REVIEW OF LITERATURE

NEUROPATHIC PAIN

The International Association for the Study of Pain defines *neuropathic pain* as "initiated or caused by a primary lesion or dysfunction in the nervous system" and due to disordered peripheral or central nerves (Merskey and Bogduk, 1994; Galluzzi, 2007; Veves et al., 2008). This disorder can be caused by compression, transection, infiltration, ischemia, or metabolic injury to neuronal cell bodies, or in combination. Neuropathic pain may be classified as either peripheral or deafferentation (central) in origin (Dworkin, 2002; Pascuzzi, 2009). Examples of the former include diabetic peripheral neuropathy (DPN), postherpetic neuralgia (PHN), antineoplastic therapy–induced or HIV-induced sensory neuropathy, tumor infiltration neuropathy, phantom limb pain, postmastectomy pain, complex regional pain syndromes (reflex sympathetic dystrophy), and trigeminal neuralgia. Deafferentation syndromes resulting in neuropathic pain include multiple sclerosis, spinal cord injury, central poststroke pain, and Parkinson disease.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>No. per 1,00,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic peripheral neuropathy</td>
<td>600</td>
</tr>
<tr>
<td>Postherpetic neuralgia</td>
<td>500</td>
</tr>
<tr>
<td>Cancer-associated</td>
<td>200</td>
</tr>
<tr>
<td>Spinal cord injury</td>
<td>120</td>
</tr>
<tr>
<td>Causalgia and sympathetic dystrophy</td>
<td>100</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>50</td>
</tr>
<tr>
<td>Poststroke pain</td>
<td>30</td>
</tr>
<tr>
<td>HIV-associated</td>
<td>15</td>
</tr>
<tr>
<td>Trigeminal neuralgia</td>
<td>15</td>
</tr>
<tr>
<td>Low back pain-associated</td>
<td>2,100</td>
</tr>
<tr>
<td><strong>Total (excluding back pain)</strong></td>
<td><strong>1,680 Million</strong></td>
</tr>
<tr>
<td><strong>Total (including back pain)</strong></td>
<td><strong>3,780 Million</strong></td>
</tr>
</tbody>
</table>

Adapted from Bennett, 1997
Bennett (1997) provided incidence estimates of common types of neuropathic pain and concluded that if neuropathic low back pain is included in the total, approximately 3.8 million individuals in the United States suffer from this disorder. Such painful conditions are likely to increase as the population grows older and age-related disorders such as herpes zoster, diabetes mellitus, cerebrovascular accidents, Parkinson disease, and cancer-diseases of aging-develop.

Diabetic peripheral neuropathy, second only to low back pain-associated neuropathy, is estimated to account for 600 cases per 100,000 (Table 1); this disorder is certain to increase as the population of citizens with diabetes mellitus also continues to expand.

**Neuropathic Versus Nociceptive Pain**

Response to an acute painful stimulus is an important adaptive mechanism that protects a person from further injury. Pain signals resulting from noxious stimuli (wounds, thermal or inflammatory insults) are converted into electrical impulses within tissue nociceptors whose cell bodies are found in dorsal root ganglions; both nociceptive and neuropathic pain signals utilize the same pain pathways (Galluzzi, 2007).

Information regarding intensity, quality, and location of pain is conveyed to the sensory cortex from the somatosensory thalamus. The central nervous system (CNS) utilizes descending inhibitory pathways via the dorsolateral fasciculus (Lissauer’s tract) of the spinal cord and periaqueductal gray matter to modulate transmission of nociceptive stimuli (Chen et al., 2004a; Kandel et al., 2000). Namaka et al. (2004) characterize this as a complex equilibrium of pain-signaling and pain-relieving pathways connecting peripheral and central nervous systems.

Efficient, rapid transmission of acute responses to a painful stimulus is a self protection process. In general, acute pain provides an “alarm” that leads to subsequent protective responses; neuropathic pain, however, signals no imminent danger. The operative difference is that neuropathic pain represents
a delayed, ongoing response to damage that is no longer acute but which continues to be expressed as painful sensations (Galluzzi, 2007).

Sensory neurons damaged by injury, disease, or drugs produce spontaneous discharges leading to sustained levels of excitability. These ectopic discharges begin to “cross talk” with adjacent uninjured nerve fibers, resulting in amplification of pain impulses (peripheral sensitization). This hyperexcitability leads to greater transmitter release causing increased response by spinal cord neurons (central sensitization). This process, known as “windup,” accounts for the fact that the level of perceived pain is far greater than what is expected based on what can be observed (Galluzzi, 2007; Ji and Strichartz, 2004; Spruce et al., 2003).
Painful nerve stimulation leads to activation of N-methyl-D-aspartate (NMDA) receptors on postsynaptic membranes in the dorsal horn of the spinal cord (Kandel et al., 2000). Release of NMDA, a modulating neurotransmitter, is coupled with subsequent release of glutamate, an excitatory neurotransmitter. The resultant extended depolarization (influx of calcium and sodium and efflux of potassium) produces much larger than usual postsynaptic potentials, known as synaptic potentiation. Spinal windup has been described as “continuous increased excitability of central neuronal membranes with persistent potentiation” (Spruce et al., 2003).

Neurons of peripheral and central nervous systems continue to transmit pain signals beyond an original injury, thus activating an ongoing, continuous central pain response (Fig. 1). Devor et al. (1993) presented evidence showing that damaged sensory fibers have a higher concentration of sodium channels, an alteration that would increase spontaneous firing.

Characterization of Neuropathic Pain

Symptoms described by patients with neuropathic pain are myriad representing a variety of possible nerve injuries implicated in causation (Veves et al., 2008; Pascuzzi, 2009). Neuropathic pain sufferers complain of numbness, burning, or tingling, or a combination; they describe electric shock–like, prickly, or pins and needles sensations. Patients completing the McGill Pain Questionnaire (Melzack, 1987) described their pain using terms such as “punishing-cruel” and “tiring-exhausting.” Boureau et al. (1990) identified six adjectives used more frequently to describe neuropathic pain; electric shock, burning, and tingling were most commonly used (53%, 54%, and 48%, respectively), in addition to cold, pricking, and itching.

Several types of abnormal sensations, or dysesthesias, may occur alone or in addition to other specific complaints in patients with neuropathic pain (Table 2). Unlike usual responses to such discomfort, these irritating or painful sensations occur in the absence of an apparent cause. A common example is the severe, aching, “toothache-like” response elicited by a cool draft of air on the cheek of a patient suffering from trigeminal neuralgia.
Allodynia is a painful response to an otherwise benign stimulus. Taken to the extreme (eg, inability to remove the stimulus), this response can result in an agonizing neuropathic symptom known as hyperpathia. Another example of this condition is “touch sensitivity” of badly sunburned skin, where even light stroking of an inflamed area causes extreme discomfort; like neuropathic pain, this response seems out of proportion to the injury (Galluzzi, 2007; Pascuzzi, 2009).

<table>
<thead>
<tr>
<th>Symptoms or Sign</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allodynia</td>
<td>Pain due to non-noxious stimuli (clothing, light touch) when applied to the affected area. May be mechanical (eg, caused by light pressure), dynamic (caused by non-painful movement of a stimulus), or thermal (caused by non-painful warm, or cool stimulus)</td>
</tr>
<tr>
<td>Anesthesia</td>
<td>Loss of sensation to the affected region</td>
</tr>
<tr>
<td>Dysesthesia</td>
<td>Spontaneous or evoked unpleasant abnormal sensations</td>
</tr>
<tr>
<td>Hyperalgesia</td>
<td>Exaggerated response to a mildly noxious stimulus applied to the affected region</td>
</tr>
<tr>
<td>Hyperpathia</td>
<td>Delayed and explosive response to a noxious stimulus applied to affected region</td>
</tr>
<tr>
<td>Hypoesthesia</td>
<td>Reduction of normal sensation to affected region</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>Nonpainful spontaneous abnormal sensations</td>
</tr>
<tr>
<td>Phantom Pain</td>
<td>Pain from a specific site that no longer exists (eg, amputated limb) or where there is no current injury</td>
</tr>
<tr>
<td>Referred Pain</td>
<td>Occurs in a region remote from the source</td>
</tr>
</tbody>
</table>

Pharmacologic induction of local anesthesia or hypoesthesia by lidocaine or transdermal fentanyl produces a predictable duration of action; this is not the case with neuropathic-induced anesthesia or hypoesthesia. The discomfort of one’s foot “falling asleep” is a common paresthesia. That uncomfortable sensation is self-limiting and resolves spontaneously, unlike the continuous, self-perpetuating, and annoying sensation of pins and needles caused by neuropathic pain (Galluzzi, 2007).
Pain Scales for Assessing Neuropathic Pain

Following adoption of pain as “the fifth vital sign” by the Joint Commission on Accreditation of Healthcare Organizations, clinicians have been exposed to varied analog assessment forms such as the Wong Baker faces scale for rating pain intensity. Recently, pain researchers have focused attention on a theory that accurate measurement of pain quality could provide insight into treatment effects too subtle to be noticed when global measures are similar (Jensen et al., 2005; Pascuzzi, 2009; Schestatsky et al., 2009). This accuracy is especially important for neuropathic pain, because specific sensory characteristics (e.g., burning, tingling) may spotlight pathophysiologic mechanisms of such pain and give clues to those types of intervention most likely to result in palliation.

Examples of standardized scales used for pain assessment include:

- Short Form McGill Pain Questionnaire (SF-MPQ) (Melzack, 1987)
- 100-mm visual analog scale (VAS) (Brown and Pilitsis, 2005)
- Numeric rating (Hardy et al., 1952) and faces scales (Wong et al., 2001)
- Pain Disability Index (Pollard, 1984)
- Pain Catastrophizing Scale (PCS) (Sullivan et al., 1995) and
- Neuropathic Pain Scale (NPS) (Galer and Jensen, 1997)

One of the most widely used current evaluations is the 0-10 rating where “0” means “I have no pain” and “10” is “the worst pain I ever had.” Investigators for the Shingles Prevention Trial devised a specific pain assessment tool for calculating a herpes zoster severity-of-illness score, the Zoster Brief Pain Inventory in which patients are asked questions to rate their pain and also are able to shade areas on a diagram to indicate the parts of their bodies that were most affected (Oxman et al., 2005). These scales underscore the fact that it may be difficult for clinicians to assess or rate a patient’s pain because the level of perceived discomfort may be much greater than what is observable. Pain scales provide useful, standardized, and validated tools for charting an individual’s response to a pain-control
intervention. In addition, detailed documentation utilizing accepted pain scales to assess a patient's level of discomfort provides protection from legal challenges regarding any prescribed pharmacotherapy.

Classification of Neuropathy

Painful neuropathies have diverse etiologies, and their clinical course varies from disease to disease. Many signs and symptoms can be common to all painful neuropathies, however, and they may even share similar, if not the same, pathophysiologic mechanisms (Backonja, 2001; Veves et al., 2008).

<table>
<thead>
<tr>
<th>Table 3. Causes of Painful Neuropathies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic</td>
</tr>
<tr>
<td>Endocrine</td>
</tr>
<tr>
<td>Painful diabetic neuropathy</td>
</tr>
<tr>
<td>Chemotherapy</td>
</tr>
<tr>
<td>Nutritional disorders</td>
</tr>
<tr>
<td>Posttraumatic</td>
</tr>
<tr>
<td>Complex regional pain syndrome</td>
</tr>
<tr>
<td>Type I</td>
</tr>
<tr>
<td>Type II</td>
</tr>
<tr>
<td>Compressive</td>
</tr>
<tr>
<td>Nerve entrapment syndromes</td>
</tr>
<tr>
<td>Lateral femoral cutaneous nerve</td>
</tr>
<tr>
<td>Common peroneal nerve</td>
</tr>
<tr>
<td>Fibular neck</td>
</tr>
<tr>
<td>Dorsal foot</td>
</tr>
<tr>
<td>Tarsal tunnel</td>
</tr>
<tr>
<td>Autoimmune</td>
</tr>
<tr>
<td>Vasculitic</td>
</tr>
<tr>
<td>Demyelinating</td>
</tr>
<tr>
<td>Neoplastic</td>
</tr>
<tr>
<td>Parainfections</td>
</tr>
<tr>
<td>Infectious</td>
</tr>
<tr>
<td>Viral</td>
</tr>
<tr>
<td>Spirochetal</td>
</tr>
</tbody>
</table>


Painful neuropathies can be classified into categories by their origin. An abbreviated list that commonly affects the lower extremity includes toxic-metabolic (eg, endocrine, chemical/chemotherapy exposure, nutritional),
posttraumatic (e.g., complex regional pain syndrome), infectious, compressive (e.g., nerve entrapment syndromes), autoimmune, and hereditary factors (Table 3, 4) (Backonja, 2001).

<table>
<thead>
<tr>
<th>Subclinical neuropathy</th>
<th>Clinical neuropathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal electrodiagnostic tests</td>
<td>Diffuse somatic neuropathy</td>
</tr>
<tr>
<td>Decreased nerve conduction velocity</td>
<td>Distal symmetric sensorimotor polyneuropathy</td>
</tr>
<tr>
<td>Decreased amplitude of evoked muscle or nerve action potential</td>
<td>Primary small fiber neuropathy</td>
</tr>
<tr>
<td>Abnormal quantitative sensory threshold</td>
<td>Primary large fiber neuropathy</td>
</tr>
<tr>
<td>Vibratory/tactile</td>
<td>Mixed</td>
</tr>
<tr>
<td>Thermal warming/cooling</td>
<td>Autonomic neuropathy</td>
</tr>
<tr>
<td>Abnormal autonomic function tests</td>
<td>Cardiovascular autonomic neuropathy</td>
</tr>
<tr>
<td>Abnormal cardiovascular reflexes</td>
<td>Abnormal papillary dilatation</td>
</tr>
<tr>
<td>Altered cardiovascular reflexes</td>
<td>Gastrointestinal autonomic neuropathy</td>
</tr>
<tr>
<td>Abnormal biochemical response to hypoglycemia</td>
<td>Gastroparesis</td>
</tr>
<tr>
<td></td>
<td>Constipation</td>
</tr>
<tr>
<td></td>
<td>Diabetic diarrhea</td>
</tr>
<tr>
<td></td>
<td>Anorectal incontinence</td>
</tr>
<tr>
<td></td>
<td>Genitourinary autonomic neuropathy</td>
</tr>
<tr>
<td></td>
<td>Bladder dysfunction</td>
</tr>
<tr>
<td></td>
<td>Sexual dysfunction</td>
</tr>
<tr>
<td></td>
<td>Hypoglycemia unawareness and unresponsiveness</td>
</tr>
<tr>
<td></td>
<td>Sudomotor dysfunction</td>
</tr>
</tbody>
</table>

DIABETIC NEUROPATHY

Neuropathy is a common and costly complication of both type 1 (T1DM) and type 2 diabetes (T2DM) (Stirban et al., 2008). The prevalence of neuropathy is estimated to be about 8% in newly diagnosed patients and greater than 50% in patients with long-standing disease (Boulton et al., 2005; Edwards et al., 2008; Gabriel, 2008; Veves et al., 2008; Fischer et al., 2009; Pascuzzi, 2009). There is increasing evidence that even pre-diabetic conditions are also associated with some forms of neuropathy (Franklin et al., 1990; Singleton et al., 2003). An estimated 15% of all patients with diabetes will develop foot ulcers and diabetic neuropathy is the leading cause of nontraumatic limb amputation (Gordois et al., 2003). The annual costs of diabetic neuropathy and its associated morbidities in the US have been estimated to exceed $10.9 billion (Gordois et al., 2003).

In recent years, considerable progress has been made toward understanding the biochemical mechanisms leading to diabetic neuropathy, and as a result, new treatment modalities are being explored.

Figure 2. Stocking-glove configuration of DPN. Diabetic neuropathy is dependent on axon length, initiating in the toes and progressing upward until reaching the calf. Neuropathy presents at the fingertips at this point (adapted from Edwards et al., 2008)
neuropathies, are generally rare, sudden in onset, often self-limited, and tend to occur in older patients

Epidemiology and Impact of Diabetic Neuropathy

Diabetic Polyneuropathy (DPN)

Estimating the prevalence, incidence, and risk of DPN depends on the criteria employed to identify the syndrome. The American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation published a consensus definition in 2005.

<table>
<thead>
<tr>
<th>Diagnostic Criteria</th>
<th>Initial Prevalence (%)</th>
<th>Time of Initial Assessment</th>
<th>Final Prevalence (%)</th>
<th>Time of later assessment</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurological examination</td>
<td>7.5</td>
<td>At diagnosis</td>
<td>50</td>
<td>25 years post-diagnosis</td>
<td>Pirart, 1978</td>
</tr>
<tr>
<td>Neurological examination and symptom score</td>
<td>20.8</td>
<td></td>
<td>36.8 T1DM: 54</td>
<td>&gt; 10 years Mean: 14.5 years</td>
<td>Young et al., 1993 T1DM, T2DM</td>
</tr>
<tr>
<td>Two or more abnormalities (symptoms, nerve conduction, QST, AFT)</td>
<td>8.3</td>
<td>At diagnosis</td>
<td>41.9</td>
<td>10 years post-diagnosis</td>
<td>Dyck et al., 1993 T1DM, T2DM</td>
</tr>
<tr>
<td>Probable polyneuropathy Absent reflex</td>
<td>28</td>
<td></td>
<td>46</td>
<td></td>
<td>Partanen et al., 1995 T2DM</td>
</tr>
<tr>
<td>Confirmed clinical neuropathy</td>
<td>2.1</td>
<td>Baseline diabetes duration 1-5 years</td>
<td>9.6</td>
<td>5 years post-baseline</td>
<td>The effect of intensive diabetic therapy on development and progression of neuropathy. (DCCT, 1995) T1DM</td>
</tr>
<tr>
<td>Abnormal nerve conduction</td>
<td>21.8</td>
<td></td>
<td>40.2</td>
<td></td>
<td>Tesfaye et al., 1996 T1DM</td>
</tr>
<tr>
<td>≥ 2 Criteria</td>
<td>12</td>
<td>&lt; 7 years duration</td>
<td>42</td>
<td>≥ 15 years duration</td>
<td></td>
</tr>
</tbody>
</table>

QST: Quantitative sensory test; AFT: Autonomic function test
Important points in the case definition include: 1) the combination of neuropathic symptoms, signs and abnormal electrodiagnostic studies is the strongest predictor of DPN, 2) symptoms alone are a poor predictor of disease, 3) electrodiagnostic studies are not required for the clinical definition of DPN, but are recommended to monitor disease in clinical research protocols (England et al., 2005; Deshpande et al., 2008; Van Acker et al., 2009). Thus, the definition of DPN may include a symptom score, a focused neurological examination and nerve conduction studies, or, optimally, all of these. Yet, regardless of the diagnostic criteria, it is clear that 1) DPN is highly prevalent in patients with diabetes, 2) its prevalence increases with the duration of diabetes and 3) strict glycemic control reduces the incidence and progression of diabetic neuropathy. Table 6 summarizes several reports on the prevalence of DPN in diabetic patients.

Figure 3. Effect of disease duration and age on prevalence of diabetic neuropathy. For both T1DM and T2DM, duration of diabetes (left) as well as age of patient (right) is correlated to the incidence of diabetic neuropathy (adapted from Young et al., 1993)
The prevalence of DPN increases with age, and tends to be more common in patients with T2DM than in those with T1DM. Figure 3 illustrates the prevalence of DPN as a function of disease duration and age interval observed in a cross-sectional study of 6 patients (37% with T1DM). In the overall population the prevalence of DPN was significantly higher in patients with T2DM (32.1%) than in T1DM patients (22.7%, P<0.001) and there was a highly significant correlation between age and prevalence of neuropathy in both T1DM and T2DM (Young et al., 1993). Similar rates of DPN were reported in the Rochester Diabetic Neuropathy Study (Dyck et al., 1993). In 1986, 380 patients were enrolled in this study; 102 (26.8%) had T1DM and 278 (73.2%) had T2DM. Patients were assessed for DPN by sign and symptom scores coupled to physiological assessments of nerve function, including nerve conduction studies and quantitative sensory testing. 54% of patients with T1DM with average disease duration of 14.5 years had DPN, while 45% of T2DM patients with average disease duration of 8.1 years had DPN.

![Figure 4. Effect of glycemic control on diabetic neuropathy in DCCT.](image)

Intensive therapy cohort which showed better glycemic control, result in lower incidences of all forms of diabetic neuropathy compared with conventional therapy (Adapted from The Diabetes Control and Complication Trial Research Group, 1995)
The EURODIAB study examined 3250 T1DM patients from 16 European countries. DPN was defined as the presence of 2 or more abnormalities in either symptoms, signs, quantitative sensory or autonomic function testing. The prevalence of DPN across Europe was 28%, with a strong correlation between duration of diabetes and level of glycemic control (Tesfaye et al., 1996).

The Diabetes Control and Complications Trial (DCCT) demonstrated that intensive treatment in patients with T1DM significantly reduced both the incidence (Teshaye et al., 1993) and progression (Teshaye et al., 1995) of neuropathy. The prevalence of neuropathy in the primary prevention cohort of the DCCT after 5 years treatment with either a conventional or intensive insulin regimen in the DCCT is depicted in Figure 4.

HbA1c levels in the intensive and conventional treatment groups were separated by about 2 percentage points throughout the follow-up (7.2% vs. 9.1%, respectively). Intensive treatment was associated with a 71% risk reduction for confirmed clinical neuropathy (abnormal history, physical examination, or both, confirmed by abnormal nerve conduction or abnormal autonomic function tests), a 54% risk reduction for clinical neuropathy, a 59% risk reduction for abnormal nerve conductions, and a 56% risk reduction for autonomic nervous system dysfunction. This figure also highlights the difference in prevalence estimates related to the stringency of criteria employed, as well as the lower prevalence of autonomic vs. peripheral neuropathy early in the disease.

In the United Kingdom Prospective Diabetes Study, 3867 newly diagnosed T2DM patients were randomized into either intensive treatment with an oral hypoglycemic agent or insulin or conventional treatment with diet. After 10 years, intensive treatment resulted in approximately 1% lower HbA1c
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vs. conventional treatment and was associated with a 25% risk reduction in microvascular endpoints (retinopathy, nephropathy and neuropathy) (Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes. UK Prospective Diabetes Study Group, 1998). However, most of this reduction was due to fewer patients in the intensive treatment arm requiring retinal photocoagulation compared to patients in the conventional arm. While there was a tendency toward a reduction in death from peripheral vascular disease and the prevalence of amputation, effects on these single endpoints failed to achieve statistical significance. Similarly, surrogate endpoints for neuropathy showed trends toward improvement in the intensive treatment group at 10 years and in a smaller group of patients followed for 15 years, the prevalence of impaired sensory perception in the lower extremities was significantly preserved by intensive vs. conventional treatment (31.2% vs. 51.7%, P=0.0052).

Height is another risk factor for DPN, suggesting that longer fibers are more vulnerable to injury. Other suggested risk factors for DPN include smoking (Tesfaye et al., 1996), excessive alcohol use (Adler et al., 1997), hypertension (Forrest et al., 1997), low plasma insulin levels (Partanen et al., 1995), and co-morbid diabetic complications (Cohen et al., 1998).

The most common morbidities related to DPN are recurrent foot infections, ulcers, and amputations, and Charcot's joints. It was estimated that upwards of 15% of patients with diabetes will develop at least one foot ulcer (Boulton et al., 2004a), and one recent study observed an annual incidence of nearly 2% (Ramsey et al., 1999). While vascular disease and ischemia contribute, it has been reported that 60 to 70% of diabetic foot ulcers are neuropathic in origin (Gonzalez and Oley, 2000). A significant proportion of neuropathic diabetic foot ulcers is accompanied by cellulitis or osteomyelitis (about 15%) and these conditions contribute to the annual incidence of lower-extremity amputation in patients with diabetes, which has been estimated to be around 0.6% (van Houtum et al., 2004). In 1999, the attributable cost for a 40- to 65-year old man with a new foot ulcer was estimated to be $28,000 for
the 2 years after diagnosis (Ramsey et al., 1999) and in 2003, the total annual cost of DPN and its complications in the US was estimated to be between $4.6 and $13.7 billion—representing up to 27% of the direct medical cost of diabetes (Gordois et al., 2003). Furthermore, there are important quality-of-life issues for patients with DPN including pain and other forms of discomfort, decreased mobility, and a variety of psychosocial impairments (Vileikyte et al., 2003).

There are relatively few data available regarding the influence of DPN on mortality. However, in their study of diabetic foot ulcers, Ramsey et al. (1999) found that 3-year survival in patients with foot ulcers was 17% less than in age- and sex-matched diabetic patients. Further, using a newly-developed accelerated failure time model which included alcohol consumption, proteinuria, race, retinopathy, sex, smoking, type of diabetes, BMI, duration of diabetes, HbA1c, and “toescore” (an age-adjusted transformation of vibration perception threshold), it was found that toescore was the most significant contributor to mortality (Coppini et al., 2000).

Diabetic Autonomic Neuropathy (DAN)

Despite its negative impact on survival and quality of life in persons with diabetes, DAN remains poorly understood. Since all organs receive input from the autonomic nervous system (ANS), DAN can affect every body system. Most organs are dually innervated, receiving fibers from the parasympathetic and the sympathetic branches of the ANS. Because the vagus nerve (parasympathetic), the longest autonomic nerve, mediates approximately 75% of all parasympathetic activity (Low et al., 2004) and neuropathy is seen first in the longest fibers, the earliest manifestations of DAN tend to be parasympathetic and are usually widespread.

Although clinical symptoms of DAN may not appear until long after diabetes onset, subclinical neuropathy may be detected within a year of diagnosis in T2DM and 2 years of diagnosis in T1DM (Pfeifer et al., 1984). Such findings highlight the importance of screening for DAN. As for DPN, the reported prevalence varies greatly depending on the criteria used to identify DAN as well as the population studied. These range from as low as 2.5% of
the primary prevention cohort in the DCCT (retinopathy and microalbuminuria-free patients with T1DM of 1 to 5 years duration) (The effect of intensive diabetes therapy on the development and progression of neuropathy. The Diabetes Control and Complications Trial Research Group, 1995) to as high as 90% of patients with long-standing T1DM who were potential candidates for a pancreas transplant (Kennedy et al., 1995).

In 1988, the San Antonio Conference on Diabetic Neuropathy provided guidelines for standardized objective measurements of neuropathy based on clinical symptoms, clinical examination, electro-diagnostic studies (EDX), quantitative sensory testing (QST), and autonomic function testing for use in clinical research studies. It was further recommended that these measures be standardized to a normative control population (Asbury and Porte, 1988), and since that time, several studies have assessed the prevalence of DAN in defined populations. Like DPN, the diagnosis of DAN depends on specific clinical and physiological assessments. Most commonly, changes in heart rate with deep breathing, position change (lying to standing), and breathing out against pressure (Valsalva ratio) are measured in each patient. Thus while DAN can involve many body systems, the cardiovascular system is most commonly tested, and frequently measures of DAN are, in reality, measures of cardiac autonomic neuropathy. In a community-based study, the overall prevalence of DAN assessed by heart rate variability was 16.7%, being somewhat more common in patients with T1DM (20.9%) than in those with T2DM (15.8%). Symptomatic DAN was much less common (2.5%) than that detected by autonomic function tests, but was significantly more frequent in patients with T1DM (5/43, 11.6%) than in patients with T2DM (1/202, 0.5%).

In a larger study (647 patients with T1DM, 524 patients with T2DM), Ziegler and colleagues defined DAN as an abnormality of ≥2 of 6 autonomic function tests. In addition to the 3 more standard tests discussed above, a spectral analysis of the EKG in the low- and mid-frequency bands and a vector analysis of deep breathing were performed to complete the battery of autonomic function tests. The investigators found that 25.3% of patients with T1DM and 34.3% of those with T2DM had abnormal findings in ≥2 of 6
autonomic function tests. If more restrictive criteria were used for diagnosis (≥3 of 6 abnormal autonomic function tests), the prevalence of autonomic neuropathy was 16.8% and 22.1% for patients with T1DM and T2DM (Ziegler et al., 1993). In another study of 110 children and adolescents with T1DM, abnormality of one or more of the 3 standard autonomic function tests was found in 42.7% of patients (Verrotti et al., 1995). In summary, DAN is a common form of neuropathy in diabetic patients. There is no convincing evidence for a difference in prevalence between T1DM and T2DM, but similar to DPN, the prevalence is highly dependent on the criteria used to define DAN.

Stepwise logistic regression analysis of potential risk factors revealed that HbA1c and duration of diabetes were independently associated with DAN (Cohen et al., 1998). The Diabetic Cardiovascular Autonomic Neuropathy study examined the potential clinical correlates of cardiac autonomic neuropathy in 647 patients with T1DM and in 524 patients with T2DM. Stepwise regression analysis showed a significant association of cardiac autonomic neuropathy and HbA1c in patients with T1DM, but not in those with T2DM (Ziegler et al., 1993). Other risk factors or significant correlates of DAN that have been reported include hypertension, female gender, LDL-cholesterol, HDL-cholesterol in patients with T1DM (Maser et al., 1990), retinopathy, DPN in patients with T1DM or T2DM (Ziegler et al., 1993), and albuminuria in patients with T2DM (Cohen et al., 1998). Due to the importance of the autonomic nervous system in regulating virtually every body function, the consequences of DAN are many and varied, ranging from bothersome, to debilitating, to deadly. Cardiac autonomic neuropathy (CAN) is the most clinically important manifestation of DAN due to its association with several negative outcomes, including increased mortality. Early markers of CAN include resting tachycardia and loss of heart rate variation during deep breathing, whereas loss of heart rate response to mild exercise is indicative of nearly complete cardiac denervation. Impaired parasympathetic function causes loss of bradycardic responses to sleep and to deep inspiration. Impaired sympathetic function, which generally occurs as the syndrome progresses, can increase cardiac adrenergic sensitivity, which may
predispose a patient to tachycardia and sudden death. A prolonged corrected QT interval (QTc) indicates an imbalance between right and left sympathetic innervation may increase risk for arrhythmias. Other common and potentially dangerous manifestations of cardiac autonomic neuropathy include exercise intolerance, orthostatic hypotension, and intraoperative cardiovascular lability (Vinik et al., 2003).

Another important syndrome associated with CAN is silent myocardial ischemia or “cardiac denervation syndrome.” Reduced appreciation of ischemic pain can impair timely recognition of myocardial ischemia or infarction and delay appropriate treatment. Many studies have compared the prevalence of silent myocardial ischemia, usually measured by exercise stress testing, in diabetic patients with and without CAN. Collectively these studies show cardiac autonomic neuropathy greatly increases the risk of silent myocardial ischemia. Conclusions from a large body of literature on cardiac autonomic neuropathy and various other diabetic cardiovascular morbidities make it abundantly clear that the presence of CAN greatly increases the risk of all-cause mortality, cardiovascular mortality, and major cardiovascular events (Freeman, 2005). There is often an association between CAN and diabetic nephropathy, suggesting that other co-morbidities likely contribute to the increased cardiovascular risk (Maser et al., 2003).

The most devastating outcome of DAN is the excess mortality associated with CAN. Many studies have explored the potential contribution of CAN to total mortality in patients with diabetes - both T1DM and T2DM. Of 15 studies included in a meta-analysis by Maser et al. (2003), 14 reported increased mortality rates in patients with CAN; in those studies the risk ratio for all-cause mortality in patients with CAN vs. those without CAN at baseline (follow-up = 0.5 to 16 years) ranged from 2.1 (Sawicki et al., 1996) to 9.2 (Jermendy et al., 1991). A statistically significant increase in mortality was reported in 12 of the studies.

Gastrointestinal manifestations of DAN are diverse and can affect any portion of the gastrointestinal tract. Esophageal dysfunction resulting from vagal neuropathy may cause heartburn and dysphagia for solids (Freeman,
Delayed gastric emptying (gastroparesis) was reported to occur in approximately 50% of patients with long-standing diabetes (Kong et al., 1999). Although diabetic gastroparesis is usually relatively benign, severe cases may cause nausea, vomiting, epigastric discomfort, and bloating which can last for extended periods or occur in cycles. Diarrhea, with or without intermittent constipation, is another common and often debilitating symptom of DAN (Low et al., 2004).

Genitourinary manifestations of DAN can include increased or decreased urinary frequency, bladder over distention, and urine retention or overflow incontinence. Patients with bladder dysfunction are predisposed to developing urinary tract infections, which may accelerate or exacerbate renal failure. Erectile dysfunction and retrograde ejaculation in men and decreased libido and decreased vaginal lubrication in women are also common and early findings in patients with DAN (Low et al., 2004; Freeman, 2005).

Autonomic sudomotor dysfunction can produce distal anhydrosis with a stocking-glove distribution similar to that of DPN. Although this manifestation of DAN is essentially asymptomatic, it can predispose a patient to heatstroke and hyperthermia and may produce a compensatory central hyperhydrosis (Freeman, 2005).

Clinical Evaluation of Diabetic Neuropathy

A consensus statement from the San Antonio Conference on Diabetic Neuropathy recommended that the diagnosis and classification of DPN for research and clinical trials be based on at least one standardized measure from each of the following categories: clinical symptoms, clinical examination, electrodiagnostic studies (EDX), quantitative sensory testing (QST), and autonomic function test (AFT) (Ziegler et al., 1993). Many currently-used techniques are based on methods developed by Dyck et al. (1992) at the Mayo Clinic. In the absence of neurological symptoms or clinically detectable neurological deficits indicative of a diffuse or focal neuropathy, subclinical neuropathy can be diagnosed as outlined in Table 4. It is important to note that the diagnosis of subclinical or clinical DPN requires that signs (e.g.,
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abnormal quantitative tests for subclinical neuropathy) and symptoms (for clinical neuropathy) are not attributable to a non-diabetic etiology. Because there are no distinguishing features unique to diabetic neuropathy, all other possible causes of the observed neuropathic disorders must be ruled out by careful history and physical examination.

Table 7. MNSI Patient Questionnaire to Evaluate Neuropathy in Diabetics

<table>
<thead>
<tr>
<th></th>
<th>Question</th>
<th>Yes</th>
<th>No</th>
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<tbody>
<tr>
<td>1</td>
<td>Are your legs and/or feet numb?</td>
<td></td>
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</tr>
<tr>
<td>2</td>
<td>Do you ever have burning pain in your legs and/or feet?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Are your feet too sensitive to touch?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Do you get muscle cramps in your legs and/or feet?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Do you ever have pricking feeling in your legs and/or feet?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Does it hurt when the bedcovers touch your skin?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>When you get in the tub/shower, are you able to tell hot water from cold water?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Have you ever had an open sore on your foot?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Has your doctor ever told you that you have diabetic neuropathy?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Do you feel weak all over most of the times?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Are your symptoms worse at night?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Do your legs hurt when you walk?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Are you able to sense your feet when you walk?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Is the skin on your feet so dry that it cracks open?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Have you ever had an amputation?</td>
<td></td>
<td></td>
</tr>
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</table>

Adapted from Feldman et al., 1994

In a recent statement by the American Diabetes Association, DPN is defined as “the presence of symptoms and/or signs of peripheral nerve
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dysfunction in people with diabetes after the exclusion of other causes" (Boulton et al., 2005). The diagnosis requires a careful history and clinical examination of the feet. Several instruments for this purpose have been developed and validated. One example is the 459 Michigan Neuropathy Screening Instrument (MNSI) (Feldman et al., 1994), shown in Table 7. This patient questionnaire consists of 15 questions about sensation, general asthenia, and peripheral vascular disease. A positive response on ≥7 of the questions is diagnostic of diabetic peripheral neuropathy and correlates well with neuropathy diagnosed by the Mayo Clinic criteria. The MNSI questionnaire is followed by a simple 8-point clinical examination involving inspection of the foot, assessment of ankle reflexes, and semiquantitative determination of vibration perception. An MNSI score ≥ 2 indicates the presence of neuropathy with a high specificity (~ 95%) and sensitivity (~ 80%) (Bax et al., 1996).

Patients with an abnormal MNSI score may undergo a more detailed examination such as that summarized by the Michigan Diabetic Neuropathy Score (MDNS), in which the severity of neuropathy is determined through a focused, 46-point neurological examination that includes measures of sensory impairment, muscle strength, and reflexes (Feldman et al., 1994). Other more complicated techniques to assess warm and cold perception thresholds, current perception thresholds, etc., have also been developed for research purposes. These are generally time-consuming and require specialized equipment, and thus are not routinely employed in a clinic setting.

Nerve conduction studies can be used to quantify the degree of nerve injury in DPN (Boulton et al., 2004b). While not usually required for the diagnosis, nerve conduction studies can help the patient and physician monitor DPN progression over a long period of time, particularly if the patient is asymptomatic. Nerve conduction studies are also useful to identify superimposed mononeuropathies, e.g., carpal tunnel syndrome. These superimposed mononeuropathies are a common problem in patients with DPN.
As for DPN, DAN is usually classified as either subclinical or clinical depending upon the presence of clinical manifestations of autonomic dysfunction. As mentioned previously, the clinical manifestations of DAN can be many and varied, but may not occur until long after DAN can be detected through autonomic function tests. Routinely, five simple, noninvasive cardiovascular reflex tests are used for diagnosis: Valsalva maneuver, heart rate response to deep breathing, heart rate response to standing up, blood pressure response to standing up, and blood pressure response to sustained handgrip (Freeman, 2005).

Abnormalities in these noninvasive tests of cardiovascular function show a strong correlation with symptoms (Low et al., 2004), and with autonomic dysfunction in other organs including pupillomotor function, gastrointestinal function, and nor-epinephrine production (Vinik and Mehrabyan, 2004; Freeman, 2005). However, abnormalities in lower-extremity sudomotor function and impotence may precede detectable impairment in the simple cardiovascular autonomic function tests (Low et al., 1986; Vinik and Mehrabyan, 2004; Freeman, 2005).

Resting tachycardia and loss of heart rate variation in response to breathing or the Valsalva maneuver are primary indicators of parasympathetic dysfunction and are among the earliest signs of CAN. Orthostatic hypotension and loss of blood pressure responses to exercise or handgrip are signs of sympathetic dysfunction and tend to occur later in disease progression. Other more complex tests of cardiovascular autonomic function may be used for research purposes, including spectral analysis of 24-hour heart rate variability, measures of neurovascular flow with Doppler technology, and scintigraphic assessment of cardiovascular sympathetic innervation.

Because many prescription and non-prescription medicines, alcohol, and tobacco can influence autonomic nervous system activity, patients should be tested in an overnight-fasted state, having refrained from alcohol and tobacco for 24 h, and they should omit even prescription drugs on the day of the test. Further, due to the influence of antecedent hypoglycemia on autonomic function (Dagogo-Jack et al., 1993), patients should not be tested...
within 24 h of a hypoglycemic episode. As with DPN, it is important to rule out possible causes of abnormal AFTs other than DAN through patient history and physical examination. Abnormalities in two or more of the simple AFTs suggest a diagnosis of DAN. In a recent statement by the American Diabetes Association, it is recommended that patients with T1DM be tested 5 years after diagnosis of diabetes and be tested yearly thereafter. Patients with T2DM should be tested at diagnosis and yearly thereafter (Boulton et al., 2005).

**PATHOGENESIS OF DIABETIC NEUROPATHY**

There may be multiple etiologies which account for the various neuropathic syndromes seen in patients with diabetes. Hyperglycemia clearly plays a key role in the development and progression of diabetic neuropathy as well as the other microvascular complications of diabetes (Tomlinson and Gardiner, 2008; Veves et al., 2008; Pascuzzi, 2009; Piriz et al., 2009). Understandably, then, investigations into the molecular and biochemical pathophysiology of diabetic neuropathy have focused on glucose metabolic pathways. Over the past 25 years animal experiments and *in-vitro* studies have identified biochemical pathways likely to be important in the development of diabetic complications and have led to possible approaches to treatment. All of these pathways are related to the metabolic and/or redox state of the cell. Pathways which are mainly driven by metabolism are: glucose flux through the polyol pathway; the hexosamine pathway; excess/inappropriate activation of protein kinase C (PKC) isoforms; accumulation of advanced glycation end products. While each pathway may be injurious alone, collectively they cause an imbalance in the mitochondrial redox state of the cell and lead to excess formation of reactive oxygen species (ROS) (Kong et al., 1999; Vinik et al., 2003; Figueroa-Romero et al., 2008) (Fig. 5). Increased oxidative stress within the cell leads to activation of the Poly (ADP-ribose) polymerase (PARP) pathway, which regulates the expression of genes involved in promoting inflammatory reactions and neuronal dysfunction.
Diabetic neuropathy is thought to occur from both hyperglycemia-induced damages to nerve cells per se and from neuronal ischemia caused by hyperglycemia-induced decreases in neurovascular flow. Much of the basic science addressing the etiology/mechanisms of microvascular complications has used non-neuronal derived cells or cell lines, but studies in animal models of neuropathy, and/or human clinical studies with specific inhibitors of each pathway suggest that each mechanism can contribute to diabetic neuropathy.

**Figure 5. Schematic presentation of hyperglycemic effects on biochemical pathways in diabetic neuropathy** (Adapted from Edwards et al., 2008). Excessive glucose metabolism generates excess NADH and leads to overload of the electron transport chain causing oxidative stress, damage to mitochondria, and activation of PARP. PARP activation by ROS acts in conjunction with the hexosamine and PKC pathways which lead to redox imbalance, gene expression disturbances, and further oxidative stress. These pathways also induce inflammation and neuronal dysfunction. NF-κB: Nuclear factor kappa B; PARP: Poly(ADP-ribose) polymerase; PKC: AGE: Advanced glycation end products; GSH: Glutathione; GSSG: Oxidized glutathione; UDPGlcNAc: UDP-N-Acetyl-glucosamine; VEGF: Vascular endothelial growth factor.

**Polyol Pathway**

The enzyme aldose reductase (AR) reduces glucose to sorbitol and sorbitol dehydrogenase (SDH) oxidizes sorbitol to fructose (Fig. 5). Both of
these enzymes are abundantly expressed in tissues prone to diabetic complications. Hyperglycemia activates the aldose reductase pathway primarily by mass action: Increased flux through the AR pathway causes increased intracellular sorbitol, a relative intracellular hypertonic state, and compensatory efflux of other osmolytes such as myo-inositol (MI, important in signal transduction) and taurine (an antioxidant) (Nakamura et al., 1999; Vincent et al., 2004). Since NADPH is consumed by aldose reductase-mediated reduction of glucose to sorbitol (Jermendy et al., 1991; Brownlee, 2005) and NADPH is required for regeneration of reduced glutathione (GSH), this too contributes to oxidative stress. The second step in the polyol pathway oxidizes sorbitol to fructose via sorbitol dehydrogenase (Feldman et al., 1997). Formation of fructose promotes glycation as well as depletes NADPH, further augmenting redox imbalance. Activation of aldose reductase may also increase formation of diacylglycerol, which activates the deleterious PKC pathway (Yamagishi et al., 2003; Uehara et al., 2004).

**Hexosamine Pathway**

In the late 1990's, the hexosamine pathway was implicated as an additional factor in the pathology of diabetes-induced oxidative stress and complications. Fructose-6 phosphate is a metabolic intermediate of glycolysis. However, during glucose metabolism some fructose-6 phosphate is shunted from the glycolytic pathway to the hexosamine pathway. The fructose-6 phosphate is converted to glucosamine-6 phosphate by glutamine fructose-6 phosphate amidotransferase (Thornalley, 2005). Glucosamine-6 phosphate is then converted to uridine diphosphate-N-acetyl glucosamine (UDP GlcNAc), a molecule that attaches to the serine and threonine residues of transcription factors (Brownlee, 2001). Hyperglycemic conditions create additional flux through the hexosamine pathway, ultimately resulting in excess GlcNAc and abnormal modification of gene expression (Sayeski and Kudlow, 1996; Kolm-Litty et al., 1998; Brownlee, 2001).

Specifically, hyperglycemic conditions and excess GlcNAc cause increased activation of Sp1, a transcription factor implicated in diabetic complications. Sp1 is responsible for the expression of many glucose-induced
“housekeeping” genes including transforming growth factor-β1 (TGF-β1) and plasminogen activator inhibitor-1 (PAI-1) (Du et al., 2000; Brownlee, 2001). Overexpression of TGF-β1 leads to increased collagen matrix production which promotes endothelial fibrosis and decreases proliferation in mesangial cells (Hirakata and Kitamura, 1996; Kolm-Litty et al., 1998). Overexpression of PAI-1 promotes vascular smooth muscle cell mitosis which plays a role in atherosclerosis (Sayeski and Kudlow, 1996). PAI-1 is not only upregulated via the hexosamine pathway but also the PKC pathway (Fig. 5). Thus, two discrete pathways leading to diabetic complications converge through the same injurious mechanism. It has additionally been shown that GlcNAc impairs β-cell function by inducing oxidative stress; increased glutamine fructose-6 phosphate amidotransferase or glucosamine leads to increased hydrogen peroxide levels and reduced expression of insulin, glucose transporter 2, and glucokinase genes (Kaneto et al., 2001). Thus, increased flux through the hexosamine pathway has been causally implicated in multiple metabolic derangements in diabetes.

**Protein Kinase C Pathway**

Elevated glucose levels stimulate diacylglycerol (DAG), which in turn activates PKC. Increased production of the PKC-β-isoform in particular has been implicated in overexpression of the angiogenic protein vascular endothelial growth factor (VEGF), PAI-1, NF-κB, TGF-β and the development of diabetic complications such as retinopathy, nephropathy, and cardiovascular disease (Fig. 5) (Veves and King, 2001; Arikawa et al., 2007; Das Evcimen and King, 2007). Data on the effects of PKC-β and VEGF on diabetic neuropathy are less clear, but generally support the concept that increased PKC pathway flux plays a role in neuropathy as well (Arikawa et al., 2007; Das Evcimen and King, 2007). PKC pathway activation alters vasoconstriction and capillary permeability, and can cause hypoxia, angiogenesis, basement membrane thickening, and endothelial proliferation (Williams et al., 1997; Edwards et al., 1999). PKC activation also alters function of the Na⁺−K⁺ATPase pump and other enzymes crucial to proper nerve conduction. Activation of different PKC isoforms has been shown to
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decrease Na⁺–K⁺ ATPase activity in smooth muscle cells and normalize activity in peripheral nerves (Greene et al., 1987; Xia et al., 1995). The link of PKC to diabetic neuropathy is supported by studies in streptozotocin (STZ) induced diabetic rats, where PKC inhibition normalizes both sciatic nerve blood flow and nerve conduction velocity (Nakamura et al., 1999). Overexpression of PKC isoforms can also directly induce insulin resistance (Cortright et al., 2000; Naruse et al., 2006).

Advanced Glycation Endproducts Pathway

Non-enzymatic reactions between reducing sugars or oxaldehydes and proteins/lipids result in advanced glycation endproducts (AGEs) (Ahmed, 2005; Toth et al., 2008). Three main pathways are responsible for the formation of reactive dicarbonyls (AGE precursors): 1) oxidation of glucose to form glyoxal; 2) degradation of Amadori products (fructose-lysine adducts); and 3) aberrant metabolism of glycolytic intermediates to methylglyoxal. AGEs are heterogeneous modified intracellular and extracellular biomolecules. Inside cells, both protein and DNA adducts alter function and cellular transport. Methylglyoxal, a highly reactive dicarbonyl, is shown to induce sensitivity to vascular damage in endothelial cells (Yao et al., 2007a). Extracellular protein AGEs include plasma and matrix proteins that disrupt cellular adhesion and activate the receptor for AGEs (RAGE) (Ramasamy et al., 2007). AGE–RAGE interaction activates the transcription factor nuclear factor kappa B (NF-κB) (Fig. 5). NF-κB regulates a number of activities including inflammation and apoptosis (Ramasamy et al., 2005). Activation of neuronal RAGE induces oxidative stress through NADPH oxidase activity (Vincent et al., 2007). Increased levels of AGE and RAGE are found in human diabetic tissue (Tanji et al., 2000). Diabetic RAGE knockout mice showed significant improvement in DPN and diminished expression of NF-κB and PKC compared to wild type diabetic model (Toth et al., 2008). While the NF-κB–PKC decrease in the knockout was present in dorsal root ganglion (DRG) and peripheral nerve, it was most pronounced in supporting Schwann cells. Collectively, the biochemical damage induced by AGEs results in impaired
nerve blood flow and diminished neurotrophic support (Wada and Yagihashi, 2005).

**Poly(ADP-ribose) Polymerase Pathway**

PARP found in Schwann, endothelial cells, and sensory neurons is also implicated in glucotoxicity. PARP is a nuclear enzyme closely associated with oxidative-nitrosative stress: free radicals and oxidants stimulate PARP activation (Fig. 5). Recent evidence also suggests that the two act in concert: PARP both causes and is activated by oxidative stress (Obrosova et al., 2005a). PARP acts by cleaving nicotinamide adenine dinucleotide (NAD+) to nicotinamide and ADP-ribose residues attached to nuclear proteins (Southan and Szabo, 2003). The results of this process include NAD⁺ depletion, changes in gene transcription and expression, increased free radical and oxidant concentration, and diversion of glycolytic intermediates to other pathogenic pathways such as PKC and AGE formation (Garcia Soriano et al., 2001; Ha et al., 2002; Du et al., 2003; Obrosova et al., 2005a). Such PARP-implicated abnormalities manifest clinically as decreased nerve conduction velocity (NCV), small fiber neuropathy, neurovascular abnormalities, retinopathy, thermal and mechanical hyperalgesia, and tactile allodynia (Pacher et al., 2002; Zheng et al., 2004; Obrosova et al., 2004; Li et al., 2005a; Obrosova et al., 2005a; Ilnytska et al., 2006).

**Oxidative Stress and Apoptosis**

The AGE, polyol, hexosamine, PKC, and PARP pathways all contribute to neuronal damage. Fig. 5 illustrates that the AGE and polyol pathways directly alter the redox capacity of the cell either through direct formation of ROS or by depletion of necessary components of glutathione recycling. The hexosamine, PKC, and PARP pathways exhibit damage through expression of inflammation proteins. The progression of diabetic neuropathy in a distal–proximal axon length-dependent manner suggests that damage is initiated in the axon (Leinninger et al., 2006b; Zherebitskaya et al., 2009). Axons are susceptible to hyperglycemic damage both due to their direct access to nerve blood supply and their large population of mitochondria (Mt). Mounting evidence suggests that the hyperglycemic environment coupled with a
compromised blood supply overloads the metabolic capacity of the Mt, producing oxidative stress (Brownlee, 2001). This oxidative stress leads to Mt damage followed by axonal degeneration and death.

**Figure 6. Oxidative stress and mitochondrial dysfunction** (Adapted from Leinninger et al., 2006b). Hyperglycemia increases production of reactive oxygen species (ROS) in mitochondria. NADH and FADH$_2$ produced from the tricarboxylic acid cycle transfer to the mitochondria, where they serve as electron donors to the mitochondrial membrane-associated redox enzyme complexes. The electrons (e$^-$) are shuttled through oxidoreductase complexes I, II, III and IV (cytochrome c), until they are donated to molecular oxygen, forming water. The electron transfer into complexes I, III and IV by NADH (and FADH$_2$ via complex II to complex III) produces a proton gradient at the outer mitochondrial membrane, generating a potential between the inner mitochondrial membrane and outer mitochondrial membrane. This potential drives ATP synthesis, and is crucial for mitochondrial viability, function, and normal metabolism. As electrons are passed from complex II to complex III, however, ROS are produced as byproducts. The levels of ROS produced during normal oxidative phosphorylation are minimal, and they are detoxified by cellular antioxidants such as glutathione, catalase and superoxide dismutase. The hyperglycemic cell, on the other hand, shuttles more glucose through the glycolytic and tricarboxylic acid cycles, providing the cell with an over-abundance of NADH and FADH$_2$ electron donors. This produces a high proton gradient across the inner mitochondrial membrane, which increases the turnover of the initial complexes, and thereby produces increased levels of radicals. Accumulation of these radicals, or ROS, is severely detrimental to mitochondrial DNA, mitochondrial membranes and the whole cell. Abbreviations: Cyto-c, cytochrome c; CoQ$_{10}$, coenzyme Q$_{10}$; e$^-$, electrons; GSH, glutathione; GSSG, oxidized glutathione; H$_2$O$_2$, hydrogen peroxide; O$_2$•–, superoxide; Pi, phosphate; SOD, superoxide dismutase
Mitochondrial damage occurs due to excess formation of ROS and reactive nitrogen species (RNS) (Nishikawa et al., 2000; Obrosova et al., 2005b, 2007). ROS, such as superoxide and hydrogen peroxide, are produced under normal conditions through the Mt electron transport chain and are normally removed by cellular detoxification agents such as superoxide dismutase, catalase, and glutathione (Fig. 6) (Leinninger et al., 2006b).

Hyperglycemia leads to increased Mt activity, raising ROS production in the Mt. Peroxynitrite, the primary RNS, is formed by the reaction of superoxide and nitric oxide (NO). RNS induce a number of cytotoxic effects including protein nitrosylation and activation of PARP (Obrosova et al., 2005a; Obrosova and Julius, 2005). Excessive ROS/RNS formation eventually overloads the natural antioxidant capacity of the cell, resulting in injuries to lipids, proteins, DNA. This damage ultimately compromises cellular function and integrity. As Mt is the origin of ROS/RNS generation, they are most susceptible to damage. While inhibitors of AR (Obrosova et al., 2002), PKC activation (PKC-DRS Study Group, 2005), AGE formation (Wada et al., 2001), and PARP (Illytska et al., 2006) can individually ameliorate hyperglycemia-induced nerve damage in animal models of diabetes, emerging evidence also suggests that these pathways converge to increase cellular oxidative stress (Fig. 5). Cellular oxidative stress is further enhanced when excessive glucose leads to overproduction of superoxide as a byproduct of mitochondrial oxidative phosphorylation (Fig. 6) (Vincent and Feldman, 2004). Overproduction of superoxide also markedly inhibits GAPDH, causing accumulation of upstream glycolytic intermediates. These intermediates further enhance AR, hexosamine, PKC, and AGE production, producing even more cellular injury.

Experimental support for this unifying hypothesis derives from studies demonstrating that inhibition of superoxide accumulation by overexpression of superoxide dismutase prevents hyperglycemia-induced increases of AR (Nishikawa et al., 2000), hexosamine pathway products (Du et al., 2000), PKC activation, and AGE formation. Thus, there exists a vicious feed-forward system in cells prone to diabetic complications, where glucose-activated
metabolic pathways converge to produce cellular oxidative stress. Decreased nerve blood flow and ischemia, resulting from the processes described above, further exacerbate tissue injury. In summary, oxidative stress and ROS link the metabolic initiators and physiological mediators implicated in progressive nerve fiber dysfunction, damage, and loss in diabetic neuropathy. The generation of ROS may initiate a feed-forward cycle in which oxidative stress itself impairs anti-oxidative defense mechanisms.

In addition to their role in metabolism, Mt are involved in the determination of cell fate and viability. Oxidative stress not only damages Mt DNA, proteins, and membranes, but it also initiates signaling pathways that result in localized mitochondrial destruction called mitoptosis. One pathway essential to mitoptosis, and subsequently apoptosis, involves Mt division via the dyanmin related protein 1 (Drp1) (Frank et al., 2001; Lee et al., 2004b). Mt normally undergo an equilibrium-driven process of fission and fusion. In times of stress, Drp1 translocates from the cytosol to the Mt so as to increase Mt fission events (Arnoult et al., 2005). Aberrant Mt fission is associated with mitoptosis and implicated in apoptosis. Increased levels of Drp1 are found in-vitro and in-vivo models of diabetic neuropathy (Leinninger et al., 2006a). This implicates Mt fission in diabetic neuropathy and renders Drp1 a potential therapeutic target. Mt are pivotal components of metabolism, oxidative stress, and programmed cell death/apoptosis (Russell et al., 1999). As such, neurons in a hyperglycemic environment display signs of both oxidative stress and apoptosis (Russell et al., 1999).

Diabetic models have shown either apoptosis in the cell body or neuroaxonal dystrophy. Some studies have shown an absence of apoptosis in high glucose treated sensory neurons (Cheng and Zochodne, 2003; Gurny et al., 2008). The current hypothesis that counts for these observations is that in-vivo, recurrent injury occurs, activating cell death pathways. Initially, the neurons with support from glia are able to undergo successful repair, however, eventually cellular defense is overcome by recurrent activation of injury pathways, causing damage to the cell body. Over time, this cycle injures
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Mt, alters Mt distribution, and initiates axons dying back toward the cell body (Vincent et al., 2004; Sullivan and Feldman, 2005).

Inflammation

Inflammatory agents including C-reactive protein and TNF-α are present in the blood of both T1DM and T2DM (Gomes et al., 2003; Gonzalez-Clemente et al., 2005; King, 2008; Schaper et al., 2008; Doupis et al., 2009). Higher levels of these proteins correlate with the incidence of neuropathy (Gonzalez-Clemente et al., 2005). Recent data from the Eurodiab Prospective Complications Study demonstrates a correlation between diabetic neuropathy and plasma levels of HSP 27 (Gruden et al., 2008). HSP 27 is a required intermediate in the pathway of TNF-α induction of the inflammatory mediators cyclooxygenase-2 (COX-2), IL-6, and IL-8. The production of the initiating inflammatory mediators TNF-α and TGF-β results from several of the glucose-induced pathways already outlined (Vincent and Feldman, 2004; Brownlee, 2005). As illustrated in Fig. 4, when excess glucose is shunted through alternative metabolic pathways such as the fructose-6-phosphate or diacylglycerol, the signaling intermediates and modified transcription factors lead to increases in TGF-β and NF-κB (Brownlee, 2001). Similarly, breakdown of glycolytic triose phosphates forms methylglyoxal, an AGE that covalently modifies transcription factors (Yao et al., 2007a). One specific consequence of these modifications is decreased binding of a repressor of angiotensin II, known as Sp3. Thus, angiotensin II increases and leads to activation of vascular endothelial cells (Yao et al., 2007a). In the endoneurium, this activation leads to inflammatory cell recruitment, local generation of cytokines, and reduced blood flow that leads to further generation of ROS (Coppey et al., 2006). Other extracellular AGEs that activate RAGE also lead to intracellular inflammatory signaling to upregulate NF-κB (Toth et al., 2008).

COX-2 is an important enzyme that is upregulated by NF-κB (Lee et al., 2004a). This upregulation is observed in peripheral nerves and vascular tissues in experimental diabetes (Kellogg and Pop-Busui, 2005). COX-2 activity appears to drive a feed-forward loop since COX-2 is upregulated by NF-κB and in turn, it generates prostaglandin E2 and ROS that activate NF-
Pharmacological blockade or gene ablation of COX-2 prevents diabetes-induced changes in peripheral nerves including depletion of GSH, increases in TNF-α, and blood flow and nerve conduction deficits (Kellogg et al., 2007; Matsunaga et al., 2007).

Another inflammatory enzyme regulated by NF-κB is inducible nitric oxide synthase (iNOS) (Kim et al., 2008a). Like COX-2, iNOS both induces and is induced by NF-κB, leading to a vicious cycle of inflammation (Hasnis et al., 2007; Kim et al., 2008a). The NO generated by iNOS directly modulates the blood supply to nerves and participates in microvascular changes following injury (Levy and Zochodne, 2004; Zochodne and Levy, 2005). NO has direct roles in axon and myelin breakdown following an injury and also contributes to the development of neuropathic pain (Levy and Zochodne, 2004; McDonald et al., 2007). Excessive local levels of NO during inflammation may damage axons and growth cones (Zochodne and Levy, 2005).

All of the inflammatory mechanisms in diabetic neuropathy appear to converge upon the activation of NF-κB. Because of chronic NF-κB activation, blood vessels and nerve cells are more susceptible to injury in ischemia reperfusion (Wang et al., 2006). Subsequent to ischemia–reperfusion there is extensive infiltration of monocyte macrophages and modest infiltration of granulocytes in diabetic peripheral nerves (Wang et al., 2006). The cytokines induced by NF-κB in endothelial cells, Schwann cells and neurons also lead to macrophage recruitment in diabetic nerves (Yamagishi et al., 2008). Macrophages promote diabetic neuropathy through a variety of mechanisms, including production of ROS, cytokines and proteases, which result in myelin breakdown and cellular oxidative damage (Conti et al., 2002; Tesch, 2007; Kawamura et al., 2008). Excessive macrophage recruitment likely impairs nerve regeneration in diabetic neuropathy (Conti et al., 2002; McDonald et al., 2007).

Growth Factors

Growth factors promote the growth and survival of neurons and direct neurite outgrowth (Leinninger et al., 2004). Given that diabetic neuropathy is
characterized by neuronal degeneration and damage to supporting Schwann cells, perturbations in growth factors such as nerve growth factor (NGF), insulin-like growth factor (IGF), and neurotrophin 3 (NT-3) have been suggested to be involved in the pathogenesis of diabetic neuropathy. These factors bind to heterodimeric tyrosine kinase receptors. The receptors for the NGF family of growth factors consist of the p75NTR and a specific trk tyrosine kinase, which confers ligand specificity.

Expression levels of multiple growth factors are altered in animal models of DPN. NGF is the most studied growth factor in diabetic neuropathy. NGF is produced by muscle and keratinocytes, and its trkA receptor is expressed on sensory and sympathetic neurons (McMahon et al., 1994, 1995; Averill et al., 1995; Fang et al., 2005). In multiple diabetic models, NGF levels are reduced as well as retrograde transport of NGF diminished (Kasayama and Oka, 1989; Hellweg et al., 1994). Interestingly, when glucose levels are returned to normal levels, NGF levels return to normal. This indicates that diabetes, either due to hyperglycemia or by lack of insulin, has the capacity to regulate growth factors (Hellweg et al., 1991). Some studies have generated conflicting results with regard to NGF expression levels (Hellweg and Hartung, 1990; Fernyhough et al., 1994, 1995a, 1995b; Delcroix et al., 1997, 1998; Hounsom et al., 1998; Arrieta et al., 2005). Despite these discrepancies, an observed decrease in the retrograde transport of NGF (both endogenous and exogenous) in diabetic rats is noteworthy in that the transport of NGF to the soma is required for its neurotrophic effects to occur (Schmidt et al., 1983, 1985; Hellweg et al., 1994; Fernyhough et al., 1995b; Arrieta et al., 2005). Similar to NGF, IGF IGF-I and II are down-regulated under diabetic conditions, though administration of insulin restored expression (Olchovsky et al., 1991; Wuarin et al., 1994; Migdalis et al., 1995). NT-3 is expressed in muscle and skin. It can signal through trkA and B to some extent, and primarily signals through trkC, suggesting broad therapeutic potential (Barbacid, 1994; Lewis et al., 2006). Like trkB, trkC is found in motor neurons, and a population of large-diameter sensory neurons responsible for proprioception and tactile sensation
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(Barbacid, 1994). As with studies of other growth factors, changes in NT-3 expression in diabetes have not been consistently documented. NT-3 proteins levels are upregulated in diabetic sural nerve, though mRNA levels have been reported as both increased and decreased (Rodriguez-Pena et al., 1995; Cai et al., 1999).

THERAPEUTIC STRATEGIES FOR DIABETIC NEUROPATHY

Glycemic control

Therapies for DPN and DAN may be divided into treatments that target the underlying pathogenetic mechanisms (Boulton, 2004; Trotta et al., 2004; Singh et al., 2005) and those aiming to relieve symptoms (Adriaensen et al., 2005). In the latter category there are numerous established approaches; in the former, the only proven method currently available to prevent DPN and DAN or slow progression is strict glycemic control (Tesfaye et al., 2005).

Type 1 diabetes mellitus

Diabetes Control and Complications Trial The DCCT compared intensive treatment (3 or more insulin injections or insulin pump) aiming to normalize HbA1c with conventional treatment (1 or 2 insulin injections aiming to prevent hyperglycemic symptoms and hypoglycemia) in 1441 patients with T1DM. After 5 years of follow-up, the prevalence of confirmed clinical neuropathy (defined by history or physical exam and confirmed by either abnormal nerve conduction or ≥ 1 autonomic function test) was 64% lower in patients receiving intensive treatment (HbA1c=7.2%) than in those receiving conventional treatment (HbA1c=9.1%). Further, nerve conduction velocities remained stable in patients receiving intensive treatment and declined significantly in those assigned conventional treatment (The effect of intensive diabetes therapy on the development and progression of neuropathy. The Diabetes Control and Complications Trial Research Group, 1995). The results of the DCCT agree with a similar study in Europe, the EURODIAB trial (Tesfaye et al., 1996).
Figure 7. Risk of diabetic complications (retinopathy) in conventional vs intensive therapy as related to HbA1C. Absolute risk of sustained retinopathy progression as a function of updated means A1C (per cent) during DCCT and the time follow-up during the study (years), estimated absolute (Poisson) regression models. (A) Conventional treatment group. Intensive treatment group. Results suggest that average glucose levels be less important to prognosis of complications than fluctuations in glycemic levels. (Adapted from Hirsch and Brownlee, 2005)

It has been reported that the benefits of tight glycemic control in reducing microvascular complications are more far-reaching than originally determined. The Epidemiology of Diabetes Interventions and Complications (EDIC) study has followed the DCCT cohort for more than 7 years after termination of the original study. EDIC has shown that despite convergence of mean HbA1c in patients originally randomized to intensive vs conventional treatment (due to institution of intensive treatment in 75% of the former conventional treatment group and a small proportion of the original intensive treatment group choosing to relax glycemic control), the rate of progress...
retinopathy and nephropathy in patients originally randomized to intensive treatment remained significantly below those in the original DCCT conventional treatment arm (Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus, 2002). This benefit was also reported for neuropathy. At the end of EDIC Year 1, mean HbA1c levels were 7.9% and 8.3% in the former intensive and conventional DCCT arms, respectively. By EDIC Year 5 the HbA1c levels were statistically indistinguishable (8.1 vs. 8.2%, p=0.09). However, there continues to be a slower rate of acquisition and progression of DPN in the patients from the former intensive treatment arm, despite over 8 years of similar control (Martin et al., 2006).

**Continuous glucose monitoring** Despite the evidence of the DCCT which suggest that HbA1c levels are strong predictors for diabetic complications; factors other than average blood-glucose levels have a profound influence on incidence of diabetic complications. Close examination of the DCCT has indicated that the prognosis of complications may need to go beyond the average blood-glucose level as indicated by HbA1c (Brownlee and Hirsch, 2006). Figure 7 shows that when the conventional therapy group of the DCCT was examined, the HbA1c shows a general correlation with incidence of diabetic complications (Hirsch and Brownlee, 2005). In contrast, when the intensive therapy group was examined, HbA1c holds only a slight relation to development of diabetic complications. As the difference between intense therapy and conventional therapy was multiple insulin injections and blood-glucose monitoring, intensive therapy cohort patients are considered much less likely to undergo dynamic glycolytic flux. These studies suggest that glycemic variability rather than average glycemic control (HbA1c) would be a better target for diminishing the onset and progression of complications.

*In-vitro* studies now show that hyperglycemia-induced superoxide resulting in oxidative stress is the major causal agent of cellular injury. Work by Monnier et al. (2006) showed no correlation between oxidative stress and 24 h mean glucose concentration, fasting plasma glucose levels or HbA1c (Monnier et al., 2006). On the other hand there was a direct correlation
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between oxidative stress and glycemic variability (as determined by mean amplitude of glycemic excursion). These data indicate that glycemic flux and variability lead to oxidative stress.

Glycemic variability is directly correlated to oxidative stress and is likely a better predictor of diabetes complications. Thus, the ability to reduce glycemic variability holds the potential of preventing complications. Until recently, real-time tracking and correction of glycemic flux was unattainable. Continuous glucose monitors (CGM) are now available which measure blood-glucose levels every 1–5 min. Glucose self-monitoring is recommended 3 or more times/day. With the implantable CGM, N 120 glucose levels/day are registered and stored (Wilson and Block, 2005; Weinzimer et al., 2008). Real-time data from the CGM allows for the patient to adjust insulin or food intake before either hyper- or hypoglycemia occurs. As such, glycemic variability may be reduced by modulating therapy (insulin/glucose intake) in response to glucose trends. Implantable CGM sensors are used for between 3 and 7 days. Widespread use of the CGM remains limited at present, likely due to the cost to the patient.

Islet transplantation Pancreas transplantations in T1DM patients are now at a sufficient success rate to assess potential therapeutic effects on diabetic complications. Pancreas/islet transplantation re-established normoglycemia in T1DM patients. Compared to T1DM patients who received only kidney transplants, T1DM patients with successful pancreas transplants (as determined by normoglycemia and insulin independence) showed improvements in motor and sensory nerve conduction and clinical neuropathy (Navarro et al., 1997). These improvements were seen after 3.5 years for clinical examinations, between 1 and 55 years for sensory nerve conduction and after 10 years for motor nerve conduction. In fact, 50% of islet transplant patients with DPN exhibited stabilization or improvement of their neuropathy (Lee et al., 2005). Improvements were seen only for DPN and not for DAN.

Type 2 diabetes mellitus

Evidence that good glycemic control can delay or prevent progression of diabetic neuropathy in patients with T2DM is more limited. Neuropathy
findings from the UKPDS were discussed previously. In brief, although it was clearly shown that intensive treatment significantly reduced the risk of an aggregate endpoint of microvascular complications, and single endpoints of retinal photo-coagulation, and cataract extraction, there was only a trend for reduction in the risk of amputation (P=0.099) in the intensively-treated patients (intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UK Prospective Diabetes Study (UKPDS) Group, 1998). There was, however, a significant reduction in the risk of sudden death (P=0.047) in which it is possible that autonomic neuropathy plays a role.

In the feasibility study for the Veterans Administration Cooperative Study on T2DM (VA CSDM), the effects of 2-year intensive treatment on clinical DPN and on DAN were examined in 153 men with T2DM (average duration= 7.8 ± 4 years) (Azad et al., 1999). Intensive treatment (stepwise introduction of multiple insulin injections plus daytime glipizide) achieved a separation in HbA1c between intensive and conventional treatment of 2.1%, with intensively-treated patients at or below 7.3%. The baseline prevalence of clinical DPN was 50% and of abnormal autonomic function tests, was 35%. The prevalence of clinical DPN and abnormal autonomic function tests increased significantly and similarly in the two treatment groups, as did the prevalence of erectile dysfunction. Although there was a nearly- significant decrease in the prevalence of cranial neuropathy and more frequent preservation of touch sensation in the upper extremities in the intensive vs.conventional treatment group, this study provided only minimal evidence that good glycemic control improves neuropathic outcomes in patients with T2DM (Azad et al., 1999).

In summary, the interventional evidence relating hyperglycemia and neuropathy in patients with T2DM is less overwhelming than that in patients with T1DM. Yet, in view of the strong association between HbA1c and the incidence, prevalence, and progression of neuropathy in all forms of diabetes, aggressive treatment aiming for normalization of both fasting and postprandial
glucose levels remains the first and most important step in treating patients with any form of diabetic neuropathy.

There is an uncommon form of neuropathy that may occur in poorly-controlled diabetic patients shortly after instituting aggressive insulin therapy. This acute painful neuropathy has also been described in other situations, particularly in male patients following rapid weight loss (Windebank and Feldman, 2001). This differs from DPN in that sensory loss is minimal and weakness does not occur. In the cases arising from rapid weight loss, improving glycemic control and recovery of weight lead to symptomatic improvement, and temporarily relaxing glycemic control leads to symptomatic relief in those cases appearing to arise from instituting aggressive insulin therapy. In this form of neuropathy, complete resolution usually occurs over 6 to 24 months.

SYMPTOMATIC TREATMENT OF PERIPHERAL NEUROPATHY

The lack of understanding of the pathogenesis of this disorder precludes the development of mechanism-specific therapies (Feldman et al., 2002, 2005). Therefore, currently accepted medical approaches are only partially successful and are often ineffective (Dworkin et al., 2005, 2007a). These include the use of anticonvulsants, antidepressants, topical agents, and opioid based therapies (Ziegler, 2006, 2008; Jain, 2008) that all have undergone placebo-controlled studies in patients with DPN.

Antidepressants

Tricylic and tetracyclic reagents The tricylic and tetracyclic antidepressants (TCA) are considered as the first line treatment for neuropathic pain. These antidepressants control pain and pain related symptoms such as insomnia and depression. The therapeutic actions of these agents are mediated by inhibition of the reuptake of norepinephrine and serotonin. In a study reported by Max et al. (1992) amitriptyline (150 mg/day) is superior to placebo in relieving DPN after 6 weeks of treatment. However, amitriptyline is associated with significant side effects, including dry mouth, sedation, and blurred vision. Desipramine is better tolerated at 111 mg/day.
and is as effective as amitriptyline in alleviating DPN (Max et al., 1992). Randomized control trials for imipramine demonstrated that a dose of 50 and 75 mg/day significantly improves DPN (Sindrup et al., 1989, 1990b; Sindrup and Jensen, 1999). In addition, clomipramine relieves the symptoms of DPN (Sindrup et al., 1990c).

Pooling the data from all of these trials suggests that approximately 1 in 3 patients experience at least 50% relief from pain by using these drugs (Collins et al., 2000). The use of TCA is limited by their side effects (Jann and Slade, 2007). Overall, secondary amines (nortriptyline, desipramine) are better tolerated than tertiary amines (amitriptyline, imipramine) (Dworkin et al., 2007b). TCAs are not well tolerated in older patients. The TCAs should be used with great caution (or avoided altogether) in patients with cardiac arrhythmias, congestive heart failure, orthostatic hypotension, urinary retention, or angle-closure glaucoma (Simmons et al., 2002). It is important to note that TCAs are contraindicated in patients taking monoamine oxidase (MAO) inhibitors. The usual dosage schedule for TCAs is 10 to 25 mg at bedtime initially, titrating as tolerated up to 100 or 150 mg as a single bedtime dose. In addition, their analgesic effects require several weeks to develop which limits their utility for acute pain (Max et al., 1987).

Selective serotonin reuptake inhibitors and Serotonin Norepinephrine Reuptake Inhibitors The selective serotonin reuptake inhibitors (SSRIs) are newer antidepressants that have largely replaced TCAs for the treatment of depression because they are better tolerated. However, in contrast to TCAs, the effects of SSRIs are limited in DPN. Fluoxetine, 40 mg/day, is not different from placebo (Max et al., 1992). In a crossover study with paroxetine and imipramine, significant benefits from paroxetine 40 mg/day are observed (Sindrup et al., 1990a, 1990b). The improvement is less than imipramine 50 mg/day but better than placebo. Citalopram 40 mg/day has also been shown to be better than placebo for treating DPN (Sindrup et al., 1992). However, the numbers of patients involved in most of these studies are small and the trial periods are all short, limiting the interpretation of these data. Pooled data for SSRI treatment of DPN demonstrates no significant
difference between SSRIs and placebo (Collins et al., 2000). In contrast, Tramadol is a weak μ-receptor agonist that inhibits reuptake of serotonin. A double-blind, randomized, placebo-controlled trial using an average dose of 210 mg/day for 6 weeks produced significantly reduced pain scores in patients with DPN (Harati et al., 1998). Nausea, constipation, headache, and dyspepsia were common side effects. Another trial also demonstrated tramadol 200-400 mg/day significantly relieves DPN over placebo (Sindrup et al., 1999).

**Tramadol** is well tolerated with only mild side effects. In addition, a combination of tramadol/acetaminophen (37.5/325 mg) when taken as 1–2 tablets four times a day is effective in alleviating DPN (Freeman et al., 2007). The serotonin-norepinephrine reuptake inhibitors (SNRI) have greater efficacy against DPN than SSRIs. Duloxetine has been approved by the Food and Drug Administration (FDA) for treating DPN following three large randomized placebo control trials (Goldstein et al., 2005; Raskin et al., 2005; Wernicke et al., 2006). In these trials, duloxetine 60 mg and 120 mg daily provided significant relief from DPN. The higher dose provides greater relief from DPN but is associated with increased side effects (Sultan et al., 2008). In general, duloxetine is better tolerated, in terms of gastrointestinal and cardiac side effects, than other serotonin-norepinephrine reuptake inhibitors. Venlafaxine 150–225 mg/day alleviates DPN but produces unacceptable cardiac side effects with increased risk of electrocardiographic changes (Rowbotham et al., 2004).

**Anticonvulsants**

Anticonvulsants control neuronal excitability by blocking sodium and/or calcium channels (Wiffen et al., 2005). Originally developed for preventing seizures, they are in broad use for the treatment of neuropathic pain. Phenytoin and carbamazepine primarily block the voltage gated sodium channel. At doses between 200 and 600 mg/day, both reduce DPN compared to placebo. Due to side effects and newer improved therapies, these compounds are not recommended (Rull et al., 1969; Saudek et al., 1977; Chadda and Mathur, 1978; Gomez-Perez et al., 1996).
**Sodium valproate** enhances GABA levels in the central nervous system, inhibits T-type calcium channels, and increases potassium inward currents. Again, side effects, including hair loss, weight gain, hepatotoxicity, and cognitive dysfunction are not insignificant and increase with long-term use, although a dose of 500 mg/day decreases DPN (Kochar *et al.*, 2004).

**Lamotrigine** is a new anticonvulsant which blocks voltage gated sodium channels, decreases presynaptic calcium currents to inhibit the release of glutamate, and increases GABA levels in the brain. Eisenberg *et al.* (2001) reported favorable results of lamotrigine (≤400 mg/day) against DPN. Vinik *et al.* (2007) also reported two large-scale (n=360) randomized double-blind, placebo-controlled 1210 trials. Although there is a reduction on the pain scale in one trial after 19 weeks of duration with lamotrigine (400 mg/day), there is no difference between lamotrigine and placebo group at the end of the trial. Lamotrigine is well tolerated but its efficacy against DPN is questionable.

**Topiramate** has multiple actions: 1) blocking activity-dependent voltage gated sodium channels; 2) inhibiting L-type voltage gated calcium channels; and 3) blocking postsynaptic kainite/α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) excitatory amino acid receptors. Raskin *et al.* (2004) reported a randomized, double-blind, placebo-controlled study which involved 323 patients with DPN. Topiramate ≤400 mg/day was usually well tolerated and significantly alleviated DPN in approximately 1 out of 6 patients.

**Zonisamide** blocks both voltage dependent sodium channels and T-type calcium channels. A randomized, double-blind, placebo-controlled pilot study of 25 patients with a mean dose of 540 mg/day did not significantly reduce DPN after 6 weeks of titration and maintenance treatment (Atli and Dogra, 2005). Common side effects were restlessness, GI discomfort, headache, and weight loss.

**Oxcarbazepine** is a keto-analogue of carbamazepine, which blocks sodium channels. In one study, ≤1800 mg/day oxcarbazepine significantly reduced DPN (Dogra *et al.*, 2005), but in a larger study, no significant reduction of DPN was seen with 1200 and 1800 mg/day (Beydoun *et al.*, 2004).
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2006). Oxcarbazepine has a good side effect profile and is well tolerated. However, more studies are necessary to clarify its potential for treating DPN.

**Calcium channel α2-δ ligands**

*Gabapentin* is widely used for neuropathic pain due to its effectiveness and relatively fewer side effects than TCA and other anticonvulsants. Gabapentin produces analgesia via binding to the α2-δ site of L-type voltage gated calcium channels and decreasing calcium influx (Striano and Striano, 2008). Gabapentin ≤2400 mg/day is effective in treating DPN compared to amitriptyline (≤90 mg/day) according to a randomized control trial of 165 patients (Backonja et al., 1998; Dallocchio et al., 2000). However, another study found no difference between gabapentin (900–1800 mg/day) and amitriptyline (25–75 mg/day) (Morello et al., 1999). Large head-to-head comparison studies are needed to demonstrate the superiority of gabapentin over amitriptyline. Gabapentin is usually well tolerated with slow titration. Common side effects of gabapentin include dizziness, ataxia, sedation, euphoria, ankle edema, and weight gain. Moreover, it usually takes weeks of titration to reach the maximal effective dose (Dworkin et al., 2007b). Like gabapentin, pregabalin also acts by binding to the α2-δ subunit of calcium channels. As demonstrated in four randomized placebo control trials, pregabalin (300–600 mg/day) is significantly more effective in alleviating DPN than placebo (Lesser et al.; 2004; Rosenstock et al., 2004; Richter et al., 2005). Unlike gabapentin, pregabalin has better GI absorption and can be administered twice per day. Its linear pharmacokinetics provides a rapid (2 weeks) onset of maximal pain relief (Dworkin et al., 2007b). However, side effects are similar to gabapentin with dizziness, ataxia, sedation, euphoria, ankle edema, and weight gain. Among these side effects, weight gain is especially concerning for patients with T2DM. Like duloxetine, pregabalin is approved by FDA for treating DPN (Hurley et al., 2008).

*Lacosamide* is a novel chemical entity with anticonvulsant and analgesic properties that is being developed to treat epilepsy and neuropathic pain conditions. Lacosamide has shown efficacy in many animal models of chronic pain and in several short- and long-term Phase II/III clinical trials in
humans with diabetic neuropathic pain. The mechanism of action of lacosamide differs from other drugs used to treat neuropathic pain in that it selectively enhances sodium channel slow inactivation without affecting fast inactivation, and may modulate collapsin-response mediator protein 2. The pharmacokinetic properties of lacosamide include a fast rate of absorption, little or no interaction with cytochrome P450 isoenzymes, limited effect of age and gender on plasma levels and low potential for drug-drug interactions (Biton, 2008).

**Mexilitine**

Mexilitine is an anti-arrhythmia medication and has been used for treating a variety of painful neuropathic conditions including DPN (Jarvis and Coukell, 1998). Several randomized placebo control trials have been performed, but none of the studies revealed greater than 50% reduction in pain scores. However, those patients with stabbing or burning pain, heat sensations, or formication benefit most by mexiletine therapy (Stracke et al., 1992).

**Opiates**

Slow release oxycodon 20 mg/day relieves DPN over a 6-week period (Gimbel et al., 2003). In a crossover design treatment strategy, slow release oxycodon was effective against DPN at a maximum dose of 80 mg/day (Watson et al., 2003). Although opioids are effective against DPN, long-term use of opioids will result in side effects including constipation, urinary retention, impaired cognitive function, impaired immune function, and many other issues associated with tolerance and addiction. Recently, trials used combination of therapies with opioid and gabapentin has proven there is an additive effect of pain relieve in comparison to individual treatment. Gilron et al. (2005) tested patients with neuropathic pain from DPN and postherpetic neuralgia using maximal tolerated doses of morphine, gabapentin or both. The combinations of drugs achieve higher potency of pain relieve than individual treatment. However, the maximal tolerated dose of each drug is lower in the patient group with combination therapy, suggesting increased side effects with drug combination. The combination pharmatherapy have
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been used widely in clinical practice but more studies need to be performed to establish safety, compliance and cost-effectiveness and determine optimal drug combinations and dose ratios; comparing concurrent with sequential combination therapy; and combining more than two drugs (Gilron and Max, 2005).

Non-steroidal Anti-inflammatory Drugs

Non-steroidal anti-inflammatory drugs (NSAIDS) are a class of medications that inhibits cyclooxygenases, and thus prevent the formation of prostaglandins. Usually, NSAIDS are not recommended for the treatment of DPN due to their detrimental effects to GI, renal, and cardiac functions. Risk of overdose is also high in patients with chronic pain. However, a small, single blinded study demonstrated ibuprofen 2400 mg/day and sulindac 400 mg/day significantly reduced the paresthesia scores of DPN at 24 weeks (Cohen and Harris, 1987).

Aspirin

Aspirin (acetylsalicylic acid-an NSAID) is a commonly used analgesic although long-term use in diabetic patients should be weighted against possible gastrointestinal side effects. In diabetic patients on high doses of aspirin, such as those with rheumatoid arthritis, the incidence of retinopathy is decreased compared with age-matched controls not taking aspirin, which suggested that aspirin may protect against glycation (Cottier, 1981). Indeed, aspirin reduces glycation in-vitro, and in animal experiments, potentially by acetylation of amino groups (Blakytny and Harding, 1992).

Alternatively, it is possible that aspirin does not directly alter glycation but inhibits glyoxidation and AGE cross-link formation; therefore its effects may be because of its antioxidant capacity (Fu et al., 1994). Besides the analgesic effects of aspirin, studies indicate a reduced risk of cardiovascular events for diabetic patients on low low-dose aspirin (Hennekens et al., 2004).
N-methyl-D-aspartate receptor antagonists

Two N-methyl-D-aspartate (NMDA) receptor antagonists, dextromethorphan and memantine, have been tested in DPN (Sang et al., 2002). These placebo-controlled cross over studies involve 23 patients with diabetic neuropathy. The studies were designed with a 7-week titration period followed by a 2-week maintenance period for lorazepam, an active placebo, or one of the NMDA inhibitors. Both high and low doses of the inhibitors were assessed. Treatment with dextromethorphan but not memantine produced a significant dose-dependent decrease in DPN. However, the NMDA inhibitors have significant side effects, including sedation, dry mouth, and gastrointestinal distress.

Topical agents

Capsaicin is an extract of capsicum peppers. Capsaicin binds to TRPV1 receptor and exhausts substance P in the peripheral nerves to achieve its analgesic effects. In the study published by the Capsaicin Study Group, 0.075% capsaicin cream applied three times a day for 6 weeks was more effective in alleviating DPN than placebo (The Capsaicin Study, 1992). Burning was the most common side effect which tended to decrease as therapy is continued. The therapeutic effects of capsaicin started weeks after the cream application. Recently, a patch containing high concentrated capsaicin has demonstrated promising effects in treating diabetic pain. Because impaired NO generation leading to reduced blood flow may be involved in DPN, a small trial using isosorbide dinitrate, an NO donor, was performed. In a 12 weeks, double-blind, placebo controlled, crossover study with 22 patients, isosorbide dinitrate spray significantly relieved DPN (Yuen et al., 2002). Patients in the trial reported minor headaches and a larger sized study is necessary to evaluate the potential use of this treatment for DPN.

Topical lidocaine 5% patches have been reported by several studies to relieve DPN. In an open labeled study, up to four 5% lidocaine patches applied for up to 18 h/day are well tolerated in patients with painful diabetic polyneuropathy. Lidocaine patches significantly improved pain and quality-of-life ratings, and may allow tapering of concomitant analgesic therapy.
CAUSAL THERAPIES

Tight glycemic control and symptomatic therapies are the proven therapies to prevent or slow down the diabetic neuropathy. However, research efforts have also been focused on those potential molecules which mitigate the biochemical aberrations inducing neuronal damage.

Aldose Reductase Inhibitors

Aldose Reductase Inhibitors (ARIs) have historically been the primary target of diabetic neuropathy treatment, due largely to their success in reducing cataract-forming osmotic stress associated with polyol accumulation in the diabetic lens (Kinoshita et al., 1968; Chylack et al., 1979). Additionally, most studies of the human AR gene (AKR1B1) and its polymorphisms in diabetic patients indicate that the “high AR expression” genotype is correlated with elevated diabetic vascular complications and early diabetic neuropathy indicators, whereas the “low AR expression” genotype is correlated with diminished complications and indicators (Demaine, 2003; Donaghue et al., 2005; Thamotharampillai et al., 2006). Furthermore, ARIs have been successful in preventing and reversing nerve deterioration in rodent models (Cameron et al., 1986; Yagihashi et al., 1990; Kato et al., 2000). A variety of ARIs have entered the market; while most have effectively reduced nerve polyol levels, this result has not always translated to amelioration of diabetic neuropathy symptoms. Recent thinking posits that polyol levels themselves are not the best indicators of drug efficacy. Instead, the “Metabolic Flux Hypothesis” emphasizes cofactor turnover rate as a better marker of oxidative stress alleviation (Barnett et al., 1986; Cameron and Cotter, 1994). This model suggests that much higher (~20-30 fold) doses of ARIs are needed to effectively decrease the rate of cofactor turnover and thereby alleviate common diabetic neuropathy symptoms (Oates, 2008).

Sorbinil

In 1981, Sorbinil was the prototype ARI to be developed solely for diabetic neuropathy treatment.
Table 8. Therapeutics for Polyol Pathway

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Trials / notes</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Sorbinil</td>
<td>Only slight improvement in NCV; high rates of skin rashes, trial withdrawn</td>
<td>Judzewitsch et al., 1983</td>
</tr>
<tr>
<td>Tolrestat</td>
<td>Halted mild diabetic neuropathy progression: no significant improvement in NCV, trial withdrawn</td>
<td>Boulton et al., 1990; Giugliano et al., 1993</td>
</tr>
<tr>
<td>Ponalrestat</td>
<td>No effect due to poor pharmacokinetics and pharmacodynamics, trial withdrawn</td>
<td>Stribling et al., 1985; Greene and Sima, 1993</td>
</tr>
<tr>
<td>Zopolrestat</td>
<td>Low level: slight NCV improvement; high levels: slight improvement in NCV: elevated liver enzymes, trial withdrawn</td>
<td>Arezzo et al., 1996</td>
</tr>
<tr>
<td>Zenarestat</td>
<td>Dose dependent increase; Improvement of NCV</td>
<td>Greene et al., 1999; Gabby, 2004</td>
</tr>
<tr>
<td>Lidorestat</td>
<td>Withdrawn at Phase 2 clinical trial</td>
<td></td>
</tr>
<tr>
<td>Fidarestat</td>
<td>Similar to sorbinil, suspended at phase 3 due to resource consolidation</td>
<td>Giannoukakis, 2003</td>
</tr>
<tr>
<td>As-3201/ranirestat</td>
<td>Promising phase 2 trials, phase 3 underway, high placebo effect complicated study</td>
<td>Bril and Buchanan, 2006</td>
</tr>
<tr>
<td>Epalrestat</td>
<td>Delayed progression of diabetic neuropathy, study not replicated</td>
<td>Hotta et al., 2006</td>
</tr>
<tr>
<td>Myo-inositol</td>
<td>Animal studies indicate beneficial effects, human studies needed</td>
<td>Sima et al., 1997</td>
</tr>
</tbody>
</table>

Despite successfully reducing and preventing NCV deficits in rodent models, Sorbinil failed to produce noteworthy results in humans. While Sorbinil, a spiroimide, slightly improved in nerve fiber regeneration and human diabetic NCV (by 0.7–1.2 m/s), it was associated with a high incidence of skin rash, and was withdrawn from the market in 1987 (Judzewitsch et al., 1983;
Sima et al., 1988). However, Sorbinil's moderate success paved the way for future ARI therapies.

**Ponalrestat** Ponalrestat is a carboxylic acid that effectively lowers nerve sorbitol levels *in-vitro* and in rats, but fails to do so in human diabetic nerves (Stribling et al., 1985; Greene and Sima, 1993). Ponalrestat is 99% plasma protein-bound in humans (~10-fold increase over rats), and most of the unbound acid is ionized at cellular pH. Such ions are slow to cross nerve plasma membranes, further diminishing Ponalrestat's effectiveness.

**Zopolrestat** Zopolrestat is a carboxylic acid analog of Ponalrestat that dose-dependently decreased diabetic rat nerve sorbitol and fructose levels. In human studies, low levels (250–500 mg) of Zopolrestat decreased nerve sorbitol levels, but had no effect on fructose levels or symptom alleviation, and showed little NCV improvement. At higher levels (1000 mg), Zopolrestat was significantly more effective at increasing NCV, but was associated with a higher incidence (7%) of elevated liver enzymes and eventually withdrawn (Arezzo et al., 1996). These trials illustrate that nerve sorbitol level per se is not the best indicator of nerve health, and that elevated ARI doses are likely needed to achieve significant diabetic neuropathy symptom improvement.

**Zenarestat** Zenarestat is a carboxylic acid ARI also showing a dose-dependent increase of NCV; notably, higher doses of Zenarestat continue to improve NCV, increase nerve fiber density and nerve health even after nerve sorbitol levels have stabilized (Greene et al., 1999). Though its development was terminated due to a high incidence of elevated serum creatine levels, the studies demonstrated nonetheless that sorbitol is not the best marker of drug efficacy, and provides hope that at high doses ARIs can be more effective than previously thought (Gabbay, 2004).

**AS-3201 or Ranirestat** is a well-tolerated spirosuccinimide discovered in 1998. Phase 2 trials were promising, showing few side effects and marked improvement in both NCV deficit and diabetic neuropathy symptoms. However, Q34 definitive phase 3 study conclusions could not be drawn as of July 2007 due to the trial's unusually high placebo effect. AS-3201 development is ongoing, and researchers hope that continued study and
increased dosage of Ranirestat will prove effective in future diabetic neuropathy treatment (Oates, 2008).

**Epalrestat** In 1992, Epalrestat entered the Japanese market as a carboxylic acid ARI with minimum side effects, but without conclusive evidence of efficacy backed by a randomized, double-blind placebo-controlled study. From 1997–2003 such a study was conducted, and at slightly elevated doses (150 mg), Epalrestat delayed nerve deterioration and alleviated many common diabetic neuropathy symptoms such as limb numbness and cramping (Hotta et al., 2006). Though these results have not been replicated, Epalrestat is now the standard drug therapy for diabetic neuropathy in Japan. Epalrestat 50 mg 3 times/day may improve motor and sensory nerve conduction velocity and subjective neuropathy symptoms as compared with baseline and placebo. Epalrestat is well tolerated, and the most frequently reported adverse effects include elevations in liver enzyme levels and gastrointestinal-related events such as nausea and vomiting. Epalrestat may serve as a new therapeutic option to prevent or slow the progression of diabetic neuropathy. Long-term, comparative studies in diverse patient populations are needed for clinical application (Ramirez and Borja, 2008).

**Myo-inositol** Myo-inositol is a naturally occurring secondary messenger involved in proper nerve function. Myo-inositol depletion is associated with decreased Na⁺–K⁺–ATPase function and decreased NCV, and has been implicated in early-stage diabetic neuropathy pathology (Sima et al., 1997). Evidence suggests that dietary myoinositol supplements might slow diabetic neuropathy progression, though further study is needed to assess efficacy.

**Hexosamine Pathway Modulators**

Activation of the hexosamine pathway generates UDPGlcNAc, which modulates transcription factors and ultimately induces neurovascular insult (Fig. 8). While ARIs directly target toxic pathways, modulation of the hexosamine pathway can redirect glycolytic flow away from subsequent deleterious pathways. This mode of action offers an intriguing possibility for altering pathways in metabolic disorders.
Figure 8. Diagram of benfotiamine effect on biochemical pathways in diabetic complications. Excess glucose elevates flux through hexosamine pathway creating UDPGlcNAc from F-6-P. UDPGlcNAc modifies transcription factors which lead to inflammation. Addition of benfotiamine, a thiamine analogue activates transketolase (TK) which diverts substrate away from the hexosamine pathway and into the pentose phosphate pathway (Adapted from Hammes et al., 2003)

Benfotiamine  Benfotiamine is a fat-soluble analogue of thiamine/vitamin B1 that activates transketolase, an enzyme converting fructose-6 phosphate into pentose-5 phosphates (Fig. 8). The reduced fructose-6 phosphate input decreases flux through the hexosamine pathway (as well as flux through the advanced glycation end product (AGE) and the diacylglycerol (DAG)-protein kinase C (PKC) pathways) (Hammes et al. 2003). The increased flux away from the hexosamine pathway and into the pentose-5 phosphate pathway may offer an additional benefit: increased redox capacity. One of the products of the pentose phosphate pathway is NADPH, a prime reactant in the formation of the antioxidant glutathione. Since..
NADPH is depleted in the polyol pathway, benfotiamine holds the speculative possibility of diminishing the effects of this pathway as well. Benfotiamine has successfully inhibited these pathways and prevented diabetic retinopathy in animal models (Hammes et al., 2003). In humans, Benfotiamine has been shown to improve pain associated with diabetic neuropathy and to improve NCV in conjunction with vitamins B6 and B12 (Haupt et al., 2005; Stracke et al., 1996; Winkler et al., 1999). Benfotiamine is currently available as a dietary supplement in the United States.

**Protein Kinase C Inhibitors**

*Ruboxistaurin* Ruboxistaurin is a PKC-β competitive inhibitor that has effectively managed many complications of diabetes in clinical trials. It has been particularly successful in reducing the progression of diabetic retinopathy, endothelial vasodilation, and (to a lesser extent) nephropathy (Ishii et al., 1996; Beckman et al., 2002b; Tuttle et al., 2005; Aiello et al., 2006). However, trials of Ruboxistaurin's effect on diabetic neuropathy have not shown significant improvement (Vinik et al., 2005). Ruboxistaurin was being developed for US marketing by Eli Lilly and was pending FDA approval as a pharmaceutical agent for diabetic retinopathy. However, the company withdrew its marketing application in March, 2007, and Ruboxistaurin's fate is currently unclear.

**Advanced Glycation End Products Inhibitors**

Clearly, glycemic control is the primary means for decreasing AGE formation. Given that this may be difficult to achieve, prevention of RAGE activation is an important alternative therapeutic goal for diabetic neuropathy (Fig. 9). Two possible approaches are feasible: to prevent the formation of AGEs or to block RAGE. Numerous compounds have been investigated for anti-glycation activity but their use in humans is still debatable. The following section describes compounds that have been assessed for the ability to decrease activity of the RAGE axis in diabetic neuropathy (Huijbers et al., 2008).
Aminoguanidine

Aminoguanidine (also called pimagedine) is a nucleophilic hydrazin compound and has received the most attention as a potential anti-glycatic drug (Thornalley, 2003). Initially, it was thought that aminoguanidine prevented AGE formation by blocking carbonyl groups on Amadori products although it is now known to react with carbonyl groups from reducing sugars or 3-DG (Edelstein and Brownlee, 1992; Lewis et al., 2006). In diabetic animals, aminoguanidine reduces nephropathy (Soulis et al., 1996), retinopathy (Chibber et al., 1994; Hammes et al., 1995), and neuropathy (some but not all studies (Miyauchi et al., 1996; Birrell et al., 2000). Preliminary studies in diabetic patients showed that aminoguanidine therapy for 28 days reduces hemoglobin-derived AGEs (Hb-AGE) but does not alter levels of Amadori products (Makita et al., 1992). Three other phase II and III trials...
aminoguanidine have been completed with nephropathy endpoints but produced no benefit. The last was discontinued because of side effects in patients, which include flu-like symptoms, gastrointestinal disturbances, and anemia (Bolton et al., 2004). Despite the earlier promising results with aminoguanidine, it is unlikely to be used for therapeutic purposes.

**Phenacylthiazolium bromide**

Compounds capable of cleaving AGE cross-links have been described, opening up the exciting possibility of reversing diabetic complications. These compounds include N-phenacylthiazolium bromide (PTB), which can cleave AGE cross-links by a mechanism which is still unclear. PTB has been used to cleave AGE cross-links between albumin and collagen *in-vitro* and recent studies in diabetic rats have shown that PTB can prevent or reverse the accumulation of AGEs in blood vessels (Cooper et al., 2000). However, another study found that although PTB can reduce model AGE cross-links *in-vitro*, it does not reduce AGE cross-links formed *in-vivo* (Yang et al., 2003). Whether AGE cross-link breakers are useful *in-vivo* will also depend on their long-term toxicity. Due to the unstable nature of PTB, analogues such as alagebrium chloride, also known as ALT-711, have been developed. This compound provides renoprotection in diabetic mice (Peppa et al., 2006; Coughlan et al., 2007). Patient trials to date have found that ALT-711 is well tolerated and produces significant vascular benefit in the elderly through decreased blood pressure and increased vascular elasticity (Little et al., 2005; Zieman et al., 2007).

**AGE Receptor Blockers**

There is considerable interest in compounds capable of blocking the interaction between AGEs and RAGE. RAGE can be blocked by usage of soluble RAGE (sRAGE), which is the extracellular ligand-binding domain of RAGE or by use of antibodies capable of reacting with RAGE. Studies by Schmidt and coworkers have performed multiple studies in diabetic mouse models using RAGE knockout mice and mice treated with sRAGE or anti-RAGE (Bierhaus et al., 2004; Hudson and Schmidt, 2004). They demonstrate: topical sRAGE improves wound healing (Wear-Maggitti et al., 2004), sRAGE
decreases atherosclerosis in ApoE knockout mice (Bucciarelli et al., 200: RAGE blockade prevents the final stages of diabetogenesis in non-obe diabetic mice (Chen et al., 2004b), and that RAGE blockage prevent sensory deficits (Bierhaus et al., 2004). Therefore, blockage of RAGE may an important mechanism to prevent diabetic complications and the Schm group is actively working on translation of sRAGE to a clinical trial.

**Poly (ADP-ribose) Polymerase Inhibitors**

As PARP mediates both neuronal dysfunction and inflammatic inhibition of PARP holds the potential of improving two aberrant causeways diabetic neuropathy, making it a promising target. PARP inhibitors such 1,5-isoquinolinediol and 3-aminobenzamide have successfully improved the PARP-mediated dysfunctions in STZ-induced diabetic rats (Li et al., 2005; Obrosova et al., 2005a; Ilnytska et al., 2006). Additionally, Nicotinamide (vitamin B3) has been shown to act as both a PARP inhibitor and antioxd in rodents, improving the complications of early diabetic peripheral neuropat (Stevens et al., 2007). Nicotinamide has an attractive therapeutic potent due to its limited side effects and toxicity (Gale et al., 2004). A combinati- therapy for diabetic neuropathy including nicotinamide, the xanthine oxida inhibitor allopurinol, and the antioxidant DL-α-lipoic acid is currently in trial.

**Antioxidants**

Given the known mechanisms leading to diabetic neuropathy, a logic therapeutic approach is to prevent oxidative stress by increasing antioxidan defense. Antioxidant defense arises from (Hogan et al., 2003) antioxid enzymes that catalyze the removal of ROS antioxidant molecules that preve the oxidation of other molecules (Dworkin et al., 2007a), usually because th are readily oxidized molecules that chelate transition metal ions so that cannot catalyze the generation of ROS in a cell (Dworkin et al., 2005). Major portion of the body’s antioxidant defense comes from dietary intake micronutrient molecules that facilitate one or more of these thr mechanisms. This makes oral antioxidants an attractive strategy for the prevention and treatment of diabetic neuropathy. Many clinical trials antioxidant defense therapies have been completed, mostly using a high do:
of a single antioxidant compound. The results of these trials have been largely negative, despite the strong rationale for this approach. Some lead candidates are summarized in Table 9

**Vitamin E** Vitamin E is a fat-soluble compound that exists in 8 isoforms with varying biological activity (Traber and Packer, 1995). Blood levels of vitamin E can decrease under prolonged oxidative stress and in individuals who cannot absorb dietary fat (Triantafillidis et al., 1998), are on a low fat diet, or are zinc deficient (Bunk et al., 1989). α-Tocopherol is the most active isoform and is the most common dietary supplement. This compound has been broadly tested for its ability to prevent chronic diseases involving oxidative stress including cancer and diabetes complications. While small studies have indicated that high intake of vitamin E may decrease incidence of certain cancers, large studies generally do not support the findings. One study found that regular intake of high does of vitamin E for more than 10 years decreased the risk of death from bladder cancer (Jacobs et al., 2002). These studies demonstrate the safety of long-term use of vitamin E. In addition to potent antioxidant action, vitamin E can promote immune function, DNA repair, and metabolism (Traber and Sies, 1996; Wozniak et al., 2004). Chronic vitamin E administration improves the ratio of cardiac sympathetic to parasympathetic tone in patients with type 2 diabetes when given 600 mg/day for 4 months (Manzella et al., 2001).

**α-Lipoic acid** Alpha-lipoic acid, also termed thioctic acid, is an antioxidant that is available for treatment of DPN in some countries (Ziegler et al., 1999b; Singh and Jialal, 2008). It has the potent ability to scavenge ROS, regenerate other antioxidants, and chelate metal ions (Packer et al., 1997). Some randomized controlled clinical trials have shown that intravenous infusions of α-lipoic acid (600 mg daily, 5 days/week for 3 weeks) significantly improved sensory symptoms of DPN or the Neuropathic Impairment Score (Ziegler et al., 1999a). In another small study of oral α-lipoic acid (800 mg, QD) a small (non-significant) trend toward improvement in measures of cardiac autonomic neuropathy was reported (Ziegler et al., 1997).
### Table 9. Antioxidant Therapy in Diabetic Neuropathy

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Trials / notes</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Resveratrol and 4-amino 1,8</td>
<td>Combination of resveratrol (10 mg/kg) and 4-ANI (3 mg/kg) attenuated conduction and nerve blood flow deficits and resulted in amelioration of diabetic neuropathic pain</td>
<td>Sharma et al., 2009</td>
</tr>
<tr>
<td>naphthalimide</td>
<td></td>
<td></td>
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<tr>
<td>Alpha-lipoic acid</td>
<td>Application of alpha-lipoic acid during 3 months has helped to decrease the symptoms of diabetic neuropathy</td>
<td>Burekovic et al., 2008; Ayaz et al., 2008</td>
</tr>
<tr>
<td>Stobadine</td>
<td>Treatment with the combination of stobadine and vitamin E significantly ($p &lt; 0.001$) reduced the NCV slowing in diabetic rats</td>
<td>Skalska et al., 2008</td>
</tr>
<tr>
<td>Lycopene</td>
<td>An antinociceptive activity of lycopene possibly through its inhibitory action on NO and TNF-alpha release and point towards its potential to attenuate diabetic neuropathic pain</td>
<td>Kuhad et al., 2008</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Marked reduction in DNA fragmentation observed after resveratrol treatment in diabetic rats as evident from decrease in Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) positive cells in sciatic nerve sections. Chronic treatment with resveratrol (10mg/kg orally) from week 4 to week 6 significantly attenuated the cold allodynia and thermal hyperalgesia</td>
<td>Kumar et al., 2007; Sharma et al., 2006</td>
</tr>
<tr>
<td>Trolox</td>
<td>Trolox treatment also improved the activity of anti-oxidant enzymes and inhibited lipid peroxidation in sciatic nerves of diabetic rats</td>
<td>Sharma and Sayyed, 2007</td>
</tr>
<tr>
<td>Co enzyme Q10</td>
<td>Cofactor that improves metabolism but also a potent antioxidant</td>
<td>Eriksson et al., 1999; Bonnefont-Rousselot, 2004; Ayaz et al., 2008</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>(AKA vitamin B3) is a weak PARP inhibitor, antioxidant, and calcium modulator, effective in experimental diabetes and currently in type 1 diabetes patient trial</td>
<td>Eriksson et al., 1999; Bonnefont-Rousselot, 2004; Stevens et al., 2007</td>
</tr>
<tr>
<td>Eugenol</td>
<td>From clove oil, both antiinflammatory and antioxidant, improves vascular and neural deficits in STZ-treated rats</td>
<td>Nangle et al., 2006</td>
</tr>
<tr>
<td>Taurine</td>
<td>Plasma taurine is depleted in diabetic rats and replacement decrease hyperalgeria and other neural and vascular deficits</td>
<td>Pop-Busui et al., 2001; Li et al., 2005a, 2006</td>
</tr>
<tr>
<td>U83836E</td>
<td>A synthetic ROS scavenger, effective against oxidative stress and neurovascular deficit in rats</td>
<td>Sayyed et al., 2006</td>
</tr>
<tr>
<td>Oleuropein</td>
<td>From Olive leaf, decreases blood glucose as Al-Azzawie and</td>
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**Note:** This table summarizes the effects of various antioxidant compounds in diabetic neuropathy studies. The compounds include resveratrol, alpha-lipoic acid, stobadine, lycopene, resveratrol, trolox, coenzyme Q10, nicotinamide, eugenol, taurine, and oleuropein. Each compound is noted for its potential benefits, such as reducing DNA fragmentation, improving antioxidant enzymes, inhibiting lipid peroxidation, and alleviating symptoms of diabetic neuropathy.
<table>
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<th><strong>Review of Literature</strong></th>
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<tbody>
<tr>
<td><strong>Minerals</strong></td>
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<tr>
<td><strong>Vitamin C</strong></td>
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<tr>
<td><strong>Quercetin</strong></td>
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<td><strong>Melatonin</strong></td>
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<td><strong>Apocynin</strong></td>
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<td><strong>Rutin</strong></td>
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<td><strong>Dimethylthiourea</strong></td>
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<td><strong>Evening primrose oil</strong></td>
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<td><strong>Nitecaptone</strong></td>
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<td><strong>Troglitazone</strong></td>
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<td><strong>N-acetylcysteine</strong></td>
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In a more recent open-label trial of 10 days intravenous administration followed by 50 days of oral treatment, α-lipoic acid was found to improve several manifestations of autonomic neuropathy (Tankova et al., 2004). Other clinical trials have been completed with varying doses and either parenteral or oral administration of the drug. In 1995, a review of current evidence assessed trials that met specific requirements of randomization, double masking and placebo-controlled design (Foster, 2007). The study concluded that α-lipoic acid should be considered as a treatment for diabetic neuropathy since parenteral supplementation improves neuropathic symptoms over 3 weeks and oral treatment improves neuropathic deficits and potentially sensory symptoms.

**Botanicals**

It has long been recognized that natural dietary products including red grapes, evening primrose, and cruciferous vegetables increase antioxidant status. In addition to many botanical extracts possessing antioxidant capacity, several compounds are known to increase the expression of antioxidant genes such as glutathione S-transferase and NAD(P)H:quinone oxidoreductase 1 (Halat and Dennehy, 2003). These enzymes belong to the class of phase 2 enzymes under the regulation of a specific promoter consensus region known as the antioxidant response element (ARE) (Itoh et al., 1999).

Many compounds can activate the transcription factor, known as nuclear factor-E2-related factor-2 (Nrf-2), that binds this promoter. Oxidative stress can activate the promoter via phosphorylation of the Nrf-2 cytoplasmic chaperone protein Keap-1 (Tamasi et al., 2004). Botanical compounds may activate Nrf-2 either by inducing mild intracellular oxidative stress, by directly activating Nrf-2, or by causing recruitment of Nrf-2 co-activators, such as small Maf proteins (Venugopal and Jaiswal, 1998; Dhakshinamoorthy et al., 2000; Kang et al., 2005). Several botanical compounds so effectively activate the antioxidant response they are used to treat diseases with an oxidative component, particularly cancer and Alzheimer's disease (Thimmulappa et al., 2002; Perry, 2007).
**Resveratrol** The most widely assessed botanical compound is resveratrol, extracted from red grapes. Studies in STZ-treated rats demonstrated attenuation of thermal hyperalgesia and cold allodynia as well as decreases in oxidative stress DNA damage, and nerve conduction deficits (Sharma et al., 2006; Kumar et al., 2007). Similarly, in type 1 diabetic mice resveratrol prevents neuropathic pain (Sharma et al., 2007). Resveratrol is likely to provide additional therapeutic benefits in T2DM patients because it also activates the SIRT1 genes that regulate glucose metabolism and insulin sensitivity (Zang et al., 2006; Chen et al., 2007; Sun et al., 2007). Resveratrol was assessed in a trial examining aging and cardiovascular disease and produced positive results (Labinskyy et al., 2006). Although trials in diabetes are indicated by these findings, there are none on record to date. Other botanicals that activate the antioxidant response in experimental diabetes and in patients include extract of Tinospora cordifolia (Prince and Menon, 1999), curcumin (Osawa and Kato, 2005), garlic oil (Anwar and Meki, 2003), evening primrose oil (Halat and Dennehy, 2003), and sulphoraphane (Thimmulappa et al., 2002; Perry, 2007).

**Neurotrophic Factors**

Peripheral nervous system injury in diabetes may be the result of both hyperglycemia and loss of neurotrophic support normally provided by insulin. This hypothesis is supported by reports of abnormal expression levels of growth factors in diabetes. Thus, there is growing interest in exploring the potential utility of NGFs, insulin, IGFs, and others neurotrophic factors in the treatment of diabetic neuropathy. Insulin receptors are found in the PNS on Schwann cells, pericytes, endothelial cells, and neurons, especially sensory neurons (Sugimoto et al., 2000, 2002; Brussee et al., 2004). Insulin-deficient rat models of diabetes appear to have more severely progressive neuropathy compared to T2DM models, suggesting insulin deficiency itself contributes to the development of neuropathy (Pierson et al., 2003; Kamiya et al., 2005). *In-vitro*, insulin activates survival-promoting PI3K/Akt signaling and neurite outgrowth in sensory neurons (RecioPinto et al., 1986; Huang et al., 2005). Local delivery of insulin to the spinal cords of STZ-treated rats improves nerve...
condition velocity measurements, and low-dose systemic delivery at a level that does not reduce hyperglycemia is able to decrease signs of mitochondrial distress in sensory neurons (Singhal et al., 1997; Toth et al., 2006).

C-peptide, long thought to be merely an inert peptide fragment byproduct of insulin synthesis, is now believed to have biological activity of its own, although little is known mechanistically as there is currently no identified receptor (Wahren et al., 2004). C-peptide deficiency is concomitant with insulinopenia in T1DM. When C-peptide is replaced in diabetic rats, a number of measures of peripheral nerve function improve. Proposed mechanisms for these observations include potentiation of insulin signaling, vasodilation via nitric oxide release, and stimulation of the release of other neurotrophic factors (Sima et al., 2001; Cotter et al., 2003; Pierson et al., 2003; Sima et al., 2004a; Kamiya et al., 2006; Zhang et al., 2007).

Insulin-like growth factors (IGFs) I and II have profound effects on nervous system development and survival, mediated through activation of the IGF-I receptor (IGF-IR) (Leinninger and Feldman, 2005; Fernandez et al., 2007). IGFs and the IGF-IR are expressed throughout the developing and adult nervous system. IGFs have been reported to be reduced in some animal models of diabetes, although this varies and may be dependent upon the model, type of diabetes, and tissue examined (Ekstrom et al., 1989; Wuarin et al., 1994; Zhuang et al., 1997; Craner et al., 2002; Schmidt et al., 2003; Kamiya et al., 2006). A number of preclinical studies in diabetic rats suggest systemic or intrathecal IGF therapy can improve neuropathy (Ishii and Lupien, 1995; Zhuang et al., 1997; Schmidt et al., 1999, 2000; Lupien et al., 2003; Brussee et al., 2004; Toth et al., 2006). Clinical use of IGFs may be complicated by their widespread systemic effects (Russo et al., 2005). Additionally, the complex system of IGF binding proteins (IGFBPs) may impact efficacy. The IGFBPs regulate IGF bioavailability, and studies attempting to establish the status if the IGFBPs in diabetes have been inconsistent (Crosby et al., 1992; Busiguina et al., 2000; Han et al., 2006).

The system of neurotrophins is critical for the development and maintenance of the PNS and CNS (Kaplan and Miller, 2000; Huang and
Reichardt, 2003) and includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins (NT) 3–6. NGF is not required for sensory neuronal survival in the adult PNS, but it does regulate axonal sprouting and the phenotype of sensory neurons (Schwartz et al., 1982; Lindsay, 1988; Chudler et al., 1997). Thus, preclinical studies of NGF in diabetic rats resulted in improvements in both signaling outcomes of the NGF system and PNS function, as well as positive effects on myelination (Apfel et al., 1994; Diemel et al., 1994; Fernyhough et al., 1995b; Elias et al., 1998; Christianson et al., 2003, 2007). Clinical studies have not progressed past phase 3, however, and there is developing interest in small molecule activators of trkA as a potentially more viable alternative approach (Apfel, 2002). BDNF is expressed by both peripheral neurons and muscle, and its receptor, trkB, is found on motor neurons and some sensory neurons (McMahon et al., 1994). Retrograde transport of endogenous, but not exogenous, BDNF to the neuron cell bodies is impaired in diabetic rats, suggesting there are problems with the local supply of BDNF at the peripheral nerve terminals (Mizisin et al., 1999). Exogenous BDNF is protective to large myelinated sensory fibers in STZ rats, but not smaller fibers, which is consistent with the distribution of trkB expression (Mizisin et al., 1997; Calcutt et al., 1998; Elias et al., 1998). Preclinical studies of exogenous NT-3 therapy in diabetic rats have had mixed results. One study found improvement in large sensory fibers, but not motor fibers (Mizisin et al., 1998). Another study found effects on both large sensory and motor fibers (Mizisin et al., 2004). Intrathecal NT-3 increased myelinated fibers in the skin of diabetic mice, but without noticeable improvement of function (Christianson et al., 2007).

Ciliary derived neurotrophic factor (CNTF) is a cytokine with numerous neurotrophic properties (Vergara and Ramirez, 2004). It is only expressed in Schwann cells in the peripheral nervous system (Kobayashi and Mizisin, 2000), and levels of CNTF are reduced in diabetic rats (Calcutt et al., 1992). This deficiency can be improved by ARI therapy (Mizisin et al., 1997). Exogenous CNTF itself has therapeutic benefit in diabetic rats, improving function in both sensory and motor fibers, along with increasing regenerative capabilities (Calcutt et al., 2004; Mizisin et al., 2004). Therapeutic use of
CNTF is complicated by its systemic effects, particularly on muscle, and thus more targeted delivery approaches are being considered (Bongioanni et al., 2004).

Treatment of diabetic neuropathy should always begin with efforts to optimize glycemic control and with patient education. There are now many useful pharmacologic approaches to treat painful neuropathy and most manifestations of autonomic neuropathy, but disease disease-modifying treatments other than strict glycemic control await a more complete understanding of the underlying mechanisms of diabetic neuropathy and the development of pharmacologic agents based on this emerging knowledge (van Doorn and Merkies, 2008).

**DIABETES ASSOCIATED MEMEORY DECLINE: Diabetic Encephalopathy**

There is substantial evidence that acute hypo- and hyperglycaemia have disruptive effects on the central nervous system (CNS) (Northam et al., 2006), although relatively less is known about the slowly developing end-organ damage to the CNS that may present itself by electrophysiological and structural changes and impairment of cognitive functioning (Brismar et al., 2007; Kuhad and Chopra, 2008). These cerebral complications of both type 1 and type 2 diabetes may be referred to as ‘diabetic encephalopathy’, a concept introduced several decades ago (Reske-Nielsen et al., 1965). Mijnhout et al. (2006) proposed a new term ‘diabetes-associated cognitive decline’ (DACD) to facilitate research into this area and to increase recognition of the disorder. This term is not suggestive of a particular pathogenesis, but merely describes a state of mild to moderate cognitive impairment, in particular, psychomotor slowing and reduced mental flexibility, not attributable to other causes (Mijnhout et al., 2006). The duration and age of onset of diabetes are the strongest predictors of low scores in psychomotor speed, memory, processing speed, attention, working memory, verbal ability, general intelligence, executive functions and a low global score (Brismar et al., 2007). The neurobiological role of cerebral insulin, insulin receptors,
Review of Literature

hyperglycemia, oxidative stress and neurotransmitter alterations in cognitive deficits associated with diabetes is as follows:

**Insulin and Memory Consolidation**

The insulin receptor is a tetrameric, membrane spanning protein (Olefsky, 1990). There are two types of insulin receptors found in the adult mammalian brain; peripheral types which are only found on glial cells and a neuron-specific brain type (Adamo et al., 1989). However, both types appear to be similar in insulin-signal transducing properties. Binding of insulin with the receptor induces autophosphorylation of the intracellular domain, which in turn initiates the receptor’s protein tyrosine kinase activity. Tyrosine phosphorylation of intracellular substrates such as the insulin-receptor substrate family (IRS) (Myers and White, 1993) then leads to activation of multiple signals, including phosphatidyl inositol- 3 kinase and GTPase regulators (Avruch, 1999).

Several studies have found high levels of insulin receptors in the CNS at specific locations. The highest concentrations of insulin receptors in the brain are in olfactory bulb, cerebral cortex, hippocampus, cerebellum and hypothalamus (Unger et al., 1989; 1991). Insulin modulates CNS levels of neuropeptides, monoamines and other neurotransmitters implicated in the pathophysiology of mood and dementing disorders (Craft and Watson, 2004; McIntyre et al., 2007). Insulin also inhibits the firing of neurons in the hippocampus and hypothalamus; modulates catecholamine signaling in the hypothalamus; stimulates phosphoinositol turnover in the hippocampus; and regulates norepinephrine and dopamine transporter mRNA concentration in neurons (Shapiro et al., 1991; Mcwen et al., 2002; Craft and Watson, 2004). Further, insulin also exerts important growth regulatory (e.g., promotion of neurite outgrowth and synaptogenesis) effects salient to CNS functions (Craft and Watson, 2004).

The presence of insulin and insulin receptor in the hippocampus, cerebral cortex and their functional involvement in brain cognition phenomena at behavioral, synaptic and molecular levels has been suggested. Insulin-
sensitive biochemical systems exist which has the potential to affect various
cognitive systems and this process may be independent of or secondary to a
glucoregulatory effect. Insulin has been shown to exert a memory-enhancing action on both humans and experimental animals. Microinjection of insulin into the CA1 region of rat hippocampus (12 MU but not in 0.5 and 6 MU) improved both memory retrieval and consolidation (Moosavi et al., 2007). Babri et al. (2007) reported that intrahippocampal injections of insulin enhance memory for a simple learning task which supports the concept that insulin possibly plays an endogenous role in memory formation. Administration of insulin into the third cerebral ventricles of rats shortly after a passive avoidance training experience resulted in higher memory retention levels compared to rats that received saline and a heat-inactivated insulin injection (Park, 2001). Recently, a pilot study demonstrated the benefit of intranasal insulin in 25 patients of Alzheimer’s disease (Reger et al., 2008a, 2008b) as well as improved memory in healthy adults (Benedict et al., 2008).

The brain insulin receptor is structurally and functionally different from the peripheral tissues (Zhao et al., 2004). The major actions of insulin/insulin receptor in the brain are mediated by altering receptor trafficking during synaptic maturation and neuromodulation which may modify the synaptic connections required for learning and memory. Insulin receptor mediated signal trafficking provides a molecular basis underlying learning and memory. Binding of insulin activates the protein tyrosine kinase activity of the insulin receptor β-subunit which triggers two major cascades of signal transduction through its downstream substrate molecules. These include insulin receptor substrate-1 (IRS-1)/PI-3 kinase/phosphoinositide-dependent kinase (PDK)/protein kinase B (PKB/Akt), and the SH2 and collagen containing protein (Shc)/growth factor receptor-bound protein-2 (Grb2)/mitogen-activated protein (MAP) kinase pathways. Both IRS-1 and PI-3 kinase are abundantly expressed in the hippocampus, colocalizing with insulin receptor (Folli et al., 1994). Increased IRS-1 at synaptic locations after learning may activate PI-3 kinase leading to regulation of subsequent memory processing. It has been known that insulin stimulates NO production (Montagnani et al., 2001; Vincent et al., 2003) via activation of endothelial NOS (eNOS), an IRS-1/PI-3
kinase/Akt pathway mediates this process (Montagnani et al., 2002; Zeng and Quon, 1996; Zeng et al., 2000).

Cognitive impairments associated with diabetes mellitus caused by inadequate insulin/insulin receptor functions have also been documented (Biessels et al., 1998). Zhao et al. (2004) hypothesized several mechanisms involved in insulin mediated memory formation (Fig 10):

1. Insulin/insulin receptor potentiates NMDA channel activity, functions of which depend on the presence and activation of AMPA receptor that cause synaptic membrane depolarization and removal of the Mg^{2+} blockage of the NMDA receptor leading to long-term potentiation. Increased Ca^{2+} influx via the NMDA receptor and neuronal activities may inhibit tyrosine phosphorylation of insulin receptor via a feedback mechanism. Depending on spatial and temporal specificity of
information processing, insulin receptor signaling through PI-3 kinase may be involved in long-term depression via internalization of AMPA receptors. Insulin receptor may also modulate GABA transmission by recruiting functional GABA receptor to the postsynaptic membrane. GABAergic neurons sense the excitatory transmission and regulate synaptic strength by sending feed forward and/or feedback inhibitory inputs to the principal neurons. Regulation of synaptic efficacy by integrated excitatory and inhibitory transmissions within specific neuronal network is thought to underlie memory encoding and retrieval in the hippocampus (Paulsen and Moser, 1998).

2. Activation of insulin receptor-Shc-MAP kinase pathway after learning may lead to regulation of gene expression that is required for long-term memory storage (Zhao et al., 2004).

3. Insulin receptor may interact with G-protein coupled receptor and PLC to activate PKC leading to facilitation of short-term memory encoding (Zhao et al., 2004).

4. The insulin receptor/IRS/PI-3 kinase pathway may trigger synthesis of NO via eNOS activity. NO acts as a retrograde messenger for neurotransmitter release, and may also act intracellularly on memory processing (Choopani et al., 2008).

Furthermore, insulin receptor signaling through the same pathway may promote neuronal survival that is certainly beneficial for long-term memory consolidation.

NEUROBIOLOGY OF DIABETIC ENCEPHALOPATHY

There are many pathophysiological mechanisms through which diabetes might affect the initiation and promotion of the many underlying pathologies associated with dementia (Biessels et al., 2006; Kuhad and Chopra, 2008). These mechanisms/factors include alteration in cerebral insulin, insulin receptors, insulin signaling, insulin like growth factors, C-
peptide, GLUTs, ischaemic cerebrovascular disease, hyperglycemia, oxidative stress and alteration in neurotransmitter levels (Kuhad and Chopra, 2008).

**Impairment in Insulin Signaling**

Insulin has to be transported across the blood–brain barrier to exert its effects on the brain, bind to cerebral insulin receptors and convey its signal through an intracellular signaling cascade (Brands et al., 2004). Each of these processes may be affected by diabetes. Transport of insulin across the blood–brain barrier, for example, was shown to be increased in hyperglycaemic, hypoinsulinaemic rodent models of type 1 diabetes (Banks et al., 1997), whereas it is decreased in hyperinsulinaemic, hyperglycaemic rat models of type 2 diabetes (Baskin et al., 1985). Binding of insulin to receptors in brain tissue of hyperglycaemic, hypoinsulinaemic diabetic animals does not differ from controls (Havrankova et al., 1979; Marks and Eastman, 1989), whereas it appears to be decreased in the brains of hyperinsulinaemic, hyperglycaemic rats (Figlewicz et al., 1985). Insulin signaling may be disturbed both in type 1 and type 2 diabetes, as type 1 diabetes is also associated with some degree of insulin resistance, albeit to a lesser degree than in type 2 diabetes (Pedersen and Beck-Nielsen, 1987; Miyazaki et al., 2003).

Several mechanisms underlie the potential adverse effects of defective cerebral insulin signaling. Recent data points to changes in the insulin receptor cascade in obesity-related insulin resistance, suggesting that brain insulin receptors also become less sensitive to insulin, which could reduce synaptic plasticity (Messier and Teutenberg, 2005). Expression of insulin-1 and -2 mRNA was significantly reduced, in ICV-STZ treated rats, to 11% in hippocampus and to 28% in frontoparietal cerebral cortex, respectively. Insulin receptor (IR) mRNA expression decreased significantly in frontoparietal cerebral cortex and hippocampus (16% and 33% of control). At the protein/activity level, different abnormalities of protein tyrosine kinase activity (increase in hippocampus), total IR beta-subunit (decrease in hypothalamus) and phosphorylated IR tyrosine residues (increase) became
apparent. The STZ-induced disturbance in learning and memory capacities was not abolished by icv application of glucose transport inhibitors known to prevent STZ-induced diabetes mellitus. The discrepancy between reduced IR gene expression and increase in both phosphorylated IR tyrosine residues/protein tyrosine kinase activity may indicate imbalance between phosphorylation/dephosphorylation of the IR beta-subunit causing its dysfunction. These abnormalities may point to a complex brain insulin system dysfunction after STZ ICV application, which may lead to an increase in hyperphosphorylated tau-protein concentration. Furthermore, deterioration of insulin receptor signaling appears to be associated with aging-related brain degeneration such as the Alzheimer's dementia and cognitive impairment in aged subjects suffering type 2 diabetes mellitus (Zhao et al., 2004). Brain insulin system dysfunction is a possible pathological core in the generation of hyperphosphorylated tau protein as a morphological marker of sporadic Alzheimer's disease and diabetes associated dementia (Grunblatt et al., 2007).

One hypothesis is that the link between DM and AD is related to the function of insulin-degrading enzyme (IDE), an enzyme that degrades not only insulin and pancreatic amylin but also beta-amyloid (Aβ). Thus, in diabetics, insulin and Aβ might compete for IDE and this might lead to an increase in Aβ (Alafuozoff et al., 2008). Dou et al. (2005) investigated changes in long-term memory-associated expression of the IR and downstream molecules in the rat hippocampus and suggested that insulin/IR signaling plays a modulatory role in learning and memory processing, which may be compensated for by alternative pathways in the brain when an insulin deficit occurs.

Insulin like Growth Factors

Recent literature has addressed the role of growth factors in relation to a variety of neurological diseases, including AD (Levy et al., 2005; Offen et al., 2001; Barinaga, 1994). In particular, IGF-I has been a focus of both biological and epidemiological studies regarding its potential protective role in neurodegenerative diseases (Schneider et al., 2003). The growth factors and their receptors are found in high levels in brain areas implicated as important
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for memory, including the amygdala, the hippocampus, parahippocampal gyrus and the cerebellum (Bohannon et al., 1988; Adem et al., 1989; Araujo et al., 1989). Raising blood IGF-I levels via parenteral IGF-I infusions has been shown to reduce brain amyloid-β in rodents (Carro et al., 2002, 2006) and improve learning and memory of transgenic mice expressing the AD phenotype (Carro et al., 2006). In addition, limited epidemiological data, largely from small-scale studies, suggests that higher total IGF-I levels may be associated with better cognitive performance (Aleman et al., 1999; Rollero et al., 1998; Morley et al., 1997; Paolisso et al., 1997; Papadakis et al., 1995) and lower risk of cognitive decline (Dik et al., 2003; Kalmijn et al., 2000) in older individuals. Nevertheless, bioavailable IGF-I seems most likely to impact cognition (IGF-I binds to brain IGF receptors in its free, or unbound, form) (Sonntag et al., 2005); thus, free IGF-I levels, or perhaps the molar ratio of total IGF-I to its principal binding protein [IGF binding protein (IGFBP)-3], are likely important in determining the full impact of IGF-I (Landi et al., 2007). Only one previous study has explored the association between free IGF-I and cognitive function (Kalmijn et al., 2000); none have specifically assessed the role of midlife free IGF-I levels, although a large body of evidence indicates that cognitive decline takes decades to develop and thus factors earlier in life may have the most significant impact (Elias et al., 2000; Brookmeyer et al., 1998).

IGF-1 has effects on neurotransmitter synthetic pathways (Brass et al., 1992) and neurotransmitter release (Kar et al., 1997). It can also affect calcium channel activity (Blair and Marshall, 1997). Morley et al. (1997) reported that cognitive functioning in aged humans was correlated with the serum ratio of IGF-1/growth hormone. IGF-1 also stimulates release of nitric oxide in endothelial cells (Tsukahara et al., 1994), a possible retrograde messenger that has been implicated in the production of LTP (Zorumski and Izumi, 1993).

Infusion of IGF-1 in the brain of aged rats improves performance on working memory and object recognition tasks (Markowska et al., 1998). A high-serum IGF-I was associated with a significant increase in cerebral blood
flow in the left premotor cortex during a working memory test (for easier items) and in left dorsolateral prefrontal cortex (for more difficult items), as measured by positron emission tomography (Arwert et al., 2005). IGF-1 improves performance in different tests for either short- or long-term memory in rodents. Also, it may be that learning per se enhances local brain IGF-I, such as has been shown for BDNF (Yamada and Nabeshima, 2003).

**Figure 11. A Schematic Presentation of Neurobiology of Diabetic Encephalopathy**

Infusion of an IGF-1 antisense oligonucleotide into the inferior olive impairs learning of the conditioned eye-blink response in rats (Castro-Alamancos and Torres-Aleman, 1994). Perturbed IGF system has been shown in the CNS of STZ rats (Grunblatt et al., 2004). After 2 weeks of diabetes, IGF-2 mRNA content is significantly decreased in the brain and spinal cord. Insulin replacement partially restores IGF-II mRNA levels in
cerebral, cortex, medulla, and spinal cord (Wuarin et al., 1996). Li et al. (2002)
have systematically examined the IGF system (IGF-1, 2, IGF-IR and IR) in the
BB/Wor model of type 1 diabetes and found significant reductions in the
expression of IGF-1, IGF-2, IGF-IR and insulin receptor already in 2-month
diabetic BB/Wor rats which persisted in 8-month diabetic rats, indicating that
these abnormalities precede the functional cognitive impairments and the
apoptotic neuronal loss in hippocampus (Li et al., 2002).

C-peptide

C-peptide deficiency is a contributing pathogenic factor in type 1
diabetic complications (Sima et al., 2004b; Sima, 2003a, 2003b). In patients
with type 1 diabetes, C-peptide improves renal function, reduces urinary
albumin excretion and glomerular filtration, and decreases blood retinal barrier
leakage (Zierath et al., 1991, 1996; Johansson et al., 1992, 1996; Forst et al.,
1998; Fernqvist-Forbes et al., 2001). Chronic C-peptide replacement prevents
functional and structural peripheral nerve changes in type 1 diabetic rat
models suggesting that C-peptide deficiency is a participating factor in the
causation of type 1 diabetic complications (Wu et al., 1996b; Ido et al., 1997;
Li et al., 1999; Samnegard et al., 2001; Zhang et al., 2001; Sima et al., 2001;
Huang et al., 2002; Pierson et al., 2003). C-peptide plays a prominent role in
cognitive dysfunction and hippocampal apoptosis in type 1 diabetes (Sima
and Li, 2005). It has been shown that administration of C-peptide partially
corrects perturbed insulin-like growth factor (IGF) activity and insulin receptor
expression and partially but significantly prevents neuronal apoptosis in the
hippocampus of type 1 diabetic BB/Wor rats, demonstrating a relationship
between C-peptide deficiency, insulin action, IGF perturbation and neuronal
apoptosis (Li and Sima, 2004).

Cerebral GLUTs

Although glucose is the major nutrient and energy source for brain cells
and plays critical role in brain cognitive functions (Zhao et al., 2004), its
uptake, transport and utilization in the majority of brain regions do not depend
on insulin. The adult brain appears to express two main glucose transporters
(GLUT-1 and GLUT-3) that are not insulin-sensitive. GLUT-1 is expressed in
the endothelium of cerebral microvessels and astrocytes, GLUT-3 is predominantly distributed in neurons (Vannucci et al., 1998; Duelli and Kuschinsky, 2001). GLUT-4 and GLUT8 gene expression was detected in the hippocampus (El Messari et al., 2002; Membrez et al., 2006). Insulin-stimulated trafficking of GLUT4 and GLUT8 may provide rapid and localized assessment of neuronal glucose levels and energy homeostasis in the hippocampus and thereby contribute to the rapid fluctuation in hippocampal glucose levels that occur during task learning. Winocur et al. (2005) reported that plasma membrane association of the insulin sensitive glucose transporter, GLUT4, was reduced in the hippocampus of obese rats in the absence of changes in total GLUT4 and insulin receptor expression. These results parallel those of human studies in pointing to the susceptibility of the hippocampus and related structures to the adverse environment of diabetes mellitus.

**Cerebrovascular Alterations**

Epidemiologic studies of vascular risk factors are proof of concept that cerebral hypoperfusion is one of the earliest pathological signs in the development of cognitive failure. Vascular risk factors involving heart disease and stroke in the elderly individual who already possess a dwindling cerebrovascular reserve due to advancing age contribute to further decline in cerebral blood flow resulting in unrelenting brain hypoperfusion. Brain hypoperfusion, in turn, can reach a critically attained threshold of cerebral hypoperfusion giving rise to a neuronal energy crisis via reduced ATP synthesis. The ensuing metabolic energy crisis initially carves up ischemic-sensitive neurons in the hippocampus and posterior parietal cortex setting up cognitive meltdown and progressive neurodegenerative and atrophic changes in the brain (de la Torre, 2008). Neuronal energy compromise accelerates oxidative stress, aberrant protein synthesis, ionic membrane pump dysfunction, signal transduction impairment, neurotransmitter failure, abnormal processing of amyloid precursor protein resulting in beta-amyloid deposition and axonal microtubule disruption from tau hyperphosphorylation.
The high energy metabolic changes leading to oxidative stress and cellular hypometabolism precede clinical expression of AD.

Diabetes is associated with both structural and functional alterations of the cerebral vascular system, which increases the risk of stroke (Mankovsky et al., 1996; Beckman et al., 2002a; Brands et al., 2004), and may also affect cognitive functioning. Atherosclerotic disease is the main manifestation of structural alterations of the large extra- and intracranial arteries in diabetic patients (Reske-Nielsen et al., 1965; Mankovsky et al., 1996). Age, duration of diabetes, male gender, triglycerides and nephropathy are important determinants of atherosclerosis, assessed by ultrasonographic measurement of carotid intima-media wall thickness (Frost and Beischer 1998, 2003). Structural abnormalities at the microvascular level include thickening of capillary basement membranes and decreased capillary density, as has been shown in brain autopsy studies of diabetic patients (Reske-Nielsen et al., 1965; Johnson et al., 1982). Functional alterations in the cerebral vascular system that have been associated with type 1 diabetes include regional alterations in cerebral blood flow and disturbances of vascular reactivity. Cerebral blood flow has been reported to be decreased (Keymeulen et al., 1995), with some degree of regional variation (Rodriguez et al., 1993). Others, however, report increased cerebral blood flow in diabetic subjects (Grill et al., 1990), and it has been suggested that the decrease in blood flow that is reported in studies that use positron emission tomography (Keymeulen et al., 1995) possibly reflects an artifact, due to concomitant atrophy (Sabri et al., 2000). Regional cerebral blood flow measurements using neuroimaging techniques can predict diabetes induced memory deficits and AD preclinically at the mild cognitive impairment stage or even before any clinical manifestation of dementia is expressed (de la Torre, 2008).

**Hyperglycemia-induced Toxicity**

Hypoglycemia is an alarming; actually life threatening condition, but the exposure to chronic hyperglycemia has a more detrimental effect on the brain than recurrent exposure to severe hypoglycemia (Sredy et al., 1991; Aszalos, 2007; Brands et al., 2004). The active neural response to hyperglycemia
induces changes in gene expression and function. The first steps against hyperosmolality are initially adaptive, but later hyperactivation of the hypothalamic magnocellular neurosecretory cells leads to their structural damage. Changes in hippocampal gene transcription are partially implicated in the deterioration of semantic memory. Neurologically passive shunting of excess glucose through alternative cellular metabolic pathways induces atherogenic, vascular lesions, free radicals, leukoencephalopathy and atrophy of the brain and thus leading to cognitive deficits.

Hyperglycemia irreversibly modifies long-lived macromolecules by forming advanced glycation end products (AGEs), as a function of glucose concentration and time (Brownlee, 2000; Singh et al., 2001). Increased amounts of AGEs have been demonstrated in the brain and spinal cord of diabetic rats, albeit at lower levels than in peripheral nerves (Vlassara et al., 1983; Ryle et al., 1997). AGEs have been reported to exert several CNS neurotoxic actions, increased oxidative stress and acceleration of aging and cross linking of soluble β-amyloid peptide (Vitek et al., 1994; Munch et al., 2003). Araki et al. (2004) studied several domains of cognitive function in 198 diabetic patients with a mean age of 74 years. Among the diabetic patients, HbA1c level, a marker of hyperglycemia, was associated with the cognitive impairment, while short-term glucose control partially improved the cognitive impairment. Serum AGE levels were significantly associated with the impairment of complex psychomotor skills independent of HbA1c. In conclusion, hyperglycemia and increased AGE accumulation were associated with the cognitive impairment in patients with diabetes mellitus as a model of accelerated aging (Aragno et al., 2005).

Oxidative Stress

Both micro- and macrovascular cerebral diseases occurring in diabetic patients and the direct neuronal damage caused by chronically elevated intracellular glucose concentrations are implicated in diabetic encephalopathy. However, it remains unclear how much of the neuronal impairment is caused directly by intracellular glucose. The direct glucose toxicity in the neurons is especially due to increased intracellular glucose oxidation (Nishikawa et al., 82).
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2000; Mastrocola et al., 2005), which leads to an increase in reactive species production (Bonnefont-Rousselot, 2002; Evans et al., 2002): in both man and experimentally diabetic rats, oxidative stress seems to play a central role in brain damage (Aragno et al., 2000, 2002; Arvanitakis et al., 2004).

Li and Sima (2004) demonstrated hippocampal neuronal apoptosis in type 2 hyperinsulinemic and C-peptidemic BBDRZ/Wor rats. Oxidative damage to various brain regions contributes to the long term complications, morphological abnormalities and memory impairments (Fukui et al., 2001; Kuhad and Chopra, 2008). The increased oxidative stress in diabetes produces oxidative damage in many regions of rat brain including the hippocampus. Enhanced formation of oxygen free radicals occurs in tissues during hyperglycemia (Baydas et al., 2002). These oxidant radicals contribute to increased neuronal death through protein oxidation, DNA damage, and peroxidation of membrane lipids (Hawkins and Davies, 2001). The activity of superoxide dismutase and catalase, enzymes involved in the antioxidant defence of the brain, appears to be decreased in STZ-diabetic rats (Kumar and Menon, 1993). Antioxidants such as curcumin, vitamin E and sesamol ameliorated diabetic encephalopathy in STZ-treated rats (Kuhad and Chopra, 2008).

Neurotransmitter Modulation

The most well known effect of diabetes mellitus on CNS is dysfunction of neurotransmitters, which is secondary to the metabolic disorders such as hyperglycemia and acidosis (Ramakrishnan et al., 2005). CNS abnormalities including neuronal atrophy and axonal degenerations (Rossi and Bestetti, 1981; Reske-Nielsen and Lundbaek, 1963) are also associated with diabetes. The altered levels of neurotransmitter in specific brain areas in patients with diabetes mellitus and in animals with experimental diabetes have been documented and implicated in the CNS disorders (Bitar et al., 1986; Tasaka et al., 1992; Trulson and Himmel, 1985).


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**Cholinergic Modulation**

Acetylcholine (ACh) output increases in the hippocampus of rats performing a spatial alternation task and peripheral and hippocampal injections of glucose enhance that release along with increasing scores on the behavioral task (Ragozzino *et al.*, 1996, 1998; Park, 2001). Similarly, peripheral administration of insulin also acts to increase ACh levels in the amygdala (Hajnal *et al.*, 1998) while intracerebroventricular administration increases ACh levels in the mid-brain, caudate nucleus and pons medulla (Bhattacharya and Saraswati, 1991). Messier has reported that peripheral insulin administration in mice attenuates scopolamine-induced deficits in an operant task (Messier and Destrade, 1994) suggesting an increase in cholinergic activity. On the other hand, Kopf and Baratti (1995) have reported that hyperinsulinemia in mice results in impaired retention of an inhibitory avoidance task and this appears to be mediated by a decrease in cholinergic activity. However, there appears to be an inverted-U dose effect curve for glucose effect on memory (Gold, 1995) and Ach release (Ragozzino *et al.*, 1996) and a difference in dose effect may explain the apparent contradiction.

Recently, it has been reported that few natural agents as ginseng, oleanoic acid and Hon Chi have an ability to increase the release of ACh from nerve terminals, which in turn stimulate muscarinic M(3) receptors in the pancreatic cells and augment the insulin release to result in plasma glucose lowering action (Chen and Liu, 2006; Hsu *et al.*, 2006b; Su *et al.*, 2007). In parallel to the reduction of plasma glucose, an increase of plasma level of insulin or C-peptide was also observed. Moreover, disruption of synaptically available ACh using an inhibitor of choline uptake, hemicholinium-3, or vesicular acetylcholine transport, vesamicol, abolished these actions. Physostigmine at concentration sufficient to inhibit acetylcholinesterase enhanced the actions of these natural substances of ACh release from the nerve terminals to enhance insulin secretion. Both the plasma glucose lowering action and the raised plasma levels of insulin and C-peptide induced by these agents were also inhibited by 4-diphenylacetoxy-N-methylpiperidine methiodide, but not affected by the ganglionic nicotinic antagonist, pentolinium
or hexamethonium, indicating the mediation of muscarinic M(3) receptors (Chen and Liu, 2006; Hsu et al., 2006b; Su et al., 2007). It can be concluded that enhancement of ACh secretion can prevent learning and memory deficits associated with diabetes.

**Glutaminergic Modulation**

Abnormal regulation of glutamatergic receptors appears to play an important role in diabetes-induced impairment in synaptic plasticity and may therefore contribute to the development of cognitive defects in diabetic patients. Trudeau et al. (2004) discussed the possibility that deficits in long-term potentiation during chronic diabetes might arise from dysfunction of the NMDA subtype of glutamate receptors in early stages of the disease. Biochemical experiments in non-obese diabetic mice suggest that up-regulation of NMDA receptors is associated with the early stages of diabetes mellitus. There is, of course, a need for further studies on how these changes in NMDA receptor properties may accentuate glutamate toxicity. Preliminary investigations in experimental models of Type 2 diabetes and NMDA receptors provide evidence that hyperinsulinaemia might be capable of limiting NMDA mediated toxicity.

In Xenopus oocytes expressed with NMDA receptors, brief insulin exposure triggered a rapid and significant potentiation of responses to NMDA mediated by NMDA receptor subtypes (Liao and Leonard, 1999; Chen and Leonard, 1996). This insulin-induced potentiation was blocked by a tyrosine protein kinase inhibitor, genistein and a broad-spectrum protein kinase inhibitor staurosporine, suggesting involvement of tyrosine and possibly downstream serine/threonine protein kinases such as protein kinase C (PKC) activities. In a similar experimental preparation, Skeberdis et al. (2001) demonstrated that application of insulin increases NMDA channel activities by recruiting NMDA receptors to the membrane surface. This process was blocked by a more specific insulin receptor tyrosine kinase inhibitor tyrphostin A47, and may involve function of SNAP-25 (synaptosomal associated protein 25), but does not seem to require tyrosine and serine/threonine phosphorylations at the NMDA receptor C-terminus. Other study, however,
showed that incubation of rat hippocampal slices with insulin caused increases in tyrosine phosphorylation of the NR2A and 2B subunits of NMDA receptors (Christie et al., 1999). Given the important roles that NMDA receptors may play in synaptic plasticity and learning and memory formation (Huerta et al., 2000; Nakazawa et al., 2002), modulation of NMDA transmission may represent one of the synaptic bases for roles of insulin/insulin receptor signaling in learning and memory.

In addition, insulin plays a role in synaptic plasticity by acting on alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor trafficking. Redistribution of AMPA receptors has been proposed to regulate strength of glutamatergic synapses. A mature synaptic connection at glutamatergic synapses in the brain requires conversion of silent glutamatergic synapses into functional synapses during the course of postnatal brain development (Wu et al., 1996a; Renger et al., 2001). A silent glutamatergic synapse that mediates only NMDA transmission is not functional unless AMPA receptors are delivered to such synapses (Malinow et al., 2000; Malinow, 2003). Conversion of a silent synapse to a functional synapse can be both development dependent (Wu et al., 1996a; Renger et al., 2001) and activity dependent (Malinow et al., 2000; Malinow, 2003) that have been hypothesized as a synaptic basis for learning and memory formation. In cultured differentiating neurons, insulin promoted transfer of silent AMPA synapse to functional synapse and accelerated reduction of silent synapses (Plitzko et al., 2001). In the mature brain, insulin facilitated clathrin-dependent internalization of AMPA receptors leading to long-term depression of AMPA receptor-mediated synaptic transmission in hippocampal CA1 neurons (Man et al., 2000).

GABAergic Modulation

Insulin-mediated receptor trafficking has also been found in the gamma-aminobutyric acid (GABA) receptor, which mediates synaptic inhibition important for neuronal functions associated with learning (Paulsen and Moser, 1998; Chapouthier and Venault, 2002; McGaugh, 2002; Zhao et al., 2004). When applied to HEK 293 cells transfected with the GABA-A
receptor, insulin caused rapid translocation of the GABA-A receptors to the plasma membrane (Wan et al., 1997). Insulin also recruited functional GABA-A receptors onto the postsynaptic and dendritic membranes of the CNS neurons, leading to augmented amplitudes of the GABA-A receptor-mediated miniature inhibitory postsynaptic current (Wan et al., 1997). Furthermore, insulin activation of muscarinic transmission potentiated GABA receptor currents likely occurs via a phosphoinositide-3 (PI-3) kinase-dependent mechanism (Ma et al., 2003). Thus, insulin/insulin receptor plays a role in receptor trafficking during synaptic maturation and synaptic usage, and it may also mediate interactions of different neurotransmission systems during neuronal activation, all of which may underlie modification of synaptic connections required for higher brain functions such as learning and memory.

The escalating diabetes epidemic with its neurological consequences may have crucial socioeconomic ramifications. Diabetes itself is not a neurological disease but ensuing hyperglycemia exerts adverse impact on brain function and cognition. Insulin deficiency, reduced insulin sensitivity, impairment of insulin sensitive neurotransmitter modulation, cerebrovascular alterations and oxidative stress render neurons susceptible to neurotoxic insults with resultant neurodegeneration and cognitive decline (Fig. 11). Research in recent years has significantly advanced our knowledge regarding physiological as well as pathological roles of insulin/insulin receptor in diabetes associated neuropsychiatric dysfunction especially learning and memory deficits. However, future indepth studies will unravel the complex interaction between various pathogenic factors and this may foster the development of preventive measures or treatment strategies to restore these deficits.

PHARMACOLOGICAL INTERVENTIONS EMPLOYED IN THE STUDY

TOCOTRIENOL

Vitamin E is one of the most important phytonutrients in edible oils. It consists of eight naturally occurring isomers, a family of four tocopherols (alpha, beta, gamma and delta) and four tocotrienols (alpha, beta, gamma and delta) homologues (Sen et al., 2007).
All the eight isomers share some important traits:

- The head, or chroman ring in technical term
- The tail, which is called the phytyl tail for tocopherols
- The active group, the hydroxy group on the head of the molecule

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Sources of Tocotrienols

Edible oil originating from plants are rich source for tocotrienols. Crude palm oil extracted from the fruits of oil palm (*Elaeis guineensis*) particularly contains a high amount of tocotrienols (up to 800 mg/kg), mainly consisting of gamma-tocotrienol and alpha-tocotrienol. It also contains the most potent form of all commercially available tocotrienols - delta-tocotrienol. Tocotrienols are also found in oil derived from rice bran, barley, wheat germ and rye.

**Tocotrienols from Normal Diet**

Since tocotrienols alone occur at very low levels in nature, with the highest concentration found in palm oil, so it is virtually impossible to attain the amount of tocotrienols that show beneficial effects from the normal diet alone. For example, one would need to consume a cup of palm olein (cooking oil) a day to get the level required for effectiveness as described in most studies.
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**Tocopherols (T1) vs. Tocotrienols (T3)**

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</tr>
<tr>
<td>130</td>
<td>216</td>
<td>21</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>170</td>
<td>17</td>
<td>2</td>
<td>200</td>
</tr>
<tr>
<td>51</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>51</td>
</tr>
</tbody>
</table>

Adapted from Slover, 1971

Tocotrienols possess powerful cardioprotective (Das *et al.*, 2007, 2008), neuroprotective (Khanna *et al.*, 2006; Shichiri *et al.*, 2007), radioprotective (Ghosh *et al.*, 2009), anti-angiogenic (Nakagawa *et al.*, 2007; Shibata *et al.*, 2009), potent natural super-antioxidant (Schroeder *et al.*, 2006; Maniam *et al.*, 2008; Matringe *et al.*, 2008), anti-cancer (Nesaretnam, 2008;
Review of Literature

Wada, 2009), anti-inflammatory (Wu et al., 2008), cycloxygenase-2 inhibitory (Yam et al., 2009), anti-nociceptive (Tiwari et al., 2009b), insulin sensitizing, hypoglycemic (Chen and Cheng, 2007; Budin et al., 2009), and cholesterol lowering (Chou et al., 2009) properties that often differ from the properties of tocopherols (Serbinova et al., 1991; Serbinova and Packer, 1994; Sen et al., 2007; Budin et al., 2009). The unsaturated side chain of tocotrienol allows for more efficient penetration into tissues that have saturated fatty layers such as the brain and liver (Suzuki et al., 1993; Atkinson et al., 2008).

<table>
<thead>
<tr>
<th>Sources</th>
<th>Amount Taken To Achieve the Required Levels of Tocotrienols</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBD Palm Olein (Cooking Oil)</td>
<td>1 tea cup (~ 80 g)</td>
</tr>
<tr>
<td>Rice Bran Oil</td>
<td>2 tea cup (~ 160 g)</td>
</tr>
<tr>
<td>Barley</td>
<td>3.0 kg</td>
</tr>
<tr>
<td>Wheatgerm</td>
<td>1.5 kg</td>
</tr>
<tr>
<td>Oats</td>
<td>4.0 kg</td>
</tr>
</tbody>
</table>

Experimental research examining the antioxidant effects of tocopherol and tocotrienols has revealed that tocotrienols appear superior due to their better distribution in the fatty layers of the cell membrane (Suzuki et al., 1993; Kawakami et al., 2007; Tsuzuki et al., 2007; Maniam et al., 2008). No-observed-adverse-effect level (NOAEL) for tocotrienol was found to be 120 mg/kg body weight/day for male rats and 130 mg/kg body weight/day for female rats (Nakamura et al., 2001). It has been suggested that the safe dose of various tocotrienols for human consumption is 200-1000 mg/day (Sen et al., 2007).
LYCOPENE

Lycopene, a carotenoid, is mostly found in tomatoes and other red fruits and vegetables, such as red carrots, watermelons, pink grapefruit, pink guava, papayas and rosehip (but not strawberries or cherries).

<table>
<thead>
<tr>
<th>Source</th>
<th>µg/kg wet weight</th>
<th>Source</th>
<th>µg/kg wet weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gac</td>
<td>2,000-2,300</td>
<td>Raw tomato</td>
<td>8.8-42</td>
</tr>
<tr>
<td>Tomato juice</td>
<td>86-100</td>
<td>Tomato sauce</td>
<td>63-131</td>
</tr>
<tr>
<td>Tomato Ketchup</td>
<td>124</td>
<td>Watermelon</td>
<td>23-72</td>
</tr>
<tr>
<td>Pink grape fruit</td>
<td>3.6-34</td>
<td>Pink guava</td>
<td>54</td>
</tr>
<tr>
<td>Papaya</td>
<td>20-53</td>
<td>Rosehip puree</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Unlike other fruits and vegetables, where nutritional content such as vitamin C is diminished upon cooking, processing of tomatoes increases the concentration of bioavailable lycopene. Lycopene in tomato paste is four times more bioavailable than in fresh tomatoes. Thus processed tomato products such as pasteurized tomato juice, soup, sauce, and ketchup contain the highest concentrations of bioavailable lycopene. Because lycopene is so insoluble in water and is so tightly bound to vegetable fiber, the bioavailability of lycopene is increased by food processing. Cooking and crushing tomatoes (as in the canning process) and serving in oil-rich dishes (such as spaghetti sauce or pizza) greatly increases assimilation from the digestive tract into the bloodstream.
Lycopene is a powerful antioxidant with a singlet-oxygen-quenching capacity 47 and 100 times greater than that of β-carotene and vitamin E respectively (Liu et al., 2005; Di Mascio et al. 1989). Lycopene is also a potent neuroprotective (Hsiao et al., 2004), antiproliferative, anticancer (Gunasekera et al. 2007), anti-inflammatory, cognition enhancer (Akbaraly et al., 2007) and hypocholesterolemic agent (Fuhrman et al., 1997; Heber and Lu, 2002). Lycopene also modulates cyclo-oxygenase synthesis pathway (Heber and Go, 1999; Sengupta et al., 2006) and reduces mutagenesis in the Ames test (Heber and Lu, 2002; Matulka et al., 2004). Lycopene has been under considerable investigation for its anti-oxidant benefits in treating various chronic human diseases like cancer, cardiovascular diseases, osteoporosis, and diabetes (Rao et al., 2006; Rao and Rao, 2007).

**SESAMOL**

![SESAMOL Structure](image)

3, 4 - METHYLENEDIOXYPHENOL (Sesamol)
Review of Literature

Sesamol is the major constituent of sesame seed oil, which makes it more resistant to oxidative deterioration than other vegetable oils (Parihar et al., 2006).

![Image of sesame](image)

**Sesame**

Sesamol is a powerful antioxidant and inhibits UV- and Fe³⁺/ascorbate-induced lipid peroxidation in rat brain (Uchida et al., 1996; Prasada et al., 2005). Sesamol reduces ferric ions and its unique solubility in both aqueous and oily phases increases its local concentration in cell membranes and makes it a chain-breaking antioxidant (Uchida et al., 1996). Sesamol scavenges hydroxyl and lipid peroxyl radicals and reduces radiation-induced deoxyribose degradation (Joshi et al., 2005). It also inhibits the formation of single-strand DNA breaks by γ-radiation (Prasad et al., 2005). It has been shown that sesamol inhibits several steps in the generation of neoplasia and mutagenesis (Kapadia et al., 2002). Recently, sesamol has been shown to possess neuroprotective (Hou et al., 2006), hepatoprotective (Hsu et al., 2006a), anti-inflammatory (Chavali et al., 2001; Hou et al., 2006), chemopreventive (Prasad et al. 2005) and anti-ageing properties (Sharma and Kaur 2006).