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Criteria for Creating and Assessing Mouse Models of Diabetic Neuropathy

Kelli A. Sullivan, Stephen I. Lentz, John L. Roberts Jr., and Eva L. Feldman*

University of Michigan, Departments of Neurology and Internal Medicine, USA

Abstract

Diabetic neuropathy (DN) is a serious and debilitating complication of both type 1 and type 2 diabetes. Despite intense research efforts into multiple aspects of this complication, including both vascular and neuronal metabolic derangements, the only treatment remains maintenance of euglycemia. Basic research into the mechanisms responsible for DN relies on using the most appropriate animal model. The advent of genetic manipulation has moved mouse models of human disease to the forefront. The ability to insert or delete genes affected in human patients offers unique insight into disease processes; however, mice are still not humans and difficulties remain in interpreting data derived from these animals. A number of studies have investigated and described DN in mice but it is difficult to compare these studies with each other or with human DN due to experimental differences including background strain, type of diabetes, method of induction and duration of diabetes, animal age and gender. This review describes currently used DN animal models. We followed a standardized diabetes induction protocol and designed and implemented a set of phenotyping parameters to classify the development and severity of DN. By applying standard protocols, we hope to facilitate the comparison and characterization of DN across different background strains in the hope of discovering the most human like model in which to test potential therapies.

Keywords

NOD; Akita; ob/ob; db/db; outbred mice; nerve conduction velocity; intraepidermal nerve fiber density

DIABETES MELLITUS

The term “diabetes mellitus” describes a number of conditions in which blood glucose is elevated including insulin dependent diabetes mellitus (IDDM) [1], non-insulin dependent diabetes mellitus (NIDDM) and gestational diabetes. Data gathered by the American Diabetes Association (<http://www.ada.org>) in 2005 estimate that 14.6 million people were diagnosed with diabetes while 6.2 million are currently undiagnosed in the United States. Today, over twenty million Americans are diabetic and the incidence is increasing by 5% per year.

The high level of blood glucose that defines diabetes is also the leading factor in cellular dysregulation and destruction resulting in the macro- and microvascular complications of this disease. Macrovascular complications lead to atherosclerosis and dilated cardiomyopathy [2], while microvascular complications include diabetic nephropathy [3, 4] retinopathy [5, 6] and neuropathy [7-10]. The most common of these complications is diabetic neuropathy (DN) occurring in approximately 60% of diabetic patients [11].

Beyond effective control of glucose levels, there are no treatments to prevent or cure DN. Numerous factors have impeded laboratory investigations aimed at the development of effective therapies, including an incomplete analysis of existing animal models that develop DN, lack of consistency among mouse models (diabetes induction and genetics) and lack of consensus concerning phenotyping methods. The AMDCC was formed by the NIH to develop new animal models of diabetes and its complications; its goal is to identify the most appropriate animal models to study the etiology, prevention and treatment of diabetic complications. The group has reported its findings comparing the effects of diabetes on cardiovascular disease [2], and nephropathy [12] in inbred mice from different genetic background strains and maintains a website containing its recommended phenotyping protocols. AMDCC investigators continue to refine genetic and dietary factors that affect the development of diabetes and its complications, potential interactions and overlap of complications within a genetic model, and common pathways that may influence treatment paradigms in human patients.

In order to provide a consistent method of examining DN in diabetic mouse models, our laboratory implemented a standardized diabetes induction protocol used by the AMDCC (<http://www.amdcc.org>) and developed an independent set of phenotyping parameters to classify and categorize mouse models of diabetes with regard to the development and severity of DN [13, 14]. The current review suggests a definition of murine DN, summarizes the published data on mouse models of DN and recommends a set of standardized criteria to define DN in mice. These criteria, along with detailed instructions on the methodologies used to phenotype mice for the presence of DN are available at our website at <http://www.pnrd.umich.edu>.

DIABETIC NEUROPATHY (DN): DEFINITION AND PREVALENCE

DN is defined as “the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes” [15] and is the most common complication of diabetes. Statistics gathered in 2005 indicate that 60-70% of diabetic patients have mild to severe DN (<http://www.ada.org>). DN is characterized by progressive, length dependent sensory loss [10]. DN first affects the longest axons innervating the feet; by the time symptoms reach the knees, the fingers are often affected [10]. Sensory symptoms correlate with abnormal nerve conduction studies [10, 16, 17], decreased myelinated fiber density (MFD) [10, 17-19] and decreased intraepidermal nerve fiber (IENF) densities [20-22]. Regenerating axons are present in human sural nerve [23, 24] and in skin biopsies [25], but over the course of the disease, regeneration fails. While maintenance of euglycemia may slow peripheral nerve deterioration [26, 27], no treatment is currently available to reverse the loss of nerve fibers and function in DN.

MOUSE MODELS OF DN

In human patients, the diagnosis of DN is made following the establishment of diabetes and ruling out other possible causes of neuropathy [7-10]. Methods used to assess neuropathy include a careful neurological examination of tendon reflexes, the ability to sense hot, cold or vibratory stimuli, and in select cases, nerve conduction studies using surface electrodes and skin biopsies [10, 13, 14, 28]. These measures have been successfully adapted and applied to mouse models of neuropathy including DN [13, 14, 29-32].

DN is described in mice with type 1 or 2 diabetes including spontaneous (genetic) and chemically induced (streptozotocin, STZ) models (Tables 1-3). The earliest reports of DN in mouse models centered on the BKS.Cg-*m*+/*Lep^{db}*/J mouse (BKS-db/db) a spontaneous model of type 2 diabetes. These mice express a mutation in the leptin receptor resulting in hyperphagia, severe obesity, hyperinsulinemia and hyperglycemia beginning at approximately 4 weeks of age. DN is reported as early as 4 weeks post onset of diabetes as measured by an increase in thermal latency. Both sensory and motor nerve conduction velocities are decreased [13, 14, 33-36], axonal transport is slowed [37, 38] and both neurotransmitter levels [39] and IENF densities are decreased [13, 14] (Table 1). A second spontaneous model of type 2 diabetes is the B6.V-*Lep^{ob}*/J or B6-ob/ob mouse. The B6-ob/ob mouse lacks leptin and is similar to the BKS-db/db mouse with regard to the development of type 2 diabetes. These mice also develop DN 7 weeks post onset of hyperglycemia [40, 41], characterized by increased thermal latency, decreased mechanical response and decreased motor and sensory nerve conduction velocities [40, 41]. Type 2 diabetes is inducible in the C57BL/6J mouse by feeding a diet high in fat (45-60% of calories derived from fat) [42, 43]. Under these conditions, C57BL/6J mice develop hyperglycemia and insulin insensitivity. The first examination of DN in this model was published by Obrosova and colleagues, who reported decreased motor and sensory nerve conduction velocities, increased thermal latencies and mechanical allodynia, similar to DN observed in spontaneous type 2 diabetes [43].

Models of spontaneous type 1 diabetes have resulted from insulin mutations (Table 1). Mutations in the insulin 1 (*Ins.D^{d1}*) and insulin 2 (*C57BL/6-Ins2^{Akita}/J*) genes result in hypoinsulinemia and hyperglycemia [44, 45]. Sensory nerve conduction velocities are reduced in both types of mice following 20-24 weeks of diabetes (Table 1). *Ins.D^{d1}* mice also demonstrate reduced myelinated fiber density (MFD) in the sural nerve [44]. DN was also examined in another model of spontaneous type 1 diabetes, the non-obese diabetic (NOD) mouse. Similar to human patients, in these mice type 1 diabetes develops following immune destruction of pancreatic beta cells. Sympathetic neuritic dystrophy is reported as early as 3 weeks post onset of diabetes [46]. Examination of thermal thresholds reveals both thermal hypoalgesia, 13 weeks post onset of diabetes [47], and hyperalgesia, 14 and 21 weeks post onset of diabetes [48, 49]. Measures of MFD and nerve conduction velocities are not reported in the NOD mice and IENF has yet to be analyzed in these spontaneous type 1 models (Table 1) [50].

C57BL/6J is the most commonly used strain of inbred laboratory mice and form the foundation for most gene targeting studies. Type 1 diabetes may be induced in this

background strain by a high or low dose regimen of streptozotocin (STZ) injection (Table 2). Both paradigms result in hyperglycemia; however, toxicity (high post-injection mortality) is often higher with single, high dose STZ treatment. Low dose STZ treatment results in a slow destruction of beta cells and immune activation resembling autoimmune induced type 1 diabetes [51]. Low dose STZ-treatment of short duration (6-12 weeks) results in decreased motor and sensory nerve conduction velocity, [52-54] and increased thermal latency [52]. Long term examinations of C57BL/6J mice (24 weeks) reveal no changes in nerve conduction, sensory function or IENF in this model [13, 14]. In these studies, DN was not assessed until 12 weeks post STZ treatment. While fasting blood glucose and glycated hemoglobin (GHb) were significantly elevated 24 weeks post STZ-treatment and the mice displayed significant weight loss, all hallmarks of type 1 diabetes in rodents, there was no behavioral or electrophysiological evidence of DN. This discrepancy between short term and long term assessments of DN in STZ-treated C57BL/6J mice awaits further study. This issue will only be clarified by serial assessment of DN immediately following STZ-treatment, with special attention to insulin levels, diet and gender.

DN was also examined in C57BL/6J mice following a single high dose of STZ (Table 2) [30, 47, 55-59]. Similar to the majority of reports in low dose STZ-treated mice, DN was examined following diabetes duration of 1-9 weeks. There is one report of a decrease in motor and sensory nerve conduction velocities along with elevations of glucose, sorbitol and fructose within the sciatic nerve [60] (Table 2). Investigations of DN in this model by Wright and co-workers reveal decreased mechanical sensitivity at 8 and 9 weeks post STZ-treatment with reduced IENF densities but no changes in thermal thresholds. Nerve conduction velocities were not measured (Table 2) [30, 55-57, 59]. Thus, while there is no doubt that the C57BL/6J mouse develops profound diabetes in response to single high dose STZ treatment, evidence of DN is again only described in short term diabetes. Similar to the results after low dose STZ-induction of diabetes, two studies of diabetes of 24 weeks [13, 14] found no evidence of DN in C57BL/6J animals after single high dose STZ-treatment. It is also evident from the reports summarized in Table 2 that few studies examine all 3 aspects of DN; therefore, the degree of DN remains to be fully characterized.

The outbred Swiss albino mouse (a.k.a. Swiss Webster, Swiss Wistar, Swiss NIH Albino) is the most thoroughly characterized model of DN (Table 3). Diabetes was induced by both high and low STZ treatment with similar results (Table 3). Decreased motor [61-63] and sensory [62, 64] nerve conduction velocities are reported following 8-36 weeks of diabetes. Studies vary in the response to a thermal stimulus. Arrieta *et al.* [65] report thermal allodynia 12 weeks post STZ-treatment while Demiot *et al.* [63] report thermal hypoalgesia 8 weeks post STZ-treatment. IENF density [62] and MFD [65] are reduced in diabetic Swiss mice, as is nerve blood flow, levels of nerve growth factor (NGF) in sciatic nerve and serum, and size of dorsal root ganglia (DRG) neurons [62]. Yet even in this model, there is no comprehensive report of DN including behavior, nerve function and peripheral nerve/DRG anatomy.

Other outbred strains include CD-1/ICR, ddY and Laka mice. CD-1/ICR mice demonstrate thermal allodynia following 7 to 11 days of diabetes [66-69]. Similarly, allodynia is reported in short term diabetes (2 and 4 weeks) [70, 71]. Single high dose STZ-treatment used to

induce diabetes and DN in ddY and Laka mice (Table 3) resulted in hypoalgesia in ddY mice. DN characterized by hyperalgesia was documented in Laka mice [72-74] (Table 3).

DN SCREENING

As demonstrated above, there is no single protocol for inducing diabetes or a standard set of tests used to define DN. We designed a set of assays to screen for the presence of DN, as well as a set of advanced measures to fully characterize DN. Mice are screened for DN by first establishing that they become and remain hyperglycemic using AMDCC guidelines. We adapted current methodologies used to assess neuropathy in human patients to establish the presence of DN in mice. These methods include using heat/pain thresholds to assess sensory function (hyper- or hypoalgesia), measuring nerve conduction velocities to establish the status of nerve electrophysiology (NCV) and examination of an anatomical marker of end organ innervation (IENF density). Initial screening of a new mouse model includes nerve conduction studies, behavioral measures of sensory function, and assessment of IENF density (Fig. 1). This panel of tests is performed at 3 and 6 months following induction of diabetes. If the presence of DN is verified, shorter and longer time points may be added to characterize the progression of DN.

GUIDELINES FOR DISEASE DEFINITION

Age related normal data for motor and sensory nerve conduction velocities are available for human patients; unfortunately, this type of information is not available for mice. Baseline control values for nerve conduction velocity in mice vary across age and genetic background as do the relative decreases following diabetes. This variability is in part due to diabetes duration, type of diabetes, background strain, gender and diet. Decreases in motor nerve conduction velocity range from 13% in C57BL/6J mice diabetic for 8 weeks [60] to a 50% reduction in the BKS-db/db mouse following 21 weeks of diabetes [13, 33]. Percent decreases in sensory nerve conduction velocities vary from 10% in the Swiss Wistar mouse following 36 weeks of diabetes to 53% in the BKS-db/db mouse 4 weeks after the onset of diabetes [36]. In the literature and in our own laboratory, the BKS-db/db mouse model of type 2 diabetes exhibits the most robust changes in both motor and sensory nerve conduction velocities with no less than a 20% decrease in either measure [13, 34, 36]. In models of type 1 diabetes, both inbred and outbred strains, induced or spontaneous, larger decreases are noted in motor (~24%) than sensory (~20%) conduction velocities. Because the duration of diabetes has a profound effect on nerve conduction velocity, we suggest that a 10 to 15% reduction in conduction velocity in animals with less than 8 weeks diabetes and a 15 to 20% reduction in mice diabetic for over 12 weeks be considered a robust model of DN.

Changes in sensory behavior are equally variable and time dependent, as many mouse models exhibit hyperalgesia and allodynia [48, 72, 75] in the early phases of neuropathy and hypoalgesia [13, 40] in later stages. Allodynia and thermal hyperalgesia are most often reported in mice with diabetes duration of ~4 weeks [48, 72, 75]. The percent change in thermal threshold in these studies ranged from 29-47%. Hypoalgesia is commonly reported following 8 or more weeks of diabetes and the percent increase in threshold is reported to be as high as 88% [40]. It is often the case that the mice fail to respond within a set of

temperature cut off points established to prevent injury. It is reasonable that a 20% change in mechanical (allodynia) or thermal (hypo- or hyperalgesia) thresholds would be indicative of DN.

IENF density is a newly established parameter for measuring DN in mice. Two papers by Christianson [30, 59] report a minimum decrease of 50% in C57BL/6J mice following 7 weeks of diabetes. Our laboratory published similar findings in the BKS-db/db mouse following 24 weeks of diabetes [13]. As more reports of DN are published in mice of different background strains and with diabetes of longer duration, a minimum percent decrease may be established for this measure.

ADVANCED NEUROPATHY PHENOTYPING

Once the presence of DN is verified, advanced neuropathy phenotyping protocols are used to further characterize DN severity, progression, and potential mechanisms (Fig. 2). These procedures are more time-consuming and are not required to establish the presence of DN. For example, nerve conduction assays are performed at 4 week intervals rather than only at 3 and 6 month time points. Assessments of MFD of the sural nerve and/or quantitation using electron microscopy of thinly myelinated and unmyelinated fibers yield additional anatomical confirmation of DN [35, 44]. Biochemical measures for oxidative stress adducts and enzymes are performed in plasma and tissue extracts of DRG and peripheral nerve [47, 54, 76-78]. Additional anatomical endpoints include assays of cell death (TUNEL) [77, 78], localization of oxidative stress adducts in DRG and peripheral nerve [13, 40, 47, 79] and classification of different populations of IENF based on neurotransmitter expression [80]. These biochemical and anatomical assessments are used to further understand the mechanisms underlying the development DN; they are not used to establish the presence of DN. For this reason, no reference ranges for normal and abnormal findings of these multiple assays are suggested in the overview.

AN EXAMPLE OF MURINE PHENOTYPING

Our laboratory has phenotyped multiple mouse models for the presence of DN [13]. The BKS-db/db mouse is an example of a spontaneous model of type 2 diabetes that develops a robust DN. Previous work in this model demonstrated clear deficits in NCV [33, 34, 81-85] and fiber density and/or morphology [33, 35, 81-83, 86-88] following the development of diabetes. BKS-db/db mice develop hyperglycemia between 3-4 weeks of age (<http://jaxmice.jax.org/>) and a profound neuropathy at 24 weeks post onset of diabetes [13]. At 24 weeks, BKS-db/db developed significantly elevated fasting glucose, GHb and body weights compared to their nondiabetic (BKS-db⁺) littermates (Table 1).

We utilized the criteria outlined above to confirm and extend the phenotype of these animals regarding the presence of DN. Behavioral testing of the BKS-db/db mice demonstrated elevated tail flick latencies indicating sensory loss (nondiabetic 4.8 sec $\pm$ 0.14 and diabetic 9.7 sec $\pm$ 0.07, $p < 0.001$). The BKS-db/db mice also demonstrated profound changes in NCV with decreased tail sensory NCV (nondiabetic 23.8 m/sec $\pm$ 0.2, diabetic 17.4 m/sec $\pm$ 0.3, $p < 0.001$), increased tail distal motor latencies (nondiabetic 2.97 msec $\pm$ 0.02, diabetic 4.7 msec $\pm$ 0.07, $p < 0.001$), and decreased sciatic motor NCV (nondiabetic 38.8 m/sec $\pm$ 0.8,

diabetic $26.7 \text{ m/sec} \pm 1.2$, $p < 0.05$). To complete guideline criteria, IENF densities were measured in BKS-db/db mice including in the foot pad. IENF density was significantly reduced at 24 weeks post onset of diabetes (nondiabetic $37.7 \text{ fibers/mm} \pm 2.6$, diabetic $21.0 \text{ fibers/mm} \pm 2.0$, $p < 0.01$) in the BKS-db/db mice (Fig. 3).

We also completed measurements of advanced neuropathy phenotyping. TUNEL assessment of fragmented DNA was used as a marker of glucose-mediated injury in DRG neurons. An increase in the number of TUNEL positive sensory neurons was detected at 24 weeks in the DRG of BKS-db/db mice (nondiabetic $13.7\% \pm 1.4$, diabetic $21.3\% \pm 2.0$, $p < 0.01$) [13]. Nitrotyrosine immunoreactivity is another marker of oxidative/nitrosative stress [47, 89]. An increase in the number and intensity of nitrotyrosine positive sensory neurons was detected at 24 weeks in the DRG of BKS-db/db mice (pixel intensity nondiabetic 48.11 ± 3.7 , diabetic 84.53 ± 6.1 , $p < 0.01$) [13] (Fig. 4). In summary, behavioral, electrophysiological and anatomical assessments of DN are profoundly impaired in the BKS-db/db mice. Our published data [13] agree with previous reports of DN in this model [33, 34, 81-85].

SUMMARY AND FUTURE DIRECTIONS

If the scientific community agrees upon standardized definitions of DN in mouse models of type 1 and 2 diabetes, mouse models could provide a more effective means for preclinical testing of potential therapies. The lack of a uniform definition of murine DN makes comparisons across different drug-intervention trials difficult at best. For example, our results point to the BKS-db/db mouse as a robust working model of DN in type 2 diabetes. If a therapy proved effective in the treatment of DN in this model, the use of standardized definitions of DN would allow confirmation of this beneficial effect in other mouse models of DN. Treatments that have a positive effect on DN across different mouse strains and different types of diabetes are much more likely to yield a meaningful clinical effect in man. It is clear that the lack of uniformity in diagnosing and monitoring DN in murine models is limiting needed therapeutic advances for patients. This review suggests a set of standardized phenotyping criteria for the diagnosis of DN. These criteria were extracted from the currently published literature and could serve as a “talking point” for the formation of standardized murine guidelines for the diagnosis of DN.

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Fig. (1). Time Line of Neuropathy Screening

Behavioral assessment of sensory function is completed prior to anesthesia. NCV are measured in anesthetized mice followed by immediate foot pad dissection.

Fig. (2). Advanced Neuropathy Phenotyping

As with neuropathy screening, behavioral assays are performed in conscious mice. Multiple tissues are harvested and analyzed immediately or stored for future use.

Fig. (3). IENF measures functional innervation of the skin

A) PGP9.5 immunofluorescence within a nondiabetic ($n = 5$) BKS-db⁺ foot pad demonstrating a normal pattern of innervation (dots). B) PGP9.5 immunofluorescence within a diabetic ($n = 5$) BKS-db/db foot pad demonstrating a decrease in the number of fibers (dots). C) Quantitation of IENF is presented as the number of fibers/linear mm of epidermis. d = dermis, e = epidermis, dots = intraepidermal nerve fiber, arrowhead = dermal fiber bundles. $\gamma p < 0.01$. Bar = 100 μ m. Open bars represent the nondiabetic measurements and the black bars represent the diabetic measurements. (Reprinted with permission of from Elsevier, Neurobiol Dis 2007 (13)).

Fig. (4). Damaged DNA was measured by TUNEL staining

A) TUNEL positive sensory neurons (arrows) were detected in the lumbar DRG of BKS-db/db. B) Increased number of TUNEL labeled DRG in BKS-db/db mice at 24 weeks, $\lambda p < 0.05$. Five animals per group and > 150 neurons per animal were counted. Results are expressed as the percent TUNEL positive cells of total neurons counted. Localization of nitrated proteins was measured by nitrotyrosine immunofluorescence (NT-immunofluorescence). C) NT-immunofluorescence reveals an increase in nitrated proteins within DRG neurons (arrows) from BKS-db/db compared to BKS-db⁺ mice [nuclei stained with DAPI]. D) Histograms of the fluorescence signal indicate a relative increase in the intensity of NT-immunofluorescence in DRG from BKS-db⁺ versus BKS-db/db mice ($\gamma p < 0.01$). Bar = 20 μ m. Open bars represent the nondiabetic measurements and the black bars represent the diabetic measurements. (Reprinted with permission of Elsevier, Neurobiol Dis 2007 (13)).

Table 1
Please Provide Table Heading

Model	Age (weeks)	Behavior	NCV	Anatomy	Biochemistry	Glucose	Weight grams	First Author Citation
Type 1								
Ins.D^{d1}	28	NA	↓CMAP, ↓sensory NCV	↓MFD sural nerve	NA	> 250 mg/dL	NA	Elias Diabetes 47:1637-642, 1998
C57BL/6J X Ins2^{C96Y} (Akita)	31	↑gait disturbance, Morris water maze	↓sensory NCV	NA	NA	~ 31 mmol/L	~ 22	Choeiri Behav Brain Res 157:31-38, 2005
C57BL/6-Ins2^{Akita}/J (Akita)	28	no change in thermal latency	no change in motor or sensory NCV	NA	NA	584 mg/dL		Sullivan Neurobiol Dis 28:276-285, 2007
NOD	18	↑TF latency	NA	NA	NA	19.6 mmol/L	No Change	Obrosova FASEB J 19:401-403, 2005
	23 to 25	NA	NA	sympathetic neuritic dystrophy	NA	>600 mg%	NA	Schmidt Am J. Pathol 163:2077-2091, 2003
	NA	thermal hyperalgesia	NA	NA	NA	<300 mg/dL	20-30	Davar Neurosci Lett 190:171-174, 1995
	NA	thermal hyperalgesia	NA	NA	NA	19.6 mmol/L	NA	Gabra Eur J Pharmacol 514:61-67, 2005
Type 2								
BKS.Cg-m⁺/+Lepr^{db}/J (BKS-db/db)	33	NA	↓motor NCV	NA	NA	350 mg% (fasting)	> 50	Sima Acta Neuropath 40:85-89, 1978
	33	NA	NA	transmission electron microscopy, severe axonal and Schwann cell abnormalities	NA	NA	NA	Sima Lab Invest 40:627-632, 1979
	56	NA	NA	transmission electron microscopy, severe axonal and Schwann cell abnormalities	NA	NA	NA	Carson Neuropathol App Neurobiol 6:361-374, 1980
	25	NA	↓motor NCV and distal motor latency	NA	NA	HbA _{1c} 4.48% 532.1 mg/100mL (fasting)	41.7	Moore Exp Neurol 70:548-555, 1980
	33	NA	↓motor NCV	↓axonal number ↓axonal size	NA	156 mg% (fasting)	2 standard deviations above control values	Robertson Diabetes 29:60-67, 1980
	25	NA	NA	NA	↓fucose incorporation ↓eucine incorporation ↓	NA	NA	Chez Neurochem Res 8:465-472, 1983

Model	Age (weeks)	Behavior	NCV	Anatomy	Biochemistry	Glucose	Weight grams	First Author Citation
	60	NA	NA	↓myelinated fiber area ↓axon area ↓index of circularity	NA	34 mmol/L	22	Sharma Diabetes 32:1152-1161, 1983
	31	NA	NA	NA	↓slow transport of AChE	NA	NA	Vitadello Exp Neurol 82:143-147, 1983
	~57	hearing loss of 64% at 180 days of age	↓NCV	↓axon size	↓slow transport of AChE	NA	NA	Gorio Adv Exp med Biol 174:549-564, 1984
	40	NA	NA	↓axonal area ↓NCV	NA	424 mg/100mL	54	Norido Exp Neurol 83:221-223, 1984
	17	NA	NA	NA	↓transport of actin and tubulin	NA	NA	Vitadello J Neurochem 45:860-868, 1985
	40	NA	NA	↓density of particles P face freeze fracture	NA	539 mg/dL	55	Antonella J Neurol Sci 69:301-317, 1985
	20	NA	no change in motor NCV	NA	NA	19 mmol/L	34	Whiteley Exp Neurol 89:314-321, 1985
	2.5-17 months	NA	NA	↓varicosity and size of Meissner corpuscles	NA	240-400 mg/dL	NA	Ras Exp Neurol 91:488-501, 1986
	38	NA	NA	NA	↑ ³² P phospholipid incorporation	393 mg/dL	57	Berti-Mattera Diabetes 37:1703-1707, 1988
	14-17	NA	NA	NA	↑fructose in sciatic nerve	37 mmol/dL	39	Calcutt Metabolism 37:450-453, 1988
	25-30	NA	NA	NA	↑glucose in sciatic nerve ↓accumulation of PFK and ChAT	26 mmol/L	48	Calcutt Muscle Nerve 11:1206-1210, 1988
	40	NA	NA	NA	no change in ATPase activity	~25 mmol/L >6 %, BA _{IC}	~50	Bianchi Diabetologia 33:131-136, 1990
	NA	NA	NA	↓number of CGRP-IR DRG neurons	RIA ↓CGRP no change SP	15 mmol/L	NA	Schmidt Exp Neurol 132:16-23, 1995
	12	NA	NA	↓IENF	NA	NA	NA	Gibran J Surg Res 108:122-128, 2002
	8	↑temperature (decreased sensitivity)	↓sensory NCV ↓motor NCV	↓vascularity of vasa nervorum in sciatic nerve	NA	~540 mg/dL	NA	Ii Circulation 112:93-102, 2005
	32	↑thermal latency	Ex vivo CAP no change	NA	NA	NA	25-44	Walwyn Exp Neurol 198:60-270, 2006
	28	thermal hypoalgesia	↓sensory NCV ↓motor NCV	↓IENF	NA	378 mg/dL		Sullivan Neurobiol Dis 28: 276-285, 2007

Model	Age (weeks)	Behavior	NCV	Anatomy	Biochemistry	Glucose	Weight grams	First Author Citation
B6.V <i>Lep^{ob}/J</i> (B6-ob/ob)	11	↑hind paw latency ↓mechanical response	↓sensory NCV ↓motor NCV	↓IENF ↑NT ⁺ DRG neurons ↑PAR ⁺ nuclei	NA	17.6 mmol/L	57	Drel Diabetes 55:3335-3343, 2006
	11	↑hind paw latency ↓mechanical response	↓sensory NCV ↓motor NCV	↓IENF ↑NT ⁺ DRG neurons ↑PAR ⁺ nuclei	NA	17.6 mmol/L	57	Vareniuk Exp Neurol 205:425-436, 2007

Methods for measuring blood glucose vary across laboratories and are reported as milligrams per deciliter (mg/dL), milligrams percent (mg %) and as millimoles per liter (mmol/L). AChE = acetylcholine esterase, CAP = compound action potential, CGRP = calcitonin gene-related peptide, ChAT = choline acetyltransferase, (fasting) = fasting blood glucose levels, IENF = intraepidermal nerve fiber, MFD = myelinated fiber density, NA = not reported, NCV = nerve conduction velocity, NT = nitrotyrosine immunohistochemistry, PAR = poly(ADP-ribose), PFK = 6-phosphofructokinase, RIA = radioimmunoassay, SP = substance P.

Table 2
Table Inbred C57BL/6J High and Low Dose STZ Models of DN

Model	Duration (weeks)	STZ mg/kg	Behavior	NCV	Anatomy	Biochemistry Gene Expression	Glucose	Weight grams	First Author Citation
High Dose									
C57BL/6J	6	200	NA	NA	↓peptidergic input to spinal cord lamina Ili	NA	~25 mmol/L	↓30%	Akkinä Exp Neurol 167:173-182, 2001
	7	180	NA	NA	↓IENF density	NA	405 mg/dL	21	Christianson Exp Neurol 179:188-199, 2003
	9	180	no difference thermal or chemogenic stimuli; mechanical hypoalgesia	NA	NA	NA	292 mg/dL	21	Christianson J Pain 4:493-504, 2003
	9	150	NA	↓MNCV ↓SNCV	NA	↓PCr/Cr ↑glucose ↑sorbitol ↑fructose	23.4 mmol/L	24.9	Obrosova FASEB J 19:401-420, 2004
	6	180	no difference in mechanosensory function	NA	↓DRG neuronal size	↑ATF3 expression	26.6 mmol/L	22.33	Wright J Periph Nerv Syst 9:242-254, 2004
	1	200	↓thermal sensitivity	NA	NA	NA	> 20 mmol/L	NA	Gabra Reg Pep 127:245-248, 2005
	8	180	↓mechanical sensitivity	NA	↓IENF	NA	366 mg/dL	21.3	Christianson Neuroscience 145:303-313, 2007
	7	180	↓formalin-induced pain behavior	NA	↓number of Fos positive neurons in dorsal horn	NA	20 mmol/L	20	Johnson J Pain 8:637-649, 2007
	6	100	↓thermal sensitivity, ↓mechanical sensitivity (pressure), ↑mechanical sensitivity (non Frey filament)	NA	↓IENF ↑NT IHC ↑PAR IHC	NA	33 mmol/L	25	Drel Europ J Pharm 569: 48-58, 2007
Low Dose									
C57BL/6J	12	40 / 5	↑hind paw latency	NA	NA	↓mitochondrial activity	26.4 mmol/dL	19.3	Tam FASEB J 18:1767-1769, 2004
129/Sv X C57Bl6	~7	40 / 7	NA	↓motor and sensory NCV	↓nerve blood flow	↓PCr, PCr/Cr	22.7 mmol/L	24.5	Obrosova Diabetes 53:711-720, 2004
B6/129S7	6	40 / 5	NA	↓motor and sensory NCV	NA	↓GSH, ↓SOD, ↑MDA, ↑catalase	329 mg/dL	19.7	Kellogg Antiox and Redox Sig 7:1521-1529, 2005
C57BL/6J	NA	50 / 5	NA	NA	NA	prolonged Ca ⁺⁺ elevation in DRG neurons	21.15 mmol/L	NA	Kostyuk Neuroscience 90:535-541, 1999
C57BL/6J	24	50 / 5	no change in thermal latency	no change in NCV	no change in IENF	no change in GSH, SOD, MDA, Catalase	366 mg/dL		Vincent Exper Neurol 208:216-227, 2007

Model	Duration (weeks)	STZ mg/kg	Behavior	NCV	Anatomy	Biochemistry Gene Expression	Glucose	Weight grams	First Author Citation
C57BL/6J	24	50 / 5	no change in thermal latency	no change in NCV	no change in TUNEL ⁺ cells or IENF	NA	466 mg/dL		Sullivan Neurobiol Dis 28:276-285, 2007

ATF3 = activating transcription factor 3, IENF = intraepidermal nerve fiber, IHC = immunohistochemistry, GSH = glutathione S-transferase, MDA = malondialdehyde, NCV = nerve conduction velocity, NT = nitrotyrosine, PAR = poly(ADP-ribose), PCr/Cr = phosphocreatinine/creatinine ratio, SOD = superoxide dismutase.

Table 3

Outbred STZ-Treated Mouse Models of DN

Model	Duration (weeks)	STZ mg/kg Days	Behavior	NCV	Anatomy	Biochemistry Gene Expression Other	Glucose	Weight grams	First Author Citation
CD-1	1	200/1	thermal hyperalgesia (allodynia)	NA	NA	NA	< 20 mmol/L	25-30	Gabra Eur J Pharm 457:115-124, 2002
CD-1	11 days	200/1	thermal hyperalgesia (allodynia)	NA	NA	NA	< 20 mmol/L	25-30	Gabra Peptides 24:1131-1139, 2003
ddY	2	150/1	↓nociceptive threshold (pressure)	NA	NA	NA	< 400 mg/dL	25	Suzuki Jpn J Pharm 79:169-175, 1999
ddY	4	200/1	thermal and mechanical hyperalgesia	NA	↑VR1 in DRG neurons	NA	599.6 mg/dL	31.5	Rashid J Pharm Exp Ther 306:709-717, 2003
ddY	8	200/1	↑mechanical threshold	NA	NA	NA	< 16.7 mmol/L	> 35	Murakami J Gene Med 8:773-781, 2006
ICR	2	200/1	altered response to formalin induced nociception	NA	NA	first phase longer, second phase abolished	> 400 mg/dL	~20	Kamei Brain Res 862:257-261, 2000
ICR	2	200/1	↓mechanical nociceptive threshold	NA	NA	NA	> 400 mg/dL	~20	Onodera Jpn J Pharm 85:335-337, 2001
ICR	3	200/1	↓mechanical threshold	↑C-fiber wind up	NA	NA	< 350 mg/dL	31-42	Kimura J Pharm Sci 97:195-202, 2005
Laka	4	200/1	thermal hyperalgesia	NA	NA	NA	293 mg/dL	19.5	Anjaneyulu Progress Neuro-Psycopharm Bio Psych 27:1001-1005, 2003
Laka	4	200/1	thermal hyperalgesia	NA	NA	NA	465.26 mg/dL	17.4	Anjaneyulu Eur J Pharm 497:285-292, 2004
Laka	4	200/1	thermal hyperalgesia	NA	NA	NA	412 mg/dL	17.42	Sharma Phytother Res 21:278-283, 2007
Swiss Wistar	14	50/3	NA	↓MCV	no change in MFD	delayed nerve regeneration	41.1 mmol/L	36.2	Kennedy Brain 123:2118-2129, 2000

Model	Duration (weeks)	STZ mg/kg Days	Behavior	NCV	Anatomy	Biochemistry Gene Expression Other	Glucose	Weight grams	First Author Citation
Swiss-Webster	6-8	100/2	NA	↓FSAs	NA	NA	22.9 mmol/L	31	Goss Diabetes 51:2227-2232, 2002
Swiss mice	8	3/70	NA	no significant change in SCV	NA	NA	12.8 mmol/L	32	Zochodne Brain 127:2193-2200, 2004
Swiss Wistar	36	85, 70, 55		↓MCV ↓SCV ↓M wave	↓IENF ↓DRG neurons	NA	31.9 mmol/L	34.5	Kennedy Diabetes 54:830-837, 2005
Swiss NIH albino mice	12	50/5	↓thermal latency ↑pressure latency	NA	↓MFD ↓Schwann cells/mm ²	↓NGF serum ↓NGF sciatic nerve	308 mg/dL	no change from control	Arrieta EurJ Clin Invest 35:201-207, 2005
Swiss Mice	8	200/1	↑thermal latency)	↓MNCV	NA	↓blood flow	486 mg/dL	21	Demirot Diabetes 55:1478-1483, 2006
Swiss Webster	24	100/2	↑thermal latency	↓sensory amp ↓SCV, ↓MCV and motor amp	↓sweat gland innervation	↓pilocarpin-induced sweating	22 mmol/L	26	Chattopadhyay Diabetologia 50:1550-1558, 2007

IENF = intraepidermal nerve fiber, GSH = glutathione S-transferase, MDA = malondialdehyde, MFD = myelinated fiber density, MCV = motor conduction velocity, NA = not reported, NCV = nerve conduction velocity, NGF = nerve growth factor, PCr/Cr = phosphocreatine/creatinine ratio, SCV = sensory conduction velocity, SOD = superoxide dismutase, VR1 = Vanilloid receptor 1.