Published in final edited form as:

Int Rev Neurobiol. 2016 ; 127: 89–112. doi:10.1016/bs.irn.2016.03.002.

Alternatives to the Streptozotocin-Diabetic Rodent

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Abstract

The study of diabetic neuropathy has relied primarily on the use of streptozotocin-treated rat and mouse models of type 1 diabetes. This chapter will review the creation and use of other rodent models that have been developed in order to investigate the contribution of factors besides insulin deficiency to the development and progression of diabetic neuropathy as it occurs in obesity, type 1 or type 2 diabetes. Diabetic peripheral neuropathy is a complex disorder with multiple mechanisms contributing to its development and progression. Even though many animal models have been developed and investigated, no single model can mimic diabetic peripheral neuropathy as it occurs in humans. Nonetheless, animal models can play an important role in improving our understanding of the etiology of diabetic peripheral neuropathy and in performing preclinical screening of potential new treatments. To date treatments found to be effective for diabetic peripheral neuropathy in rodent models have failed in clinical trials. However, with the identification of new endpoints for the early detection of diabetic peripheral neuropathy and the understanding that a successful treatment may require a combination therapeutic approach there is hope that an effective treatment will be found.

1. INTRODUCTION

For years the standard animal model for the study of diabetic neuropathy has been the streptozotocin-treated rodent. In rats depending on the species, age, and delivery, a single dose of streptozotocin ranging from 40 to 75 mg/kg is usually sufficient to destroy enough β cells to cause an insulin-deficient form of diabetes (Rees & Alcolado, 2005; Tesch & Allen, 2007). Such rats fail to gain weight and often lose weight unless supported with a low-dose insulin treatment regime, which can allow such animals to be maintained for extended periods of time in a hyperglycemic state (Calcutt, 2004). In mice, the dose of streptozotocin required to create a model of type 1 diabetes is generally higher than that used for rats, with single doses ranging from 100 to 200 mg/kg (Rees & Alcolado, 2005; Tesch & Allen, 2007). More recently, multiple low dosing of streptozotocin has become the preferred method to induce an insulin-deficient form of diabetes in mice (O'Brien, Sakowski, & Feldman, 2014; Rees & Alcolado, 2005; Tesch & Allen, 2007). Using the latter approach creates a type 1

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diabetic mouse model that is more stable in regard to maintaining their initial weight and will even gain weight compared to mice treated with a single high dose of streptozotocin (personal observation). The potential for high doses of streptozotocin to cause nonspecific effects on nerve and kidney has for many years been a criticism of this type 1 diabetic model, even though studies have shown that neurotoxicity is not the cause of slowing of nerve conduction velocity or changes in thermal nociception in streptozotocin-treated diabetic rats or mice (Davidson et al., 2009; Wiese, Matsushita, Lowe, Stokes, & Yorek, 1996). Nonetheless, investigators seeking the ultimate animal model to mimic the human development and progression of diabetic neuropathy have developed additional models in order to decipher the complex pathogenesis of neuropathy in obesity and types 1 and 2 diabetes. The purpose of this chapter is to review some of the characteristics of these other rodent models of diabetes and diabetic neuropathy.

Peripheral neuropathy is a multifaceted complication of diabetes that frequently leads to foot ulcers and may progress to limb amputations (Kim, Kim, & Yoon, 2012). Even though it is the most common complication of diabetes, the only recommended clinical treatment is good glycemic control which, at best, only delays the onset and slows progression (Callaghan, Little, Feldman, & Hughes, 2012; Figueroa-Romero, Sadidi, & Feldman, 2008). Diabetic peripheral neuropathy has been described by some investigators to be a disease of the vasculature leading to nerve ischemia and altered nerve function (Cameron, Cotter, Archibald, Dines, & Maxfield, 1994; Cameron, Cotter, Dines, et al., 1994; Cameron, Cotter, & Low, 1991; Nukada & Dyck, 1984). Other investigators have proposed that diabetic peripheral neuropathy is caused by a combination of metabolic defects associated with an increased flux of glucose through the aldose reductase pathway leading to a defect in Na^+/K^+ -ATPase activity and an alteration of signal transduction pathways in the nerve (Cameron, Cotter, Dines, & Love, 1992; Cotter, Dines, & Cameron, 1993). Additional pathologic contributors to diabetic peripheral neuropathy have been reported to include increased formation of advanced glycation endproducts, reduced neurotrophic support, and increased inflammatory and oxidative stress (Pop-Busui, Sima, & Stevens, 2006; Sima, 2006). These and likely other mechanisms, which are reviewed in detail elsewhere in this volume, cause damage to neurons, Schwann cells, and the vasculature. Ultimately, relentless damage to the nerve complex and surrounding vasculature leads to diabetic peripheral neuropathy. Given the complex etiology of diabetic peripheral neuropathy, creating an animal model that mimics all the pathologies and developmental pattern of neuropathy seen in human diabetic subjects is probably an impossible task. Therefore, investigators have focused on gaining what information they can from the multiple animal models that have been created before determining whether there is some translational value in these data.

2. RODENT MODELS OF OBESITY

2.1 High-Fat Fed Sprague-Dawley Rats and C57Bl6/J Mice

Feeding rats or mice a high fat or Western diet to induce obesity has recently become a more common approach to creating models of prediabetes (Coppey, Davidson, Lu, Gerard, & Yorek, 2011; Davidson, Coppey, Calcutt, Oltman, & Yorek, 2010; Groover et al., 2013; Nowicki, Kosacka, Serke, Bluher, & Spaniel-Borowski, 2012; O'Brien, Sakowski, et al.,

2014; Obrosova et al., 2007; Yorek et al., 2015). There have been many different formulations of these diets, but in general they cause excess weight gain, raise circulating lipid levels, and cause insulin resistance. Our studies have shown that rats fed a high-fat (45% kcal) diet rapidly gained weight, became hyperinsulinemic, and developed insulin resistance in 4–6 weeks (unpublished data). However, fasting blood glucose and hemoglobin A_{1c} levels were not increased (Davidson et al., 2010; Davidson, Coppey, Dake, & Yorek, 2011; Davidson, Coppey, Kardon, & Yorek, 2014). In 12 weeks the rats developed sensory neuropathy, as indicated by slowing of sensory nerve conduction velocity and onset of thermal hypoalgesia (Davidson et al., 2010; Davidson, Coppey, Dake, et al., 2011; Davidson, Coppey, Kardon, et al., 2014). C57Bl6/J mice fed a high-fat diet also develop a prediabetes phenotype including insulin resistance, impaired glucose utilization, fasting hyperglycemia, and sensory neuropathy (Coppey et al., 2011; Yorek et al., 2015). In our studies, diet-induced obese mice, like their rat counterparts, had a normal motor nerve conduction velocity after 12 weeks of a high-fat diet. Others have reported both motor and sensory neuropathy in C57Bl6/J mice fed a high-fat diet for 16 weeks (Obrosova et al., 2007) or longer (Anderson, King, Delbruck, & Jolival, 2014). Reasons for these differences could include the type of high-fat diet used or study duration. It has been reported that exercise can alleviate some of the neuropathic deficits associated with diet-induced obesity (Groover et al., 2013). C57Bl6/J mice have been commonly used by investigators. However, it should be noted that other strains of mice are also used and may respond differently to high-fat-containing diets. For example, Swiss Webster mice develop insulin resistance and cognitive impairment but do not gain weight or show indices of peripheral neuropathy (Anderson et al., 2014). Human subjects determined to have impaired glucose tolerance have also been reported to develop a sensory neuropathy (Asghar et al., 2014; Kannan et al., 2014; Papanas, Vinik, & Ziegler, 2011; Papanas & Ziegler, 2012; Smith & Singleton, 2008).

2.2 Zucker Rats

The Zucker (fa/fa) rat is the best-known and most widely used rat model of genetic obesity. The fa mutation was discovered by Zucker and Zucker (Kava, Greenwood, & Johnson, 1990) in crosses between Sherman and Merck stock M rats (13M strain). Animals homozygous for the fa allele become noticeably obese by 3–5 weeks of age, and by 14 weeks of age their body composition is over 40% lipid. Obese Zucker rats do not become hyperglycemic, but are hyperlipemic, hypercholesterolemic, hyperinsulinemic, and develop adipocyte hypertrophy and hyperplasia (Alderson et al., 2003; Bray, 1977; Kurtz, Morris, & Pershadsingh, 1989). The Zucker rat has also occasionally been used in investigations of obesity-associated noninsulin-dependent diabetes mellitus. In our studies with this model, we found that vascular and neural dysfunction developed at a slower rate in obese Zucker rats than in the Zucker diabetic fatty (ZDF) rat model of type 2 diabetes (see later). In both models, vascular impairment preceded slowing of motor nerve conduction velocity, which occurs at 12–14 weeks of age in ZDF rats and 32 weeks of age in obese Zucker rats (Oltman et al., 2005).

3. RODENT MODELS OF TYPE 2 DIABETES

3.1 Zucker Diabetic Fatty Rats

There is a wide variety of models duplicating various pathologies associated with type 2 diabetes. In rats and mice the most commonly used to study neuropathy are those that lack a functional leptin receptor (ZDF rat, spontaneously diabetic Torii (SDT) rat, and db/db mouse) or are leptin deficient (ob/ob mouse) (Sasase, Yokoi, Pezzolesi, & Shinohara, 2015). The ZDF rat is recognized as a standard model for metabolic syndrome and type 2 diabetes and has been extensively used. It develops obesity and hyperglycemia at 8–10 weeks of age due to the leptin-receptor deficit and a second mutation that developed in a colony of obese Zucker rats that was isolated through inbreeding (Peterson, Shaw, Neel, Little, & Eichberg, 1990). The ZDF rat has been used to study pain mechanisms associated with early diabetic neuropathy (Gao & Zheng, 2014; Lirk et al., 2012, 2015; Otto, Wyse, Cabot, & Smith, 2011). It has also been used to examine effect of various treatments on diabetic neuropathic endpoints (Li et al., 2006; Lupachyk, Watcho, Hasanova, Julius, & Obrosova, 2012; Lupachyk, Watcho, Obrosova, Stavnichuk, & Obrosova, 2013; Oltman et al., 2008; Oltman, Davidson, Coppey, Kleinschmidt, & Yorek, 2009). We found that monotherapy treatment of ZDF diabetic fatty rats once complications had developed using different classes of drugs for vascular and neural dysfunction did not achieve expected efficacy levels suggesting a complex etiology that includes multiple mechanisms (Oltman et al., 2008).

3.2 Spontaneously Diabetic Torii Rat

The SDT rat is an inbred strain of Sprague-Dawley rat that has been established as a nonobese model of type 2 diabetes (Sasase et al., 2013). Male SDT rats show high plasma glucose levels by 20 weeks of age with pancreatic islet histopathology. Prior to the onset of diabetes, glucose intolerance with hypoinsulinemia is observed (Sasase et al., 2013). SDT rats develop profound complications including retinopathy, nephropathy, and neuropathy (Sasase et al., 2013). However, another group has reported that the time of onset of glucosuria is different between male and female SDT rats with male rats demonstrating a 100% incidence of diabetes at 40 weeks of age, while it is only 33% for female rats (Shinohara et al., 2000). In order to shorten the period of development of hyperglycemia in the SDT rat, a second SDT type 2 diabetic rat model was created by introducing the fa allele of the Zucker fatty rat into the SDT rat genome. This created a new obese model of type 2 diabetes with both male and female SDT fatty rats showing overt obesity, hyperglycemia, and hyperlipidemia at an early age (Kemmochi et al., 2013). Diabetic peripheral neuropathy including decreased nerve conduction velocity and a decrease in sural nerve fibers has been reported in this model (Yamaguchi et al., 2012).

3.3 Zucker Diabetic Sprague-Dawley Rat

As discussed earlier, masking the effects of leptin by either decreasing the leptin receptor or eliminating leptin production has been used to create models of obesity-related type 2 diabetes. One concern for the applicability of the ZDF rats to humans is the recessive homozygous mutation in the leptin receptor (fa) that causes loss of function and induces severe hyperphagia (Davis, Cain, Banz, & Peterson, 2013; Reinwald, Peterson, Allen, & Burr, 2009). Leptin has been shown to have an array of effects that may influence

development of the metabolic syndrome (Beltowski, 2012; Gade, Schmit, Collins, & Gade, 2010; Kaur, 2014; Ricci & Bevilacqua, 2012). Thus, loss of leptin signaling in the ZDF rat makes it a less than ideal model for complications of type 2 diabetes in humans, who generally do not have a leptin-receptor deficit. These concerns led to the creation of the Zucker diabetic Sprague-Dawley (ZDSD) rat (Davis et al., 2013; Peterson et al., 2015; Reinwald et al., 2009). The ZDSD rat was developed by crossbreeding the Charles River Laboratory diet-induced obese rat (Sprague-Dawley-derived) with lean ZDF $-/-$ rats. Selective inbreeding produced animals with a predisposition to obesity and a propensity to develop overt diabetes between 15 and 21 weeks of age with nutritional intervention (Reinwald et al., 2009). Importantly, ZDSD rats have an intact leptin-signaling pathway and more modest accumulation of body fat compared to ZDF rats (Reinwald et al., 2009). We obtained ZDSD rats with the objective of characterizing endpoints associated with diabetic neuropathy in comparison to age matched Sprague-Dawley rats (Davidson, Coppey, Holmes, et al., 2014). We found that chronic hyperglycemia in ZDSD rats caused vascular and neural dysfunction similar to that documented in other diabetic rat models and consistent with the development of diabetic neuropathy (Coppey, Davidson, Dunlap, Lund, & Yorek, 2000; Coppey, Gellett, Davidson, Dunlap, & Yorek, 2002; Davidson et al., 2010; Oltman et al., 2005; Terata et al., 1999). The one exception was that chronic hyperglycemia in ZDSD rat did not cause a decrease in intraepidermal nerve fibers (IENF) in the skin, as has been reported for other rat models of diabetes. There was, however, a decrease in epidermal Langerhans cells. Overall, we found that the ZDSD rat is an easily maintained animal model for type 2 diabetes that spontaneously develops hyperglycemia upon dietary manipulation. This was followed by the development of diabetic neuropathy, including loss of nerves in the subepithelial layer of the cornea and decreased corneal function. Since determination of corneal nerve density and function are noninvasive surrogate markers for diabetic neuropathy (Quattrini et al., 2007), the ZDSD rat with its functional leptin pathway may be a good model for preclinical testing of treatments for diabetic neuropathy.

3.4 Goto–Kakizaki Rat

The Goto–Kakizaki (GK) rat is considered one of the best animal models of nonobese type 2 diabetes (Akash, Rehman, & Chen, 2013) as they exhibit many characteristics in common with diabetic patients. The GK rat spontaneously develops type 2 diabetes due to the complex interaction of multiple mechanisms that include the presence of several susceptibility genes, gestational metabolic impairment inducing an epigenetic programming of the offspring pancreas and the major insulin target tissues, and environmental-induced loss of β -cell differentiation due to chronic exposure to hyperglycemia/hyperlipidemia, inflammation, and oxidative stress (Portha, Giroix, Turrel-Cuzin, Le-Stunff, & Movassat, 2012). Investigations using GK rats have demonstrated a number of diabetes-related neuronal defects in this model compared to the Wistar rats that are commonly used as controls. GK rats have impaired glucose tolerance and progressive insulinopenia and go on to develop peripheral nerve abnormalities in the absence of overt hyperglycemia (Murakawa et al., 2002). Onset of hyperglycemia worsens the development/progression of peripheral neuropathy in GK rats. Several studies have demonstrated that sustained improvement in hyperglycemia can improve diabetic peripheral neuropathy in GK rats (Ueta et al., 2005; Wada et al., 1999). Recently, Wang, Gao, Yin, and Yu (2012) demonstrated that diabetic

neuropathy also occurs in the cornea of type 2 diabetic GK rats. They concluded that defects in the sensory nerve and/or tear film may contribute to diabetic keratopathy and delayed wound healing in diabetic corneas. Their finding that subepithelial corneal nerve loss in GK rats was more prominent in the central cornea than in the limbal region is similar to our own findings using the high-fat fed, low-dose streptozotocin-treated rat model as described later (Davidson, Coppey, Kardon, et al., 2014; Wang et al., 2012).

3.5 BioBreeding Zucker Diabetic Rat

The BioBreeding Zucker diabetic rat (BBZDR)/Wor rat was created by breeding the insulin-resistant Zucker fatty rat into the inbred Bio-Breeding/Worcester (BB/Wor) strain in order to introduce the defective leptin receptor gene (Tirabassi et al., 2004). BBZDR/Wor rats develop hyperglycemia at about 7 weeks of age and become obese with elevated triglyceride and cholesterol levels. Lipid levels can be further increased by feeding a diet containing high fat and sucrose. These rats are insulin resistant with hyperinsulinemia and develop microvascular- and macrovascular-related complications including retinopathy, neuropathy, nephropathy, and coronary artery disease with hypertension (Gao & Zheng, 2014; Tirabassi et al., 2004). With regard to neuropathy, the diabetic BBZDR/Wor rat shows a slowly progressive nerve conduction defect accompanied by mild myelinated fiber atrophy, mild changes of the node of Ranvier, and significant segmental demyelination and Wallerian degeneration (Sima et al., 2000). These changes were much less dramatic than those observed in the type 1 spontaneously diabetic BB/Wor rat maintained with the same degree of hyperglycemia (Kamiya, Murakawa, Zhang, & Sima, 2005; Sima et al., 2000).

3.6 Otsuka Long-Evans Tokushima Fatty Rat

OLETF rats, when fed a diet with or without sucrose, were hyperglycemic compared to control rats (Kamenov, Higashino, Todorova, Kajimoto, & Suzuki, 2006). At 10 months of age motor nerve conduction velocity and thermal nociception were significantly decreased, and all parameters deteriorated further when OLETF rats received a sucrose-supplemented diet. Other studies with sucrose-fed OLETF rats have demonstrated a reduction in sciatic nerve blood flow and decreased Na^+/K^+ -ATPase activity in the sciatic nerve (Nakamura et al., 2001).

3.7 Ob/ob and db/db Mice

The most common mouse models of type 2 diabetes are those with the ob/ob or db/db mutations. These mice are deficient in leptin (ob/ob) or lack a functional leptin receptor (db/db). They are also obese, insulin resistant and develop spontaneous hyperglycemia. Studies by Grote et al. (2013) demonstrated that insulin resistance occurred in muscle, liver, and sciatic nerve. Characterization of peripheral diabetic neuropathy in ob/ob mice revealed that after 9–13 weeks of hyperglycemia motor and sensory nerve conduction velocity are decreased, and mice have thermal hypoalgesia, tactile allodynia, and a significant loss of IENF (Drel et al., 2006; O'Brien, Hur, et al., 2014). Treating ob/ob mice with an aldose reductase inhibitor or with peroxynitrite decomposition catalysts improved diabetic peripheral neuropathy endpoints (Drel et al., 2006; Varenjuk et al., 2007) suggesting that activation of the aldose reductase pathway as well as increased oxidative stress contribute to the development and progression of diabetic peripheral neuropathy in the ob/ob mouse.

The leptin-receptor deficiency present in db/db mice has been crossed into a number of background strains allowing for studies of multiple pathological conditions (Sullivan et al., 2007). The db/db mouse, like the ZDF rat, is obese and insulin resistant. It develops a severe hyperglycemia and peripheral neuropathy with decreased nerve conduction velocity and morphological changes in peripheral nerves (Cho et al., 2014; Norido, Canella, Zanoni, & Gorio, 1984; Nowicki et al., 2012; Pande et al., 2011; Robertson & Sima, 1980; Shi et al., 2013; Sima & Robertson, 1978). In a study comparing the effect of hyperglycemia in 4-month-old C57BL6 (control), ob/ob, and db/db mice it was found that myelin thickness was significantly reduced in small, medium-sized, and large axons of db/db mice compared with control C57BL6 mice (Nowicki et al., 2012). In contrast, only large fibers showed a decrease in myelin sheath thickness in ob/ob mice, while the number of nonmyelinated nerve fibers was lower in ob/ob mice than in db/db mice. A thickened basal lamina of Schwann cells also occurred only in ob/ob mice. The basement membrane of endoneurial microvessels was thickened in both ob/ob and db/db mice. Thus, fundamental differences in some endpoints of diabetic neuropathology exist between ob/ob and db/db mice.

3.8 Tsumura Suzuki Obese Diabetes Mouse

Other mouse models of obesity and spontaneous type 2 diabetes include the Tsumura Suzuki Obese Diabetes (TSOD) mouse (Lizuka et al., 2005). The male mice exhibit polydipsia and polyuria at about 2 months of age followed by hyperglycemia and hyperinsulinemia. Sensory and motor neuropathy deficits can be observed at 12 and 14 months of age, respectively, with weakness of front and hind paws at about 17 months of age (Lizuka et al., 2005). At this time, light microscopic and electron microscopic examination of the sciatic nerve shows a decrease in the density of nerve fibers as well as degenerative changes of myelinated fibers. Interestingly, a study by Kawada et al. (2010) showed that TSOD mice even after 18 months of age did not develop any form of cardiac dysfunction.

3.9 Combined High-Fat Fed, Low-Dose Streptozotocin Models of Type 2 Diabetes

A rodent model for type 2 diabetes that I have found to be useful and easy to work with is the high-fat fed, low-dose streptozotocin-treated rat, or mouse (Gao & Zheng, 2014; Skovso, 2014). The rationale behind this approach is that the high-fat diet renders the rodent insulin resistant, and the low dose of streptozotocin destroys enough of the β cells to induce a constant state of hyperglycemia.

Reed et al. (2000) were the first to use the high-fat fed streptozotocin-treated rat as a model for type 2 diabetes. Sprague-Dawley rats were fed a high-fat diet for 7 weeks and then treated with streptozotocin (50 mg/kg). These rats were hyperglycemic, insulin resistant, and sensitive to the glucose-lowering effects of metformin and troglitazone. Others have also found this model to be suitable for testing agents for the treatment of type 2 diabetes (Ding et al., 2005; Gaikwad, Viswanad, & Ramarao, 2007; Reed et al., 2000; Srinivasan, Viswanad, Asrat, Kaul, & Ramarao, 2005; Wang et al., 2009; Zhang, Lv, Li, Xu, & Chen, 2008; Zhu, Peng, Liu, Zhang, & Li, 2010). It should be noted that the literature contains some variability in the insults used to create this model. A study by Wang, Li, Liu, Liu, and Sun (2011) examined parameters for creating the high-fat diet streptozotocin-diabetic rat model and found that duration of high-fat diet, dosage of streptozotocin, and the age of rats

were the most important factors for establishing this model. Generally the high-fat diet is consistent at 40–50% calories as fat for a period of 4–8 weeks (Gao & Zheng, 2014). However, the dose of streptozotocin used ranges from 20 to 50 mg/kg (Reed et al., 2000; Wang et al., 2011). It has been our experience using rats that were 12 weeks of age at the onset of the study, that after 8 weeks on a high-fat diet, 20 mg/kg streptozotocin resulted in a low success rate for inducing hyperglycemia. When we used 40 mg/kg streptozotocin, our mortality rate was high and rats began dying 2–4 weeks following the injection of streptozotocin. With 30 mg/kg streptozotocin our success rate was over 90%, and the mortality rate was less than 5%. Rats do not require insulin treatment to maintain weight, unlike streptozotocin-induced type 1 diabetic rat models (Davidson, Coppey, Holmes, et al., 2011; Davidson, Kleinschmidt, Oltman, Lund, & Yorek, 2007). An important item to remember is that the potency of streptozotocin can vary widely between vendors and between different lots purchased from the same vendor. We agree with Reed et al. (2000) that the high-fat fed streptozotocin–diabetic rat models late stage type 2 diabetes in patients. The diabetes in these rats is analogous to the development of human type 2 diabetes when the decline in hyperinsulinemia is not able to compensate for insulin resistance and hyperglycemia occurs (Reed et al., 2000).

My laboratory was the first to characterize diabetic neuropathy in the high-fat fed low-dose streptozotocin-treated rat (Davidson, Coppey, Holmes, et al., 2011). In our studies, Sprague-Dawley rats were fed a high-fat diet for 8 weeks before treating with streptozotocin. Our initial study examined vascular and neural endpoints at 16 and 24 weeks after the onset of hyperglycemia and high-fat diet, respectively (Davidson, Coppey, Holmes, et al., 2011). Compared to high-fat fed rats we found that following induction of hyperglycemia glucose utilization was further impaired. At the end of the study period, the diabetic rats weighed about the same as control rats, insulin and leptin levels were near control values, and they were hyperlipidemic. Furthermore, we found that at the end of the study period weight of the epididymal fat pad was significantly less in the high-fat streptozotocin–diabetic rats compared to high-fat fed rats. However, epididymal fat pad weight in the high-fat streptozotocin–diabetic rats was still greater than control rats. Serum insulin and leptin levels were significantly decreased in high-fat streptozotocin–diabetic rats compared to high-fat fed rats but similar to control rats. Two notable differences between high-fat fed rats and high-fat streptozotocin–diabetic rats were that in diabetic rats motor nerve conduction velocity was significantly decreased and superoxide levels and nitrotyrosine staining were increased in epineurial arterioles (Davidson et al., 2010; Davidson, Coppey, Holmes, et al., 2011). This indicates that oxidative stress in the microvasculature is increased by hyperglycemia. In epineurial arterioles from high-fat fed rats superoxide levels and nitrotyrosine staining were not increased and motor nerve conduction velocity not significantly impaired (Davidson et al., 2010). Sensory nerve conduction velocity, thermal nociception, and intraepidermal nerve fiber profiles were impaired in both high-fat fed rats and high-fat streptozotocin–diabetic rats. Interestingly, acetylcholine- and CGRP-mediated vascular relaxations of epineurial arterioles were impaired to a similar degree in high-fat fed rats and high-fat streptozotocin–diabetic rats. It was expected that because of the increase in superoxide and nitrotyrosine staining in epineurial arterioles from high-fat streptozotocin–diabetic rats compared to high-fat fed rats that vascular relaxation to acetylcholine would be

impaired to a greater degree in high-fat streptozotocin–diabetic rats. This suggests that mechanisms in addition to oxidative stress contribute significantly to impairment of vascular function in epineurial arterioles from high-fat fed rats and high-fat streptozotocin–diabetic rats. More recent studies have revealed loss of corneal nerves and decrease in corneal nerve sensitivity in diet-induced obese rats and high-fat streptozotocin–diabetic rats (Davidson, Coppey, Kardon, et al., 2014).

We have also used the high-fat diet low-dose streptozotocin approach to create a mouse model of type 2 diabetes (Yorek et al., 2015). Unlike rats, feeding C57Bl6/J mice a high-fat diet causes an elevated level of fasting blood glucose (Coppey et al., 2011; Davidson, Coppey, Holmes, et al., 2011). However, hyperglycemia in the high-fat fed mouse is modest and not associated with an increase in hemoglobin A₁C levels. Moreover, blood glucose was not increased in the fed state. Using a low dose of streptozotocin with a high-fat fed C57Bl6/J mouse creates a higher level of blood glucose, which is present in both the fasted and fed state. This type 2 diabetic mouse model has been previously used to examine pharmacological interventions, vascular biology and atherosclerosis, cardiomyocyte hypertrophy, and β -cell function (Bansal et al., 2012; Lin et al., 2015; Lv et al., 2010; Mali et al., 2014; Ullevig, Zhao, Zamora, & Asmis, 2011; Wang, Hsu, Lin, & Chen, 2014; Xue, Ding, & Liu, 2010; Zhu et al., 2014). In our studies comparing the effect of diet-induced obesity, type 1 and type 2 diabetes on neuropathy, we found the severity of hyperglycemia was significantly different between the types 1 and 2 diabetic mouse models (Yorek et al., 2015). However, motor and sensory nerve conduction velocity, thermal and mechanical sensitivity, and intraepidermal nerve fiber density in the skin were all impacted similarly in diet-induced obesity and types 1 and 2 diabetic mice. Loss of corneal nerves in the subepithelial layer and penetrating the corneal epithelium occurred more rapidly in the diet-induced obesity mice and type 2 diabetic mice compared to type 1 diabetic mouse. This suggests that hyperglycemia is not the only factor contributing to nerve fiber loss in the cornea and that loss of intraepidermal and corneal nerve fibers is mediated by different factors and/or occurs at different rates.

Overall, these studies demonstrate that the high-fat fed low-dose streptozotocin-treated rodents are a good animal model for preclinical studies for discovery and evaluation of new treatments for diabetic neuropathy especially in relation to changes in nerve structure in the skin and cornea.

3.10 Streptozotocin–Nicotinamide Rat

For this model the rat is treated with a dose of streptozotocin generally used to create type 1 diabetes followed by nicotinamide (Sharma et al., 2012; Szkudelski, 2012). Nicotinamide is a poly(ADP-ribose) polymerase inhibitor and has been shown to reverse neurological and neurovascular deficits in streptozotocin–diabetic rats (Negi, Kumar, Kaundal, Gulati, & Sharma, 2010; Stevens et al., 2007). When nicotinamide is used in combination with streptozotocin, it protects β cells from the effects of streptozotocin and the result is a model of type 2 diabetes (Szkudelski, 2012). In this model the severity of diabetes strongly depends on the doses of streptozotocin and nicotinamide given to the animal (Szkudelski, 2012). It

has been reported that the streptozotocin–nicotinamide-treated rat develops neuropathy (Sharma et al., 2012).

4. RODENT MODELS OF TYPE 1 DIABETES

4.1 Spontaneously Hypertensive Rat

Use of streptozotocin has been the most often used approach to induce type 1 diabetes in rodents. However, inducing type 1 diabetes in Sprague-Dawley or Wistar rats does not cause hypertension, which is an independent risk factor for neuropathy in diabetic patients. Streptozotocin has therefore been used to induce type 1 diabetes in spontaneously hypertensive rats (Gregory, Jolival, Goor, Mizisin, & Calcutt, 2012; Sanada et al., 2015) to study the combined effects of diabetes and hypertension on peripheral neuropathy.

4.2 BioBreeding/Worcester Rat

There are several genetic rat and mouse models that have been used to replicate type 1 diabetes. The BB/Wor rat has been stated to model human insulin-dependent diabetes mellitus (Whalen, Mordes, & Rossini, 2001). This rat requires daily insulin treatment to survive. It has been reported that motor and sensory nerve conduction velocities are decreased after 4 and 6 weeks of diabetes and continue to decline for up to 9 months (Kamiya, Zhang, & Sima, 2009). Myelinated sural nerve fibers show progressive decreases in fiber number and size. Other deficits include thermal hyperalgesia, loss of content of neuropeptides in dorsal root ganglia, and reduced endoneurial blood flow (Kamiya, Zhang, & Sima, 2004; Stevens, Zhang, Li, & Sima, 2004). Interestingly, Sima and colleagues have demonstrated that many of the neurological deficits caused by diabetes in the BB/Wor rat can be prevented/reversed with C-peptide treatment (Kamiya et al., 2004; Sima et al., 2001; Stevens et al., 2004; Zhang et al., 2001).

4.3 Ins2Akita Mouse

The heterozygous Ins2Akita mouse spontaneously develops insulin-dependent diabetes, including hyperglycemia, hypoinsulinemia, polydipsia, and polyuria, which is more severe in males than females. The Akita mouse has been widely used to introduce a diabetic background into other mouse genetic models. For instance, crossing the Akita mouse with the apoE (–/–) mouse created a mouse model used for studying the effect of diabetes on atherosclerosis (Jun, Ma, & Segar, 2011). The C57BL/6-Akita mouse has been proposed as a nonobese model for type 2 diabetes (Yaguchi, Nagashima, Izumi, & Okamoto, 2003) and has been used to examine the effect of diabetes on the central nervous system. Studies using the Akita mouse for determination of the effect of diabetes on autonomic neuropathy have shown that the Akita mouse develops abnormal cardiac function, with a loss in nerve density (Yang & Chon, 2011). In regard to peripheral neuropathy, 16-week-old Ins2Akita mice develop sensory neuropathy including reduced nerve conduction velocity, thermal and mechanical hypoalgesia, and tactile allodynia (Drel et al., 2011). Motor nerve conduction velocity showed a nonsignificant trend to slowing, whereas others (de Preux Charles et al., 2010) have reported a decrease in motor nerve conduction velocity in Ins2Akita mice at 12 weeks of age.

4.4 Nonobese Diabetic Mouse

The nonobese diabetic (NOD) mouse is susceptible to the development of autoimmune diabetes but also multiple other autoimmune diseases (Bour-Jordan et al., 2013). Over 20 susceptibility loci linked to diabetes have been identified in NOD mice (Bour-Jordan et al., 2013), and the strain has been used extensively to cross the diabetic background onto other mice (Gross et al., 2008). Invasive insulinitis, seen in NOD mice, causes early sympathetic islet neuropathy, but it remains to be determined whether this is responsible for the impaired glucagon secretion (Taborsky et al., 2009). In addition to the sympathetic neuropathy developed by the NOD mouse, it has also been reported to develop sensory neuropathy with hypoalgesia and correction by use of a peroxynitrite decomposition catalyst (Obrosova et al., 2005).

5. OTHER ANIMAL MODELS

This chapter has focused primarily on rodent models of obesity and diabetes and peripheral neuropathy. A number of other animal models have also been used successfully in the study of the effect of diabetes on peripheral and autonomic neuropathy. These include guinea pigs, rabbits, cats, dogs, pigs, and monkeys (Cohen, Tesfamariam, Weisbrod, & Zitnay, 1990; Estrella et al., 2008; Islam, 2013; Mesangeau, Laude, & Elghozi, 2000; Mizisin et al., 2007; Morgan, Vite, Radhakrishnan, & Hess, 2008). However, ethical, practical, and financial costs concerns will likely continue to limit the extent to which these other models are used.

6. CONCLUSIONS

In 1997, Dr. Tomlinson coauthored a review article titled “Does neuropathy develop in animal models?” (Hounsom & Tomlinson, 1997). Investigators have been addressing this question for the past 18 years. In the abstract of that paper the authors wrote in reference to diabetic neuropathy, “currently the cornerstone of treatment lies with the maintenance of euglycemia and development of effective treatments for diabetic neuropathy is urgently needed.” The same statement could be made today. The only accepted treatment for diabetic neuropathy remains good glycemic control, but it is now clear that this is ineffective, especially in patients with type 2 diabetes (Callaghan et al., 2012). Hounsom and Tomlinson further noted that animal models have been developed to investigate the pathogenesis of diabetic neuropathy and evaluate potential therapeutic agents. However, no model is perfect and no one would suggest that diabetic rodents can replicate the human condition fully. Since this article was written, animal models, including those discussed earlier, continue to be used to investigate and identify potential new treatments for diabetic neuropathy. However, translation of findings to humans with diabetic peripheral neuropathy has continued to fail. Many articles have been written to rationalize these failures. We know that diabetic neuropathy is a complex disease with multiple etiologies. It is my belief that animal models of diabetes and diabetic neuropathy can still play a role in discovery of a treatment. However, this treatment will likely be a combination therapy that will delay progression and induce nerve repair when used in combination with glycemic control. It should also be accepted that the preclinical efficacy of any treatment be tested in several animal models utilizing multiple endpoints. A consensus statement regarding the phenotyping of rodent

models for diabetic peripheral neuropathy was published following a joint meeting of the Diabetic Neuropathy Study Group of EASD (Biessels et al., 2014). Following these guidelines will help in standardizing studies coming from multiple laboratories.

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