ABSTRACT: Diabetes targets the peripheral nervous system with several different patterns of damage and several mechanisms of disease. Diabetic polyneuropathy (DPN) is a common disorder involving a large proportion of diabetic patients, yet its pathophysiology is controversial. Mechanisms considered have included polyol flux, microangiopathy, oxidative stress, abnormal signaling from advanced glycation endproducts and growth factor deficiency. Although some clinical trials have demonstrated modest benefits in disease stabilization or pain therapy in DPN, robust therapy capable of reversing the disease is unavailable. In this review, general aspects of DPN and other diabetic neuropathies are examined, including a summary of recent therapeutic trials. A particular emphasis is placed on the evidence that the neurobiology of DPN reflects a unique yet common and disabling neurodegenerative disorder.

DIABETES MELLITUS AND THE PERIPHERAL NERVOUS SYSTEM: MANIFESTATIONS AND MECHANISMS

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Diabetes mellitus imposes substantial burdens on the nervous system and is the most common cause of neuropathy or peripheral nerve damage. Moreover, diabetic neuropathies are rising in prevalence with the growing global burden of type II diabetes mellitus. Although this review emphasizes peripheral nerve disorders, there is now recognition that diabetes also targets the central nervous system, especially white matter (diabetic leukoencephalopathy). Within the peripheral nervous system alone, however, diabetes renders several types of nerve damage, including diffuse damage (polyneuropathy) and focal damage (mononeuropathy). Both contribute to sensory and motor deficits and both are associated with significant disability in patients. In polyneuropathy it is now recognized that impaired glucose tolerance, even without overt diabetes mellitus, may be a risk factor.

The San Antonio Consensus criteria are commonly used to define diabetic neuropathy for research purposes. For clinical neuropathy, the guidelines require symptoms and signs, or one of these with abnormal testing (nerve conduction, quantitative sensory testing, or autonomic testing). Subclinical neuropathy is identified by abnormal testing only. More specific staging of diabetic polyneuropathy (DPN) has also been described by Dyck and Dyck: NO, no neuropathy; N1, asymptomatic neuropathy without (N1a) or with (N1b) findings on neurological examination; N2, symptomatic; N3, disabling.

Both pathophysiology and therapy for diabetic neuropathies remain challenging. There has been a long history of failed clinical trials for polyneuropathy, in part related to issues of what was targeted, what was being measured, and how well the trial was designed. Despite these problems, there are new and exciting thoughts about how these disorders develop and what avenues may offer significant hope. Because of the size of the topic, a number of aspects are only covered briefly in this review and the bias is...
toward emphasizing aspects of its neurobiology. Three excellent and comprehensive texts addressing diabetic neuropathy have been published in addition to recent reviews addressing slightly different points of view, and diagnostic criteria have recently been published by the American Diabetes Association.

**CLASSIFICATION AND PREVALENCE**

Diabetic neuropathies comprise diabetic polyneuropathy (DPN), a symmetric diffuse disorder that particularly targets sensory neurons with long axons, and focal neuropathies or mononeuropathies. The latter include classic entrapment neuropathies that are more common in diabetes such as carpal tunnel syndrome (CTS), ulnar neuropathy at the elbow (UNE), meralgia paraesthetica (entrapment of the lateral femoral cutaneous nerve of the thigh) at the inguinal ligament, or peroneal neuropathy at the fibular head. Other mononeuropathies much more specifically identified in diabetic patients include intercostal and abdominal segmental radiculopathies, oculomotor palsies, and lumbosacral radiculoplexus neuropathies. Brown and Asbury subdivided DPN clinically into subtypes, with the group of mixed motor, sensory, and autonomic neuropathy representing 70% of patients. A predominantly sensory phenotype was found in 39% that was yet further divided into large-fiber, small-fiber, or mixed neuropathies. Pure motor DPN or autonomic DPN were uncommon (<1% each). In the author’s experience, pure sensory DPN on the basis of clinical evaluation alone (some have subclinical electrophysiological motor involvement) represents the large majority of patients, particularly early in their course. Some have added a category of an acute sensory DPN with rapid onset (likely overlapping with a condition known as “insulin neuritis” or neuropathy after the onset of insulin use), an association with acute hyperglycemia, the presence of prominent pain, and a shorter overall duration related to control of hyperglycemia.

The reported prevalence of DPN varies with the type and the intensity with which it is sought. In the classic Diabetes Control & Complications Trial (DCCT) of diabetic complications in intensively rather than conventionally treated patients with type I diabetes mellitus, clinical neuropathy was defined as an abnormal clinical neurological examination plus either abnormal nerve conduction in at least two peripheral nerves or unequivocally abnormal autonomic-nerve testing. In patients without neuropathy at baseline, 9.8% of conventional and 3.1% of intensively treatment patients had developed it by 5 years. For patients in the secondary intervention cohort with retinopathy at baseline but not neuropathy, the rates were 16.1% for conventional and 7.0% for intensive treatment. Overall, when looking at a variety of studies (summarized by Shaw et al.), type I diabetic prevalence figures vary from 13%–17% in hospitalized patients based on symptoms and signs, and 8%–54% with more comprehensive batteries in primary care or population-based screening. For type II diabetic patients, similar figures run from 19%–58% in hospital-based studies with some ancillary testing and 13%–46% in primary care or population-based screening more heavily weighted toward testing. There are likely significant flaws, however, from relying on hospital-selected data.

With very comprehensive and extensive batteries of evaluation, such as that applied to the Rochester Diabetic Cohort (n = 380), evidence of DPN was identified in 54% of type I diabetics and 45% of type II diabetics. Using the strict criteria of an abnormal neuropathy impairment scale (NIS) and seven abnormal laboratory studies, 21% of the Rochester diabetic cohort had DPN. Symptomatic DPN was identified in a smaller proportion, 15%–15%. In other cohorts, such as the Pittsburgh epidemiology of diabetes complications (n = 400), DPN was identified in 34% of type I diabetics, whereas in the San Luis Diabetes Study DPN was present in 26% of type II diabetics (n = 279). In patients with impaired glucose tolerance only, as a precursor of type II diabetes, the prevalence figures have been more controversial.

The prevalence of cardiovascular autonomic neuropathy detected by heart-rate interval studies (including the response to Valsalva’s maneuver, or deep breathing) has ranged from ~16%–25% in type I and II diabetic patients, with a smaller proportion having symptoms. Several studies have suggested that cardiovascular autonomic neuropathy is a risk factor for increased mortality. For gastrointestinal symptoms, prevalence figures are also variable, with numbers for constipation or diarrhea ranging between 3% and 35%. Impotence has been identified in 23%–57% of type I and II diabetic men, with higher rates with increasing age. Overall, a population-based study from the Rochester diabetic cohort (n = 231 diabetics) identified a prevalence of autonomic dysfunction (using a composite scale of laboratory-based autonomic tests known as CASS) of 54% in type I diabetics and 73% in type II diabetics, with postural hypotension in 8.4% and 7.4%, respectively.
Further work in the Rochester diabetic cohort identified symptomatic CTS in 7.6%. In an older Mayo Clinic study by Mulder et al., symptomatic CTS was identified in 8.7% of 103 diabetic subjects and 4.9% had UNE.

Risk factors for the development of DPN have included the degree and duration of hyperglycemia, smoking, and the presence of other complications such as retinopathy and nephropathy. Tesfaye et al., representing 31 centers participating in the European Diabetes (EURODIAB) Prospective Complications Study, examined risk factors for the development of DPN in patients with type 1 diabetes mellitus over a 7–10-year period. At endpoint, DPN had developed in 276 of 1,172 patients without it at onset (23.5%) and its cumulative incidence was related to the glycosylated hemoglobin value and duration of diabetes. Independent risk factors were higher levels of total and low-density lipoprotein cholesterol and triglycerides, a higher body mass index, higher von Willebrand factor levels, urinary albumin excretion rate, hypertension, and smoking. Cardiovascular disease at baseline independently doubled the risk of neuropathy. Overall, the findings identified a striking correlation between risk factors for vascular disease and the development of DPN.

It is possible that the severity and impact of DPN on individual patients is easing, given better control regimens, more education, and improved pain therapy. This possibility, however, has not been rigorously addressed and such improvements may be uneven among populations and regions.

**CLINICAL MANIFESTATIONS**

To appreciate fully the clinical features of DPN, the reader is directed to one of the more interesting and comprehensive articles on the subject, written in 1945 by Rundles. Symptomatic DPN usually presents with spontaneous positive (paresthesias described as prickling, tingling, “pins and needles,” burning, crawling, itching, abnormal sensation to temperature, pain), or negative (numbness, injury insensitivity) sensory symptoms in the toes. With time, such sensations may advance up the foot and involve the fingers and hands. Neuropathic pain is a prominent early feature of DPN and can be severe despite minimal signs of DPN. Patients describe their feet as “tight,” having painful prickling, burning, electrical, sharp, or jabbing sensations. Symptoms may be prominent at night, and overall quality of life is significantly compromised. There may be allodynia (provocation of pain by simple mechanical stimuli) from bed covers or from walking. Walking thus may be painful and hesitant or unsteady. Although diabetic patients do not generally exhibit a wide-based classic sensory ataxia, loss of touch and pressure sensitivity on the soles, and later loss of more specific proprioceptive axons, may predispose them to falling. The sensory abnormalities of DPN are “stocking and glove” in distribution, reflecting the susceptibility of longer nerves.

Clinical neurological examination by experienced examiners has inherent variability but its advantages are its low cost, multimodality evaluation, and rendering of a direct examiner–patient interaction. Sometimes this kind of interaction is essential in identifying early foot ulcerations or other problems and should not be replaced by laboratory measures such as computerized QST (quantitative sensory testing). An additional screening tool for sensory loss is the Semmes–Weinstein 10 g monofilament test. The monofilament is pressed against the dorsum of the large toe (plantar application has also been described) until it bends and the patient (with eyes closed) is asked to identify when the stimuli are applied (for example, 5–10 times on each toe).

In addition to sensory loss which may involve the toes, whole foot, or leg below the knee, for example, there may be loss of ankle reflexes despite reinforcement, and in more advanced neuropathies, loss of the knee reflexes or upper-limb deep tendon reflexes. In DPN the earliest motor manifestation is atrophy of the EDB muscle. Clinical weakness of toe and foot dorsiflexors occurs later. Subtle motor axon involvement may lead to abnormalities of foot posture that contribute to foot ulceration and gait imbalance. Charcot joints, for example, at the ankle, are destructive arthropathies secondary to repetitive injury of which the patient may be unaware (Fig. 1).

Symptoms of diabetic autonomic neuropathy include distal anhidrosis or inappropriate truncal sweating and rarely gustatory sweating (facial and trunk sweating from certain foods). Constipation, sometimes alternating with diarrhea, is a symptom of diabetic visceral autonomic neuropathy. Other gastrointestinal autonomic symptoms are less common and include frank fecal incontinence, dysphagia, heartburn, nausea and vomiting, early satiety, and bloating and fullness after meals. Patients may have unawareness of hypoglycemia. Impotence is a common manifestation of autonomic neuropathy in men (vascular factors also play a role). Postural hypotension with orthostatic dizziness or occasional fainting results from loss of sympathetic control to resistance arterioles. Loss of bladder sensitivity occurs in diabe-
tes leading to overflow incontinence, but this is more strictly classified as a visceral sensory deficit.

Diabetic foot ulceration is a major forerunner of eventual amputation. DPN contributes to the development of foot ulcers in several ways. There is loss of protective sensory sensation in the feet, with consequent repetitive injury. Patients may injure their foot without realizing it. Foot atrophy distorts how gravitational forces are transmitted to foot and leg bones and leads to undue pressure on bony prominences. Loss of sweating in the feet leads to skin drying and cracking. Clinical evaluation, including use of monofilaments, is valuable in predicting the risk of foot ulceration and some have advocated yearly screening.\textsuperscript{1,19}

CTS is most commonly associated with positive sensory symptoms and pain in the territory of the median nerve. Many patients complain of symptoms in all digits of their hand. Symptoms may be prominent at night or on awakening and are provoked by repetitive activities involving the hand or by pregnancy. Concurrent hypothyroidism is important to exclude. A high index of suspicion for treatable CTS in diabetic patients who also have polyneuropathy is valuable: in some patients CTS may emerge later with increased use of the hands when walking is difficult. Longer standing and more severe CTS may be associated with hand weakness from denervated thenar muscles.

Diabetic lumbosacral plexopathy (DLSP), sometimes also known as radiculoplexus neuropathy, diabetic amyotrophy, Bruns–Garland syndrome, or proximal diabetic neuropathy, is less common but highly debilitating.\textsuperscript{12,61} Rarely, nondiabetics can develop a similar syndrome that appears to respond to high-dose corticosteroid therapy.\textsuperscript{60} DLSP is often associated with significant weight loss or cachexia. Surprisingly, it may emerge early in the course of diabetes or following institution of insulin therapy in type II diabetic patients. It is usually asymmetric and associated with deep boring or aching pain in the thigh. Over the next few weeks after development of pain, weakness and wasting of proximal thigh muscles develops in iliopsoas, quadriceps, and thigh adductors. Some patients become unable to walk. Spontaneous recovery occurs but is slow, over a number of months, and in some cases contralateral DLSP emerges. Variations of DLSP include symmetric involvement, foot drop, or apparent worsening of generalized DPN.

Other causes of polyneuropathy must always be considered in a diabetic patient. Patients, for example, with a more subacute and a prominent motor disorder may have chronic inflammatory demyelinating polyneuropathy (CIDP) with more prominent features of primary demyelination on neurophysiological testing (see below). CIDP is recognized to accompany diabetes in some patients.\textsuperscript{217} In the author’s practice, additional patients with diabetes
have been identified to have POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes), multifocal motor neuropathy, mitochondrial cytopathy, anti-MAG (myelin-associated glycoprotein) neuropathy, and others. Associated hypothyroidism or vitamin B12 deficiency should also be excluded. Although debated and advocated in some models, unequivocal clinical differences in DPN of patients with type I or II diabetes independent of age or duration of diabetes have not been demonstrated. The combination of lumbar spinal stenosis and CTS may present features that resemble polyneuropathy with lower- and upper-limb involvement. “Pseudopolyneuropathy” from this combination can be distinguished by careful clinical, neurophysiological, and imaging approaches.

**NEUROPHYSIOLOGY**

Neurophysiological measurements are gold standards in the evaluation of DPN. They are widely available, generally carried out in standardized formats, and have been rigorously examined in clinical trials. Among the measures available, the amplitude of the sural sensory nerve action potential (SNAP), measured behind the ankle 140 mm from the calf stimulating site with a surface skin temperature of at least 30°C, is the earliest alteration in DPN. A decline in amplitude below 6-µV when measured from baseline to peak is considered abnormal, and the amplitude correlates with myelinated fiber density. Other sensitive indices are the declines in sural nerve conduction velocity and peroneal nerve motor conduction velocity. In clinical trials, centralized monitoring of nerve conduction results and technique with review of waveforms has substantially improved the quality of the material and of the associated trials. Most adequately powered and designed trials involve training over specific trial procedures and assessment of normal controls. High-quality trials have involved repeated measures at the trial onset and endpoints.

DPN is associated with some conduction slowing, although not as severe as in CIDP or CMT1a (Charcot–Marie–Tooth disease type 1A), suggesting primary demyelination, and reduction in amplitude or loss of CMAPs (compound muscle action potentials) and SNAPs, indicating axon dropout. Distal motor latencies may be asymptotically prolonged at sites of entrapment such as the carpal tunnel. Frank conduction block is uncommon, except at entrapment sites. Temporal dispersion can be identified, but usually in the setting of entrapment or severe disease. DPN involving the upper limbs may be more difficult to establish, since CTS and UNE are frequently admixed and may cloud the interpretation of upper-limb studies. Conduction slowing or loss of amplitude of the radial SNAP recorded at the base of the thumb is a useful index of sensory DPN in the upper limbs, and this nerve is not generally prone to entrapment. Needle electromyography may detect distal denervation. Renal failure superimposed on DPN is thought to suppress abnormal spontaneous activity such that denervation may be more difficult to recognize. Motor unit recruitment is reduced in distal muscles of patients with DPN and remaining units are remodeled and enlarged, indicating chronic denervation with reinnervation.

There is a large literature on the use of a number of other neurophysiological techniques in DPN, but these are not reviewed here. They include elevated jitter on SFEMG (single-fiber electromyography), F-wave latencies, H-reflex latencies, somatosensory evoked potentials, refractory period testing, macro-electromyography, axonal threshold accommodation, resistance to ischemic conduction failure (RICF), and motor unit number estimates. Extensive reviews of neurophysiological testing in DPN and other diabetic neuropathies have been published.

The neurophysiological characteristics of DLSP include focal denervation of muscles innervated by the lumbosacral plexus, e.g., iliopsoas, quadriceps, and adductor muscles. CMAPs recorded over vastus medialis may be smaller on the side of involvement—their recovery parallels clinical improvement. Paraspinal muscle denervation is also common, indicating involvement of proximal roots. Foot drop from peroneal nerve involvement may occur, and the overall pattern of neurological involvement may be quite patchy. Most patients also demonstrate widespread changes of DPN, largely chronic but occasionally exacerbated during DLSP.

Autonomic testing is important in identifying either selective autonomic involvement or associated autonomic dysfunction in DPN. Forms of testing include evaluation of cardiovascular autonomic neuropathy (RR interval variation at rest, with deep breathing, and with Valsalva maneuver; blood pressure response to standing, Valsalva maneuver, or isometric exercise; spectral analysis). Prolonged QTc intervals in type I diabetes may predict an increased risk of mortality.

Quantitative sensory testing includes approaches that span Semmes–Weinstein 10-g monofilament sensation to sophisticated computerized interfaces such as the CASE IV (computer-assisted sensory examination) developed by Dyck and colleagues. Modalities tested for by QST include vibration per-
ception threshold (VPT; probably the most commonly used form of QST in clinical trials) but also touch-pressure, warm and cool detection thresholds (WDT and CDT), and heat as pain (HNDT, or heat-nociception detection threshold). CASE IV can be used for VPT, WDT, CDT, and HNDT. QST abnormalities in clinical trials correlate with other measures of DPN. From a report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology, QST has been considered a useful test for the diagnosis of DPN for clinical and research purposes, but not alone and the evidence for its sole use for diagnosis had not yet achieved the highest level.

MORPHOLOGICAL AND STRUCTURAL FEATURES

The most common abnormality of sural nerves studied morphologically in patients with DPN is axon loss. The numbers of remaining myelinated axons correlate well with the amplitude of the SNAP recorded electrophysiologically prior to biopsy. In humans, it is uncertain whether such loss reflects retraction of branches of sensory neurons or loss of the complete neuronal tree including perikarya at the ganglion. In some patients there may be evidence of active axonal degeneration, but this is less common given the chronicity of the neuropathy. Axon loss is patchy or multifocal (unless it is so severe that most axons are absent), a pattern that has suggested an ischemic or microangiopathic mechanism. In some other forms of neuropathy, however, such as inherited neuropathy unrelated to microvascular disease, multifocal loss has also been identified. Unmyelinated axon loss and regeneration may also be observed. It is probably a common misconception that painful diabetic polyneuropathies are predominantly due to loss of small fibers. Most pathological studies of DPN have identified widespread fiber involvement, with both large- and small-fiber involvement. In addition to undergoing axonal degeneration, myelinated axons also exhibit segmental demyelination and remyelination. Clusters of small regenerating axons, with thin myelin sheaths, may superficially resemble onion bulbs (“pseudo-onion bulbs”). Atrophy of axons, a feature of DPN models, has been difficult to demonstrate in human biopsies, possibly because of preexisting dropout. Alterations in the vasa nervorum have been an important finding in human DPN nerve samples. A number of alterations have been described including perivascular basement membrane thickening, endoneurial capillary closure, microthrombosis, endothelial cell reduplication, smooth muscle proliferation, and pericyte degeneration. Declines in endoneurial capillary luminal area and rises in basement membrane thickness develop very early in patients, even those with impaired glucose tolerance prior to frank diabetes. Overall, the findings confirm the importance of concurrent microvascular disease in DPN, but do not necessarily indicate cause and effect, since it may represent parallel involvement of nerves and vessels.

Punch biopsies of the skin (3 mm), used to examine epidermal innervation of axons, is a sensitive index of DPN. An alternative approach has been the use of a skin blister. Samples are examined using immunohistochemistry with an antibody directed to the axon marker PGP 9.5. Standardized approaches toward the sampling and processing of skin biopsies and the measurement of the linear density of intraepidermal skin fibers (IENF) have been proposed by the European Federation of Neurological Societies. DPN is associated with a reduction in the density of IENF and other changes, such as the presence of degenerative axonal ovoids. The loss of small epidermal axons is important because it has a significant impact on wound healing. Reductions can also be observed, however, in patients with impaired glucose tolerance without frank diabetes. Kennedy also identified gastrointestinal tract denervation in patients with autonomic neuropathy.

Recently, a sensitive and noninvasive approach toward assessing DPN has been developed by examining corneal innervation using corneal confocal microscopy. Malik et al. described the technique in 18 diabetic patients and 18 age-matched control subjects that involved scanning the cornea for the nerve plexus of Bowman’s layer. Corneal nerve fiber density, length, and branch density were reduced in diabetic patients, with a trend toward greater reductions in patients with more severe DPN.

Surprisingly, there are few morphological studies in humans of dorsal root or autonomic ganglia in diabetes. A postmortem study, now dated, was carried out by Greenbaum et al. involving 6 patients. Some, though not extensive, loss of sensory neurons replaced by nests of Nageotte were reported. The authors also described vacuolar changes in neurons but their significance is uncertain, since vacuolar changes from swollen mitochondria can emerge as a postmortem artifact. Schmidt et al. described dystrophic changes of proximal sensory neuron axons within dorsal root ganglia of diabetic humans but also with advanced age.
NEUROBIOLOGY

General Considerations. There are some very interesting aspects of diabetes that reflect a unique neurobiology. Although some parts of the nervous system, such as sensory neurons, are targeted, others (e.g., motor neurons) are less involved. There have been substantial problems in bringing together diverse ideas and hypotheses as to how DPN develops. Investigators have not done a good job at interpreting the range of findings and their implications or in linking divergent ideas. At one end of the spectrum is the hypothesis that neuropathy is an exclusive microvascular disease, brought about by reductions in nerve blood flow and ischemia. At another end, to which the present author leans, is the idea that the targeting is directly neuronal. At the level of the neuron, the degeneration does not reflect, for example, the pattern of alterations that occur after an axotomy or with primary neuron dropout. It seems that neurons are beset by several forms of insult, including ischemia from microangiopathy, oxidative stress, and polyol flux. Neurons fail to resist such input because their support mechanisms are impaired from attenuated growth failure support (including that of insulin). Some of these proposed mechanisms are considered below.

Experimental diabetes has been studied in several models, particularly after administration of a single or short course of streptozotocin (STZ) to mice or rats, a specific pancreatic beta-cell toxin that leads to hyperglycemia within a few days. The STZ model is closest (although not identical, since rodents may survive for long periods without insulin) to type I human diabetes. In rats, STZ models have been widely used, although often for only short periods, whereas a more complete repertoire of diabetic models, particularly after administration of a single or short course of streptozotocin (STZ) to mice or rats, a specific pancreatic beta-cell toxin that leads to hyperglycemia within a few days. The STZ model is closest (although not identical, since rodents may survive for long periods without insulin) to type I human diabetes. In rats, STZ models have been widely used, although often for only short periods, whereas a more complete repertoire of changes may take 6–12 months of diabetes. Similarly, STZ diabetes can be studied in mice after somewhat shorter durations (e.g., 4–9 months). There are a number of diabetic models, not all listed here, including the BB/W rat with type I diabetes discovered by Sima (discussed below), a more recent BB/Z type II diabetic rat examined in that laboratory, the ZDF (Zucker diabetic fatty) rat, the db/db or ob/ob mouse with leptin receptor or leptin mutations, respectively, and others as reviewed elsewhere. Functional abnormalities of the vasa nervorum may develop early in models of DPN and are thought to presage later structural changes. Such changes then lead to ischemic damage of neurons and axons in diabetes.

Mechanisms for microangiopathy center on endothelial damage with features that include impaired vasodilation by nitric oxide (NO), damage from oxidative stress, and alterations generated by polyol flux. The abnormalities of NO release and function by the endothelium are particularly important and include excessive quenching of NO by advanced glycosylation endproducts (AGEs). Overall, diabetic vessels have more prominent impairment of vasodilatory mechanisms, favoring excessive functional vasoconstriction. Microangiopathy is then exacerbated by hyperviscosity, loss of red-cell deformability, increased platelet aggregation, and alterations in local oxygen release, all likely contributing to an eventual cascade of hypoxia and ischemia.

An important debate evolves around whether microangiopathy and associated abnormalities of oxygen delivery explain the early development of polyneuropathy. As already discussed, structural changes of microvessels are prominent in human sural nerve biopsies. Although some of this work involved patients with long-standing diabetes and increased age, work by Malik et al. has identified changes early during the course of DPN. Newrick et al. demonstrated that human sural nerves have decreased oxygen tensions in diabetic subjects. In contrast, Theriault et al. measured human sural nerve blood flow (intraoperative laser Doppler flowmetry) in patients with early DPN scheduled for research trial–related nerve biopsies. Some patients later also underwent contralateral nerve blood-flow measures prior to follow-up biopsies, at the end of the year-long trial. Interestingly, blood flow did not decline with increasing severity of DPN but tended instead toward higher values in more severely affected patients, or in the same patients over time (Fig. 2). It is unlikely that arteriovenous shunting explains such a change. Patients with vasculitis of their peripheral nerves, in contrast, had significant reductions in local blood flow.

A number of investigators have identified very early reductions in nerve blood flow in animal models of DPN. Some of these studies can be criticized on technical grounds, as discussed in detail elsewhere. and not all investigators have identified such declines in nerve blood flow. An extensive battery of pharmacological approaches, largely in short-term rat models, have reversed conduction and blood-flow abnormalities in tandem. It is uncertain, however, whether at least some of these approaches target other functional abnormalities, such as blood flow or neuronal function in ganglia. Early
nerve-trunk ischemia may therefore not account for diabetic neuropathy; instead, parallel forms of involvement may eventually become synergistic. In our laboratory, we did not identify declines in nerve blood-flow (under strict physiological conditions with near-nerve temperature control) in various models of DPN, using a number of independent and blinded examiners and a variety of blood-flow measurement techniques.\(^{104,269–271,273,274,277}\) We also observed that sympathectomy and vasodilatation, in contrast to other work, was associated with worsened indices of experimental DPN, not improvement.\(^{271}\) Moreover, direct examination of unfixed vascular profiles in DPN suggests that microvessels increase in caliber and may exhibit angiogenesis.\(^{277}\) These findings have also suggested that the use of vascular endothelial growth factor (VEGF), advocated for increasing nerve blood-flow in human clinical trials, may be unhelpful. For example, preclinical work addressing its use in diabetic models has been criticized on technical grounds regarding the approach used to evaluate both flow and vessels.\(^{196}\) There is also concern that VEGF may exacerbate diabetic retinopathy, where elevated levels are thought to induce angiogenesis.\(^3\) Declines in dorsal root ganglion (DRG) blood flow, where the impact of ischemia might cause greater damage, can be identified in some models and we did confirm some shifts to lower oxygen tensions in both nerve and dorsal root ganglia.\(^{273,274}\) Both nerve trunks and DRG also exhibit abnormalities of blood-vessel function, for example, with prominent sensitivity to endothelin-induced vasoconstriction and ischemic damage.\(^{254,267,269}\)

Overall, then, although microangiopathy is an undoubted accompaniment to DPN, the evidence for an exclusive role in the etiology of DPN is mixed. It seems unlikely that DPN patients, already frequently treated for vascular disorders, will experience benefit from simple approaches such as the use of vasodilators. Such approaches also pose a risk of exacerbating postural hypotension from concurrent autonomic neuropathy.

### Accumulation of Polyols

Accumulation of polyols, especially sorbitol, into peripheral nerves occurs by excessive flux through the aldose reductase (AR) pathway. This critical metabolic abnormality has generated intense attention for several decades and has led to a series of clinical trials of aldose reductase inhibitors (ARIs). AR is localized largely to glial cells (Schwann, DRG supporting cells; also endothelial cells), and polyol flux is thought to damage supporting cells and then axon function.\(^{142}\) In axons, then, there are associated depletions of nerve myo-inositol, changes in protein kinase C (PKC) subunits, and dysfunction of nerve Na\(^+/K\) ATPase.\(^{17,20,80,82,141,172,207}\) The changes in Na\(^+/K\) ATPase activity, in turn, have been linked to a rise in intra-axonal Na\(^+\) content with associated changes of channel properties ultimately manifest as slowing of nerve conduction velocity.\(^{43,80,207}\) Conduction slowing is a fundamental, widespread, and early marker of DPN that occurs in motor and sensory, myelinated and unmyelinated axons. ARIs or PKC inhibitors thus derive their rationale for clinical trials.\(^{2,43,94,145,193,201,207,202}\)

Whether excessive polyol flux induces neurodegeneration, however, is unknown. Similarly, there are complexities in how PKC subunits are distributed and behave in peripheral nerves that make it unclear whether inhibition is beneficial or harmful.\(^{256,257}\)

### Free Radical Oxidative and Nitrergic Stress May Damage the Peripheral Nervous System

Several investigators have provided evidence that free radical liberation damages axons, Schwann cells, and probably perikarya of neurons through oxidative and nitrergic stress.\(^{37,127,128,261}\) as reviewed elsewhere.\(^{239}\) Polyol flux and advanced glycosylated endproducts are thought to play a major role in the generation of free radicals.\(^{153,157}\) This pathway involves auto-oxidation of glucose, and glycation products of glucose gener-

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**FIGURE 2.** Measurements of blood flow (flags) in intact sural nerves were made in patients using an intraoperative laser Doppler flowmeter following sural nerve conduction recordings and prior to nerve biopsy. The patients were participants in a clinical trial for mild DPN requiring sural biopsies at outset and on the opposite side at endpoint. While the plot indicates correlation between sural nerve myelinated fiber density and the amplitude of the SNAP, blood flow values tended toward higher, rather than lower levels in patients with more severe DPN (higher flow with lower action potential amplitudes and lower myelinated axon density). Similarly, in the patient who completed the 1-year follow-up contralateral biopsy tended to have higher flow values despite more neuropathy. Overall, the findings suggested that declines in sural nerve blood flow were not a requisite for early DPN. Other mechanisms of disease likely contribute toward early axon loss. (Reproduced with permission from Theriault M, et al. Brain 1997;120:1131–1138.)
ate oxygen free radicals that include the hydroxyl radical (OH), superoxide anion (O\textsuperscript{2−}), hydrogen peroxide, singlet oxygen, and organic analogs. Concurrently, antioxidant defenses are lowered in diabetes, allowing targeting of lipids, DNA, and proteins.\textsuperscript{240} The depletion of the amino acid taurine with antioxidant properties has been postulated to aggravate oxidative stress in experimental diabetes.\textsuperscript{120,154,156,165}

Nitricergic stress from the free radical NO may arise from the targeting of proteins with thiol groups, DNA, or activation of PARP [Poly (ADP-ribose) polymerase] that depletes intracellular energy reserves. NO combines with superoxide to generate peroxynitrite (ONOO\textsuperscript{−}), a highly potent oxidizing agent that nitrates protein tyrosines and can eventually lead to cell death.\textsuperscript{136} It has been suggested that nitricergic stress plays a significant role in the development of neuropathy. Nitric oxide synthase activity in ganglia and nerve is increased, and there are footprints of peroxynitrite toxicity in ganglia (nitrotyrosine).\textsuperscript{40,102,279} PARP inhibitors or PARP-null mice had attenuated experimental DPN but the mechanism was thought to be through arresting microangiopathy.\textsuperscript{121,152,155}

The idea that free radical damage underlies neuron damage in DPN has thus tentatively linked several known abnormalities in diabetes. For example, excessive AR flux may deplete the antioxidant GSH within cells.\textsuperscript{239} AGEs may generate reactive oxygen species and deplete GSH. Reactive oxygen and nitricergic species may also target mitochondria.\textsuperscript{239}

AGEs (advanced glycosylation endproducts) are products of the nonenzymatic reactions (glycation) of glucose with amino groups on proteins through the Maillard reaction.\textsuperscript{13,28} During an initial glycation reaction that is potentially reversible, intermediates known as Schiff bases are formed and then converted into an Amadori adduct known as fructoselysine. Further irreversible glycation and oxidation generates glycoxidation products or AGEs, which are permanently deposited. Proteins are structurally modified. The prototypes (although over a dozen are known) measured most often are CML [N\textsuperscript{ɛ}-carboxymethyl] lysine] and pentosidine. AGEs are thus formed by oxidative stress but also contribute to it by catalyzing lipid peroxidation. In turn, AGEs may alter myelin, the extracellular matrix, vasoreactivity (quenching of nitric oxide), and nerve structural proteins. They also directly act on specific receptors, one of which is RAGE (receptor of AGEs) identified by Schmidt et al.\textsuperscript{179,180} AGE−RAGE signaling then activates NF-κB, a nuclear transcription factor that is critical to survival signaling of peripheral neurons,\textsuperscript{68} although excessive activation may mediate cellular dysfunction\textsuperscript{144} (reviewed elsewhere\textsuperscript{213,248}). There are early indicators that polyol flux, nitrergic stress, and AGE−RAGE signaling are linked in important ways. For example, consumption of NO by AGEs in diabetes may contribute to loss of vasodilation (functional microangiopathy) in diabetic microvessels, as discussed earlier.\textsuperscript{32,33,47,49,87,111,117,229,245}

**Mitochondrial Dysfunction.** Mitochondria may be primary targets of oxidative stress and perhaps growth factor deficiency in diabetes. Mitochondria are essential for the bioenergetic reserve of cells including neurons and the formation of permeability transition pores can release cytochrome-c and initiate an apoptotic cascade.\textsuperscript{83} The presence of morphological changes in neuronal mitochondria such as vacuolation is questionable, however, and may be artifactual.\textsuperscript{123} Alternatively, molecular changes in mitochondrial function triggered by oxidative stress may be critical. Along these lines, alterations in mitochondrial depolarization and calcium flux, reversible with growth factors including insulin, have been described.\textsuperscript{49,192} Interestingly, insulin receptors have been localized to mitochondria in sensory neurons.\textsuperscript{219}

**Neurodegeneration.** DPN is a neurodegenerative disease. It is truly a “dying back” disorder with loss of distal axons before dropout of the entire neuron tree. The idea that large-scale catastrophic apoptosis of parent neuron soma or perikarya develops early in the disease has not been supported by a number of investigations.\textsuperscript{50,176,280} Instead, there is gradual withdrawal of terminals of peripheral neurons from their target organs, with later gradual loss of parent perikarya (Fig. 3). This does differ somewhat from the original concepts of “dying back” where the lesion was considered exclusively distal or axonal. Instead, there are concurrent alterations of perikaryal function and gene expression that help support distal axons. Evidence for the idea of a unique diabetic neurodegeneration has not been easy to acquire, but has emerged from more recent models. For example, in long-term experimental STZ-induced diabetes in rats, neither neuron nor sural nerve axon dropout can be unequivocally documented using rigorous three-dimensional counting approaches.\textsuperscript{280} Axons and sensory neurons in ganglia atrophy, and there may be loss of very distal epidermal skin axons. Concurrent with atrophy is loss of neurofilaments investing axons, an important determinant of their caliber. These changes do take some time to develop; they may not be evident, for example, after
only 2 months of experimental diabetes but instead are found after 6–12 months. Both myelinated and unmyelinated axons exhibit this change, and its development correlates with downregulation of mRNA levels of all three neurofilament subunits in the cell body. Abnormalities of neurofilament phosphorylation, as well as deficits in neurofilament synthesis and investment, are also a feature of DPN. Parenthetically, axon atrophy likely does not explain the much earlier slowing of conduction velocity demonstrable in the models, more likely to be related to changes in axon excitability, accumulation of intraneuronal sodium, and declines in Na/K ATPase activity.

As discussed earlier, different models offer differing insights into the pathogenesis of DPN. DPN in rats rendered diabetic with STZ is manifest as motor and sensory conduction velocity slowing, RICF, mild sural sensory axon atrophy, but relative preservation of neuron and axon numbers, even until late stages of the disease (e.g., 12 months). Conduction slowing develops early, within 2 weeks of the onset of hyperglycemia. Rises in “apoptosis or stress markers” without frank neuron loss may occur, and there are shifts of neuron sizes to smaller size categories with only an apparent loss of large neurons. Markers of cellular “stress” expression include activated caspase 3 and elevations of HSP-27 (heat shock protein-27), and PARP. Long-term models of STZ rat diabetes also highlight a number of molecular changes that precede dropout. In addition to loss of neurofilaments, there is loss of α1-tubulin mRNA, the building block of microtubules; αCGRP, a peptide that has roles in pain neurotransmission and microvascular function; and GAP43/B50, the growth cone protein. Molecules altered in sensory neurons by diabetes also include declines in neurotrophin receptors, peptides, Na1.8, PKCβ-II, and CREB with rises in ERK, JNK, p38, insulin receptor, RAGE, and Na1.4, 1.6, and 1.9. Recent work using microarrays has also mapped early changes serially in rat DPN models for up to 8 weeks. Significant downregulation was noted for a ubiquinone subunit, MAG, and three as

FIGURE 3. Hypothetical scheme of sensory neuron degeneration in progressive DPN. (A) The neuron structure is intact but there are early metabolic and electrophysiological changes such as slowed conduction velocity. (B) There is early axon atrophy and loss of epidermal axons. At this stage early changes in perikaryal gene expression occur. (C) There is loss of distal axon integrity and perikaryal atrophy. (D) There is further axon loss and loss of central branches of the sensory neuron. Perikarya at this stage have rises in apoptotic markers. (E) There is irretrievable neuron loss.
yet unclarified molecules. There was upregulation of tau, a sodium channel (type IV beta), a potassium-channel interacting protein, and two unclarified additional molecules. Additional genes were categorized as being influenced by the duration of diabetes, and changes were categorized for those involved in glucose metabolism, synaptic vesicle formation, and extracellular matrix formation. Overall, these findings are of considerable interest but require verification. A variety of approaches will be required that confirm protein expression and functional significance in several long-term models and humans. Also, microarrays of ganglia do not strictly distinguish whether genes of interest are altered in neurons or other cells within them, such as satellite cells.

In contrast to the STZ rat model of DPN, STZ diabetes in mice may be more akin to human disease despite lower polyol levels. The pancreatic beta cell toxicity of STZ can be variable in mouse strains, with some resistance to its actions. Although this does not indicate resistance to the development of DPN, differing injection regimens of STZ may be needed to induce comparable levels of hyperglycemia. Once this is accomplished, however, the advantage of mouse models is the ability to superimpose diabetes in mouse strains with specific molecular alterations. STZ diabetes in simple Swiss–Webster mice or CD1 mice is associated with slowed motor and sensory conduction velocity, loss of very distal sural axons, and denervation of sweat glands. In models of sufficient duration (6–9 months), unlike STZ rats, mice have overt and unequivocal loss of DRG sensory neurons (~20%). At the same timepoints, however, motor neuron caliber and numbers are well preserved, highlighting how DPN can be selective. Finally, STZ mice with DPN also exhibit thinning of myelin sheaths, not generally observed in STZ rats, but a feature of human disease as well. Interestingly, a small subset of mice given STZ recover pancreatic beta cell function after a few months and revert to euglycemia. This subset was of particular interest, since it offered some ideas on what humans undergoing successful islet beta cell transplant might experience. Electrophysiological changes, myelin thinning, epidermal denervation, and sweat gland denervation were improved in the mice that recovered (Fig. 4). Sensory neuron dropout, in the DRG, however, was not reversed. These observations indicated that collateral reinnervation of target tissues, such as the epidermis, from preserved sensory neurons can be associated with clinical recovery. Strategies aimed at preserving sensory neurons and allowing them to reinnervate collaterally may offer hope for patients even with severe DPN.

From the transgenic mice studied to date with superimposed diabetes, several interesting observations have emerged. In a mouse model in which axons were not invested with any neurofilaments, electrophysiological and morphological features of DPN were accelerated. This is important because it suggested that diabetic neurofilament abnormalities, rather than generating the changes of DPN, may instead protect axons to some extent from its progression. DPN has been studied in thy1-YFP mice that have fluorescent axons: cutaneous axons from these mice, evaluated noninvasively, progressively disappeared during 6 months of experimental diabetes.

Experimental models of autonomic neuropathy in rodents have been extensively examined in the Schmidt laboratory. A finding of particular interest has been the discovery of axonal dystrophic changes (neuroaxonal dystrophy), particularly in proximal axons within sympathetic ganglia but also in distal axons. These dystrophic changes, also identified in humans, may represent accumulations of neurofilaments without frank cellular loss and are in keeping with a progressive neurodegenerative disorder. They may represent aberrant intraganglionic sprouts. Abnormalities of diabetic autonomic ganglia in models also correlated with changes in gene expression. Overall, differential changes included transcripts involved in synapse and mitochondrial structure and function, oxidative stress, and glycolysis.

An important set of models of DPN in rats has been discovered and generated by the Sima laboratory. Although more expensive and not studied widely in other laboratories, these models have been rigorously examined. The BB/W rat, a type 1 model, develops diabetes at about 70 days of age because of an autoimmune-mediated destruction of pancreatic beta cells. These rats develop slowing of motor and sensory conduction velocity as well as more marked structural alterations of axons (including loss) than STZ rats. A particular structural abnormality is axoglial dysjunction, in which paranodal axon and Schwann cell contacts are disrupted. There are associated electrophysiological and molecular changes linked to this alteration. More recently, this BB/W model has been compared to a newer model of type II diabetes known as BB/Z. Differences in this model include a slower evolution of electrophysiological abnormalities, less axonal structural damage, no axoglial dysjunction, and more targeting of Schwann cells and myelin.
Insulin Deficiency and Neuronal Growth Factors.

DPN may involve failed signaling from growth factors that act on neurons\(^2\) but the results of clinical trials using them have been disappointing.\(^7,247\) A number of neuronal growth factors share downstream signaling cascades that promote survival and outgrowth, but any presumed efficacy in treating DPN would depend on whether the involved neurons express relevant receptors. For example, in diabetes it is unlikely that a single growth factor such as NGF (nerve growth factor) protects all types of neurons against damage because most cases of DPN involve both large- and small-fiber involvement. Elegant work targeting DRG sensory neurons with herpes simplex-mediated transfer of NGF has, however, demonstrated substantial benefit in a mouse DPN model.\(^78\)

Insulin itself is a highly potent neuronal growth factor that is capable of acting on receptors expressed on most sensory neurons and on their axons. To influence neuronal function, insulin receptors signals share signaling cascades with neurotrophin growth factors.\(^31,99,220,255\) Insulin-receptor expression is upregulated by injury, and its ligation by insulin can promote regeneration of distal axon branches, even if given intrathecally.\(^78,225\) Insulin receptors have been identified on mitochondria, and insulin can reverse their inappropriate depolarization during diabetes.\(^91\) Overall, then, insulin receptors are autophosphorylated by insulin binding, develop tyrosine kinase activity, and signal through the IRS-1 and 2 docking protein pathway.\(^250\) IRS-1 contains multiple serine/threonine and tyrosine phosphorylation sites that signal the survival kinases PI-3K-Akt, as well as Shc,Grb-2,S6 kinase, PKC\(\epsilon\) kinase, MAP2 kinase, Raf1 kinase, and c-fos.\(^66,69,86\) PI3K-PDK1-Akt block apoptosis by interacting with BAD, caspase-9, NF-\(\kappa\)B, and the forkhead transcription factor FKHRL1.\(^30,62,146\) (reviewed elsewhere.\(^70,84\)) Since inappropriate activation of NF-\(\kappa\)B by RAGE and

**FIGURE 4.** Examples of epidermal axon innervation of the skin of footpads of mice with experimental DPN. (A) Diabetic (note loss of axons); (B) recovered from diabetes; (C) nondiabetic controls; (D) negative control (no primary antibody). Despite loss of ganglion sensory neurons in this model, collateral innervation permitted regrowth of epidermal axons. (Copyright © 2005 American Diabetes Association. From Diabetes, 2005;54:830–837. Reprinted with permission from The American Diabetes Association.)
caspases are implicated in diabetic neuron degeneration, this survival pathway is of particular interest. The PI3k-Akt pathway is also locally activated in distal axon terminals and growth cones to promote outgrowth. Another important set of intracellular signals that influence survival, the MAPK pathway, is also altered in diabetes.

Insulin is related to insulin-like growth factors (IGFs) in that they can cross-occupy each other’s receptors and share downstream survival transduction pathways. IGFs circulate but are also produced in glial cells and IGF-1Rs are also expressed on Schwann cells, where they promote myelination. IGF-1 knockout mice have features similar to DPN. C-peptide, the cleaved fragment of the insulin prohormone, has also shown benefit in DPN in preclinical and clinical work. The mechanism may be through enhancement of insulin (or possibly IGF-1) signaling on neurons or axons.

In rats with DPN, studied in our laboratory, low-dose intermittent insulin applied near nerve reversed sciatic motor conduction slowing independently of blood glucose levels; the contralateral nerve, exposed to the carrier, but not to insulin itself, applied identically in the same animals developed the expected conduction slowing. Similar reversal of conduction slowing, axonal atrophy, and epidermal axon loss in rats with DPN occurred when low-dose insulin was applied intrathecally (Fig. 5). Insulin applied intrathecally accessed motor and sensory neurons, yet had no impact on systemic hyperglycemia. Identical doses administered subcutaneously over the back without intrathecal placement did not reverse DPN. In additional work, sequestering of endogenous local CSF insulin using an anti-insulin antibody generated conduction slowing and atrophy resembling DPN in nondiabetic rats, whereas unrelated antibody infusions had no impact.

Overall, these findings have suggested that normal CSF insulin has an important role in the support of peripheral neurons. Among patients receiving insulin therapy, insulin may yet be relevant to development of DPN because of inadequate doses or

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**FIGURE 5.** Intrathecal administration of insulin to rats is capable of accessing sensory neurons in lumbar ganglia (A). Insulin was labeled with the fluorochrome FITC; sensory neurons in the ganglia are outlined by insulin labeling of their membranes. This route of administration reversed electrophysiological and structural features of experimental DPN without influencing systemic glucose levels. Identical section under light microscopy (B). Scale bar, 20 μm. Insulin can signal through receptors expressed on peripheral sensory neurons (C). Sections of mouse dorsal root ganglia are labeled with an antibody to the β subunit of the mouse insulin receptor. Axons in the ganglia are also labeled. (A,B: Copyright © 2004 American Diabetes Association. From Diabetes, 2004;53:1824–1830. Reprinted with permission from The American Diabetes Association.)
because the type of intermittent dosing applied is nonphysiological. Insulin may not be available to signal neurons partly protected by a blood–tissue barrier. Finally, resistance to insulin action, a defect in type II diabetes, could potentially operate at the neuronal level.

**Impaired Regeneration.** Diabetes mellitus imposes a “double hit” on the peripheral nervous system because it is associated with neuropathy with axon damage, and also impairs the ability of axons to regenerate. The topic of failed nerve regeneration in diabetes has been recently reviewed elsewhere. If therapy is identified that is capable of arresting diabetes-induced neurodegeneration, an important challenge will then be to support the regeneration of axons necessary to restore function. In addition, recovery from common diabetic focal neuropathies, such as CTS or UNE, may critically depend on how regeneration fares. Potential mechanisms of impaired regeneration in diabetes, briefly listed, include impaired sprouting of injured axons, a delay in how axons alter their synthesis of regeneration proteins in response to injury, slowed Wallerian and Wallerian-like degeneration including macrophage invasion of the distal stump of injured nerves, accelerated retrograde loss of neurons, alterations in adhesive extracellular matrix, failure of microvascular plasticity after injury necessary to support regrowth, altered elaboration of local NO for vasodilation and myelin clearance, failure of insulin or IGF-1 support, and failed wound healing of neighboring tissues necessary to support axons.

There has not been an extensive literature on the development of mononeuropathies in experimental diabetes. Rats reared on wire cage flooring, instead of sawdust or shaving covered plastic flooring, develop electrophysiological evidence of an entrapment neuropathy of their very distal paw motor nerves (Fig. 6). Diabetics had earlier involvement, similar to the predisposition to entrapment observed in humans. Nerves in experimental diabetes are also more susceptible to ischemia than those of nondiabetics, as demonstrated in nerve ischemia models using microspheres or exposure to the vasoconstrictor endothelin. In our laboratory we have also examined a model of dorsal root ganglia ischemia from local endothelin exposure. The ischemia induced neuron necrosis and apoptosis, intraganglionic axonal degeneration with neuron cell body responses, and downstream degeneration of sensory axons. Diabetics were more sensitive to damage.

**Translation Therapy**

**Primary Forms of Therapy.** No form of therapy in DPN has been identified that provides unequivocal, safe, and effective stabilization or reversal of the condition. There have been many trials, most providing only modest disease stability or no improvement. The reasons for this disappointing record can be identified both in the preclinical work and choice of agent (e.g., short-duration conduction velocity models, use of NGF despite its targeting of only small sensory axons or autonomic fibers) but also in trial design and endpoints. Intensive control of hyperglycemia slows the progression of DPN and prevents its appearance in both type I and type II diabetic patients, discussed above. Successful pancreas transplantation associated with euglycemia has been associated with a slow and gradual improvement in DPN.

Results of a number of recent trials are summarized in Table 1. Given the lack of current agreement about their benefits, none at this time are classified as providing level I evidence. Several classes of agents have been examined. There has been, for example, several decades of experience with ARIs, aimed to prevent excessive sorbitol flux in nerve. A meta-analysis has identified some improvements in conduction velocity with a number of ARIs. These trials will not be reviewed in depth here as they have been reviewed elsewhere. In general, ARI trials have been confounded by systemic...
toxicity (renal, hepatic, skin), lack of potency, lack of penetration into the endothelium, and problematic trial design (selection, duration, size). Only one ARI, epalrestat, is currently marketed in Japan.9 The most recent ARIs used in clinical trials have been fidarestat and ranirestat.

Roboxistaurin mesylate was used as a PKCβ inhibitor in 205 patients in a recent Phase II trial but the results were disappointing, with benefits mainly in a subset of patients with less severe DPN.242 Two antioxidants have been identified with some benefits: gamma linolenic acid101 or evening primrose oil and alpha lipoic acid.265 The use of neurotrophic family growth factors has not been beneficial in trials of rhNGF (Phase III) and rhBDNF.7,247 C-peptide administered to human type I diabetic patients was

Table 1. Some recent randomized* double-blinded controlled clinical trials for diabetic polyneuropathy.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Duration (n)</th>
<th>Mechanism</th>
<th>Impact (modality)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therapy for neuropathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ranirestat</td>
<td>12 wks (86)</td>
<td>ARI</td>
<td>Improved (TNS, VPT, CV)</td>
<td>23</td>
</tr>
<tr>
<td>Fidarestat*</td>
<td>52 wks (279)</td>
<td>ARI</td>
<td>Improved (F wave CV, symptoms)</td>
<td>90</td>
</tr>
<tr>
<td>Ruboxistaurin mesylate</td>
<td>52 wks (205)</td>
<td>PKCβ inhibitor</td>
<td>Benefit in Subgroup only</td>
<td>242</td>
</tr>
<tr>
<td>Alpha-lipoic acid IV&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3 wks (1258)</td>
<td>Anti-oxidant</td>
<td>Improved (symptoms, sensation)</td>
<td>265</td>
</tr>
<tr>
<td>MIRE</td>
<td>4 wks (39)</td>
<td></td>
<td>None</td>
<td>44</td>
</tr>
<tr>
<td>QR-333</td>
<td>4 wks (34)</td>
<td>Topical flavanoid (?ARI), ascorbyl palmitate, Vitamin D3</td>
<td>Improved (symptoms, QOL)</td>
<td>236</td>
</tr>
<tr>
<td>rhBDNF</td>
<td>12 wks (30)</td>
<td></td>
<td>No benefit</td>
<td>247</td>
</tr>
<tr>
<td>Acetyl-L-carnitine</td>
<td>52 wks (1257)</td>
<td>Growth factor</td>
<td>Improved (morphometry, VPT, ?pain, not CV)</td>
<td>202</td>
</tr>
<tr>
<td>C-peptide</td>
<td>12 weeks (49)</td>
<td>?insulin sensitizer</td>
<td>Improved (sensory CV, VPT)</td>
<td>64</td>
</tr>
<tr>
<td>Gamma-linolenic acid</td>
<td>52 wks (111)</td>
<td>Unknown</td>
<td>Improved (conduction, sensation, strength)</td>
<td>101</td>
</tr>
<tr>
<td>rhNGF</td>
<td>52 wks (1019)</td>
<td>Growth factor</td>
<td>No benefit</td>
<td>7</td>
</tr>
<tr>
<td>Pain Therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duloxetine†</td>
<td>12 wks (348)</td>
<td>SSNRI</td>
<td>↓ pain scales</td>
<td>169</td>
</tr>
<tr>
<td>Duloxetine†</td>
<td>12 wks (457)</td>
<td>SSNRI</td>
<td>↓ pain scales</td>
<td>77</td>
</tr>
<tr>
<td>Oxcarbazepine</td>
<td>16 wks (146)</td>
<td>AED</td>
<td>↓ VAS</td>
<td>48</td>
</tr>
<tr>
<td>Oxcarbazepine</td>
<td>16 wks (347)</td>
<td>AED</td>
<td>Primary endpoint negative</td>
<td>14</td>
</tr>
<tr>
<td>CR Oxycodeone&lt;sup&gt;f&lt;/sup&gt;</td>
<td>6 wks (159)</td>
<td>Opioid</td>
<td>↓ pain scales</td>
<td>76</td>
</tr>
<tr>
<td>Zonisamide</td>
<td>12 wks (42)</td>
<td>AED</td>
<td>none</td>
<td>8</td>
</tr>
<tr>
<td>Lamotrigine&lt;sup&gt;f&lt;/sup&gt;</td>
<td>6 wks (59)</td>
<td>AED</td>
<td>↓ pain scales</td>
<td>63</td>
</tr>
<tr>
<td>Pregabalin†</td>
<td>12 wks (42)</td>
<td>Ca channel blocker</td>
<td>↓ pain scales but lumped with nonDPN</td>
<td>72</td>
</tr>
<tr>
<td>Pregabalin†</td>
<td>6 wk (246)</td>
<td>Ca channel blocker</td>
<td>↓ pain scales</td>
<td>171</td>
</tr>
<tr>
<td>Pregabalin†</td>
<td>8 wks (146)</td>
<td>Ca channel blocker</td>
<td>↓ pain scales</td>
<td>173</td>
</tr>
<tr>
<td>Pregabalin†</td>
<td>5 wks (338)</td>
<td>Ca channel blocker</td>
<td>↓ pain scales</td>
<td>119</td>
</tr>
<tr>
<td>Sodium valproate</td>
<td>12 wks (43)</td>
<td>AED</td>
<td>↓ pain scales</td>
<td>112</td>
</tr>
<tr>
<td>Sodium valproate</td>
<td>6 wks (31)</td>
<td>AED</td>
<td>No benefit</td>
<td>160</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>6 wks (244)</td>
<td>SSNRI</td>
<td>↓ pain scales</td>
<td>174</td>
</tr>
<tr>
<td>Topiramate&lt;sup&gt;g&lt;/sup&gt;</td>
<td>18–22 wks (1259)</td>
<td>AED</td>
<td>No benefit</td>
<td>225</td>
</tr>
<tr>
<td>Topiramate</td>
<td>12 wks (323)</td>
<td>AED</td>
<td>↓ pain scales</td>
<td>170</td>
</tr>
<tr>
<td>Isosorbide dinitrate spray</td>
<td>4 wks (22)</td>
<td>NO donor</td>
<td>↓ pain scales</td>
<td>280</td>
</tr>
<tr>
<td>Tramadol&lt;sup&gt;f&lt;/sup&gt;</td>
<td>42 days (131)</td>
<td>Weak opioid, SSRI</td>
<td>↓ pain scales</td>
<td>85</td>
</tr>
<tr>
<td>Morphine,† gabapentin or both</td>
<td>5 wks (35 DPN)</td>
<td>Opioid and AED</td>
<td>↓ pain scales</td>
<td>75</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>2 wks (29)</td>
<td>SSRI</td>
<td>↓ pain scales</td>
<td>211</td>
</tr>
</tbody>
</table>

AED antiepileptic drug; ARI, aldose reductase inhibitor; CV conduction velocity; MIRE monochromatic infrared energy; n total number of patients in trial, all groups; QOL quality of life; SSNRI selective serotonin and norepinephrine reuptake inhibitor; VAS visual analogue pain scale; VPT vibration perception threshold.

*This trial was not labeled as randomized; † Level 1 evidence of efficacy.
<sup>a</sup>This was a meta-analysis.
<sup>b</sup>Endpoint criteria for this negative study have been criticized. An open-label trial showing benefit has been published in abstract form.243

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associated with improved sensory conduction velocity and VPT but no change in heat or cold sensation after 12 weeks in a small study of 49 patients. Multiple surgical decompression of peripheral nerves in diabetic patients is of no proven benefit and should be discouraged.

For DLSP, effective therapy has not been established in published randomized controlled trials. A randomized controlled trial of methylprednisolone was reported in abstract form as improving symptoms (especially pain) but not disability in DLSP. Despite claims otherwise, no definite role for intravenous gamma globulin has been demonstrated. Our own experience in a small series of three patients with DLSP was disappointing, with overt progression despite immunosuppression and IVIG.

**Therapy for Neuropathic Pain.** It is hoped that approaches toward the reversal of DPN would concurrently improve neuropathic pain. As reversal DPN therapy is currently not available, however, specific therapy for neuropathic pain is required in many patients. Most currently available treatments are used for all forms of peripheral neuropathic pain and will only be briefly summarized here (for a comprehensive and recent review, see Vinik). Table 1 lists some recent double-blind randomized controlled clinical trials for newer agents, including those with level 1 evidence of benefit. Those treatments not listed, but with level 1 evidence to support their use, include tricyclic antidepressants, mezipaline, and anti-epileptic agents including phenytoin and carbamazepine. Gabapentin, a weak anti-epileptic agent, also with level 1 evidence for efficacy, is a safe treatment for neuropathic pain with a wide therapeutic range. Many clinicians consider it first-line therapy. In patients with significant renal failure from diabetic nephropathy, the dose of gabapentin should be titrated to a lower therapeutic range, as it is excreted renally. Opioids including oxycontin have level 1 evidence to support their use in diabetic neuropathic pain.

Exacerbated constipation and cognitive dysfunction are side effects that limit their use. The combination of morphine and gabapentin had synergistic benefits in a group of patients that included those with painful DPN. Tramadol is a weak opioid and SSRI (selective serotonin reuptake inhibitor) that improves neuropathic pain. Newer agents also hold some promise for diabetic neuropathic pain and include pregabalin, and duloxetine, a serotonin and norepinephrine uptake inhibitor. The role for cannabinooids is as yet undetermined and local skin application of local anesthetics or capsaicin have had mixed benefits.

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**REFERENCES**


88. Isho Y, Chang K, Lejeune W, Tilton RG, Monafo WW, Wil- liamson JR. Diabetes impairs sciatic nerve hyperemia in-


testing and peripheral nerve electrophysiology. Acta Neuro-

136. Mallozzi C, Di Stasi AM, Minetti M. Peroxynitrite modulates
 tyrosine-dependent signal transduction pathway of human

137. Max MB, Lynch SA, Muir J, Shofa SE, Smoller B, Dubner R.
Effects of desipramine, amitriptyline, and fluoxetine on pain

138. McQuay H, Carroll D, Jadad AR, Wiffen P, Moore A. Anti-
convulsant drugs for management of pain: a systematic re-

139. Merlot S, Firtel RA. Leading the way: directional sensing
through phosphatidylinositol 3-kinase and other signaling

140. Middelms A, Delcroix JD, Sayers NM, Tomlinson DR,
Fernyhough P. Enhanced activation of axonally transported
stress-activated protein kinases in peripheral nerve in dia-
betic neuropathy is prevented by neurotrophin-3. Brain
2003;126:1671–1682.

141. Mizisin AP, Li L, Calcutt NA. Sorbitol accumulation and
transmembrane efflux in osmotically stressed SJL schwan-

142. Mizisin AP, Powell HC. Schwann cell changes induced as
early as one week after galactose intoxication. Acta Neuro-
pathol 1997;93:611–618.

143. Mizisin AP, Powell HC, Myers RR. Edema and increased
endoneurial sodium in galactose neuropathy. Reversal with

144. Mohamed AK, Bierhaus A, Schiekofer S, Tritschler H,
Pop-Busui R, Marinescu V, Van Huysen C, Sullivan K,
Greene DA, et al. Dissection of metabolic, vascular, and
nerve conduction interrelationships in experimental dia-
betic neuropathy by cyclooxygenase inhibition and acetylL-

145. Middlemas A, Delcroix JD, Sayers NM, Tomlinson DR,
Fernyhough P. Role of poly(ADP-ribose) polymerase activation in

146. Merlot S, Firtel RA. Leading the way: directional sensing
through phosphatidylinositol 3-kinase and other signaling

Neuropathies associated with diabetes mellitus. Neurology

148. Nukada H. Mild ischemia causes severe pathological
changes in experimental diabetic nerve. Muscle Nerve 1992;
15:1116–1122.

Sural nerve oxygen tension in diabetes. Br Med J 1986;293:
1053–1054.

150. Nukada H. Mild ischemia causes severe pathological
changes in experimental diabetic nerve. Muscle Nerve 1992;
15:1116–1122.

151. Oates PJ. Polyal phoy pathway and diabetic peripheral neuropa-

152. Ochoosova IG, Drel VR, Pacher P, Ilnytska O, Wang ZQ,
Stevens MJ, et al. Oxidative-nitrosative stress and poly(ADP-
ribose) polymerase (FARP) activation in experimental dia-
betic neuropathy: the relation is revisited. Diabetes 2005;
54:3435–3441.

153. Ochoosova IG, Fathallah L, Stevens MJ. Taurine counteracts
oxidative stress and nerve growth factor deficit in early ex-
perimental diabetic neuropathy. Exp Neurol 2001;172:211–
219.

154. Ochoosova IG, Li F, Abatan OF, Forsell MA, Komjati K, Pacher
P, et al. Role of poly(ADP-ribose) polymerase activation in

155. Ochoosova IG, Van Huysen C, Fathallah L, Stevens MJ. Effect of dietary taurine supple-
mintion on GSH and NAD(P)-redox status, lipid peroxi-
dation, and energy metabolism in diabetic precataractous

156. Ochoosova IG, Van Huysen C, Fathallah L, Cao XC, Greene
DA, Stevens MJ. An aldose reductase inhibitor reverses early
diabetes-induced changes in peripheral nerve function, meta-
tabolism, and antioxidative defense. FASEB J 2002;16:123–
125.

157. Orchard TJ, LLoyd CE, Masen RE, Kuller LH. Why does
diabetic autonomic neuropathy predict IDDM mortality? An
analysis from the Pittsburgh Epidemiology of Diabetes Com-
S165–S171.

158. Oskarsson P, Ljunggren JG, Lins PE. Efficacy and safety of
melixetine in the treatment of painful diabetic neuropathy.
1597.

159. Otto M, Bach FW, Jensen TS, Sindrup SH. Valproic acid has
no effect on pain in polyneuropathy: a randomized, con-

160. Perkins BA, Oalaye D, Zimman B, Bril V. Simple screening
tests for peripheral neuropathy in the diabetes clinic. Dia-
betes Care 2001;24:1225–1226.

161. Pirart J. Diabetes mellitus and its degenerative complica-
tions. A prospective study of 4,400 patients observed between

162. Pirart J. Diabetes mellitus and its degenerative complica-
tions. A prospective study of 4,400 patients observed between

163. Poldedefis M, Hauer P, Griffin JW, McArthur JC. Skin biopsy
as a tool to assess distal small fiber innervation in diabetic

164. Pop-Busui R, Marinescu V, Van Huysen C, Li F, Sullivan K,
Greene DA, et al. Dissection of metabolic, vascular, and
nerve conduction interrelationships in experimental dia-
betic neuropathy by cyclooxygenase inhibition and acetylL-

Identification of changes in gene expression in dorsal root
ganglia in diabetic neuropathy: correlation with functional

166. Pugliese G, Tilton RG, Speedy A, Chang K, Santarelli E,
Providence MA, et al. Effects of very mild versus overt diabetes
on vascular haemodynamics and barrier function in rats.

167. Quatrini C, Jeziorska M, Malik R. Small fiber neuropathy in
diabetes: clinical consequence and assessment. Int J Low

168. Raskin J, Pritchett TL, Wang F, D’Souza DN, Waninger AL,
Iyengar S, et al. A double-blind, randomized multicenter
trial comparing duloxetine with placebo in the management

DM, Xiang J, et al. Topiramate vs placebo in painful diabetic
neuropathy: analgesic and metabolic effects. Neurology

170. Raskin P, Donofrio PD, Rosenthal NR, Hewitt DJ, Jordan
DM, Xiang J, et al. Topiramate vs placebo in painful diabetic
neuropathy: analgesic and metabolic effects. Neurology

171. Richter RW, Portenoy R, Sharma U, LaMoreaux L, Bock-
brader H, Knapp LE. Relief of painful diabetic peripheral
neuropathy with pregabalin: a randomized, placebo-con-

172. Roberts RE, McLean WG. Protein kinase C isoform expres-
sion in sciatic nerves and spinal cords of experimentally

173. Rosenstock J, Tuchman M, LaMoreaux L, Sharma U, Pre-
gabalin for the treatment of painful diabetic peripheral
neuropathy: a double-blind, placebo-controlled trial. Pain

174. Rowbotham MC, Goli V, Kunz NR, Lei D. Venlafaxine ex-
tended release in the treatment of painful diabetic neurop-
110:697–706.
175. Rundles RW. Diabetic neuropathy—general review with re-
178. Said G, Slama G, Selva J. Progressive centripetal degenera-
181. Schmidt RE. The role of nerve growth factor in the patho-
186. Schmidt RE, Dorsey DA, McDaniel ML, Corbett JA. Charac-
187. Schmidt RE, Dorsey DA, Roth KA. Immunohistochemical character-
190. Schmidt RE, Plurad DA, Roth KA. Effects of chronic experimen-
191. Schmidt RE, Plurad SB. Ultrastructural and biochemical character-
193. Schmidt RE, Plurad SB, Sherman WR, Williamson JR, Tilton RG. Effects of aldose reductase inhibitor sorbinil on neuro-
199. Sima AA. Peripheral neuropathy in the spontaneously dia-
201. Sima AA, Hay K. Functional aspects and pathogenetic considera-
206. Sima AA, Lustro AC, Thibert P. Distal symmetric polyneu-


269. Zochodne DW, Ho LT. Diabetes mellitus prevents capsaicin from inducing hyperaemia in the rat sciatic nerve. Diabetologia 1993;36:493–496.


