

# Diabetic neuropathy: cellular mechanisms as therapeutic targets

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**Abstract** | In patients with diabetes, nerve injury is a common complication that leads to chronic pain, numbness and substantial loss of quality of life. Good glycemic control can decrease the incidence of diabetic neuropathy, but more than half of all patients with diabetes still develop this complication. There is no approved treatment to prevent or halt diabetic neuropathy, and only symptomatic pain therapies, with variable efficacy, are available. New insights into the mechanisms leading to the development of diabetic neuropathy continue to point to systemic and cellular imbalances in metabolites of glucose and lipids. In the PNS, sensory neurons, Schwann cells and the microvascular endothelium are vulnerable to oxidative and inflammatory stress in the presence of these altered metabolic substrates. This Review discusses the emerging cellular mechanisms that are activated in the diabetic milieu of hyperglycemia, dyslipidemia and impaired insulin signaling. We highlight the pathways to cellular injury, thereby identifying promising therapeutic targets, including mitochondrial function and inflammation.

Vincent, A. M. *et al.* *Nat. Rev. Neurol.* 7, 573–583 (2011); published online 13 September 2011; doi:10.1038/nrneurol.2011.137

## Introduction

Neuropathy is the most common complication of diabetes, affecting approximately 50% of patients over the course of their disease.<sup>1–3</sup> Patients with diabetes can develop several peripheral nerve disorders, but the vast majority exhibit a distal, symmetric polyneuropathy that starts in the feet and progresses proximally.<sup>4</sup> Neuropathy occurs in both type 1 diabetes (in which the pancreas does not produce enough insulin) and type 2 diabetes (in which either the production of or cellular response to insulin is impaired).<sup>5</sup> Typical symptoms of diabetic neuropathy include pain, numbness, tingling, weakness, and difficulties with balance. The disease is associated with substantial morbidity, including depression, susceptibility to foot or ankle fractures, ulceration and lower-limb amputations.<sup>5–8</sup>

Recent reviews have focused on progress in clinical trials and how to apply current knowledge to the development of rational treatment regimens for patients with diabetes in order to prevent or improve neuropathy. In this article, we aim to move the research forward by discussing our understanding of how metabolic factors influence the development of neuropathy, how the various mechanisms interact, and the potential implications of these insights for future therapeutic interventions.

## Pathophysiological features

Typical diabetic sensory peripheral neuropathy is characterized pathologically as an axonopathy with distal predominance.<sup>9</sup> The disease first affects the longest axons, which innervate the feet; by the time symptoms reach the

knees, the fingers are often affected.<sup>10</sup> Axonal changes are present in both myelinated and unmyelinated fibers, with early development of ‘honeycombed’ Schwann cell–axon networks, and later axonopathy with corrugated myelin breakdown.<sup>11</sup> Regenerating axons are present in human sural nerve and skin biopsy samples but, over the course of the disease, regeneration fails.<sup>12</sup> Reduced blood flow through loss of autonomic nerve functions may contribute to the progression of diabetic neuropathy,<sup>13</sup> and alterations in microvessels, similar to the pathogenic neovascularization described in diabetic retinopathy and nephropathy, also are observed in peripheral nerves.<sup>14</sup>

## Biochemical features

Reduced glycemic control is clearly associated with the development of diabetic neuropathy: both direct glucose measures and levels of glycosylated hemoglobin correlate with the occurrence of neuropathy.<sup>9,15,16</sup> However, the cause of diabetic neuropathy is more complex than dysregulated glucose levels alone. Several contributing factors have been postulated and have received differing degrees of acceptance. In this section of the Review, we emphasize the importance of glucose-mediated injury in the pathogenesis of diabetic neuropathy. However, the presence of many of these mechanisms—such as accumulation of sorbitol, oxidative stress, and 12/15-lipoxygenase activation—before the development of overt hyperglycemia and diabetes in patients with the metabolic syndrome indicates that additional factors must link diabetes with peripheral neuropathy.<sup>17</sup>

Oxidative stress has been considered the final common pathway of cellular injury in hyperglycemia<sup>18</sup> but, as highlighted in this Review, the mechanisms leading to

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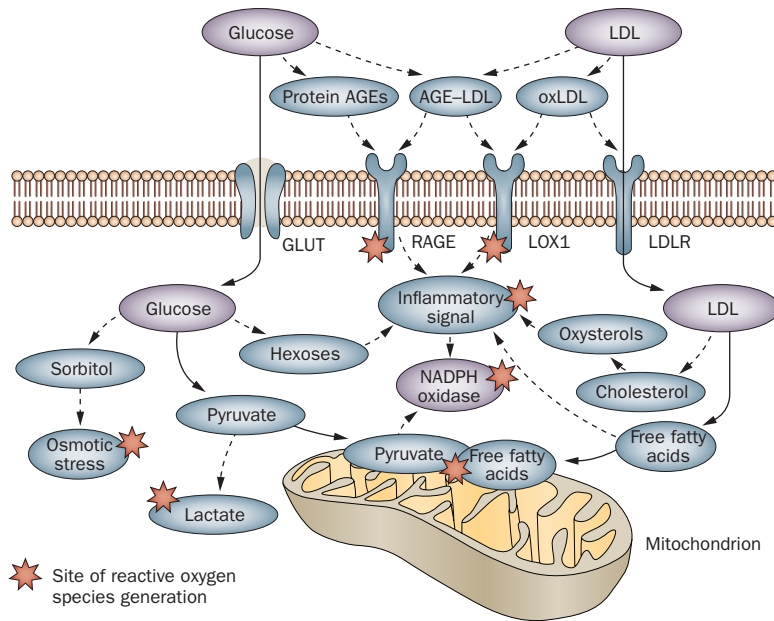
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## Competing interests

The authors declare no competing interests.

**Key points**

- Multiple metabolic imbalances underlie the development of diabetic neuropathy
- Hyperglycemia, dyslipidemia and cardiovascular dysfunction are each independent risk factors for neuropathy
- Targeting risk factors as well as cellular oxidative stress and inflammation will be important in future treatment approaches
- Injury to neurons, Schwann cells and microvascular endothelial cells in the diabetic milieu contributes to the pathogenesis of neuropathy



**Figure 1** | Hyperglycemia and hyperlipidemia activate multiple injury mechanisms in sensory neurons. Glucose and lipoproteins interact with various receptors on neurons and microvascular endothelial cells. Diabetes-modified (that is, oxidized and glycated) proteins and lipoproteins bind additional receptors. These receptors include transporters that internalize glucose and lipids, which can accumulate intracellularly and disrupt mitochondrial metabolic pathways. The receptors also initiate inflammatory signaling mechanisms that directly produce oxidative stress and increase expression and activity of oxidative and nitrosative enzymes. Oxidative stress damages mitochondria and other cellular components, leading to neuronal injury. Abbreviations: AGE, advanced glycation end product; GLUT, glucose transporter; LDLR, LDL receptor; LOX1, oxidized LDL receptor 1; oxLDL, oxidized LDL; RAGE, receptor for advanced glycation end products.

diabetic neuropathy are more complex, and antioxidants alone do not prevent this disorder. Data from preclinical and clinical studies show that in diabetes, oxidative and nitrosative stress are increased in plasma and tissues.<sup>19–23</sup> One exception to this trend is a study suggesting that hyperglycemia does not increase oxidative stress in the dorsal root ganglia,<sup>24</sup> but the data are weak and other studies disagree.<sup>19–23</sup> In this section, we describe biochemical factors that result from the diabetic state and contribute to oxidative stress and nerve damage in diabetic neuropathy (Figure 1).

**Hyperglycemia**

*Disposal of excess glucose*

Several mechanisms of hyperglycemia-induced cellular injury were first described in the vascular endothelium.<sup>18</sup>

Subsequently, these mechanisms were observed in peripheral sensory neurons.<sup>22,25–27</sup> Glucose uptake is less rapidly regulated in neurons than in endothelial cells, which may account for the high susceptibility of neurons to glucose-mediated injury.<sup>28</sup>

Intracellular glucose is principally removed through the process of glycolysis, which generates pyruvate for mitochondrial catabolism to form ATP. Excess pyruvate from glycolysis is thought to injure neurons through two mechanisms. First, the overload of metabolites to the mitochondrial electron transfer chain leads to increased generation of reactive oxygen species,<sup>22</sup> which inhibit the activities of key mitochondrial components such as acinittate hydratase and complex I, resulting in mitochondrial dysfunction.<sup>29</sup> Second, excess pyruvate is shunted to the lactate pathway when oxygen is limiting, as this pathway yields NAD<sup>+</sup>, which is required for continued glycolysis.<sup>30</sup> However, the lactate pathway is a temporary response to low oxygen: lactate cannot be further metabolized and must be converted back to pyruvate when oxygen levels recover. If lactate accumulates and NAD<sup>+</sup> is depleted, glycolysis is inhibited and neuronal functions are impaired.<sup>31</sup>

If glycolysis does not adequately dispose of intracellular glucose, a number of alternative pathways are activated. Aldose reductase reduces glucose to sorbitol, and sorbitol dehydrogenase oxidizes sorbitol to fructose.<sup>32</sup> These activities increase cellular osmolarity and deplete NADPH, both of which lead to oxidative stress.<sup>33,34</sup> Activation of this osmotic stress pathway, involving increased expression of the taurine transporter, is evident in cultured human Schwann cells exposed to hyperglycemia.<sup>35</sup> Excess glucose can also be shunted to the hexose pathway, in which the glycolytic intermediate fructose-6-phosphate is converted, via glucosamine-6-phosphate, to uridine diphosphate-*N*-acetylglucosamine.<sup>36</sup> This molecule modifies serine and threonine residues of specific transcription factors, such as Sp1. These transcription factors are implicated in hyperglycemic inflammatory injury in endothelial basement membranes and pancreatic  $\beta$ -cells.<sup>37</sup> Further evidence suggests that Sp1 is activated in sural nerves of patients with diabetes.<sup>38</sup>

*Formation of advanced glycation end products*

Advanced glycation is a nonenzymatic chemical modification of proteins, lipids and nucleic acids via attachment of reactive carbohydrate groups to exposed sites. In diabetes, the oxidizing environment and increased carbohydrate accumulation accelerate the formation of advanced glycation end products (AGEs).<sup>39</sup> Furthermore, clearance of AGEs from plasma is reduced in diabetic patients with renal impairment.<sup>40</sup>

Protein AGEs produce diabetic neuropathy through two major mechanisms. First, advanced glycation tends to decrease the biological function of proteins, thus inhibiting neuronal activity.<sup>41</sup> Second, extracellular lipid and protein AGEs bind to cell surface receptors, particularly the receptor for AGE (RAGE), initiating an inflammatory signaling cascade that further increases

oxidative stress and neuronal cell injury through the activity of NAD(P)H oxidase<sup>42</sup> and also increases nitrosative stress.<sup>43</sup> In experimental diabetes in rats and mice, the expression of RAGE is elevated in peripheral epidermal axons, sural axons, Schwann cells and dorsal root ganglia neurons, following the pattern of electrophysiological and structural abnormalities associated with neuropathy.<sup>44</sup>

The importance of AGEs in the development of diabetic neuropathy has been largely confirmed using AGE inhibitors such as aminoguanidine and benfotiamine<sup>45</sup> and through studies of RAGE-knockout mice.<sup>44</sup> The AGE–RAGE axis seems to mediate a sustained cellular proinflammatory response that is involved in chronic injury in diabetic complications.<sup>46</sup> These sustained changes involve long-term activation of the proinflammatory transcription factor nuclear factor  $\kappa$ B, and upregulation of RAGE expression following initial RAGE activation. Inhibition or genetic deletion of RAGE significantly reduces diabetic neuropathy in mice and may be a viable therapeutic target in humans, although the physiological role of RAGE is not known.

### Dyslipidemia

Increased plasma lipids, particularly triglycerides and cholesterol, are a feature of the metabolic syndrome, which can lead to diabetes. It has long been known that increased plasma lipids, known as dyslipidemia, are a major risk factor for cardiovascular disease. Clinical epidemiological studies have now demonstrated a similar strong association between dyslipidemia and microvascular complications, including neuropathy in both type 1 and type 2 diabetes. Dyslipidemia is, therefore, an important modifiable parameter in the prevention and treatment of neuropathy in diabetes.<sup>47–49</sup> The mechanisms by which plasma lipids produce neuronal injury are not fully known, but several factors that play a part in lipid-mediated neuropathy have been identified.

Some evidence exists that neuropathy, particularly when it involves loss of autonomic control of the cardiovascular system, is closely associated with vascular disease factors, including obesity, high plasma levels of cholesterol and triglycerides, and high blood pressure.<sup>50,51</sup> In a small study of patients with type 1 diabetes, cardiac autonomic neuropathy was associated with impaired ventricular function but not associated with systemic markers of vascular endothelial dysfunction, suggesting that vascular disease itself may not directly lead to neuronal injury.<sup>52</sup> However, a more thorough examination of risk factors and complications in more than 1,400 patients with type 1 diabetes revealed that a decreased vibration perception threshold, which predicts foot ulceration and amputation, was strongly associated with a previous history of cardiovascular disease.<sup>53</sup> Further work is needed to determine whether elevated lipids have direct effects on peripheral neurons and/or Schwann cells.

Studies of patients with type 2 diabetes more frequently demonstrate a correlation between peripheral sensory neuropathy and peripheral vascular disease than

do studies of patients with type 1 diabetes.<sup>54</sup> In rodents, a high-fat diet leads to accumulation of sorbitol, oxidized lipids and poly ADP-ribose polymerase (PARP), and activation of lipoxygenases in peripheral nerves before the development of diabetes.<sup>17</sup> Metabolic pathways that might mediate neuronal injury in dyslipidemia are described below and illustrated in Figure 1. Cell culture studies suggest that downstream of inflammation and oxidative and nitrosative stress, protein damage may lead to mitochondrion-mediated activation of cell death mechanisms in neurons.<sup>55,56</sup> These molecular modifications also activate the endoplasmic reticulum unfolded-protein response in many cell types, which can lead to cell death through endoplasmic reticulum stress.<sup>57,58</sup>

### Free fatty acids

Free fatty acids cause lipotoxicity in cultured neuronal and Schwann cell lines.<sup>59</sup> This toxicity is mediated through lysosomal dysfunction via mechanisms that are not well understood, but are thought to involve permeabilization of the lysosomal membrane by cathepsin L, leading to oxidative stress and mitochondrion-activated injury.<sup>55,60</sup> Palmitic acid may be the primary free fatty acid that promotes injury of cultured Schwann cells.<sup>61</sup>

In addition to directly affecting cells of the PNS, elevated plasma levels of free fatty acids produce systemic effects that may also promote diabetic neuropathy. Cultured adipocytes and tissue macrophages release inflammatory cytokines that are known to produce peripheral nerve inflammation.<sup>59</sup> Furthermore, increased intramuscular free fatty acids in humans promotes insulin resistance via impaired insulin signaling, thereby blocking glucose disposal.<sup>62</sup> This suggests a mechanism whereby plasma lipids promote hyperglycemia, leading to a synergy of dyslipidemia and hyperglycemia that increases diabetic complications.

### Oxidized and glycated LDLs

In diabetes, plasma lipoproteins are subject to an oxidizing environment. Peripheral sensory neurons, like vascular endothelial cells, express scavenger receptors for oxidized LDLs (oxLDLs), including oxidized LDL receptor 1 (LOX1) and Toll-like receptor 4.<sup>21,63–65</sup> These neurons also express RAGE, which binds glycated LDL.<sup>42</sup> The receptors internalize oxLDL and glycated LDL, releasing potentially injurious triglycerides and fatty acids, and initiate an inflammatory signaling pathway that results in activation of NADPH oxidase.<sup>21,66,67</sup> This enzyme produces substantial cellular oxidative stress by generating superoxide radicals and by depleting NADPH levels. Oxidative stress in diabetes leads to increased expression of oxLDL and RAGE via p38 mitogen-activated protein kinase (MAPK) signaling, producing a positive-feedback mechanism of injury.<sup>44,64</sup>

### Oxysterols

An oxidizing environment increases the oxidation of cholesterol to oxysterols. Oxysterols are known to accumulate in the brain in neurodegenerative diseases such as Alzheimer disease.<sup>68</sup> Recent studies in PC-12 cells have

shown that oxysterol derivatives of cholesterol cause neurotoxicity through mitochondrion-mediated cell death pathways.<sup>69</sup> The concept that oxysterols increase neuronal injury and could have a pathological role in diabetic complications is gaining traction, as oxysterols readily form in patients with diabetes,<sup>70</sup> although there is little clinical evidence to date.

### Insulin resistance

Insulin resistance is the hallmark of type 2 diabetes. Although neurons do not depend on insulin signaling for glucose utilization, a growing body of evidence suggests that peripheral insulin resistance contributes to neuropathy. Notably, neuropathy frequently occurs in patients with impaired glucose tolerance, before the development of diabetes.<sup>71,72</sup>

Recent *in vitro* studies showed that application of insulin to human primary cortical neurons blunts intracellular signaling in response to subsequent exposure to insulin.<sup>73</sup> Moreover, clinical data show that the degree of insulin resistance, as determined by the glucose disposal rate, correlates with the onset of complications including neuropathy, independently of glycemia levels.<sup>74</sup>

Insulin resistance can also develop in type 1 diabetes and is associated with the presence of microvascular complications such as neuropathy.<sup>74</sup> Free fatty acids cause insulin resistance in liver and muscle,<sup>62,75</sup> and are also responsible for cellular inflammation and endoplasmic reticulum stress.<sup>58</sup> Both inflammation and endoplasmic reticulum stress have been observed in neurons under the influence of various pathogenic mechanisms. We suggest, therefore, that free fatty acids that accumulate as a result of insulin resistance may injure peripheral neurons. Peripheral insulin resistance induced by a high-fat diet in rats is mirrored by decreased insulin receptor activity in neurons in the brain, which leads to neuronal stress and injury, and a loss of neurotrophic signaling.<sup>76</sup>

### Current therapeutic strategies

Therapies aimed at blocking the symptoms of painful neuropathy are available, but few options target the root causes of the disease. The immense physical, psychological and economic cost of diabetic neuropathy underscores the need for causally targeted therapies. Antioxidant strategies have been most widely explored, but only  $\alpha$ -lipoic acid, which is part of the standard of care for diabetes in Germany, has shown promise, albeit limited.<sup>77</sup>

### Targeting hyperglycemia

The most informative studies of patients with type 1 diabetes are the Diabetes Control and Complications Trial (DCCT) and the subsequent Epidemiology of Diabetes and its Complications (EDIC), which involved follow-up evaluations of the same cohort of 1,300 patients for over 20 years. Data from these studies have provided important insights into the optimal approach to glycemic control for type 1 diabetes. In the DCCT, intensive insulin therapy early in the disease course to maintain a mean hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) of 7.2% reduced the cumulative incidence of diabetic neuropathy by 60% at 5 years

compared with conventional treatment, which involved less-intensive glycemic therapy (mean HbA<sub>1c</sub> 9.0%).<sup>78</sup>

EDIC, a 20-year follow-up study of the patients in the DCCT, yielded unanticipated results. Within 1 year of beginning EDIC, glycemic control in the two treatment groups equalized to an average HbA<sub>1c</sub> of 8%. All 1,300 patients were assessed annually for diabetic neuropathy. After 8 years, patients from the intensive-therapy DCCT cohort had a lower incidence of diabetic neuropathy than patients from the conventional-therapy DCCT cohort,<sup>79</sup> despite 8 years of comparable glycemic control. These findings underscore the importance of early, intensive glucose control for the prevention of complications, including neuropathy, in diabetes.<sup>78</sup> This study led researchers to propose the idea of glucose or metabolic memory, because early intensive glycemic control results in fewer complications decades later, despite reducing the level of control. Metabolic memory is the subject of ongoing research and probably arises from long-term alteration of gene expression owing to epigenetic changes or onset of vascular disease.<sup>80–82</sup>

The UK Prospective Diabetes Study, which followed a cohort of more than 3,000 patients with type 2 diabetes for over 15 years, provides important insights into the optimal approach to glycemic control in this form of diabetes.<sup>83</sup> In this study, patients who received intensive glycemic therapy (insulin or sulfonylurea) experienced a reduction in the incidence of neuropathy—as measured with a biothesiometer—of approximately 12% compared with patients assigned to a conventional regimen. The difference between the two treatment groups was even greater when all microvascular complications were considered. In type 2 diabetes, however, attempts to intensively control glycemia are associated with increased severe myocardial events and are not recommended for standard care.<sup>83</sup>

### Pain management

Pain is the most severe consequence of neuropathy in terms of patient quality of life, yet it remains undertreated.<sup>84</sup> Often, patients are unaware that pain is a symptom of diabetic neuropathy and fail to report it. In addition, therapies remain variable, and only one-third of patients report at least 50% reduction in pain with therapy.<sup>85</sup> The prevalence of painful diabetic neuropathy is estimated to be 18% in type 2 diabetes and 6% in type 1 diabetes, and the incidence increases with age and diabetes duration.<sup>84</sup> An evidence-based review of clinical trial data established that pregabalin is reasonably effective for treating pain in diabetic neuropathy, and showed that venlafaxine, duloxetine, amitriptyline, gabapentin, valproate, opioids and capsaicin are probably effective and should be considered for patients who are unresponsive to pregabalin.<sup>86</sup>

Current first-line therapies involve monotherapies or drug combinations that target the specific type of pain, and comprise anticonvulsants (gabapentin and pregabalin), serotonin–noradrenaline reuptake inhibitors (duloxetine), and tricyclic antidepressants (amitriptyline, nortriptyline and desipramine). In clinical trials, these medications have shown a similar number needed



to treat (between three and six patients) to observe a 50% reduction in pain.<sup>87–90</sup>

Clearly, more-efficacious and neuropathy-specific medications are needed. New analgesics show some promise for improved efficacy against pain symptoms. One such compound is tapentadol, a dual-action compound that acts as a  $\mu$ -opioid receptor agonist and nor-adrenaline reuptake inhibitor. Compared with placebo, extended-release tapentadol significantly improved pain and was well-tolerated in 588 randomly assigned patients with type 1 and type 2 diabetes who were dissatisfied with at least 3 months of prior treatment with opioid and/or non-opioid analgesics.<sup>91</sup> A treatment algorithm for the management of pain has been developed and is becoming accepted as the standard of care in patients with painful diabetic neuropathy (Figure 2).<sup>85</sup>

### New therapeutic targets and strategies

A substantial proportion of patients with diabetes develop neuropathy despite intensive glycemic control. This was clearly shown in the Steno-2 study, in which patients treated with a multifactorial intervention including aspirin, statins, renin–angiotensin blockers, glycemic control, and lifestyle modifications nevertheless developed diabetic complications at a high rate.<sup>92</sup> An unmistakable need exists, therefore, for new treatment strategies. A selection of the most promising of these strategies is discussed below, and a summary of compounds in development is presented in Table 1. Most clinical trials have produced disappointing results, but they have often been confounded by a high rate of improvement in the placebo group, or other unanticipated effects.<sup>93</sup> Furthermore, failure of new drugs in the long term probably results from the multiple mechanisms that contribute to neuronal injury in diabetes (Figure 1).

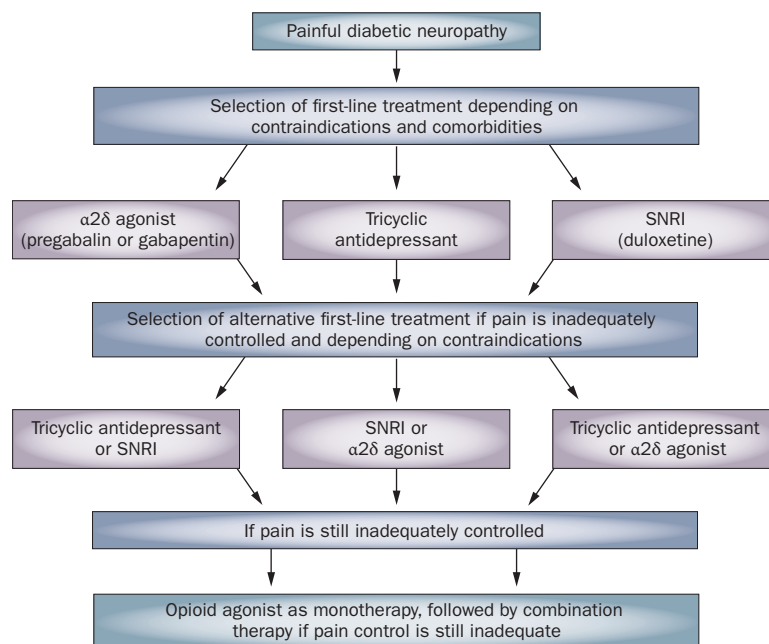
### Cytoprotective therapies

#### Reducing cell death

Opinion in the field of diabetic neuropathy is divided on whether neuronal loss occurs and, if it does, whether cell death involves apoptosis, nonapoptotic programmed cell death, or necrosis. Evidence of apoptosis has been demonstrated in both rodent and cell culture models of neuropathy,<sup>22,60,94–98</sup> but other studies failed to reproduce these findings.<sup>19</sup> The involvement of mitochondria in neuronal injury is generally accepted, implicating an apoptotic mechanism, despite limited evidence for a decrease in the number of dorsal root ganglion neurons *in vivo*.<sup>99</sup> Both lysosomes and mitochondria are implicated in fatty-acid-induced neuronal injury via cathepsin-mediated membrane permeabilization.<sup>60</sup> Activation of caspase 3, a key molecular component of the apoptotic pathway, is generally observed in models of diabetic neuropathy, and several protective compounds with different mechanisms of action, including lipid receptors or antioxidants, block the activation of this apoptosis effector enzyme.<sup>65,100</sup>

#### Inhibiting poly ADP-ribose polymerase

PARP is activated following oxidative damage to DNA. This enzyme adds poly(ADP-ribose) subunits to DNA strand



**Figure 2** | Proposed algorithm for treating pain in diabetic neuropathy. Available data suggest that a tricyclic antidepressant, an SNRI, or an  $\alpha 2\delta$   $\gamma$ -aminobutyric acid receptor agonist (anticonvulsant) should be considered for first-line pain treatment in diabetic neuropathy. Selection of the relevant agent depends on contraindications for a given patient and cost. Following initiation of therapy, patients should be interviewed at each follow-up visit to determine whether their pain, depression, and quality of life have changed. If pain is inadequately controlled, another first-line agent may be considered. Combination therapy may be considered if the patient still reports pain. No clinical trial-based data or guidelines are available for combination therapy, but the combination of an opioid receptor agonist with a tricyclic antidepressant, duloxetine, pregabalin or gabapentin is considered to be appropriate. Abbreviation: SNRI, selective serotonin–noradrenaline reuptake inhibitor. Permission obtained from Sage Publications © Jensen, T. S. *et al. Diab. Vasc. Dis. Res.* **3**, 108–119 (2010).

breaks, converting DNA damage into intracellular signals that activate either DNA repair by the base-excision pathway, or cell death.<sup>101</sup> Inhibition of PARP may slow the progression of diabetic neuropathy by blocking the activation of cell death and by preventing PARP-mediated depletion of NAD<sup>+</sup> and ATP.<sup>102,103</sup> This potential is particularly interesting because PARP inhibitors are currently in clinical trials for cancer treatment<sup>104–106</sup> and, if effective, could rapidly translate to the diabetes clinic.

#### Providing trophic support

Recent studies have used gene and stem cell transfer in rodent models of diabetes to provide neuronal trophic support or promote neovascularization in order to prevent or improve diabetic neuropathy. Clinical data are not yet available, although trials are in progress. In mice with streptozotocin-induced diabetes, gene transfer of neurotrophin-3 using herpes simplex viral delivery into the footpads provided long-term protection against neuropathy.<sup>107</sup> After 5.5 months, mice that had received the construct displayed preservation of action potential amplitudes and conduction velocity in sensory and motor nerves, response to a paw heat stimulus, pilocarpine-induced sweating, and intraepidermal nerve fiber density, unlike mice that received a control herpes simplex viral vector.

**Table 1** | Recent drug trials in diabetic neuropathy

Drug	Proposed mechanism	Preclinical studies	Clinical trial results
Aleglitazar	Dual PPAR $\alpha$ / $\gamma$ agonist	In rats, decreased plasma glucose and LDL cholesterol levels; increased glucose clearance and HDL cholesterol levels; improved insulin resistance <sup>120</sup>	Reduced glycemia in phase II trials; currently in phase III trial for diabetic cardiovascular end points <sup>121</sup>
L-arginine	Improves circulation in microvessels	Produces vasodilation of isolated vessels of all species <sup>122</sup>	No effect on endothelial function or neuropathy score <sup>123</sup>
Zenarestat, epalrestat, ranirestat, fidarestat and five related compounds	Aldose reductase inhibitors	Zenarestat prevented abnormal neurotrophin receptor expression; <sup>124</sup> fidarestat prevented oxidative stress and neuropathy in diabetic rats <sup>125</sup>	Epalrestat is well-tolerated long term <sup>126,127</sup> and approved in Japan; <sup>128</sup> most compounds produce modest improvements in nerve conduction and pain scores; ranirestat seems to improve motor nerve function in mild to moderate disease; <sup>123</sup> fidarestat showed some adverse effects in long-term treatment <sup>33</sup>
$\alpha$ -Lipoic acid	Antioxidant; pyruvate dehydrogenase activator; other unknown mechanisms	Improved nerve and cardiac disorders in diabetic rats <sup>129</sup>	Approved for standard of care in Germany; <sup>77</sup> some evidence that the compound decreases oxidative stress, <sup>130</sup> prevents AGE formation <sup>131</sup> and improves neuropathic deficits; US trials remain inconclusive <sup>132</sup>
Actovegin	Increases cellular metabolism through an unknown mechanism; increases glucose and oxygen uptake and use; increases ATP turnover	Improved brain metabolic defects in rats with experimental stroke <sup>133</sup>	Sequential intravenous and oral delivery over 160 days improved neuropathic symptoms, vibration perception threshold, sensory function, and quality of life <sup>134</sup>
Fibrates	Lipid lowering	Fenofibrate improves insulin sensitivity <sup>135</sup> and other parameters that affect neuropathy, such as vascularization <sup>136</sup> and lipid metabolism <sup>137</sup>	Clofibrate decreases neuropathy; <sup>138</sup> fenofibrate decreases eye and kidney complications; <sup>138</sup> fenofibrate decreases risk of amputation in patients with diabetes but without macrovascular disease <sup>139</sup>
Gabapentin	GABA analogue that blocks new synapse formation <sup>140</sup>	No preclinical data or known mechanism; use of anticonvulsants based on similarities between pathophysiology of diabetic neuropathy and epilepsy <sup>141</sup>	Blocks pain and improves symptoms of cardiac autonomic neuropathy <sup>142</sup>
Acetyl-L-carnitine	Restoring possibly depleted levels in diabetes; required for mitochondrial function	Improved blood flow and sciatic motor nerve conduction velocity in rats with type 1 diabetes <sup>143</sup>	Early treatment may decrease pain; one of two large studies suggested improvement in NCV and nerve regeneration <sup>144</sup>
Pentoxifylline and pentosan polysulphate	Improves circulation in microvessels by blocking phosphodiesterase; antioxidant	Cliastazol, another phosphodiesterase inhibitor, improved NCV in rats with type 1 diabetes <sup>145</sup> but was ineffective in humans <sup>146</sup>	In combination, these compounds improved cardiovascular autonomic function and vibration perception in type 2 diabetes <sup>147</sup>
Benfotiamine	Blocks AGE formation	Decreased AGE levels and diabetic complications in rats <sup>148,149</sup>	Reviews propose testing in patients, but clinical trials have not been instigated <sup>40,41</sup>
C-peptide	Lacking in type 1 diabetes; binds to a G protein-coupled receptor and alters metabolism <sup>150</sup>	Improved blood flow and early neuropathy in rats with type 1 diabetes <sup>151,152</sup>	Short-term use (<3 months) decreased early evidence of NCV slowing, sensory deficits and autonomic neuropathy in patients with type 1 diabetes <sup>153</sup>
Nerve growth factor	Neurotrophic factor	Decreased neuropathy in rats <sup>154</sup> and mice; <sup>155</sup> however, the endogenous form may be responsible for pain in neuropathy <sup>156</sup>	Some efficacy against sensory deficits, but produced painful adverse effects <sup>157,158</sup>
Ruboxistaurin	Akt inhibitor	Decreased microvascular complications in rodents <sup>159</sup>	Seems to be effective against diabetic retinopathy, but no effect on neuropathy in phase III trials <sup>160</sup>
Basic fibroblast growth factor	Stimulates angiogenesis and nerve cell regeneration	Intravenous administration in rats modestly improves blood flow, NCV deficits and hypoalgesia <sup>161</sup>	Not determined

Abbreviations: AGE, advanced glycation end product; GABA,  $\gamma$ -aminobutyric acid; NCV, nerve conduction velocity; PPAR, peroxisome proliferator-activated receptor.

Two studies have investigated the effect of intramuscular injection of endothelial progenitor cells into the hindlimbs of streptozotocin-treated rodents.<sup>108,109</sup> In both cases, the treatment increased neovascularization and blood flow, and preserved sciatic nerve function. In one of the studies, the progenitor cells were obtained from the bone marrow of C57Bl/6J mice and injected into other C57Bl/6J mice that had been diabetic for 12 weeks.<sup>108</sup> Loss of microvessels was reversed, and the injected cells preferentially engrafted

into peripheral nerves and increased the expression of angiogenic neurotrophic factors. Autologous transplants would avoid the risk of rejection by the recipient, making this technique particularly attractive for the reversal of neuropathy in patients.

#### Inhibiting NADPH oxidase

As highlighted in Figure 1, NADPH oxidase is a key mediator of oxidative and nitrosative stress in diabetic

neuropathy, and inhibition of this enzyme could, therefore, be a therapeutic strategy to reduce the cellular injury that may underlie this disease. Moreover, numerous studies in rodents have suggested a role for NADPH oxidase in diabetic neuropathy.

In hypertensive rats, expression of the NADPH oxidase 4 isoform (NOX4; also known as renox) is increased in the kidney in diabetic nephropathy. Administration of angiotensin blocks NOX4 activation, oxidative injury and renal dysfunction without altering blood pressure, suggesting that NOX4 might mediate pathological changes independently of blood pressure.<sup>110</sup> We have demonstrated increases in NADPH oxidase activity in rodent dorsal root ganglion neurons following hyperglycemia, or RAGE or LOX1 activation,<sup>21,22,42</sup> and we recently found that expression of both NOX2 and NOX4 is increased *in vitro* and *in vivo* in states of hyperglycemia and dyslipidemia (A. M. Vincent *et al.*, unpublished work). We are further exploring the pathological role and regulation of these NADPH oxidase isoforms in diabetic mice.

NADPH oxidase inhibitors are not yet available for clinical use. Nonspecific NADPH oxidase inhibitors—for example, diphenylene iodonium and apocyanin—inhibit other flavoenzymes such as nitric oxide synthase and xanthine oxidase.<sup>111</sup> Apocyanin has been used as an experimental inhalant to assess airway disease but has no therapeutic applications.<sup>112</sup> In cell culture and rodent models of experimental diabetes, apocynin and diphenylene iodonium can prevent the development of diabetic complications,<sup>113–115</sup> but the lack of specificity of these compounds limits conclusions regarding the direct role of NADPH oxidase in these effects. Development of a specific inhibitor is needed to enable investigation of the therapeutic potential of targeting NADPH oxidase in diabetic neuropathy.

### Reducing inflammation

Figure 1 indicates a central role for inflammatory mechanisms in neuropathy. We predict, therefore, that successful treatment or prevention of diabetic neuropathy will require inflammation to be blocked at the systemic and cellular levels. Activation of receptor-mediated inflammatory signaling by AGE and oxidized lipoproteins<sup>46</sup> leads to oxidative and nitrosative stress, which can cause microvascular disease. Consistent with a key role for inflammation in diabetic neuropathy, sciatic and sural nerve blood flow and conduction velocities were protected by the anti-inflammatory effects of erythropoietin in diabetic rats.<sup>116</sup>

Pain pathways in diabetic neuropathy involve the inflammatory mediator p38 MAPK. Recent clinical trials of a novel p38 MAPK inhibitor in neuropathic pain demonstrated rapid pain relief,<sup>117</sup> with decreased systemic inflammation after 14 days of treatment. These findings suggest that blocking of inflammation is feasible and could form an effective component of strategies to treat diabetic neuropathy. However, extended treatment with a different p38 MAPK inhibitor did not effectively block systemic or chronic inflammation in patients with

### Box 1 | Monitoring disease progression in diabetic neuropathy

Clinical measurement of neuropathy using a neuropathy disability scoring system involves assessment of sensitivity to temperature, vibration and touch, visual examination of the feet, and examination of tendon reflexes.<sup>162</sup> More-objective evaluation using quantitative sensory testing involves measuring the responses to vibrating and thermal stimuli applied to the feet in order to determine sensation and pain thresholds.<sup>163</sup> Suspicion of peripheral neuropathy generally leads to attempts to define the extent of damage and rate of progression using electrophysiological nerve conduction studies.<sup>164,165</sup> These studies measure the nerve conduction velocity, response amplitudes and latencies for the major peripheral sensory and motor nerves. This assessment is considered to be the most precise determinant of peripheral neuropathy, but it is an unpleasant procedure for the patient and does not assess damage to small fibers.<sup>166</sup>

Injury to small fibers is determined using biopsy. Nerve biopsy is considered too invasive for routine diagnosis, but skin biopsy is increasingly used, especially in clinical trial settings. Skin biopsies are considered accurate and can provide early diagnosis for peripheral neuropathy, but they are invasive and painful, and can result in infection in the sampled area.<sup>163,167</sup> Despite these drawbacks, skin biopsies can objectively demonstrate early nerve damage and detect evidence of early regeneration.<sup>168</sup>

Comparison of neuropathy clinical trials is difficult because of the wide array of clinical end points selected; the majority of end points focus on patient-reported improvements in pain and/or quality of life. A thorough review of the literature discussed problems with trial design, including the often-observed large placebo effect in the patient population.<sup>93</sup> The review concluded that most of the effective treatments for diabetic neuropathy have adverse effects that limit their usefulness, and that few studies have sufficient information on the effects of treatment on function and quality of life.<sup>86</sup>

rheumatoid arthritis, suggesting that the role of this kinase in cellular inflammation is complex.<sup>118</sup> Selective inhibition of the proinflammatory enzyme cyclo-oxygenase-2 prevents cardiac autonomic neuropathy in mice with type 1 diabetes,<sup>119</sup> providing further support for the use of anti-inflammatory agents to prevent neuropathy.

### Conclusions

Crucial advances in our understanding of and approach to the treatment of diabetic neuropathy have been made. It is now widely recognized that physicians and patients must aim to counteract multiple risk factors in order to improve both daily care and clinical trial outcomes. Standardization of assessment methods for monitoring disease progression will improve current weaknesses in patient care and clinical trial design (Box 1). Particular attention to dyslipidemia and cardiovascular risk factors, in addition to hyperglycemia, is likely to improve all diabetic macrovascular and microvascular complications. In the future, novel therapeutic targets at the level of mitochondrial metabolic control and inflammatory pathways are likely to further decrease the incidence of diabetic neuropathy.

### Review criteria

The National Library of Medicine PubMed database was searched for the term “diabetic neuropathy”. Information was extracted from full-text versions of articles published in English. Mechanistic data obtained since 1985, and data from clinical trials conducted since 2004, were included.

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#### Author contributions

A. M. Vincent researched data for the article. All authors contributed to discussions of the content, writing of the article, and review and/or editing of the manuscript before submission.