

## Nutritional supplementation for diabetic retinopathy

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### Abstract

Oxidative stress is increased in the diabetic retina, and this could be due to increased generation of free radicals, or an impaired anti-oxidant defence system. The potential role of the nutraceuticals Pycnogenol<sup>®</sup>, Ginkgo biloba extract, vitamin E, vitamin C, lipoic acid, genistein, *Aloe vera*, and chromium in the treatment or prevention of diabetic retinopathy is discussed. Although epidemiological evidence does not support a relationship between antioxidant intake and reduced risk for diabetic retinopathy, intervention trials indicate that further research is needed to clarify the role of Pycnogenol<sup>®</sup>, *Ginkgo biloba* extract, and vitamin E in the management of this condition.

## **Introduction**

This literature review will provide an insight into the potential role of nutritional supplementation in type 2 diabetes. Diabetes is a disorder of the metabolism of carbohydrates, lipids, and proteins in which the body cannot produce insulin or cannot use it to its full potential. This can lead to damage of several different tissues, particularly those that are insulin insensitive such as the retina, kidney and nerves (Kowluru and Kennedy, 2001)

Type 1 diabetes occurs in children and young adults, 40 – 50 % of whom will go on to develop significant microvascular complications. Type 2 diabetes is most common, accounting for 90 – 95 % of all diabetic patients (Watkins *et al.*, 2003b).

Both forms cause long-term complications such as cataract, retinopathy, neuropathy (peripheral nerve damage), and nephropathy (progressive loss of renal function). Over 135 million people are affected by diabetes worldwide. In the US, 0.3 % of the population develop type I diabetes by the time they are 20 years of age and 800 000 new cases of type II diabetes are diagnosed annually (American Diabetes Association, 2004). Diabetes therapy is centred upon the control of blood sugar. Retinopathy is the most common complication, resulting in registrable blindness for over 10 000 diabetic people per year in the USA (Fong *et al.*, 2004). All forms of diabetes are characterised by chronic hyperglycaemia that results in disease-specific microvascular changes. These changes result in retinopathy, nephropathy, and a variety of debilitating neuropathies. Accelerated macrovascular atherosclerotic change is also associated with diabetes; people with diabetes are at higher risk of myocardial infarction, stroke, and limb amputation (Brownlee and Gd, 2001).

## **Histological changes in diabetic retinopathy**

The structure of the retinal microvessels is directly related to maintenance of an effective blood-retinal barrier and is formed by the tight junctions between endothelial cells. The endothelial cells of retinal vessels are non-fenestrated and are joined by tight junctions. The retinal microvessel wall also contains pericytes, which provide mechanical support and stability, as well as a basement membrane that surrounds the endothelial cells and pericytes, and is made up of glycoprotein and collagen.

The three types structural microvessel changes that occur in diabetes are basement membrane thickening, selective pericyte loss, and endothelial cell changes.

Basement membrane thickening is a known complication of diabetes (Ashton and T, 1974) and studies of diabetes-affected versus control eyes have shown that there is a 2 – 5 fold increase in thickness in people affected by diabetes. This process is thought to be related to acceleration of the normal ageing process, although the underlying cause is not clear. Vacuole formation and altered collagen synthesis may also occur. Autopsy studies have found a 1:1 ratio between pericytes and endothelial cells in non-diabetic eyes compared with a 1:4 ratio in diabetic eyes (Cogan and Kuwabara, 1967).

A localised, limited proliferative response of endothelial cells is thought to result in small, round outgrowths from the vessel wall (Forrester *et al.*, 1993). These are known as saccular aneurysms, or dot haemorrhages, and are the one of the earliest ophthalmoscopic signs of retinopathy (Ashton and T, 1974). Pericyte loss may play a part in this process, either by creating a weak point in the vessel wall, or via removal of their inhibitory control of endothelial proliferation (Forrester *et al.*, 1993).

### **Biochemical events relating to the development of diabetic retinopathy**

Hyperglycaemia refers to a condition in which there is an excessive amount of glucose circulating the blood plasma. Glucose levels vary throughout the day, and before and after meals, however, the normal blood glucose level in fasting adults is 4 – 7 mmol/L. A patient who consistently measures above 7 mmol/L is considered hyperglycaemic, and one who consistently measures below 4 mmol/L is considered hypoglycaemic. There is a relationship between hyperglycaemia and microvascular diabetic complications in both type 1 and type 2 diabetes (Shamoon *et al.*, 1993, Turner *et al.*, 1998); both hyperglycaemia and insulin resistance appear to play a part in the development of these complications (Turner *et al.*, 1998, Wei *et al.*, 1998, Ebara *et al.*, 2000, Ginsberg, 2000).

Hyperglycaemia is thought to lead be involved in the development of diabetic complications via four pathways; increased formation of advanced glycation end-products (AGEs), the polyol pathway, activation of protein kinase C (PKC) isoforms, and increased hexosamine pathway flux.

### *Increased formation of AGEs*

The formation of AGEs is thought to be a hyperglycaemia-related process (Brownlee and Gd, 2001) that is implicated in the development of diabetic retinopathy (McCance *et al.*, 1993, Murata *et al.*, 1997, Chibber *et al.*, 1997, Yamagishi *et al.*, 2002, Nakamura *et al.*, 2003, Boehm *et al.*, 2004, Fosmark *et al.*, 2006). Diabetic micro- and macrovascular disease has been shown to improve with removal of AGEs (Forbes *et al.*, 2003, Bierhaus *et al.*, 2004, Hammes *et al.*, 2003, Stracke *et al.*, 2001). Formation of AGEs involves the attachment of glucose (or other sugar molecules) to a protein or lipid molecule in the absence of the controlling action of an enzyme. When the addition of sugars to protein or lipid molecules is controlled, it is termed glycosylation; glycation is an uncontrolled process that negatively effects the functioning of biomolecules. Glycation that takes place within the body is termed endogenous glycation, and occurs in the bloodstream to absorbed simple sugars such as glucose, fructose and galactose. Red blood cells are involved in glycation, and glycated haemoglobin levels are use in the monitoring of blood sugar in diabetes.

Following glycation, a series of reactions take place, resulting in the irreversible development of AGEs (John and Lamb, 1993, Raj *et al.*, 2000), some of which are involve in the development of age-related conditions such as type II diabetes. This chain-reaction can also result in the production reactive oxygen species (ROS) (Huebschmann *et al.*, 2006) including the highly oxidative radical, hydrogen peroxide. Higher amounts of AGEs are found in diabetic retinal vessels (Stitt *et al.*, 2002) than in controls. AGE formation can occur within a week in endothelial cells exposed to high glucose levels (Brownlee and Gd, 2001). Proteins modified by AGEs in endothelial cells include basic fibroblast growth factors (Giardino *et al.*, 1994) and those involved in macromolecular endocytosis (Shinohara *et al.*, 1998). The increase in endocytosis induced by hyperglycaemia is prevented by overexpression of the enzyme glyoxalase 1 (Shinohara *et al.*, 1998), which is also implicated in pericyte loss and capillary regression (Maisonpierre *et al.*, 1997).

### *Polyol pathway*

This pathway has been implicated in diabetic complications, especially retinopathy, nephropathy, and neuropathy, and is also known as the sorbitol-aldose reductase pathway. Aldose reductase is the first enzyme in the polyol pathway, and it catalyses the NADPH-dependent reduction of a wide range of carbonyl compounds, including glucose (Brownlee, 2001). Unused glucose enters the polyol pathway, when aldose reductase reduces it to sorbitol. High blood glucose levels result in excessive production of sorbitol. Activation of the polyol pathway results in decreased production of reduced NADP<sup>+</sup> and oxidised NAD<sup>+</sup>, which are co-factors in redox reactions within the body. This may result in increasing concentrations of triose phosphate (Williamson *et al.*, 1993), which may in turn increase production of AGEs (Brownlee, 2001). Reduction of glucose to sorbitol results in consumption of NADPH, which is required for regenerating reduced glutathione and nitric oxide. Nitric oxide is also known as 'endothelium derived relaxing factor', or EDRF, and signals dilation of blood vessels, and glutathione acts as an antioxidant. This may result in induced intracellular oxidative stress, and is a likely mechanism by which the increased activity of the polyol pathway contributes to disease pathogenesis (Lee *et al.*, 1999). Sorbitol may also glycate nitrogens on protein such as collagen resulting in the production of advanced glycation end products (AGEs).

Most cells of the body require insulin to allow glucose to gain entry to them, but the cells of the retina, kidneys, and nervous system are insulin independent, which means that glucose moves freely across their cell membranes. Glucose that is not used for energy production enters the polyol pathway. When glucose levels are normal, this does not cause a problem as aldose reductase has a low affinity for glucose at normal levels. However, when glucose levels are high, sorbitol is able to accumulate, NADP is used up, and so there is less NADPH available for other cellular processes. It has been proposed that when sorbitol accumulates within cells, water is drawn in due to osmosis, although the sorbitol concentrations measured in diabetic vessels are too low to cause osmotic damage (Brownlee, 2001).

In summary, excessive activation of the polyol pathway, in a hyperglycaemic state, leads to increased accumulation of sorbitol, increased production of reactive oxygen species, and decreased levels of glutathione and nitric oxide. These factors are thought to be involved in the development of damage to retinal microvessels in diabetic retinopathy.

### **Activation of protein kinase C**

The protein kinase C (PKC) family is made up of eleven isoforms, nine of which are activated a lipid second messenger called DAG. Intracellular hyperglycaemia increases the amount of DAG in the retina of diabetic animals. This increased level of DAG in cultured vascular cells (Xia *et al.*, 1994) and in the retina of diabetic animals (Koya and King, 1998), activates PKC. Hyperglycaemia may also indirectly increase activation of PKC via ligation AGE receptors (Portilla *et al.*, 2000) and increased activity of the polyol pathway (Keogh *et al.*, 1997). It has been suggested that abnormal activation of PKC is involved in the decreased production of nitric oxide in experimental diabetes (Craven *et al.*, 1994), as well as decreased production of nitric oxide in smooth muscle cells that is induced by hyperglycaemia (Ganz and Seftel, 2000). Inhibition of nitric oxide production may also contribute to increased microvascular matrix protein accumulation (Craven *et al.*, 1997).

### **Hexosamine pathway**

Throughflow of excess glucose through the hexosamine pathway may also contribute to the complications of diabetes (ref 74). Hyperglycaemic activation of this pathway may result in changes to gene expression and protein function, which together may contribute to the pathogenesis of diabetic complications (Brownlee and Gd, 2001)

### **Hyperglycaemia and oxidative stress**

Hyperglycaemia and diabetes have been associated with increased levels of oxidative stress (Guigliano *et al.*, 1996). Hyperglycaemia has been shown to increase the production of superoxide by endothelial cells, in a study of cultured endothelial aortic cells (Giardino *et al.*, 1996). Superoxide may activate all four pathways of hyperglycaemic damage. Superoxide partially inhibits the glycolytic enzyme GAPDH, which results in a build up of glucose, glucose-6-phosphate, and fructose-6-phosphate and means that they are available to enter into the polyol and hexosamine pathways. Inhibition of GAPDH also results in a build-up of glyceraldehydes-3-phosphate, which in turn results in increased production of DAG from DHAP, resulting in increased activation of PKC. Glyceraldehyde-3-phosphate is also converted to methylglyoxal, the main intracellular AGE precursor.

Increased flux through the hexosamine pathway increases protein modification, and increased flux through the polyol pathway consumes NADPH and depletes GSH (Brownlee and Gd, 2001).

Oxidative stress has been found to be associated with diabetic complications in some studies (Sinclair *et al.*, 1992, Sinclair *et al.*, 1991). Chemically, oxidation refers to the removal of electrons and, when it occurs within the body, it can result in the formation of cytotoxic chain reactions. Reactive oxygen species (ROS) is a term used to describe some types of free radicals, hydrogen peroxide and singlet oxygen, which are all capable of damaging membrane lipids, proteins, nucleic acids, and carbohydrates via oxidation (Beatty *et al.*, 2000).

There are exogenous and endogenous substances that can provide protection from ROS damage. These are often referred to as antioxidants. Singlet oxygen is scavenged by vitamin A and other retinoids, as well as beta-carotene (Levin *et al.*, 1997) and other carotenoids (Di Mascio *et al.*, 1989). The superoxide anion radical is efficiently removed by the enzyme superoxide dismutase (SOD), which in the cell mitochondria contains manganese, and in the cytosolic compartment contains copper and zinc. Hydrogen peroxide is metabolised by the selenium-containing enzyme, glutathione peroxidase. When there is an imbalance between the amounts of ROS being produced and the amount of exogenous and endogenous antioxidants available i.e. more ROS than antioxidants, a state of oxidative stress exists (McCord, 2000).

Ocular tissues are particularly susceptible to oxidative damage. The transparency of the cornea, aqueous humour, lens and retina allow continuous exposure to light, which along with aging, inflammation, air pollutants and cigarette smoke, has been shown to result in an increase in the production of ROS (Machlin and Bendich, 1987, Borish *et al.*, 1987).

The retina is particularly vulnerable to oxidative damage because of its abundance of polyunsaturated fatty acids, the occurrence of phagocytosis within the retinal pigment epithelium, and the fact that it has much higher blood flow than other tissues. Polyunsaturated fatty acids are predominantly found in photoreceptor outer membranes, and are readily oxidised (Machlin and Bendich, 1987, Beardsley, 1991, Van der Hagen *et al.*, 1993). Phagocytosis results in the production of ROS and high blood flow increases the availability of oxygen.

In diabetes, oxidative stress may result from:

- Excessive oxygen radical production from the auto-oxidation of glucose (Woff *et al.*, 1991)
- Glycated proteins (Mullarkey *et al.*, 1990) (glycation is the result of a reducing sugar molecule, such as fructose or glucose, bonding to a protein or lipid molecule without the controlling action of an enzyme).
- Stimulation of cytochrome P450-like activity as a result of excessive nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) production via glucose metabolism (Jain, 1989)
- Ketone body acetoacetate (Jan *et al.*, 1998)
- Glycation of antioxidant enzymes (Baynes and Thorpe, 1996, Guigliano *et al.*, 1996).

#### *The vitamin antioxidant network*

A system of vitamin antioxidant nutrients exists to neutralize ROS. Vitamin C reacts with superoxide in the aqueous phase and Vitamin E reacts with peroxy radicals in the lipophilic phase. In the process, vitamins C and E themselves become free radicals. However, they have aromatic ring structures, which delocalize the unpaired electron and are therefore less reactive. They also form part of the antioxidant network, in which vitamin C is able to regenerate vitamin E from the vitamin E radicals (Packer, 2001a).

Alpha lipoic acid is a vitamin-like antioxidant that is soluble in fat and water (Kagan *et al.*, 1990). It is manufactured within the body and is also found in food such as liver and yeast. Dihydrolipoic acid (DHLA), a powerful metabolic antioxidant, is formed when alpha lipoic acid is reduced by enzymes. It has an important role in antioxidant defence through maintenance of high levels of the reduced (potent) forms of vitamins C and E. Alpha-lipoic acid also boosts cellular levels of glutathione, a major cellular antioxidant. Levels of glutathione decline with age, and low levels are associated with higher incidence of disease in older people. Glutathione concentrations are recognised as a predictor of the susceptibility to disease as well as a marker of biological age (Packer, 2001b).

#### **Diabetic retinopathy**



Hyperglycaemia is a major causative factor in the development of endothelial dysfunction, and is a precursor to peripheral vascular complications including changes to vascular permeability and retinal blood flow and thickening of basement membranes (Kowluru and Kennedy, 2001). Capillaries are lined with endothelial cells that are closely associated with pericytes. Hyperglycaemia results in loss of endothelial cells and pericytes, which are contractile and provide tone to the capillaries. If these vascular changes are confined within the retina, then they are termed background retinopathy. If, however, new vessels break through the internal limiting membrane of the retina, the change is termed proliferative retinopathy. In this case, new vessels grow on the surface of the retina and adhere to the posterior layer. Contraction of the vitreous can lead to traction on these vessels and subsequent retinal detachment (Watkins *et al.*, 2003a).

#### Method: literature review

We identified pertinent articles on use of nutritional supplementation for type 2 diabetes published in peer-reviewed journals, through a multi-staged, systematic approach. In the first stage, a computerized search of the PubMed database (National Library of Medicine) and the Web of Science database was performed to identify all relevant articles published between 1950 and December 2007. Terms and words used for the search included 'diabetes mellitus, type 2', 'diabetes mellitus', 'nutrition', 'antioxidants', 'Pycnogenol', 'chromium', 'magnesium', 'selenium', 'vanadium', 'niacinamide', 'vitamin B<sub>3</sub>', 'cobalamin', 'vitamin B<sub>12</sub>', 'ascorbic acid', 'vitamin c', 'tocopherol', 'vitamin E', 'carnitine/ or acetylcarnitine/ or palmitoylcarnitine', 'l carnitine', 'folic acid/ or pteroylpolyglutamic acids/ or tetrahydrofolates'. Searches were limited to 'humans' and 'optimised therapy'. In the second stage all abstracts were examined to identify articles that described randomised controlled trials investigating nutritional intervention in people with type 2 diabetes. Studies were excluded if there intervention contained more than one nutrient and/or was not for oral use. Copies of the entire articles were obtained, where possible. Bibliographies of the retrieved articles were manually searched with use of the same search guidelines. In the third stage, articles were reviewed and information relating to the use of nutritional supplementation for type 2 diabetes was incorporated in to the manuscript. The literature search was not limited to the English language, although no translation was required

#### **Nutraceuticals and diabetic retinopathy**

The term 'nutraceutical' refers to chemical components of common foods that are claimed to have a medicinal effect.

#### **Outcome measures**

### Plasma and urine F(2)-isoprostanes

The isoprostanes are [prostaglandin](#)-like compounds formed *in vivo* from the free radical-catalyzed peroxidation of [arachidonic acid](#) without the activity of [cyclooxygenase](#) enzyme. They are accurate markers of [lipid](#) peroxidation in both animal and human models of oxidative stress (Morrow 1996). Their association with coronary artery calcification is thought to be supportive of the hypothesis that oxidative damage is involved in the early development of atherosclerosis (Gross *et al.*, 2005).

### Fasting plasma glucose and two-hour oral glucose tolerance test

These tests measure of how well glucose is cleared from the blood. In order to carry out a fasting plasma glucose test, the patient must have been fasting for 8 -12 hours, at which time a baseline blood sample is taken. In order to measure two-hour oral glucose tolerance (OGT), the patient is then given a glucose solution to drink. The standard dose since the late 1970s has been 1.75 grams of glucose per kilogram of [body weight](#), to a maximum [dose](#) of 75 g. A blood sample for measurement of glucose is taken at 2 hours, and sometimes at further two-hour intervals depending on the setting.

The World Health Organisation classifies diabetes mellitus as:

- Fasting plasma venous glucose  $\geq 7.0$  mmol/l (126 mg/dl)  
OR
- 2-hour OGTT (with 75g glucose) plasma venous glucose  $\geq 11.1$  mmol/l (200 mg/dl) (World Health Organisation, 2006).

### Red blood cell sorbitol levels

Accumulation of sorbitol is thought to occur in response to hyperglycaemia and be one of the biochemical events implicated in the development of diabetic complications (Brownlee, 2001).

### Insulin sensitivity/resistance

Insulin resistance occurs when the body doesn't respond as well to the insulin that the pancreas is making to help glucose leave the blood and enter the body's cells. An insulin resistance test measures the amount of [glucose](#) necessary to compensate for an increased [insulin](#) level without causing [hypoglycemia](#) (DeFronzo *et al.*, 1979).

### Thiobarbituric acid reactive substances (TBARS)

These substances are [low-molecular-weight end products](#), [probably malondialdehyde](#), that are [formed](#) during the [decomposition](#) of [lipid peroxidation products](#). The plasma or urine concentration of the TBARS is used as an index of lipid peroxidation and oxidative stress (Cherubini *et al.*, 2005)..

### Serum albumin and urine albumin excretion rates

Proteins from the blood can escape into the urine when the filters of the kidney, called glomeruli, are damaged. Sometimes the term albuminuria is used when a urine test detects albumin specifically. Microalbuminuria is defined as excretion of between 30 and 300mg of albumin a day in the urine. Microalbuminuria is an important prognostic marker for kidney disease in [diabetes mellitus](#). It is also a useful biological marker for individuals who are at high risk of cardiovascular events (Yuyun *et al.*, 2005). Microalbuminuria is diagnosed either on 24-hour urine collections (20 to 200 µg/min) or, more commonly, if elevated concentrations (30 to 300mg/L) on at least two occasions. Low blood albumin levels ([hypoalbuminemia](#)) can also result from excessive urinary excretion of albumin (Kaysen *et al.*, 1995).

Fasting blood insulin levels

Can indicate loss of insulin sensitivity

HbA1c

HbA1c is a test that measures the amount of glycosylated hemoglobin in the blood. Glycosylated hemoglobin is a molecule in red blood cells that attaches to glucose. Higher levels of glycosylated hemoglobin are related to higher levels of glucose in the blood. The test gives a good estimate of how well [diabetes](#) has been controlled over the last 2 or 3 months.

Malondialdehyde levels

Malondialdehyde is the end-product of [lipid peroxidation](#) by [reactive oxygen species](#). It is therefore used as a biomarker for [oxidative stress](#)

Glutathione peroxidase

Glutathione peroxidase is the general name of an [enzyme](#) family with [peroxidase](#) activity whose main biological role is to protect the organism from oxidative damage..

#### Superoxide dismutase

The enzyme superoxide dismutase catalyzes the [dismutation](#) of [superoxide](#) into [oxygen](#) and [hydrogen peroxide](#). As such, it is an important [antioxidant](#) defense in nearly all cells exposed to oxygen.

#### Ex vivo assessment of eicosanoid synthesis

Eicosanoid is the collective term for oxygenated derivatives of essential fatty acids (Beare-Rogers *et al.*, 2001)

#### *Pycnogenol*<sup>®</sup>

Pycnogenol<sup>®</sup> is a standardised extract of bark of the French maritime pine (*Pinus pinaster*), and has been shown to protect endothelial cells against oxidant-induced injury (Wei *et al.*, 1997). Pycnogenol promotes a protective antioxidant state by upregulating important enzymatic and nonenzymatic oxidant scavenging systems (Wei *et al.*, 1997). It is one of the most potent natural antioxidants, and also has an affinity for proteins such as collagen (Roghdewald, 2002). Pycnogenol<sup>®</sup> significantly decreases nitrogen monoxide generation, and it is thought that this function may result from a combination of several biological activities including its ROS and nitrogen monoxide scavenging activity, inhibition of the enzyme nitric oxide synthase (iNOS), and inhibition of iNOS-mRNA

expression (Virgili *et al.*, 1998). Pycnogenol® can readily cross the blood-brain barrier to provide antioxidant protection to central nervous system tissue. This property differentiates it from other known antioxidants. Its antioxidant potential is associated with anti-inflammatory action, whereas its affinity for collagen may be responsible for the reduction of pathologically increased capillary permeability (Roghdewald, 2002). Supplementation of conventional diabetes treatment with Pycnogenol® has been shown to lower glucose levels and improve endothelial function (Liu *et al.*, 2004). Pycnogenol® is licensed in France for the treatment of diabetic retinopathy.

Pycnogenol® has been investigated for use in the treatment and prevention of diabetic retinopathy in several trials over the past 50 years. All of these studies showed that Pycnogenol® slowed retinopathy progression and result in a partial recovery of lost visual acuity.

In one investigation, a six-month double masked trial of Pycnogenol® versus Dexium 500 (calcium dobesilate) was carried out with 32 type 1 and 2 diabetic participants. The pharmacology of Dexium is similar to that of Pycnogenol® except that it does not have such a high binding affinity collagen.

The Pycnogenol® group were instructed to take 120 mg/day for the first six days and then 80 mg/day for the remainder of the trial. Pycnogenol® was found to reduce exudates in both eyes of five participants, compared with just one Dexium-treated participant. The authors concluded that Pycnogenol® may also be beneficial for improvement of the visual field (Leydhecker, 1986).

Another double-masked, placebo controlled trial investigated the effect of 150 mg/day Pycnogenol® on objective parameters in patients with retinopathies associated with diabetes, atherosclerosis, and hypertension (Spadea and Balestrazi, 2001). Examination of the ocular fundus showed a significant improvement in those treated with Pycnogenol® ( $p = 0.003$ , left eyes;  $p = 0.043$ , right eyes), compared with the placebo, and electrophysiology and fluoroangiography was improved in the treatment group ( $p < 0.001$ ). The group taking Pycnogenol® also experienced a significant reduction in vascular permeability and an improvement in the blood-retinal barrier. No significant change in visual field was found and no side effects were reported by either group.

In 1988, Plantorgan conducted a multi-centre field study with 1169 participants with diabetic retinopathy (Arzneimittel, 1988). It was of six-month duration and the main objective was to assess the effect of Pycnogenol® on the progression of visual acuity. Deterioration of visual acuity ceased, and in some cases improved. The improvement was noted after three months of supplementation and continued over a further three months. Side effects were reported by 1.45 % of participants, and included stomach problems, headache, nausea, and dizziness.

### *Vitamin E*

Alpha-tocopherol is the most effective antioxidant of the vitamin E group, and protects against lipid peroxidation (Machlin, 1980). Vitamin E exists in four common forms: alpha-tocopherol, beta-tocopherol, gamma-tocopherol, delta-tocopherol (Drevon, 1991).

The most predominant form in the human retina and plasma is alpha-tocopherol (Alvarez *et al.*, 1987, Handelman *et al.*, 1985), which is also the most effective scavenger of free radicals (Burton. and Ingold, 1984). Vitamin E is found in high concentrations within the retina (Dilley, 1970, Hunt, 1984), and as the major lipid-soluble antioxidant present in all cellular membranes, it protects against lipid peroxidation (Machlin, 1980) and may also have a role as a quencher of singlet oxygen Plasma concentration is known to reduce with age while high dietary intake of vitamin E can increase concentration in the retina (Seddon, 1999).

The potential role of vitamin E supplementation in the control of diabetic retinopathy relates to increased levels of oxidative stress in the vascular endothelium of diabetic patients (Baynes and Thorpe, 1996, Guigliano *et al.*, 1996). Antioxidant treatment may improve endothelial cell dysfunction in diabetes (Timimi *et al.*, 1998).

An eight-month randomised controlled crossover trial reported that oral vitamin E treatment (1800 IU/day) appeared to be effective in normalising retinal haemodynamic abnormalities and improving renal function in type I diabetic patients with a short disease duration without affecting glycaemic control (Bursell *et al.*, 1999).

Epidemiological studies have shown that vitamin E improves insulin sensitivity in short-term supplementation trials (Paolisso *et al.*, 1994, Paolisso *et al.*, 1993).

### *Vitamin C*

Vitamin C (ascorbic acid) is a chain-breaking antioxidant, i.e. it scavenges ROS directly and prevents the propagation of chain reactions that would otherwise lead to a reduction in protein glycation. It also prevents the breakdown of nitric oxides, and decreases the oxidation of low density lipoproteins (LDL) (Young *et al.*, 1995). Vitamin C has been shown to affect retinal blood flow in animal models and human supplementation trials (Cunningham *et al.*, 1994, Visnon *et al.*, 1989, Wang *et al.*, 1995, Yue *et al.*, 1989). It is water soluble, reduces platelet aggregation (Wilkinson *et al.*, 1999), and may be important in lowering blood pressure (Taddei *et al.*, 1998).

Vitamins C and E both protect against oxidative stress caused by non-enzymatic glycation, autoxidative glycosylation, and metabolic stress in diabetic people (Baynes and Thorpe, 1999). Vitamin C also reduces diabetes-induced sorbitol accumulation in the erythrocytes and significantly reduces lipid peroxides in the erythrocytes and plasma (Cunningham *et al.*, 1994).

However, epidemiological studies investigating the relationship between micronutrient intake and retinopathy do not support supplementation as a treatment or prevention strategy. The San Luis Valley Diabetes Study reported no protective effect and highlighted the potential for deleterious effects of antioxidant (including vitamin C) use in diabetic retinopathy (Mayer-Davis *et al.*, 1998). The Third National Health and Nutrition Examination Survey reported no association between serum ascorbic acid and alpha-tocopherol concentrations and retinopathy in people with type 2 diabetes (Millen *et al.*, 2003). Both studies were, however, limited by cross-sectional designs,

while the San Luis Valley study was further limited by the collection of 24-hour recall data, which is unlikely to reflect long-term food intake.

A prospective study was designed using participants from the Atherosclerosis Risk in Community Study, to investigate the relationship between diabetic retinopathy and intake of vitamins C and E (Millen *et al.*, 2004). No relationship was demonstrated, although misclassification of nutrient intake for food frequency questionnaires, exclusion of subjects for whom data was missing, misclassification of specific lesions of retinopathy and a lack power to detect an association when the effect of vitamins C and E on retinopathy was modest, are very likely to have limited the findings of this study. (Millen *et al.*, 2004).

### *Lipoic acid*

Lipoic acid is a naturally occurring antioxidant with potent ROS-scavenging activity (Packer *et al.*, 1995). It has the unusual property of being an ROS scavenger in its oxidised state (Chidlow *et al.*, 2002), and has been shown to quench:

- Hydroxyl radicals
- Hypochlorous acid
- Singlet oxygen
- Nitric oxide radical
- Peroxynitrite
- Hydrogen peroxide.

Alpha-lipoic acid and dihydrolipoic acid work in a redox couple, and together have other antioxidant properties including chelation of transition metals and the regeneration of other antioxidants such as glutathione, vitamin C and vitamin E (Biewenga *et al.*, 1997). Alpha-lipoic acid has been shown to protect the retina against ischaemia-reperfusion injuries *in vivo* and *in vitro* (Chidlow *et al.*, 2002). Ischaemic injury to the retina is considered to be one of the major causes of visual loss, and occurs in conditions such as retinopathy of prematurity and glaucoma (Hayreh, 2001, Osborne *et al.*, 2001), as well as diabetic retinopathy. Alpha-lipoic acid is available on prescription in Germany and is widely used in the treatment of diabetic neuropathy.



The effect of lipoic acid on nitric oxide-mediated vasodilation in diabetic patients has been investigated, and compared with the well-recognised effect of vitamin C (Heitzer *et al.*, 2001). Alpha-lipoic acid has been shown to increase insulin sensitivity in type-2 diabetes (Jacob *et al.*, 1999), and has the added benefits of being readily absorbed from the diet in a bioavailable form and being well-tolerated (Packer *et al.*, 1995). Large doses (at least 600 mg/day) are well tolerated and no serious adverse consequences have been recorded. A review of the clinical trials of alpha-lipoic acid in the treatment of diabetic neuropathy suggests that there is some evidence to support its use and beneficial effects on acute symptoms and progression have been reported (Ziegler *et al.*, 1999).

#### *Phytoestrogens: Genistein*

Genistein, is an isoflavonoid that is found in soybeans (Naim *et al.*, 1973). It is a naturally occurring tyrosine kinase inhibitor (Akiyama *et al.*, 1987), but also has several other properties including acting as an antioxidant (Wei *et al.*, 1993, Wei, 1994, Wei *et al.*, 1996) by protecting against lipid peroxidation and by scavenging superoxide and superoxide anions (Jha *et al.*, 1985, Wei *et al.*, 1993).

Genistein has been shown to inhibit proliferation of vascular endothelial cells and tumour cells, (Akiyama *et al.*, 1987, Fotsis *et al.*, 1995, Steele *et al.*, 1995).

Long-term oral administration of genistein in diabetic rats inhibits retinal vascular leakage (Nakajima *et al.*, 2001). This finding supports the use of tyrosine kinase inhibition in the treatment of diabetes-induced retinal vascular leakage. Genistein may also be useful in the prevention of neuronal degeneration in the inner retina that results from ischaemic injury, as systemic administration has been shown to improve ischaemia-reperfusion-induced retinal degeneration (Hayashi *et al.*, 1997).

#### *Chromium*

It is known that chromium deficiency will lead to impaired glucose tolerance due to insulin resistance i.e. insulin is produced in correct amounts but is not taken up properly. A chromium deficiency can also contribute to hyperglycaemia (Cefalu and Hu, 2004). It has been suggested that chromium supplements might have beneficial

effects in the management of type 2 diabetes where the primary pathological change is a decline in insulin response (insulin resistance).

Some reports have suggested that chromium supplements may increase insulin sensitivity and improve glucose tolerance and other measures of diabetic control in patients with type 2 diabetes. A placebo-controlled, double-masked randomised pilot study demonstrated that supplementation with chromium picolinate in combination with biotin in patients with poorly controlled diabetes receiving anti-diabetic therapy improved glucose management and several lipid measurements (Singer and Geohas, 2006). The investigators concluded that this type of supplementation may have the potential to improve lipid metabolism in people with poorly controlled diabetes. A meta-analysis of randomised controlled trials investigating the effect of chromium supplementation on glucose and insulin response on healthy individuals and those with diabetes reported no evidence of effect in non-diabetic subjects. The data for diabetic subjects were reported to be inconclusive (Althuis *et al.*, 2002).

However, a more recent review of clinical studies of chromium picolinate supplementation in diabetes mellitus found that 13 out of 15 studies reported a significant improvement in at least one outcome of glycaemic control. Pooled data showed substantial reductions in hyperglycaemia and hyperinsulinaemia, equating to a reduced risk of disease complications, with chromium picolinate supplementation (Broadhurst and Domenico, 2006). There is support in the literature for the safety and therapeutic value of chromium picolinate supplementation for the management of cholesterolaemia and hyperglycaemia in people with diabetes.

#### *Plant extracts- Aloe vera*

The leaves of the *Aloe vera* plant contain a high concentration of anthraquinone compounds that have been used historically as a cathartic. Within the leaves there is a gel, which has been used as a food and to treat burns and other wounds (Grindlay and Reynolds, 1986).

*Aloe vera* gel extract has been shown to have antioxidant properties in diabetic rats (Rajasekaran *et al.*, 2005). Oral administration of *Aloe vera* gel extract to diabetic rats resulted in a reduction in fasting blood glucose levels,

plasma and tissue cholesterol, triglycerides, free fatty acids, phospholipids, and a significant improvement in plasma insulin (Rajasekaran *et al.*, 2006). Aloe vera gel and phytosterols derived from the gel are reported to have a long-term blood glucose level control effect (Tanaka *et al.*, 2006). Randomised controlled trials are needed to determine any treatment effect of *Aloe vera* for diabetes mellitus.

## **Conclusions**

Epidemiological evidence does not support a relationship between antioxidant intake and reduced risk for diabetic retinopathy, however, intervention trials indicate that further research is needed to clarify the role of Pycnogenol<sup>®</sup> (Leydhecker, 1986, Spadea and Balestrazi, 2001, Arzneimittel, 1988), *Ginkgo biloba* extract (Huang *et al.*, 2004) and Vitamin E (Bursell *et al.*, 1999). It has been suggested that the lipid-soluble vitamin E could be combined with a water-soluble antioxidant such as glutathione or N-acetylcysteine, to increase its efficacy in improvement of blood flow (Jain, 1999). Pycnogenol<sup>®</sup> is reported to protect vitamin E from oxidation (Cossins *et al.*, 1998), and also prevents platelet activity in humans without increasing bleeding time (Puttner *et al.*, 1999). There is also support for use of vitamin C supplementation (van den Enden *et al.*, 1995, Williamson *et al.*, 1993, Baynes and Thorpe, 1999), and potential for development of a nutritional supplement aimed specifically at diabetic patients, containing Pycnogenol<sup>®</sup>, *Ginkgo biloba* extract, vitamin E, or vitamin C, or a combination of these.

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