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DIABETES: A MODEL OF OXIDATIVE ACCELERATED AGING

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Medial thickening of arterioles has long been recognized as the hallmark of the angiopathic microvascular disease associated with diabetes mellitus. Deposits of glycoproteins and glycolipids as seen in early stages of atherosclerosis and accelerated large vessel disease are characteristic in diabetes. More recently, the role played by peroxidation of plasma lipids has been recognized as a contributing pathogenic factor in the accelerated atherosclerosis which is typical of the diabetic state.

Hyperglycemia stresses the cellular microenvironment and disrupts cell membrane function. Prolonged hyperglycemia reduces the endogenous intracellular antioxidants catalase, superoxide dismutase and glutathione peroxidase (Vajayalingam et al., 1996; Jain and McVie., 1994). Glycosylated hemoglobin (HgbA1c) is widely regarded as a good index of the average level of hyperglycemia a patient exhibits over a three month period, and an increase in HgbA1c is inversely correlated with the level of protective antioxidants actively maintained in red blood cells. Antioxidant deficiencies have been shown to be present at diagnosis of Impaired Glucose Tolerance (IGT), Insulin Resistance (IR) and Non-Insulin Dependent Diabetes Mellitus (NIDDM) (Vajayalingam et al., 1994). Reduced serum antioxidant levels are known to contribute to the increased oxidative stress in Insulin Dependent Diabetes Mellitus (IDDM)(Asayama et al., 1993). The human body has multiple natural antioxidants which reduce the constant onslaught of oxidative radicals resulting from aerobic respiration. The naturally occurring enzyme, glutathione peroxidase, is regarded to be an acutely important antioxidant system in IGT, IR and NIDDM. Persons with IGT and IR are known to have a glutathione deficiency averaging about 15%, while NIDDM patients exhibit about a 20% decrease in intracellular glutathione (Vajayalingam et al., 1994). Glutathione peroxidase activity is enhanced by selenium, and requires reduced nicotinamide adenine dinucleotide phosphate (NADPH) for the reductive regeneration of the active antioxidant state. In diabetes, NADPH is consumed by the competing reduction of glucose to sorbitol in insulin dependent tissues under high glucose conditions (Packer, 1993). NADPH is also consumed during the conversion of glucose to other monosaccharides. These increased demands on NADPH may decrease the antioxidant capacity of glutathione peroxidase in IGT, IR, NIDDM and IDDM individuals.

Extracellular radical-scavenging antioxidants, such as uric acid, ascorbic acid (vitamin C), tocopherols (vitamin E), sulfhydryl and natural preventive antioxidants, and the iron transporting/binding proteins in plasma, are typically deficient in IDDM. Serum ascorbic acid, α-tocopherol, and sulfhydryl are reported to be decreased in both IDDM and NIDDM (Asayama et al., 1993). Antioxidants in the diet derived from fruits, wine, teas, vegetables, and other plant products are primarily scavenging antioxidants (Rice-Evans et al., 1995). The ascorbic acid metabolism defects exhibited by insulin dependent diabetics are correctable by insulin treatment. Insulin dependent diabetics also exhibit platelet dysfunction secondary to vitamin E deficiency that is partially correctable with ascorbate supplementation (Asayama et al., 1993). Enhancement of serum, plasma, and intracellular antioxidant levels may be protective against lipid peroxidation and free radical damage, and against the secondary complications of chronic diabetes: coronary artery disease, cerebrovascular disease, peripheral vascular disease, neuropathy, retinopathy, nephropathy, and possibly aging. Free radicals formed by the auto-oxidation of glucose and glycosylated proteins initiate oxidation-reduction reactions and promote oxidative stress which has been implicated in the pathogenesis of diabetes. The degree to which oxidative radicals are present in chronic diabetes directly parallels the severity of diabetic complications (Godin et al., 1988). The greatest damage from reactive oxygen species (ROS) and free radicals is to phospholipids and fatty acids in cell membranes. Free radicals oxidize proteins causing interference with sodium, potassium and adenosine triphosphate (ATP) ion channels resulting in failure of cell membrane receptors. Free radical oxidants also disrupt the equilibrium of biological systems by damaging major cell constituent macromolecules. Deoxyribonucleic acid (DNA) undergoes single and double strand breaks as free radicals bind phosphate groups, destroying deoxyribose sugars and bases. Excessive oxidation is implicated in mutagenesis, carcinogenesis, diabetes, aging and many other disease processes due to the oxidation-initiated role in tissue degeneration and cell death (Maxwell, 1995; Barhoumi et al., 1997; Parke, 1996; Sevenian and Hochstein, 1985).

Natural antioxidant defenses have been found to be defective in a number of different diseases (Vajayalingam et al., 1996; Asayama et al., 1993; Maxwell, 1995). If disease is, in fact, associated with an imbalance between oxidative stressors and antioxidant defenses, it should be possible to limit oxidative damage and prevent or retard disease progression by supplementing and/or enhancing natural antioxidant defenses (Maxwell, 1995),

because antioxidants, even in relatively low concentrations, significantly inhibit the rate and extent of oxidative damage. Potential health-promoting antioxidant interventions might support natural enzyme antioxidant systems and/or scavenging antioxidants.

Natural enzyme antioxidant systems are found in the intracellular environment and include superoxide dismutase (SOD), catalase, and glutathione peroxidase. Catalase is functionally associated with the heme molecule and reduces peroxides. Glutathione peroxidase contains selenium and helps prevent formation of the highly destructive hydroxyl radical. In addition, it participates in regeneration of vitamin C, which in turn regenerates vitamin E, requiring NADPH for reducing equivalent (Maxwell, 1995).

Natural preventative antioxidants found in blood plasma include transferrin, a major iron transporting protein; lactoferrin, an iron binding protein in milk; ceruloplasmin, a copper-containing protein that enhances iron binding to transferrin; and albumin, a weak copper-binding protein that is the major antioxidant of plasma (Maxwell, 1995).

Scavenging antioxidants are found in the plasma and/or serum. They include uric acid, bilirubin, sulfhydryl or thiols, vitamin C, vitamin E, β-carotene, ubiquinol, flavonoids, melatonin, possibly vitamin A, and some estrogens (Maxwell, 1995; Reiter, 1995). Scavenging antioxidants may be either water or lipid soluble. Uric acid comprises at least 44% of the antioxidant activity of plasma. Vitamin C is a major water soluble antioxidant that inhibits lipid peroxidation and is regenerated intracellularly by glutathione peroxidase. Thiols are contributed by albumin in the plasma. Thiols such as acetylcysteine are involved in regeneration of glutathione peroxidase. The tocopherols, vitamin E, are major lipid-soluble antioxidants that prevent lipid peroxidation in lipoproteins and biological membranes. Vitamin E is regenerated by vitamin C. β-Carotene is a lipid-soluble vitamin A precursor that is synergistic with vitamin E and prevents lipid peroxidation. Ubiquinol, the reduced form of coenzyme Q10, is also lipid soluble and regenerates vitamin E (Maxwell, 1995). Flavonoids are polyphenolic compounds present in foods such as fruits, vegetables, tea and wine that may play a role in the prevention of disease (Rice-Evans et al., 1995; Scholes, 1983; Watkins, 1997). Melatonin is a naturally occurring indole that scavenges the highly toxic hydroxyl radical, neutralizes the peroxyl radical, and stimulates glutathione peroxidase in the brain (Reiter, 1995). The estrogens, estradiol, estrone, and estriol, are primary ovarian steroid hormones that may possess some antioxidant activity.

Dietary antioxidants are commonly found in tea, wine, fruits and vegetables (Rice-Evans, 1995; Scholes, 1983; Watkins, 1997). Green tea, called "sencha", has particularly large amounts of vitamin C and phenolic compounds (Scholes, 1995). Wines contain polyphenols, vitamin C, and some B vitamins (Watkins, 1997). Fruits and vegetables harvested at maturity contain a variety of flavonoids, carotenoids, lycopenes, zeaxanthins, phenols, indoles, and luteins (DeCava, 1996). Antioxidants derived from these dietary sources are the safest for long term consumption.

Pharmacological agents that have direct or indirect antioxidant activity include acetylcysteine, which donates thiols; SOD, the activity of which is prolonged by conjunction with albumin; catalase; glutathione peroxidase, which is enhanced by selenium supplementation; deferoxamine, which is an iron chelator; Probucol, a lipid soluble drug that prevents oxidation of lipoproteins and is regenerated by vitamin C; salicylates, which are free radical scavengers; lazaroids, or 21-aminosteroids, that are inhibitors of iron-dependent lipid peroxidation; mannitol, which is a hydroxyl radical scavenger; dimethyl sulfoxide, DMSO, which is a hydroxyl radical scavenger; dimethyl thiourea, or DMTU, which is a hydroxyl radical scavenger; Captopril, an angiotensin converting enzyme inhibitor; Amiodarone, a cardiac anti-arrhythmic agent; Allopurinol, used in treating gout and/or hyperuricemia; Adenosine, a cardiac anti-arrhythmic; albumin; and Carvedilol, a vitamin E-containing beta-blocker.

There are complex, interdependent relationships between antioxidant molecules and what the human body requires to maintain health and ward off disease. Oxidative injury to cellular macromolecules and biomembranes has been implicated as a pathophysiological mechanism in a variety of human diseases (Maxwell, 1995) and oxidation has been implicated as a cause of, or a result of, many diseases (Barhoumi et al., 1997). It appears that dietary antioxidant supplementation may be an achievable and valid means to the goal of improving health and retarding aging. Antioxidant benefits of food and the importance of diet are rapidly becoming recognized and antioxidants are being utilized for their potential to promote health, prevent disease and retard aging.

The glutathione system of intracellular antioxidant protection is stressed in diabetics by hyperglycemia, auto-oxidation of excess glucose, and is altered by metabolism. The glutathione peroxidase system in cells is considered the most important biochemical mechanism by which phospholipids in cell membranes and serum lipids are protected from oxidative damage under normal metabolic conditions and the balance of antioxidant levels and deleterious oxidative activity is essential for cellular life. Excessive oxidative activity contributes to the aging process, as well as to many degenerative diseases, and increases significantly in diabetes. Increasing the antioxidant protective capacity of the intracellular environment may be a primary basis for clinical improvements reported by diabetics (McDaniel et al., 1997) and for those with other oxidation-associated diseases, as well as for accelerated aging.

A variety of antioxidants is important to prevent the onset of diseases with potential origins in the interaction of reactive chemicals with cellular components. Many of these diseases have an increased incidence associated with increased age in humans, including atherosclerosis, bursitis, transplant rejection, diabetes, some can-

cers, Parkinson's disease, hypertension, trauma, bacterial sepsis, hypoxia, stroke, rheumatoid arthritis, inflammatory bowel disease, cataracts, senile macular degeneration and chronic obstructive pulmonary disease (Maxwell, 1995). For some of these disorders there is a question of whether the disease initiated elevated levels of free radicals resulting in damage to macromolecules or oxidative damage to macromolecules by free radicals resulted in the onset of disease.

In some diseases natural mechanisms preventing damage to cells by reactive molecules have been compromised prior to cellular injury and the onset of symptoms. Dr. Denham Harmon initially introduced the concept that aging might be manifested as a collection of diseases resulting from free radical and electrophilic radical-associated cell and tissue damage due to down-regulated, or at least decreased, function of naturally occurring mechanisms by which cells protect themselves from highly reactive compounds (Harmon, 1956; Halliwell and Gutteridge, 1985; Sohal, 1987; Sohal et al., 1990; Pacifici and Davies, 1991; Harris, 1977; De Hann et al., 1992). In specific disease states, such as the inflammatory disease rheumatoid arthritis, the occurrence of oxidative damage to tissues resulting from reactive chemicals is firmly established, while in other disease states oxidative damage to macromolecules and biomembranes has been thought to be a potential mechanism of pathology occurring subsequent to onset of the disease. Thus, it is highly likely that some disorders result in increased levels of oxidative radicals, while in others, elevated levels of reactive chemicals may act to stimulate the onset of disease.

Cellular systems that counter the effects of oxidative radicals decline in function in some disease states and with increased age include: superoxide dismutase, catalase, glutathione (GSH), and glutathione peroxidase (Cand and Verdetti, 1989; Hussain et al., 1995). Of these, glutathione peroxidase acts to remove reactive chemicals from circulation by interaction of the chemicals with glutathione sulfhydryl groups. Glutathione depletion occurs in instances where cells are exposed to sources of reactive chemicals. As long as the target cell has adequate endogenous mechanisms to eliminate these reactive chemicals, cellular macromolecules are minimally damaged. However, when dietary compounds such as vitamins C or E, which reduce oxidized GSH, are depleted or when endogenous antioxidant systems such as SOD, catalase, or GSH peroxidase fail to function or are not maintained at normal levels, damage to cellular macromolecules increases dramatically (Ames, 1989). Decreased capacity to counter the membrane-damaging effects of free radicals may be a factor predisposing to the onset of symptomatic IDDM.

The ability of serum antioxidants to delay the peroxidation of lipids has been expressed as the total peroxyl radical-trapping antioxidant parameter (TRAP). This has been determined primarily by the serum content of the "scavenging" antioxidants vitamin C and E, uric acid, and protein sulfhydryl (Wayner, 1987). Glutathione can also be measured in living lymphocytes using Functional Intracellular Analysis™. The antioxidant enzyme system glutathione peroxidase differs in fubction from other scavenging antioxidants in that it catalyzes the reduction of oxidants utilizing a sulfhydryl-containing protein, GSH, as the reducing equivalent donor, resulting in GSH oxidation. Cellular functions resulting in the synthesis of glutathione, reduction of the oxidized protein, or sparing of glutathione oxidation may significantly increase the protection of cells from damage in an environment rich in reactive chemicals. Enhancing cellular glutathione peroxidase, by whatever means, should not only protect macromolecules under oxidative stress and/or disease processes such as diabetes, it should act to retard aging.

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