THE PREVALENCE, IMPACT, AND MULTIFACTORIAL PATHOGENESIS OF DIABETIC PERIPHERAL NEUROPATHY

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ABSTRACT

Neuropathy is the most common complication associated with diabetes, and sensorimotor diabetic peripheral neuropathy (DPN) is the most common form of diabetic neuropathy. Although prevalence estimates of DPN vary based upon the criteria used for diagnosis, it is generally held that at least 50% of all patients with diabetes have DPN and between 30% and 50% of patients with prediabetes may also have neuropathy. The severity of DPN is related to the duration of diabetes and the patient’s level of glycemic control. Proper management of blood glucose and good foot care are the only methods of treatment for DPN. Animal and in vitro experiments implicate 4 major pathways of glucose metabolism (polyol, advanced glycation end product, protein kinase C, and hexosamine) in the development of DPN. Initially thought to be unrelated mechanisms, recent evidence suggests there is a connection in that each of these pathways contributes to the formation of reactive oxygen species (ROS). This increased production of ROS, along with decreased scavenging of ROS, leads to cellular oxidative stress, which in turn leads to metabolic and vascular imbalances that initiate and promote DPN. Additionally, evidence suggests that the reduced availability of neurotrophic growth factors, which normally protect cells from ROS-triggered cell injury, oxidative stress, and cell death, contributes to the pathogenesis of DPN. Treatment strategies that halt oxidative stress decrease cell injury and in many cases restore function in cell culture and animal models of DPN; therefore, oxidative stress is a therapeutic target in the treatment of diabetic complications. Therapies targeted at the individual pathways or at cellular oxidative stress hold promise for the treatment of DPN.

In 2000, the prevalence of diabetes was estimated at 17.7 million in the United States. Within a 30-year period, this number is expected to increase to over 30 million. In 2002 alone, direct and indirect costs of diabetes in the United States were estimated at $132 billion. Even though this is an enormous cost, it omits the intangibles associated with the disease, for example, pain and suffering—which means it understates the burden of the disease. Patients with diabetes and its complications are more likely to be unemployed, disabled, and depressed. This article will focus on one of the more disabling complication of diabetes, neuropathy, discussing its prevalence, impact, and pathogenesis.

Neuropathy is the most common complication associated with diabetes. The most common form of diabetic neuropathy is sensorimotor diabetic peripheral neuropathy (DPN). Individuals with DPN first experience loss of distal sensation in the feet. In 80% of patients this leads to a numb or insensate foot, with no pain. When the loss of sensation reaches mid-calf, patients begin to experience sensory loss of the distal fingertips. In the feet, the lack of tactile and pain sensations predispose patients to callus formation, fissures, and

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In the absence of proper foot care, patients with DPN can develop non-healing infections and foot ulcerations, requiring amputation. DPN is the most common cause of non-traumatic amputations and 15% of all individuals with diabetes will experience an amputation over the course of their lifetime.

While estimates vary depending on criteria used for diagnosis, it is generally held that at least 50% of all patients with diabetes have DPN. The severity of DPN generally correlates with the duration of diabetes and the patient's level of glycemic control. In a well-cited study, Pirart followed over 4400 of his own patients for 25 years, beginning with their initial diagnosis. At time of diagnosis, 12% of patients had DPN. This increased to greater than 50% after 25 years of diabetes.

In the United Kingdom, DPN was evaluated in 6487 diabetic patients by assessing ankle reflexes, vibration, pinprick, and temperature sensation coupled with a 9-point symptoms score. Five percent of the individuals between the ages of 20 and 29 years had DPN. This increased with age, reaching 44.2% in subjects between 70 and 79 years of age. Similar results were reported when DPN was diagnosed by examining ankle reflexes and great toe sensation in 8757 diabetic patients between the ages of 18 to over 70. In this large cohort, 33% of the population was neuropathic, with an increased incidence of over 50% in elderly subjects.

While the number of patients with DPN increases with duration of diabetes, DPN is also present in up to 10% to 18% of newly diagnosed diabetic patients. Patients with impaired glucose tolerance (IGT), known as prediabetes, may also have neuropathy. Prospective screening of patients with an oral glucose tolerance test reveals that 30% to 50% of individuals with otherwise “idiopathic” painful sensory neuropathy have IGT. IGT neuropathy is similar to early DPN, with predominant sensory symptoms and signs.

At present, treatment for DPN centers on good foot care and controlling blood glucose. In contrast to diabetic retinopathy, where laser treatment is available, and diabetic nephropathy, where angiotensin-converting enzyme inhibitors or angiotensin receptor blockers are used, there are no treatments for DPN other than treating the diabetic condition per se. In 2001, the healthcare costs associated with DPN are estimated to range from $4.6 to $13.7 billion US dollars. DPN comprises a substantial portion of the total cost of diabetes as well. Up to 27% of direct medical costs of diabetes can be attributed to DPN.

**Pathophysiology of DPN**

The signs and symptoms of DPN reflect the pathological changes present in the diabetic nervous system. There is loss of large and small myelinated nerve fibers, evidence of segmental remyelination and demyelination, and variable amounts of axonal degeneration. The reported pathologic findings demonstrate that fiber loss is primary and demyelination with remyelination is secondary.

Changes in nerve structure occur in parallel with changes in blood vessels surrounding them, for example, capillary basement thickening, endothelial hyperplasia contributing to diminished oxygen tension and hypoxia, and capillary narrowing involving small myelinated or nonmyelinated C fibers. Vascular changes are present before diabetes develops—in individuals at risk of developing type 2 diabetes, microvascular reactivity is impaired and endothelium-dependent vasodilatation is compromised. In diabetes, vessel tone evidences an early physiological shift that favors vasoconstriction: vasodilatation becomes blunted while vasoconstrictor activity increases.

Studies in experimental diabetes in rats demonstrate a blood flow reduction to the nerves of 41% to 57% beginning 1 week after onset of disease. These findings parallel other reports: endoneurial hypoxia is present, peripheral nerves are susceptible to hypoxia, and oxygen treatment will prevent and reverse certain abnormalities associated with experimental DPN. More studies are needed in humans to clarify the association between microvascular reactivity and the early changes present in nerves in diabetes. A study by Theriault et al in patients with mild diabetes was unable to demonstrate a correlation between sural nerve blood flow, prebiopsy sural nerve amplitude, sural nerve fiber density, hemoglobin A1c, duration of diabetes, or age of patients. Data were again collected a year later, and there was a mild trend toward decline in nerve fiber density, but sural nerve blood flow was similar.

**Pathways Associated with Hyperglycemia and Oxidative Stress**

Since the Diabetes Control and Complications Trial established that hyperglycemia underlies the development of DPN, the last 10 years of research in experimental diabetes has focused on understanding the relationship between glucose-mediated metabolic...
and vascular disturbances in DPN. Animal and in vitro experiments implicate 4 major pathways of glucose metabolism in the development of DPN. These include: 1) increased polyol pathway activity leading to sorbitol and fructose accumulation, NAD(P)-redox imbalances and changes in signal transduction; 2) nonenzymatic glycation of proteins yielding “advanced glycation end-products” (AGEs); 3) activation of protein kinase C (PKC), initiating a cascade of stress responses, and 4) increased hexosamine pathway flux.

While initially conceived as disparate mechanisms, accumulating evidence suggests that these defects are interrelated and collectively responsible for the development and progression of DPN. One unifying mode of injury lies in the ability of these changes to both increase production as well as decrease scavenging of reactive oxygen species (ROS) leading to cellular oxidative stress. Unchecked, oxidative stress leads to the metabolic and vascular imbalances that initiate and promote DPN. Each of these pathways is discussed below in relationship to their role in DPN.

THE POLYOL PATHWAY

In complications-prone tissues, excess glucose not metabolized by glycolysis enters the polyol pathway (Figure 1). In the polyol pathway, glucose is converted to sorbitol, then to fructose; and it involves the oxidation of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) to NADP+. Increased activity of this metabolic pathway soon depletes the NADPH needed to regenerate the antioxidant glutathione. Without adequate glutathione, nerves have a diminished ability to scavenge ROS, promoting oxidative stress. In addition, as glucose is metabolized via the polyol pathway, it causes sorbitol to accumulate. Excessive amounts of sorbitol can lead to cellular osmotic stress; this alters the antioxidant potential of the cell; further increasing ROS accumulation. Excessive fructose produced in the polyol pathway also leads to nonenzymatic glycation/glycoxidation that accelerates ROS-mediated damage of cellular proteins and lipids.

THE AGE PATHWAY

Intracellular hyperglycemia appears to be the primary initiating event in the formation of AGEs via the advanced glycation end product pathway (Figure 2). Glycation/glycosylation occurs as glucose combines with proteins (Schiff bases) forming early glycation products at a rate proportional to glucose concentra-

![Figure 1. The Polyol Pathway](image1.png)

![Figure 2. The AGE Pathway](image2.png)
ucts undergo a slow, complex series of chemical reactions, becoming AGEs. Because AGEs are irreversible, they do not normalize when hyperglycemia is corrected, but rather accumulate overtime.

The formation of AGEs in cells leads to intra- and extracellular cross linking of proteins and protein aggregation which alters tertiary structures, impairing function. Hyperglycemia and greater polyol flux accelerate this process. AGEs can cause neuronal-specific injury by inhibiting axonal transport which leads to degeneration of the axon in proportion to its length. Because the processes associated with AGE formation require transition metals and ions as catalysts, the process has the potential to deplete the transition metal capacity, resulting in even more AGE formation.

AGEs bind to a number of receptor proteins including the receptor for advanced glycation end products (RAGE). In mesangial and endothelial cells, activation of RAGE by AGEs results in a burst of ROS production. The exact mechanism for this is unknown, but is thought to involve NADPH oxidase. This event alone could contribute to cellular oxidative stress and dysfunction. In addition, RAGE signals via phosphatidylinositol-3 kinase (Pl-3 kinase), Ki-Ras and mitogen activated protein kinases (MAP kinases) which initiate and sustain the translocation of NF-κB from the cytoplasm to the nucleus in a number of cell types including circulating monocytes and endothelial cells. The RAGE receptor gene contains 2 NF-κB binding sites within its promoter region, therefore, activation of RAGE leading to translocation of NF-κB results in the amplification of RAGE and promotes a cycle of damage and continued oxidative stress.

The PKC Pathway

The effect of diabetes on the PKC pathway is complex (Figure 3). PKC is responsible for the activation of essential proteins and lipids in cells that are needed for cellular survival. The abnormal physiologic balance caused by diabetes increases extracellular osmotic stress. Normal cells compensate for the stress by increasing intracellular osmolality, for example, accumulating nonperturbing organic osmolytes, including sorbitol, myo-inositol, and taurine. This process depletes taurine and myo-inositol. Depletion of taurine (a chelator and inhibitor of PKC) diminishes antioxidant defense; depletion of myo-inositol interferes with intracellular phosphoinositide signaling, decreasing PKC activation. However, increased activity in the polyol pathway activates PKC, as does the osmotic stimulation of stress-activated protein kinases.

PKC activity is closely tied to a cell’s redox status. The binding of antioxidants to the catalytic domain of PKC inhibits its activity; when PKC interacts with prooxidants, it becomes activated. PKC activation leads to MAP-kinase activation and phosphorylation of transcription factors that increase gene expression of multiple cellular stress-related genes (c-Jun kinases and heat shock proteins) that damage the cells. Although the role of the PKC activity is better established in the retina, kidney, and microvasculature than in the nerves, its effects on the pathogenesis of DPN are believed to result from its effect on vascular blood flow. Total PKC activity is reduced or unchanged rather than increased by diabetes in rat sciatic nerve, yet specific isoforms of PKC may be elevated. The role of PKC is further complicated by the fact that activation of vascular PKC promotes vasoconstriction and tissue ischemia. Activation of PKC may thus have a bifunctional effect in DPN: low PKC activity may alter nerve blood flow and nerve conductions in DPN, while high activity impairs nerve function possibly by interfering with neurochemical regulation (Figure 3).

The Hexosamine Pathway

The hexosamine pathway is activated when excessive metabolites of glycolysis accumulate (Figure 4).
Intermediates in the pathway lead to changes in gene expression and protein function that contribute to the pathogenesis of diabetic complications.43 For example, many of the acylglycoslated proteins that are produced in the pathway are transcription factors that increase proteins associated with complications of diabetes. These proteins are often inflammatory intermediates and include transforming growth factor-β1 that promotes nephropathy and plasminogen-activator inhibitor that inhibits normal blood clotting, increasing vascular complications.62 Thus, the activation of this pathway increases nerve oxidative stress through vascular disease leading to microvascular occlusion that produces ROS.

The hexosamine pathway appears to be particularly important in type 2 diabetes through 2 major mechanisms.62 The rate-limiting enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT) is specifically increased in the muscles of spontaneously diabetic mice. The overexpression of GFAT promotes insulin resistance, and hyperinsulinemia.62 In addition, activation of the hexosamine pathway induces oxidative stress via the generation of intracellular hydrogen peroxide.63 Several hexosamine pathway-mediated changes can be suppressed by treatment with antioxidants.63

**Oxidative Stress: A Possible Unifying Mechanism**

Each of the 4 pathways discussed contribute to the formation of ROS. Reactions that occur through the polyol pathway increase oxidative stress, as well as deplete cofactors needed for antioxidant defense. Byproducts of AGE formation promote generation of ROS, increasing oxidative stress. PKC activation results in decreased blood flow, angiogenesis, capillary occlusion, inflammation, and ROS.61 The hexosamine pathway leads to macro and microvascular occlusion, ischemia, and ultimately ROS.61

In normal neurons, the production of ROS is tightly controlled. The free radicals of superoxide and hydrogen peroxide are essential for normal cell function, but in excessive amounts they are detrimental. Superoxide is generated by mitochondrial electron transfer chain when nicotinamide adenine dinucleotide (NADH) is oxidized to NAD+.40 When the amount of glucose present is excessive, it impairs the mitochondrial electron transfer chain by inhibiting adenosine triphosphatase synthase. This leads to slowing of mitochondrial electron transfer, increasing the release of electrons available to combine with molecular oxygen to produce superoxide.43 Slowed mitochon-

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**Figure 4. The Hexosamine Pathway**

PAI-1 = plasminogen activator inhibitor-1; TGF-β = transforming growth factor-beta; ROS = reactive oxygen species; GFAT = glutamine-fructose-6-phosphate amidotransferase.

**Figure 5. Mechanisms Leading to Neuronal Degeneration in Hyperglycemia**

AGE = advanced glycation end product; GSSG = oxidized glutathione; NADPH = nicotinamide adenine dinucleotide phosphate hydrogen; NF-κB = nuclear factor κB; PKC = protein kinase C; ROS = reactive oxygen species; TCA = tricarboxylic acid; TGF-β = transforming growth factor beta; VEGF = vascular endothelial growth factor.
drial electron transfer also leads to activation of NADH oxidase. Activated NADH oxidase generates superoxide as a byproduct.

Superoxide is metabolized to hydrogen peroxide and water by superoxide dismutase enzymes. 40 Hydrogen peroxide, in turn, can easily oxidize multiple cellular components and is readily diffusible across membranes. 40 When hydrogen peroxide reacts with free iron, it produces hydroxyl radicals that react with lipids. 40 These lipid peroxides are directly toxic to cells and mediate cell death. Both excess superoxide and hydrogen peroxide are injurious to neurons. 40 When superoxide reacts with nitric oxide it forms peroxynitrite that attacks and disrupts proteins and lipids. 67 Superoxide can also inhibit enzymes by attacking the iron sulfur center; enzymes that are vulnerable to this reaction include complexes 1-3 of the electron transfer chain and aconitase of TCA cycle. 67

Proteins and nucleic acids that undergo peroxidation and nitrosylation can accumulate and overload the cellular mechanisms to recycle them. 40 Also, the damage that occurs to nucleic acids can activate mechanisms of apoptosis. 66 Together, these processes lead to loss of neuronal function. 40

In addition, oxidative modification by superoxide and hydrogen peroxide decreases expression of some transcription factors of key proteins needed for survival (i.e. complex 1 and bcl-2) 40 while increasing gene expression of several proapoptotic proteins (JNK kinase, poly adenosine diphosphate polyribose polymerase, and cyclooxygenase-2). 69 Mitochondria in neurons appear to be particularly sensitive to oxidative damage, which results in impaired energy regulatory function that leads to loss of neuronal function and the development of DPN. 70, 71

In summary, research in experimental and clinical diabetes indicates that glucose-mediated cellular oxidative stress is a unifying mechanism underlying the metabolic and vascular dysfunction in DPN. 39, 70, 72-74 Treatment strategies that halt oxidative stress decrease cell injury and, in many cases, restore function in cell culture and animal models of DPN. 41, 61, 75-82 Oxidative stress therefore is a therapeutic target in the treatment of diabetic complications. Therapies may be designed to block; 1) each of the individual 4 pathways leading to increased ROS accumulation, 2) the free radicals that are generated by oxidative stress, or 3) the cellular damage produced by the accumulation of free radicals. Effective therapies, currently under development, may reduce the morbidity of DPN, by controlling the disease process itself.

**Conclusion**

The intellectual challenge to basic and clinical scientists exploring the pathogenesis of DPN is an understanding of the important common components underlying the various hypotheses proposed to explain the development of these diabetic microvascular complications. Glucose-induced generation of ROS and oxidative stress link virtually all of the physiological mediators implicated in this process. In each of these pathogenetic elements, generation of ROS initiates a feed-forward cycle; since oxidative stress itself impairs antioxidative defense mechanisms, a vicious cycle occurs resulting in metabolic, vascular, and structural damage (Figure 5). Therapies targeted at the individual pathways or at cellular oxidative stress hold promise for the treatment of DPN.

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