Abstract
Diabetic neuropathy (DN), an important microvascular complication of diabetes, is one of the major causes of morbidity in patients with diabetes. Studies have confirmed that glycaemic control and duration of diabetes are important factors for the development of DN. However, the exact mechanism of damage to peripheral nerves due to prolonged hyperglycaemic exposure is still not clear. Recent epidemiological studies have highlighted the importance of cardiovascular risk factors such as hypertension, smoking, raised serum cholesterol, triglycerides and lipoprotein a to the development of DN. Various mechanisms of microvascular and haemodynamic dysfunction of capillaries of nerve fibres have been postulated in the development of DN. Similarly endothelial dysfunction due to activation of protein kinase C, oxidative stress, advanced glycation end products and polyol pathway activation has been shown to be related to DN in human and experimental DN. Other possible mechanisms such as platelet dysfunction, essential fatty acid deficiency, immunological mechanisms, nerve growth factor deficiency and presence of adhesion molecules have been described in relation to DN. However, no single theory can explain the exact pathogenic mechanism of the development of DN. This review looks into different theories that have been proposed and describes the inter-relationship of various factors that can lead to the development of DN. (Int J Diabetes Metab 13:135-140, 2005)

Key words: Diabetes mellitus, neuropathy, human, pain

Introduction
Diabetic Neuropathy (DN) is a frequent complication of diabetes, but its true prevalence is still not clear. The reported prevalence of DN varies a great deal from 14% to 63% depending upon the type of population studied and criteria used to define DN. In the Rochester Diabetic Neuropathy Study, DN affected almost 60% of subjects although it was symptomatic only in about 15%. In the EURODIAB IDDM Complication Study, which included 3250 patients from 16 European countries, the overall prevalence of neuropathy in 16 European countries was 28%.

Pirart observed in a cohort of 4400 subjects that the prevalence of DN increased from 7% within 1 year of diagnosis to 50% for those with diabetes for more than 25 years. Pirart also observed that, among patients with type 1 diabetes who developed DN, 65% already had diabetic retinopathy and 26% had diabetic nephropathy. This suggests that a similar mechanism may be involved in the genesis of these microvascular complications. Many epidemiological and prospective studies have shown both increasing patient age and duration of diabetes to be associated with DN. Despite this, the exact pathogenic mechanism of the development of diabetic neuropathy is not fully understood and a number of observations and postulations have been described.

Poor Glycaemic Control
A number of clinical and epidemiological studies suggest that the magnitude and duration of hyperglycaemia is an essential element for the development of chronic complications of diabetes mellitus including DN. This has been supported by the findings of the DCCT, 1993 in type 1 diabetes where DN was reduced by 69% in subjects in the primary-prevention cohort and by 57% in the secondary-intervention cohort. Similarly in type 2 diabetes, the UKPDS, 1998 demonstrated a 40% reduction in DN (Vibration perception threshold >25) in the intensive control group. There have been numerous other studies showing the relationship between raised glycosylated haemoglobin and DN. Despite this, the exact pathogenic mechanism of damage to peripheral nerves following hyperglycaemia is far from clear.

Cardiovascular risk factors
Forsblom et al, 1994 in a prospective study of type 2 diabetic patients followed for 9 years observed 29% mortality in subjects with DN, the majority of whom died from cardiovascular disease suggesting common risk factors. These findings have been supported by data from the Pittsburgh Epidemiology of Diabetes Complications Study in which hypertension has been associated with the development of DN. Similarly, associations between DN and serum cholesterol, triglycerides and lipoproteins have been reported by different workers to be statistically significant. Mitchell et al found that the risk of developing DN was more than three times in type 1 subjects who smoked than non smokers, but smoking was not a risk factor for DN in type 2 diabetes. In the Sheffield Prospective Study, Rajbhandari et al also found smoking to be significantly associated with DN in subjects with type 1 diabetes.

Microvascular and haemodynamic dysfunctions
Fagerberg was the first to describe microvascular changes
microangiopathy. With observations in retina and kidney of diabetic with sub-clinical neuropathy. This observation is in keeping suggestive of increase blood flow in sural nerves of patients oxygen saturation and reduced fluorescein rising time Scott et al nerve suggestive of ischaemia, have been shown in the sural saturation and raised fluorescein rising time, which are polarography is reduced in diabetic subjects with diabetic neuropathy. Similarly, reduced microvascular oxygen tension measured by microelectrode blood flow in epineural vessels and there were arteriovenous shunts on the surface of nerves. Morphological study of the nerve biopsy samples with mild diabetic neuropathy showed reduced endoneurial capillary density. This correlated significantly with reduced myelinated fibre density. Both basement membrane area and endothelial cell profile number per capillary were increased in diabetic patients and correlated significantly with both neurophysiological and neuropathological measures of neuropathic severity. Newrick et al showed that the sural nerve oxygen tension measured by microelectrode polarography is reduced in diabetic subjects with neuropathy. Similarly, reduced microvascular oxygen saturation and raised fluorescein rising time, which are suggestive of ischaemia, have been shown in the sural nerve of subjects with diabetic neuropathy. Interestingly Scott et al have shown a paradoxical rise in microvascular oxygen saturation and reduced fluorescein rising time suggestive of increase blood flow in sural nerves of patients with sub-clinical neuropathy. This observation is in keeping with observations in retina and kidney of diabetic microangiopathy.

Endothelial Dysfunction
Nitric Oxide (NO) has been increasingly recognised as a major player in the pathogenesis of DN. Endothelium dependent relaxation of smooth muscle, mediated by endogenous NO, has been shown to be impaired in type 2 diabetic patients with peripheral sensory neuropathy. The exact mechanism of the underlying deficit is unclear but may involve depletion of NO or altered smooth muscle sensitivity to NO. The build up of advanced glycation end products (AGE) in subendothelial collagen may quench NO released by the endothelium before it reaches smooth muscle cells. Similarly formation of oxidative free radicals may also inactivate NO. More recently topical isosorbide dinitrite spray has been shown to be useful in the treatment of neuropathic pain in chronic painful diabetic neuropathy. Similarly von Willebrand factor (vWF), a glycoprotein synthesised by endothelium and released when endothelial cells are damaged, is raised in patients with diabetes. Plater et al found raised vWF in subjects whose peroneal motor and sural sensory conduction velocities were reduced in comparison to those whose nerve conduction remained stable on follow up after 2 years. Increased plasma vWF antigen is raised in neuropathic compared to nonneuropathic diabetic patients. On the other hand in a study of full thickness skin biopsies from the dorsum of the foot of DN and control subjects, Veves et al did not find any differences between the two groups in the staining intensity of vWF.

Platelet dysfunction
Platelets have been implicated in the pathogenesis of vascular disease that accompanies diabetes. Markers of platelet activation such as beta-thromboglobulin and platelet factor 4 are higher in subjects with type 2 diabetes than non-diabetic controls. The platelets from diabetic subjects are also hypersensitive to a variety of agonists including adenosine diphosphate (ADP), thrombin and collagen, which cause platelet aggregation at a lower level than platelets from non-diabetic subjects. Borsey et al demonstrated raised serum levels of beta-thromboglobulin and platelet factor 4 in subjects with proliferative retinopathy. Rajbhandari et al showed that both serum beta-thromboglobulin and platelet factor 4 were raised in subjects at baseline who developed DN after 9 years.

Diacylglycerol - Protein Kinase C activation
Recent studies have identified that the activation of protein kinase C (PKC) and an increase in diacylglycerol (DAG) levels, initiated by hyperglycaemia, are associated with many vascular abnormalities in retinal, renal and cardiovascular tissues. Among the various isoforms of PKC, the B and D forms are shown to be preferentially activated in the vasculature of diabetic animals. These PKCs are DAG sensitive, unlike the other isoforms, which are sensitive to calcium. The increase in cellular DAG is thought to be caused by agonist-stimulated hydrolysis of phosphatidylcholine by phospholipase D or from glycolytic intermediates. The glucose induced activation of PKC has been shown to increase the production of extracellular matrix and cytokines, to enhance contractility, permeability, and vascular endothelial cell proliferation, and to induce the
activation of cytosolic phospholipase A2 and to inhibit Na⁺-K⁺ ATPase. Thus, activation of the DAG-PKC pathway by hyperglycaemia has been proposed to cause micro- and macrovascular dysfunction. Recent clinical trials in human subjects have shown PKC-b inhibitors to be useful in the treatment of diabetic retinopathy and DN.39

**Oxidative/reductive stress**

The earliest metabolic imbalance linked to increased blood flow in animal models of diabetes is cytosolic reductive stress, that is an increased ratio of free NADH/NAD⁺. This 'hypoxia-like' redox imbalance is caused by increased oxidation of substrate, coupled to reduction of the co-factor NAD⁺ to NADH.40 Excessive glucose can be transported intracellularly mainly by the glucose transporter GLUT-1, and metabolized to change redox potential.41 The hypothesis of oxidative stress causing microvascular complications is supported by the evidence that many biochemical pathways strictly associated with hyperglycaemia (glucose autooxidation, polyol pathway, protein synthesis, protein glycation) can increase the production of free radicals.42 A recent clinical trial of intravenous alpha-lipoic acid, a potent antioxidant, has shown to be effective in improving neuropathic symptoms and other neuropathic end points.43

**Advanced Glycation Products**

Glucose has been demonstrated to react non-enzymatically with primary amines of proteins to form glycated compounds. Glycation occurs in virtually all proteins exposed to hyperglycaemia. Although initial glycation is reversible, chronic exposure to hyperglycaemia leads to irreversible formation of advanced glycation end products (AGE). A number of AGEs have been identified in human tissue and have been found to accumulate with age and diabetes. The presence of AGEs in plasma and tissues has been linked to the development of complications in diabetes.44 The cellular interactions of AGEs are mediated by receptors for AGE and by AGE-specific cell surface binding proteins. These receptor-mediated signaling processes stimulate endothelial function, activate monocytes and attenuate lipid metabolism thus playing an important role in vascular dysfunction.45 In animal experiments, inhibitors of glycation have been found to be useful in the prevention of neuropathy.46

**Polyol pathways**

The microvascular complications of diabetes primarily affect tissues of retina, nerves and kidney where glucose transport across the cell membrane is independent of insulin. In these tissues the intracellular glucose concentration mirrors the levels found in extracellular space. Therefore, chronic hyperglycaemia of diabetes results in elevated intracellular glucose levels which has to be metabolised in the alternative pathway. Sorbitol pathway consists of a two step enzymatic process in which glucose is reduced to sorbitol by aldose reductase, and subsequently to fructose by sorbitol dehydrogenase. The role of aldose reductase in catalyzing the intracellular accumulation of sugar alcohols such as sorbitols in these tissues has been recognized for almost 30 years.47 Increased influx through this pathway leads to impairment of Na⁺ dependent myoinositol uptake system and subsequently decreased intracellular myoinositol concentrations and increased osmotic pressure in tissues such as the lens, retinal pericytes and neural tissue. This, in combination with glycosylation, is able to alter intracellular redox balance, and might therefore have tremendous pathophysiological importance.48 Long-term treatment with an aldose reductase inhibitor fidarestat in streptozotocin induced diabetic rats improved nerve conduction and reduced demyelination and axonal atrophy.49 However, some experimental intervention studies and clinical trials of these inhibitors appeared disappointing because of either their inadequate design or the inability to reverse established functional and metabolic deficits.50

**Essential fatty acids deficiency**

In human and experimental diabetes, the level of linoleic acid is normal. However, gamma linolenic acid is reduced as a result of a deficit in the enzyme delta 6 desaturase. This deficit is thought to be responsible for many of the microvascular changes in diabetic neuropathy.51 Similarly, synthesis of prostaglandins PGI₂ and PGE₂ have been shown to be reduced in the sciatic nerve of experimental diabetic animals due to diminished arachidonic acid content.52 Dietary supplementation of gamma linolenic acid has been shown to be effective in increasing nerve conduction velocity in diabetic rats.53 However, its use in human diabetic neuropathy has been disappointing (author’s unpublished observation).

**Autoimmunity**

Immunological mechanisms have been proposed in the aetiology of autonomic neuropathy.54 This is based on the findings of a clinical association with iritis, increased levels of circulating immune complement breakdown products and activated T-lymphocytes, and the detection of complement fixing antibodies to nervous tissues in patients with diabetic autonomic neuropathy. Furthermore, inflammatory infiltrates with lymphocytes and macrophages have been demonstrated but autoantibodies against sympathetic ganglia are not increased in long-term diabetic patients.55 Zanone et al56 assessed autoantibodies to autonomic structures in 92 adolescent diabetic subjects and found that there was subtle autonomic dysfunction in the group that had positive antibodies to sympathetic and parasympathetic nervous structures and their sera had cytotoxic effect on human adrenergic neuroblastoma cells. Gustatory sweating, one of the features of autonomic neuropathy, has been shown to be resolved in patients who are immuno-suppressed following renal transplantation adding weight to this argument.57 However, whether these are coincidental findings or if they play a pathogenic role is not clear.

**Nerve Growth factors**

Nerve growth factor (NGF) is one of several neurotrophic factors that are known to play an important role in the development, maintenance and survival of neuronal tissue.58 In diabetic neuropathy, the nerve is thought to be deprived of NGF as shown by reduced levels in skin biopsy.59 However, Zanone et al.60 did not find any difference in NGF antibody levels between subjects with and those without
DN. Furthermore, subcutaneous NGF did not reverse the nerve damage during the treatment of DN.\textsuperscript{39}

**Adhesion Molecules**

Adhesion molecules are widely distributed in the body and have been associated with a number of pathological conditions including microvascular complications of diabetes. Jude et al.\textsuperscript{60} studied the role of adhesion molecules by following 28 diabetic patients over a 5 year period. Out of these, 13 developed neuropathy and in this group P-selectin and intercellular adhesion molecule-1 were significantly increased at baseline in comparison to those diabetic subjects who did not develop DN on follow up. However, after 5 years there were no differences in the adhesion molecule level between these two groups suggesting its role was only in the pathogenic process but not when neuropathy was fully established. Further clarification is needed to establish its role in relation to DN.

![Figure 2: Possible interaction of various pathogenic mechanisms for the development of diabetic neuropathy.](image)

**Conclusion**

Diabetic neuropathy is one of the major problems in patients with diabetes mellitus, and is the major cause of morbidity and mortality. Good glycaemic control, with maintenance of normoglycaemia, may be able to prevent chronic complications. It is extremely difficult to achieve continuous normoglycaemia with currently available treatment and so other modalities of therapy to prevent complications should be explored. In order to do this, the pathogenic mechanism of microvascular complications needs to be fully understood. A number of mechanisms have been described, but none of them can satisfactorily explain the exact pathogenesis of microvascular complications in diabetes. Many of the proposed mechanisms are interdependent and it is likely that more than one mechanism is involved in the development of the chronic complications of diabetes (Fig. 2). In addition, there may be genetic influences in either protecting or making them susceptible to the development of complications. Unless we have detailed knowledge of the mechanisms responsible for diabetic complications, it will not be easy to develop strategies to prevent or treat them.

**References**

Pathogenesis of human diabetic nephropathy


