in BBDP/Wor rats than in BBZDR/Wor rats. Primary changes in the neurons of both strains result in decreased Na^+/K^+-ATPase, inactivation of Na^+-channels, and intra-axonal Na^+ accumulation at the node resulting in paranodal swelling and eventual nerve degeneration. BBZDR/Wor rats display a more severe Na^+/K^+-ATPase defect. Disruption of the paranodal ion-channel barrier by axoglial dysjunction and paranodal demyelination is common in both human and BBDP/Wor type 1 diabetes. However, these structural changes are not observed in human type 2 diabetics or in BBZDR/Wor rats. The process of peripheral nerve regeneration is a multistep process requiring participation from myelin-producing Schwann cells, macrophages, fibroblasts, neurons, neurotrophic factors, cytokines, and extracellular matrix proteins. DPN in BBZDR/Wor and BBDP/Wor rats shares features of human DPN, including Wallerian degeneration (the recruitment of macrophages to clear the debris from degenerating axons), which must precede the initiation of axonal regrowth. To initiate nerve regeneration, early response genes stimulate the recruitment of macrophages by Schwann cells, which in turn stimulate release of interleukins and clear myelin debris. Expression of early response genes is delayed in BBDR/Wor rats but maintained at near-normal levels in BBZDR/Wor rats (Pierson et al. 2002). This difference may explain the increased efficiency of nerve regeneration seen in type 2 diabetic patients. It has been hypothezied that the perturbed insulin signaling due to insulin and/or C-peptide deficiency in type 1 BBDP/Wor rats may account for the differences seen between these two diabetic models (Sima et al. 2000).

Diabetic Retinopathy

The frequency of diabetic retinopathy increases proportionally to the duration of diabetes and blood glucose control (Jiang et al. 1996). Diabetic retinopathy can be divided into three clinical stages, all primarily due to retinal hypoxia (Howard 1996). The first two stages, background retinopathy and preproliferative retinopathy, are characterized by microvascular abnormalities. Microaneurysms are the earliest clinically visible manifestation of background retinopathy, followed by retinal hemorrhages, focal leakage of proteins, and capillary nonperfusion that can lead to retinal edema. Additional microvascular abnormalities result from significant vascular occlusion and characterize the preproliferative retinopathy stage. These changes result in more severe retinal ischemia, including new blood vessels arising from the retina or optical disc, which define the third stage, proliferative retinopathy. Approximately 50% of patients who reach the preproliferative stage will progress to proliferative retinopathy within 15 mo (Jiang et al. 1996). Overgrowth of these vessels can lead to hemorrhage, retinal tears, and retinal detachment. Treatment options for diabetic retinopathy are limited to repeated laser surgery to stem new vessel growth (Aiello 2003).

The BBZDR/Wor rat has been pivotal in shedding light on initial pathways involved in diabetic retinopathy. BBZDR/Wor rats progress to late stages of preproliferative retinopathy but do not demonstrate proliferative aspects of the disease. The first morphological studies of the type 2 BBZDR/Wor model of diabetes showed a hyperglycemia-dependent increase in NADH oxidase activity in the retinas, indicative of oxidative injury and endothelial cell dysfunction in the retina (Ellis et al. 1998, 2000, 2002). It was demonstrated that hyperglycemia-induced pseudohypoxia results in an imbalance in cytosolic NADH/NAD+ produced by vascular endothelial cells and pericytes leading to the generation of superoxide radicals that dismutate to H_2O_2 (Ellis et al. 1998). High H_2O_2 levels correlate with increased vascular endothelial growth factor (VEGF[1]). The retinal levels of H_2O_2, VEGF, and its receptors VEGF-R1 and VEGF-R2 were investigated to document disruption of the blood retina barrier (BRB[1]) in diabetic rats compared with nondiabetic rats. VEGF and VEGF-R1 immunoreactivity is statistically higher in the inner and outer BRB of diabetic rats, whereas VEGF-R2 is statistically higher in the inner BRB but not in the outer BRB. These studies were the first

to document the initial sequence of events leading to early stages of diabetic retinopathy. They are relevant to human disease, which also displays upregulation of VEGF and increased reactive oxygen species (Frank et al. 2004).

Diabetic Nephropathy

Diabetic nephropathy is the most common cause of end-stage renal disease in the United States, and it affects both type 1 and type 2 diabetic patients. The annual cost of caring for these patients is in the billions of dollars (CDC 2003). Not all individuals who develop diabetes progress to renal disease, which suggests a genetic component that predisposes some individuals to the development of diabetic nephropathy (Quinn et al. 1996). Structural changes in the diabetic kidney include overall increase in kidney size and glomerular volume, mesangial cell proliferation, accumulation of glomerular extracellular matrix, glomerular sclerosis, and tubular fibrosis (Osterby et al. 2001). The early stages of decreased function in the kidney result in increased urinary albumin excretion. Fulminant kidney disease in the diabetic is characterized by proteinuria, hypertension, and progressive renal failure (Trevisan and Viberti 2004). Diabetic nephropathy in humans is correlated with faulty activity of transforming growth factor-beta (TGF-β^1) family members, key regulators of cell proliferation, and the metabolism of extracellular matrix proteins (ECM[1]; Huang et al. 2002). In addition, upregulation of tissue transglutaminase (TGase[1]) in diabetic patients leads to increased deposition of ECM (Skill et al. 2001). Overexpression and/or decreased breakdown of ECM proteins such as collagen lead to the basement membrane thickening that is characteristic of the early stages of nephropathy (Tsilibary 2003).

To validate the BBZDR/Wor animal as a model for human disease, we compared kidney sections taken from diabetic obese male BBZDR/Wor rats with lean littermate controls (unpublished observations). Results showed that lean nondiabetic BBZDR/Wor rats had essentially normal-appearing kidneys, whereas the kidneys of the diabetic animals showed the presence of both nodular and diffuse glomerulosclerosis and increased glomerular size with concomitant mesangial and basement membrane thickening. Moreover, the BBZDR/Wor rat displayed endothelial cell proliferation, interstitial fibrosis, and arteriolosclerosis. Disease severity correlated with duration of diabetes. Collectively, these changes are similar to those reported in human diabetic type 2 patients (Osterby et al. 2001). Furthermore, diabetic animals showed a 3- to 4-fold increase in collagen expression compared with nondiabetic animals. Increase in the collagen-positive area of the kidney proportionally correlated with the duration of diabetes, suggesting expansion of ECM components. Chronic diabetic animals (>10 mo) showed >30% of the kidney area positive for collagen, including the glomerular and tubulointerstitial regions of the kidney. In contrast, most of the collagen was found in the glomerulus, with little or no collagen in the tubulointerstitial regions of the animals with acute diabetes (unpublished data). Likewise, BBZDR/Wor rats are consistent with progressive changes in biomarker expression of tissue TGase with the advancement of diabetes. TGase expression was increased in the glomerulus of diabetic animals compared with nondiabetic controls (unpublished observations). TGase was significantly elevated in animals with diabetes duration >200 days. Although it is necessary to perform additional work to validate this model, these data collectively suggest that BBZDR/Wor rats will be a valuable model for the study of diabetic nephropathy.

Macrovascular Complications (Coronary Artery Disease [CAD[1]])

Morbidity and mortality associated with macrovascular complications of diabetes include atherosclerosis in the carotid, cerebral, and large arteries of the lower limbs. Statistics indicate that CAD is the number one cause of death in the United States, affecting more than 12 million Americans of whom 2 million will experience a second coronary episode within 1 yr. The annual cost of CAD in the United States exceeds $112 billion (Califf et al. 1991). Diabetes is a leading risk factor (2-4 times higher) for CAD through exacerbation of several risk factors associated with atherosclerosis including hypertension, dyslipidemia, hyperinsulinemia, and insulin resistance (Howard 1996).

Dyslipidemia, in the presence or absence of hyperglycemia, is associated with atherogenesis. Alterations include elevated triglycerides, a low level of HDLs and increased levels of both low-density lipoproteins (LDLs[1]) and total cholesterol. Additional factors unique to diabetes (e.g., glycation and oxidation of LDLs; glycation of HDLs, lipoproteins, and excessive collagen) appear to potentiate the effects of each known risk factor for CAD and contribute to the mechanism of diabetes-associated vascular disease. Hyperglycemia-induced changes in vascular permeability, platelet metabolism, and adhesion also increase the risk of thrombosis (Stamler 1989). In addition, microangiopathy in diabetics is associated with cardiac dysfunction, subendocardial fibrosis, and glycoprotein deposits (Skrha 2003).

Surgical Interventions for CAD

Currently, percutaneous coronary artery angioplasty (PTCA[1]) with the placement of stents is the major intervention used to treat CAD (O'Meara and Dehmer 1997). It was reported in 2001 that more than 500,000 percutaneous interventions were performed in the United States annually (Pompa and Wang 2001). PTCA involves insertion of an expandable balloon catheter against a primary atherosclerotic plaque or secondary restenosed lesion to increase vessel patency and blood flow (Christopher et al. 1996;

Gruentzig 1978). Nevertheless, PTCA is only temporarily successful in treating CAD, because restenosis of the artery limits the long-term benefits. Restenosis (or neointimal hyperplasia) is due to the proliferation of the intima, a layer of smooth muscle cells that line the lumen of the vessel. After PTCA treatment, restenosis results in complete blockage of the original artery. The placement of stents after angioplasty to prevent early restenosis revolutionized interventional cardiology (Sigwart 1990). The placement of a stent reduces the rate of restenosis between 20 and 35% in nondiabetics; however, restenosis still remains particularly robust in patients with diabetes (Rozenman et al. 1997, 2000). Of the diabetic patients, 50% experience restenosis within 6 mo after PTCA or stent placement (Califf et al. 1991; Feuerstein, 1997; Marso et al. 1999). This problem has significantly affected clinical applications of PTCA for treatment of diabetics with vascular disease.

BBZDR/Wor and CAD

The diabetic BBZDR/Wor rat shares most known risk factors of clinical cardiovascular disease, including hyperglycemia and central obesity, mild hypertension, and dyslipidemia (unpublished observations). Dyslipidemia is one of the most important risk factors for atherosclerosis and arterial restenosis after PTCA and the stenting procedure (Anderson et al. 2001). We observed that the BBZDR/Wor obese rats of both sexes developed hypercholesterolemia and hypertriglycerolemia within 60 days of age (manuscript in preparation). Blood chemistry profiles demonstrated an increased level of cholesterol (>300 mg/dL) and triglycerides (>550 mg/dL). These results suggested that the BBZDR/Wor rat would be a good model for the study of PTCA and stent placement in a diabetic animal.

Induced Arterial Stenosis Models

Balloon injury-induced neointima formation (stenosis) in the rat carotid artery, first described by Clowes and coworkers (1983), is a widely accepted research model. We have recently evaluated stenosis formation in BBZDR/Wor lean and obese rats after balloon injury (unpublished data). Stenosis was induced (via the insertion of a balloon catheter) in the right common carotid artery of diabetic BBZDR/Wor obese male rats (minimum 4-mo duration of diabetes), nondiabetic obese female rats, and lean nondiabetic controls.

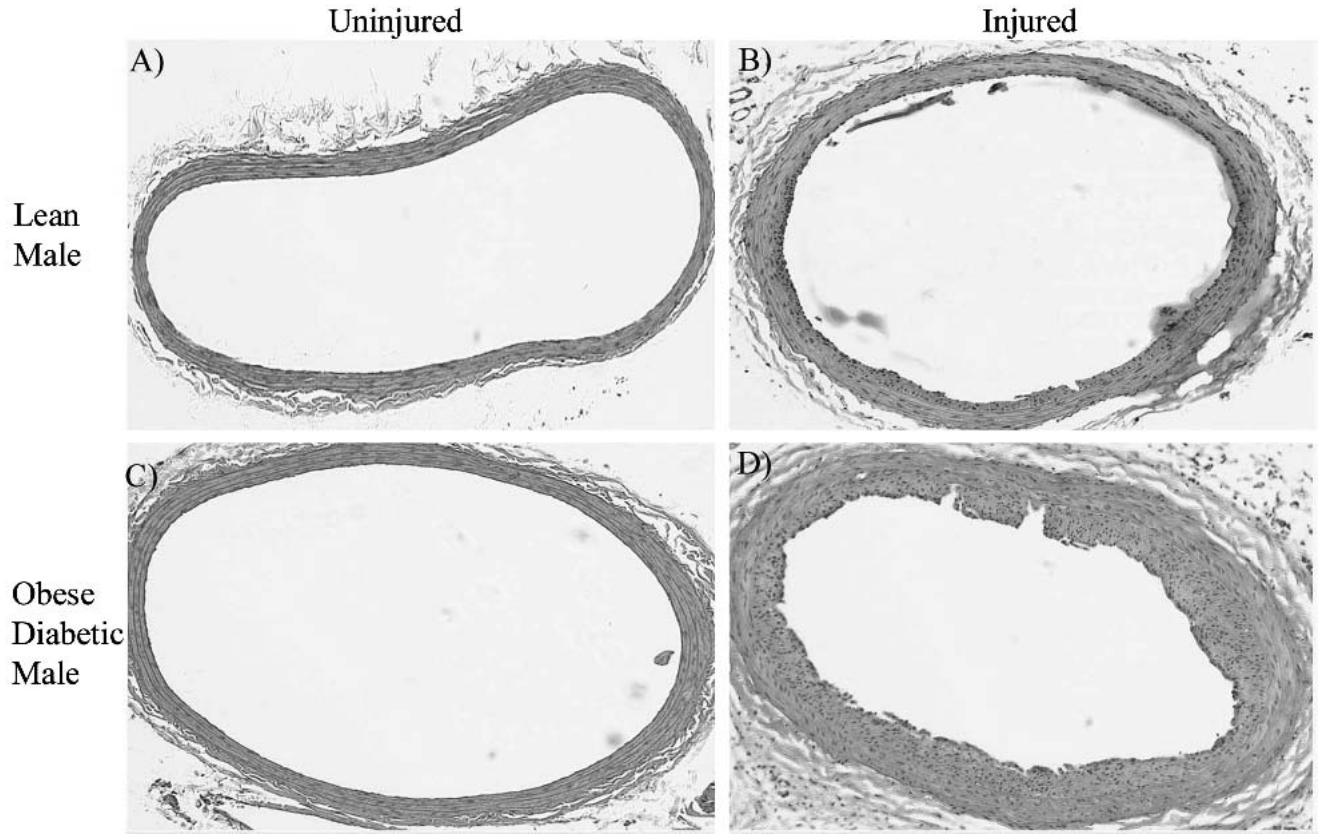


Figure 2 Stenosis morphology in BBZDR/Wor carotid artery. Cross-sectional area of carotid arteries isolated from BBZDR/Wor lean (A-B) and BBZDR/Wor obese male diabetic rats (C-D). Both injured (B, D) and uninjured (A, C) carotid arteries were removed and fixed by cardiac perfusion with 10% neutralized formalin and subsequent immersion in 10% formalin. Three histological sections of the intermediate and central regions of injury (right carotid) were selected for analysis. All histological sections (5 μm) were stained with hemotoxylin/eosin before analysis. Representative pictures are shown.

Left carotid arteries were not manipulated for comparison. At necropsy, both arteries were removed and processed for histopathology and morphometry. Neointima formation in the injured artery was observed in all animals; however, we consistently observed a difference in obese animals compared with lean animals (Figure 2). Carotid arteries isolated from obese diabetic male and obese female (data not shown) animals displayed decreased lumen areas and increased neointima formation at all time points after balloon injury. This evidence was particularly pronounced 4 wk after injury (illustrated in Figure 2). Experiments are ongoing to determine whether these differences are also observed at extended times after surgery. Differences were also observed in the composition of the areas with new growth. The neointima consisted mainly of newly proliferated smooth muscle cells and some inflammatory cells in BBZDR/Wor lean and obese female rats at all time points after balloon injury (data not shown). However, in BBZDR/Wor obese male type 2 diabetic animals, the neointima tissue also featured necrotic centers, fatty depositions, cholesterol crystals, foam cells, and infiltration of inflammatory cells beginning 3 wk after surgery (data not shown). These morphological characteristics are similar to those seen in human atherosclerotic plaques.

Conclusion

BB rats have proved invaluable in understanding the pathogenesis of type 1 diabetes. We have reviewed herein the development and characterization of a new type 2 diabetic rat with the same genetic background as BB/Wor type 1 diabetic rats. The BBZDR/Wor obese male diabetic rat displays all features commonly associated with type 2 diabetes whereas obese female BBZDR/Wor rats phenotypically resemble individuals with syndrome X. In addition, the overall genetic make-up of these animals also makes them excellent models for the study of diabetic complications. Published work using the BBZDR/Wor rat includes DPN and retinopathy studies. Our unpublished observations extend the utility of these animals toward studying nephropathy and CAD, two areas of diabetic research that are hampered by appropriate models. Further work comparing and contrasting type 1 and type 2 diabetic BB rats should help elucidate detailed molecular mechanisms behind diabetic complications, and help lead to the development of better therapeutics to treat the complications associated with diabetes.

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