CHAPTER II

LITERATURE REVIEW

I. Definition of Diabetes Mellitus

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with dysfunction and failure of various organs, especially the eye, kidney, nerves, heart and blood vessels (The expert committee on the diagnosis and classification of diabetes mellitus, 2001).

Several pathogenic processes are involved in the development of diabetes these range from autoimmune destruction of the pancreatic β-cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Impairment of insulin secretion and defects in insulin action are the primary cause of the hyperglycemia, symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome (Kashiwagi A, 2001).

The classification of diabetes mellitus can be divided into five groups which are as follows: (The expert committee on the diagnosis and classification of diabetes mellitus, 2001)

- insulin-dependent diabetes mellitus (IDDM) or Type 1 diabetes

- non insulin-dependent diabetes mellitus (NIDDM) or Type 2 diabetes

- gestational diabetes mellitus (GDM)
- impaired glucose tolerance (IGI) and impaired fasting glucose (IFG)
- other specific types of diabetes mellitus

1.1 Type 1 diabetes mellitus (formerly called Type 1, IDDM or Juvenile diabetes)

Type 1 diabetes is characterized by β-cells destruction caused by an autoimmune process, usually leading to absolute insulin deficiency (The expert committee on the diagnosis and classification of diabetes mellitus, 2001). The onset is usually acute developing over a period of a few days to weeks. Over 95 percent of persons with Type 1 diabetes mellitus develop the disease before the age of 25 most of these patients have the "immune-mediated form" of Type 1 diabetes mellitus with islet cell antibodies and often have other autoimmune disorders such as Hashimoto's thyroiditis and Addison's disease.

1.2 Type 2 diabetes mellitus (formerly called Type 2, NIDDM, or Adult-onset)

Type 2 diabetes mellitus is characterized by insulin resistance in peripheral tissue and an insulin secretory defect of the β-cells (Bethesda.National diabetes data group, 1995). This is the most common form of diabetes mellitus and is highly associated with a family history of diabetes, older age, obesity and lack of exercise. It is more common in woman, especially women with a history of gestational diabetes. Insulin resistance and hyperinsulinemia eventually lead to impair glucose tolerance. Defective β-cells become exhausted, further fueling the cycle of glucose intolerance and hyperglycemia (Mayfield J, 1996).

Diabetes mellitus is a multifactorial disease associated with high risk for vascular complication. The morbidity associated with long-standing diabetes results microangiopathy, retinopathy, nephropathy and neuropathy. Microangiopathy is the higher prevalent of complication in Type 1 or in Type 2 with the macroangiopathy (Virsaladze D et al., 2001).

II. Vascular complication in diabetes mellitus

The major course of morbidity and mortality in both Type 1 and Type 2 diabetic patients is vascular diseases. These vascular diseases include small (microangiopathy) and large vessels (macroangiopathy).

Microangiopathy is the hallmark of retinopathy, neuropathy, and nephropathy (Standl E et al., 1996) whereas macroangiopathy in diabetes is manifested by accelerated atherosclerosis which affects vital organs, heart and brain. Diabetic microangiopathy is characterized both morphological and functional alterations of microvessels (Barnell AH, 1991). Morphological, diabetes produces thickening of vascular basement membrane, enhanced endothelial-leukocyte interaction (Hadcock S et al., 1991).

It is now well established that endothelial dysfunction play a major role of diabetic micro-and macroangiopathy. In recent years a body of evidence has accumulated that endothelial dysfunction is closely associated to microangiopathy and atherosclerosis in both Type 1 and Type 2 diabetes mellitus (Cosentino F and Luscher TF, 1998).

2.1 Normal Endothelial function

The endothelial cell (EC) forms the lining of blood vessels wall separating the lumen from the vascular smooth muscle cell (VSMC). In addition to serving as a physical barrier between the blood and tissue, the

EC facilitates a complex array of functions in intimate interaction with the VSMC.

The last two decades of research have established unambiguously that the EC has a critical role in overall homeostasis. The system exerts effects on both the surrounding VSMC and the cells in the blood that lead to one or more of the following alterations.

- vasodilatation or vasoconstriction to regulate organ blood flow
- growth and/or changes in the phenotypic characteristics of VSMC
- proinflammatory or antiinflammatory changes
- maintenance of fluidity of blood and avoidance of bleeding (Wautier JL et al., 1996).

Endothelial cells (ECs) activity regulate basal vascular tone and vascular reactivity in physiological and pathological conditions, by responding to mechanical forces and neurohumoral mediators with the release of a variety of relaxing and contracting factors (Furchgott RF, 1993). The endothelium-derived relaxing factors (EDRFs) include nitric oxide (NO), prostacyclin and endothelium hyperpolarizing factor (EDHF) (Furchgott RF, 1993).

Nitric oxide (NO)

Endogenous NO is produced through the conversion of the amino acid L-arginine to L-citrulline by enzyme, nitric oxide synthase (NOS) from which several isoforms; NOS type I (isolated from brain) and type III (isolated from ECs).

NOS type I (nNOS) is important in neurotransmission, the central control of vascular homeostasis, and possible learning and memory. In peripheral nervous system, NOS appears to be linked to nonadrenergic noncholinergic (NANC) neuronal pathway.

NOS type III (eNOS) is essential for the control of vascular tone in response to several stimuli, including mechanical (e.g., shear stress) receptor dependent (e.g., acetylcholine) and receptor independent (e.g., calcium ionophore) (Furchgott RF, 1993). NO produced by NOS type III in the endothelium diffuses to the VSMC where it activates the enzyme guanylatecyclase. The concomitant increase in cyclic GMP then induces relaxation of the VSMC. Thus, the net effect of an increase in NO is vasodilatation. NOS type III also contributes to the prevention of abnormal platelet aggregation (Lopez-Jaramillo P et al., 1990; Griendling KK et al., 1996).

NOS type II (isolated from macrophages) are Ca^{2+} calmodulin independent and are termed "inducible-NOS"or iNOS, since their activation is only promoted under pathophysiological situations in which macrophages exert cytotoxic effects in response to cytokines (e.g., sepsis) (Brune B et al., 1998).

2.1.1 Measurement of NO-mediated vasodilatation

Typically, NO-dependent vasodilatation is probed by the vasodilatory response to infusion of a compound (e.g., acetylcholine or methacholine), which increases the synthesis and release of NO via a receptor- mediated response that is calcium dependent (Calver A et al., 1992) or in response to reactive hyperemia which stimulates shear stress-induced NO production. This response is compared with the vasodilatation evoked by specific chemical compounds that directly act on VSMC (e.g., sodium nitroprusside).

2.1.2 Angiotensin II (ANG-II)

The EC also produces mediators that induce vasoconstriction, including endothelin (Haefling IO, 1992), prostaglandin (Goldin E et al., 1996) and ANG-II (Hsueh WA et al., 1993). EC regulates vascular tone by maintaining a balance between vasodilatation (NO production) and vasoconstriction (e.g., ANG II generation). ANG-II binds to and regulates VSMC tone via specific angiotensin (ANG) receptors. Depending upon specific receptor activated, ANG-II can exert regulatory effects upon several VSMC functional activities including contraction (i.e., vasoconstriction) and growth, proliferation, and differentiation. Overall, the actions of ANG-II oppose those of NO.

2.1.3 The EC as a regulator of homeostasis

Functions of the EC extend beyond those pertaining to vascular tone. The EC has a prominent role in maintaining blood fluidity and restoration of vessel wall integrity (when injured) to avoid bleeding. The systems that maintain homeostasis in the vasculature include: 1. vasoconstriction and/or vasodilation effects; 2. antiplatelets aggregation; 3. coagulation and 4. fibrinolysis. The EC plays a key role in the balance between the coagulation and fibrinolytic system.

2.1.4 The EC as a mediator of VSMC growth and inflammation

The EC also plays a key role in growth and differentiation of the VSMC through the release of either promoters of growth and/or inhibitors of growth, differentiation and on vascular remodeling. A large number of peptides have been proposed as the main messengers for growth signals [insulin-like growth factor 1 (IGF-1), PGF, basic fibroblast growth factor (BFGF), etc.] (Natarajan R et al., 1997).

The EC is also involved in the production of specific molecules that have a regulatory role in inflammation. The most important are leukocyte adhesion molecule, intracellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM).These molecules are denominated "**adhesion molecules**" and function to attract and "anchor" those cells involved in the inflammatory reaction. Very recently if has been demonstrated that the atherosclerotic process is associated with an increased blood level of inflammation markers (Bieglsen ES, 1999).

2.2 Endothelial cell dysfunction in diabetes mellitus

Several studies have demonstrated that large and resistance arteries are presence of endothelial dysfunction, which mostly characterized by the impaired of endothelium-dependent vasodilation a physiological marker of decreased bioavailability of NO (Cohen RA, 1995) and increased leukocyte adhesion to ECs (Yang X-D et al., 1996; Schroder S et al., 1991) in experimental models of diabetes and humans with Type 1 and Type 2 diabetes.

2.2.1 Impaired endothelium-dependent vasodilation in diabetes

Impaired endothelium-dependent vasodilation is a common feature of large and resistance arteries of experimental diabetic animals (Mayhan WG et al., 1989). This impairment arise from several mechanisms: decreased production one of EDRFs especially NO, enhanced inactivation of EDRFs and enhanced generation of endothelium-derived constricting factors (EDCFs). In alloxan diabetic rabbit, decreased endothelium-dependent vasodilation to acetylcholine (Ach) and adenosine diphosphate of the isolated abdominal aorta have been demonstrated after 6 weeks of diabetes (Tesfamariam B et al., 1989). Additional, Nitenberg A et al., (1993) demonstrated that the endothelium-dependent relaxation of

coronary arteries is also impaired in both Type 1 and type 2 diabetic patients.

2.2.2 Vascular endothelial cell interaction in diabetes mellitus

There is now a large body of evidence, which focus on role of leukocyte adhesion to vascular endothelium and their subsequent activation and migration into subendothelium in diabetes mellitus. *In vivo* leukocyte adhesion on the endothelium was increased in rabbits with alloxan diabetes (Hadcocks S et al., 1991) while capillary occlusions by monocytes and granulocytes presented destruction of capillary bed in diabetic retinopathy of rats (Schroder S et al., 1991). Moreover, indirect evidence from recent studies seems to indicate that a number of abnormalities in leukocyte-endothelium interaction of diabetes can be related to hyperglycemia. Leukocytes adhere to the endothelium initially via specific cell surface adhesion molecules (selectins) (Tanaka Y et al 1993) followed by further adhesion via integrin-cell adhesion molecules interactions (ICAM-1) and vascular cell adhesion molecule (VCAM). After a variable period of time, the leukocyte penetrated the basement membrane and migrated in to the surrounding tissue (Springer TA, 1990; Adams DH et al., 1994)

The sequence of events that allows the traveling of leukocytes to site host defense is designated the multistep paradigms of leukocyte recruitment. Important events of the transvascular movement of leukocytes are composed of: 1) margination and captering of free-flowing leukocytes, 2) leukocyte rolling, 3) activation and firm adhesion 4) transendothelial diapedesis and chemotactic migration of the leukocytes (Figure1.). Different mechanisms appear to mediate leukocyte rolling and adhesion, the leukocyte rolling is dependent on selectins expressed on endothelium (P-selectin) and leukocyte (L-selectins), where as the latter is

dependent on the intergrins (CD11/CD18) found on leukocytes and their ligands (ICAM-1, VCAM-1) on endothelial cell (Yang X-D et al., 1996).
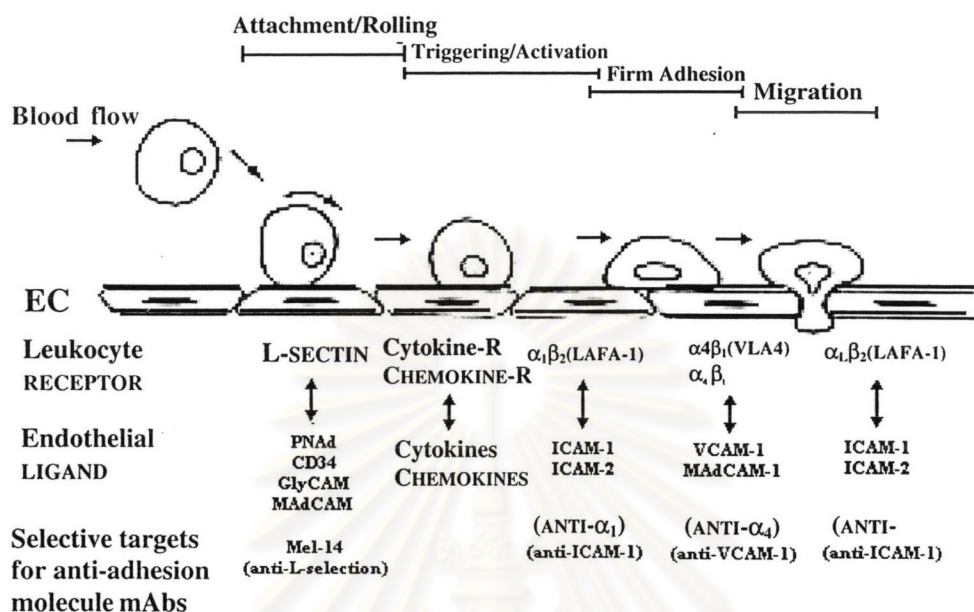

Figure1. Sequential multistep model of leukocyte/endothelial adhesion. extravasculation of leukocyte from blood into tissues mediated by cascade of adhesive interactions between leukocytes and endothelial cells.

III. Oxidative stress and antioxidant system

Free radicals are atoms or molecules that have one or more unpaired electrons in their atomic structure and highly reactive. Free radicals are constantly being generated in the body, as a result of the normal metabolic processes. Under physiological conditions, damage due to free radicals is countered by antioxidants. Oxidative damage can therefore be consequence of raised free radical production, insufficient antioxidant potential, or both (Baynes JW., 1991). Sometimes, excessive free radical formation occurs in the body, and the antioxidant system in the body cannot cope with situations, i.e. the pro-oxidants overwhelm the

antioxidants (Baynes JW., 1991). This situations known as oxidative stress. Thus, oxidative stress is general term used to describe a state of potential oxidative damage caused by free radicals. By far, the most common free radicals formed in the body are oxygen derived and are therefore also known as reactive oxygen species (ROS). These include superoxide radical (O_2), hydroxy radical (OH) and hydrogen peroxide (H_2O_2).
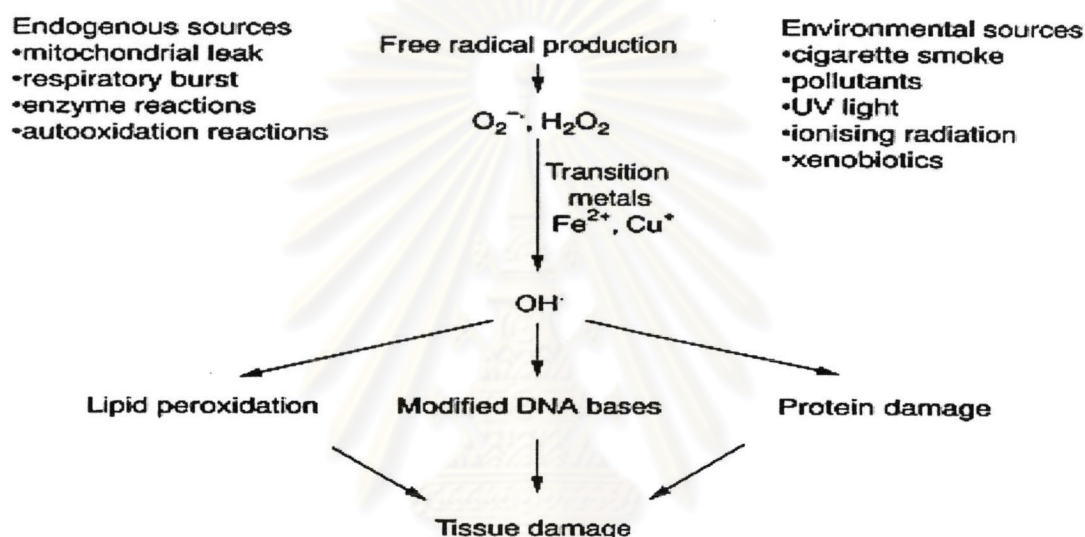

Figure 2. Major sources of free radicals in the body and the consequences of free radical damage. (Baynes JW, 1991)

Uncontrolled production of ROS often leads to damage of cellular macromolecules (DNA, lipids and protein) and other small antioxidant molecules. A number of major cellular antioxidant defense mechanisms exist to neutralize the damaging effects of free radicals.

Antioxidant system

Our body is equipped with a sophisticated antioxidant system to deal with the production of reaction oxygen species (ROS). The system includes enzymatic antioxidant and non-enzymatic antioxidant.

1. enzymatic antioxidant

Antioxidant enzymes or "scavenging enzymes" provide the first line of defense against ROS by converting them to more reduced and more stable lines. Such enzymes include the following :

-Superoxide dismutase (SOD), the enzyme that convert endogenous O_2^- into hydrogen peroxide, including Cu, Zn-SOD, Mn-SOD, and extracellular SOD (Kashiwagi A, 2001).

- Catalase (CAT), this enzyme reacts very efficiently with H_2O_2 to form H_2O and molecular oxygen and with H^+ donors with peroxidase activity (Young IS and Woodside JV, 2001) although catalase isn't essential for survival it plays and important role in the acquisition of tolerance to oxidadive stress (Hunt CR et al., 1998)

-Glutathione peroxidase (GPX), catalyze the oxidation of glutathione and the expense of H_2O_2 with catalase, it can also react effectively with lipid and other organic hydroxyperodeoxides. Thus, this enzymes play an important role and protecting cell against lipid peroxidation (Young IS and Woodside JV, 2001). Most GPX are localized mainly to the cytosol and mitochondria, suggesting that these enzymes are the main scavengers of H_2O_2 in these subcellular compartments.

2. non-enzymatic antioxidant

A second line of defense is provide by non-enzymatic or exogenous antioxidants primarily obtained as nutrients or nutritional supplements such as

-Vitamin E

Vitamin E belongs to a family of naturally occurring lipid-soluble compounds with different antioxidant properties of which 2-tocoperol is the most abundant in the human body. It is a physiological membrane-bound chain-breaking antioxidant that protects cell membrane lipid from oxidant damage by free radicals. This resonance-stabilized radical can be subsequently oxidized to from tocopherol quinone, or react with another 2-tocopheroxy radical to form stable dimers.

Alternatively, 2-tocopherol can be regenerated by reaction with other antioxidants, such as ascorbate or GSH (Young IS and Woodside JV, 2001)

Enzymatic system (Cu-, Zn- and Mn-superoxide dismutase (SOD), catalase, glutathione (GHS), glutathione peroxidase (GPX), and GSH reductase (GR)) function by direct on sequential removal of ROS.

Oxidative stress occurs when there is an imbalance between free radicals reactions and the scavenging capacity of antioxidant defense mechanism (Sies H, 1991).

Table 1. Micronutrients and endogenous antioxidants involved in free radical defense. (Jakus V et al., 2000)

Nutrients	Functional role
Carotenoids	Hydrophobic antioxidant
Vitamin E	Hydrophobic antioxidant
Niacin, tryptophan	Precursors to NADH/NADPH
Riboflavin	Cofactor for GR
α-lipoic acid	Cofactor for oxid. decarboxylation of pyruvate to acetyl-coenzyme
Selenium	Integral part of GPX
Zinc/copper	Integral part of SOD
Manganese	Integral part of SOD
Bioflavonoids	Hydrophobic antioxidant
Plant phenolics	Hydrophobic antioxidant
GSH	Endogenous hydrophilic antioxidant
Ubiquinol (coenzyme Q)	Endogenous hydrophobic antioxidant
NADH/NADPH	Endogenous hydrophilic antioxidant

3.1 Oxidative stress in diabetes mellitus

In diabetes, oxidative stress seems caused by both increased production of ROS, decreased production in antioxidant defense system. Although the source of oxidative stress remain unclear, it has been suggested that chronic hyperglycemia in diabetes enhances the ROS production from glucose autoxidation, protein glycoxidation (Baynes JW, 1991). Enhanced oxidative stresses in Type 2 diabetes are characterized by increasing in lipid peroxidation which can adequately reflect increased oxidative stress.

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Table 2. Possible sources of oxidative stress in diabetes mellitus (Jakus V et al., 2000)

Increased generation of ROS : autoxidation of carbohydrates, autoxidation of fatty acid in triglyceridesm phospholipids and cholesteryl esters : acute and chronic hyperglycemia : glycation, advanced glycation and glycoxidation
Decreased antioxidant defense : alterations in GSH concentration or metabolism : decreases in antioxidant system, e.g. catalase, SOD, GPX : alteration in vitamin E and ascorbate homeostasis : alteration in concentrations of other antioxidants, e.g. ubiquinol, carotene, taurine and uric acid
Alteration in enzymatic pathways : increased polyol pathway activity : decreased glyoxalase pathway activity : alteration in mitochondrial oxidative metabolism : altered prostaglandin and leukotriene metabolism
Other mechanisms : ischemia-reperfusion injury, hypoxia and pseudohypoxia

Among the mechanism proposed as mediators of the endothelial dysfunstion observed in diabetes, hyperglycemia plays a key pathogenic role in the development of diabetic vascular diseases. High blood glucose concentrations result in endothelial dysfunction that is associated with loss of endothelium derived NO, increased vascular permeability, increased endothelial adhesives, and thickening of the basement membrane of blood

vessels, and the increased generation of oxygen free radical (Jones AF et al., 1987).

Diabetes increases oxidative stress in tissue and plasma both human and experimental animals, increasing oxidative stress might play a role in the development of diabetic complication. Oxidative stress develops in the retina of diabetic animals and galactos-fed animals (Halliwell B, 1999; Gutteridge JM et al., 2000), indicating that oxidative stress is associated with the development of retinopathy.
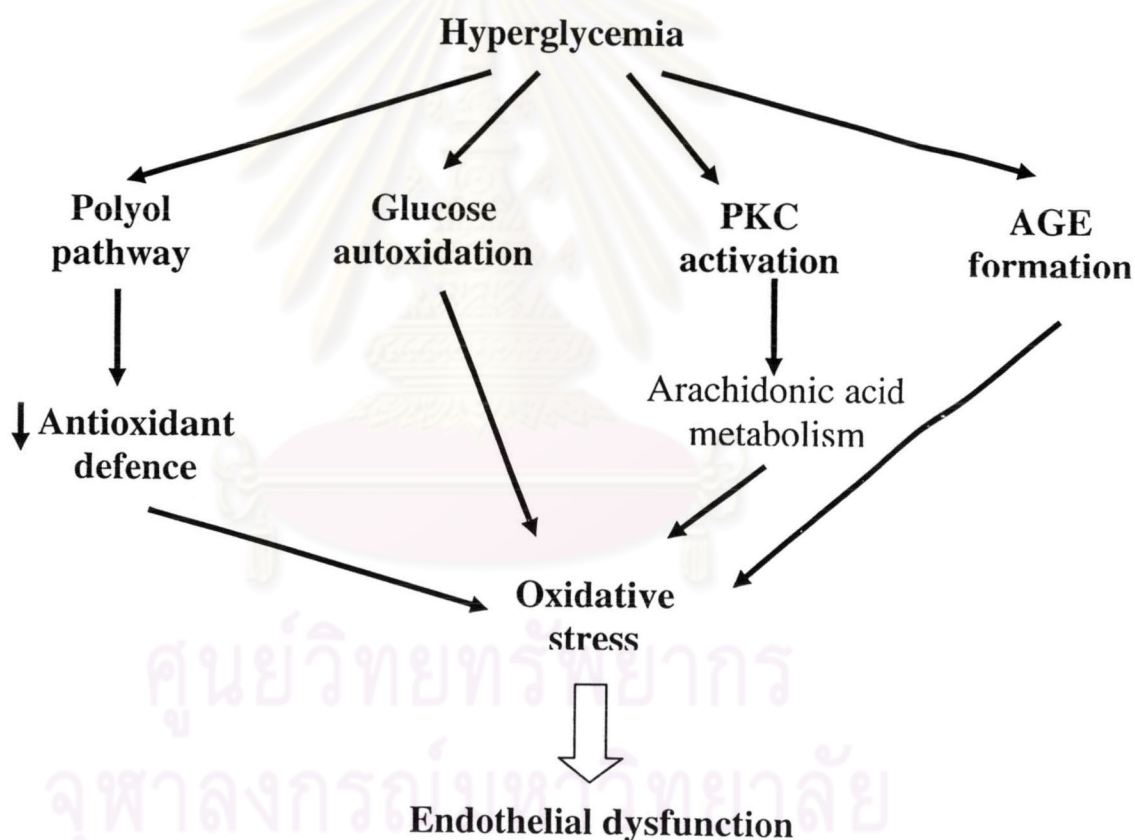
Figure 3. Mechanisms of increased oxidative stress in diabetes
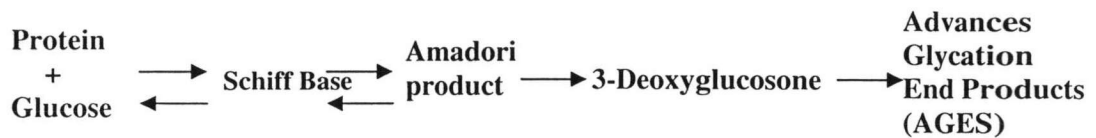
3.1.1 Advance glycation end products (AGEs)

Protein + Glucose ⇌ Schiff Base ⇌ Amadori product → 3-Deoxyglucosone → Advances Glycation End Products (AGES)

Figure 4. Nonenzymatic glycosylation of proteins. (Pickup J, 1997)

Advance glycation or glycosylation end products (AGEs) are the products of glycation and oxidation (glycoxidation), which are increased with age and at an accelerated rate in diabetes mellitus (Sell DR et al., 1992) under glycemia conditions intracellular concentration of glucose and its metabolites are elevated, which is followed by the glycation of non-enzymatic proteins, leading to the formation of glycation products.

The binding of glucose to proteins, a process referred to as glycosylation, leads to changes in the structure and function of many body proteins. In diabetes, excessive glycosylation also occurs with proteins of red blood cell, lens and myelin sheath. Excessive non-enzymatic glycosylation has many adverse effects such as inactivation of enzymes, inhibition of regulatory molecule binding, cross linking of glycosylated proteins, trapping of soluble proteins by glycosylated extracellular matrix, decreased susceptibility to proteolysis, abnormalities of nucleic acid function, altered macromolecular recognition, and increased immunogenicity (Elgawish A et al., 1996).

Non-enzymatic glycation of protein under hyperglycemic condition is accompanied by the production of active oxygen. Using the electron paramagnetic resonance method Mullarkey CJ et al (1990) demonstrated that non-enzymatic glycation of proteins hyperglycemic abundant conditions is accompanied by super oxide anion (O_2)

production. In vitro studies have suggested that glycation itself may result in production of superoxide (Jones et al., 1987).

It is now clear that AGEs may act as mediators, not only of diabetic complication but also of widespread age related pathology such as Alzheimer s disease (Munch G et al., 1998), decreased skin elasticity (Monnier VM, 1981), male erectile dysfunction (Seftel AD, 1997), pulmonary fibrosis (Matsuse T, 1998) and atherosclerosis (Stitt AW, 1997). Since many cells and tissues of the eye are profoundly influenced by both diabetes and aging, it is fitting that advanced glycation is now receiving considerable attention as a possible modulator in important visual disorders.

The important effects that advanced glycation has on ocular tissues and the role that AGEs, and their specific receptors, have in the initiation and progression of sight threatening disorders such as diabetic retinopathy, glaucoma, cataract formation, and age related macular degeneration (AMD) (Stitt AW, 2001).

3.1.2 The polyol pathway

Figure 5.   The polyol pathway (Barnett AH, 1991)

Hyperglycemia induces the polyol pathway, resulting in induction of aldose reductase (AR) and product of sorbitol. Elevated sorbitol levels are found in high concentrations in the tissues commonly involved in the major diabetic complication. This accumulation creates an osmotic gradient that draws into the cells to maintain osmotic balance. As a result, the delicate protein fiber within the lens become opaque and cataract forms (Barnett AH, 1991).

In this pathway, AR, utilizing reduced nicotinamide-adenine dinucleotide phosphate, reduces the aldehyde form of glucose to sorbitol. Sorbitol is metabolized to fructose by sorbitol dehydrogenase. Importance of the poyol pathway may vary among tissues. Induction of oxidative stress may occur through many different mechanisms, including depletion of nicotine-adenine dinucleotide phosphate (NADPH), which inturn is required for antioxidant activity of glutathione reductase and consequent disturbance of glutathione and nitric oxide metabolism. (Atalay M and Laaksonen DE, 2002)

Mean red cell GSH and NADPH level and $NADPH/NADP^+$ and GSH/GSSG ratios were decreased in 18 Type 2 Diabetic patients compared to 16 non-diabetic control subjects (De Mattia et al., 1994; Bravi et al., 1997). Similarly in a recent study aldose reductase inhibitor; sorbinil restored nerve concentrations of antioxidants reductase glutathione (GSH) and ascorbate, and normalized diabetes-induced lipid peroxidation in streptozotocin-diabetic rats (Obrosova IG et al., 2002).

3.1.3 Protein kinase C activation and active oxygen production

Protein kinase C (PKC) is a phospholipid dependent serine/threonine kinase. In diabetes mellitus, diacylglycerol (DAG) is synthesized de novo utilizing excess glucose taken up by cells, and activates PKC via the glycolysis system. It has been reported that PKC

activation is observed in many vascular tissues such as retina, heart, aorta and glomeruli which are isolate from diabetic animals. PKC activation is related to vasoconstriction, proliferation and overgrowth of smooth muscle cell as well as accelerated synthesis of extracellular matrix proteins and thus plays significant roles in the onset and progression of vascular cell dysfunction in diabetic mellitus (Koya D and King GL, 1998). Ishii H et al (1996) demonstrated that a PKC-β isoform-specific inhibitor (LY 333531) has been developed and its usefulness in inhibiting the onset and progression of diabetic complication.

It is also indicated that PKC is activated by generated active oxygen, and that the activated PKC induces the activation of phospholipase A2 (PLA2) resulting in enhanced prostaglandin metabolism, which is associated with increased production of active oxygen (Klann E et al., 1998).

3.1.4 Auto-oxidation of glucose

The term autoxidation describes the capability of glucose to enolized, thereby reducing molecular oxygen and yielding oxidizing intermediates (Hunt JV et al., 1998). The reduced oxygen products formed in the autoxidative reaction are superoxide anion (O_2^-), the hydroxyl radical ($^\cdot OH$), and hydrogen peroxide (H_2O_2). All can damage lipids, as well as proteins, through cross-linking and fragmentation (Giugliano D et al., 1996). Free radical also accelerate the formation of AGEs, which in turn supplies more free radicals; this process is termed autoxidative glycosylation, or glycoxidation (Baynes JW, 1991).

In a recent study, Nishikawa T et al., (2000) have been reported that the production of active oxygen is increased when the oxidative phosphorylation in mitochondria is enhanced. The mitochondria have been show to play an important role in active oxygen production particularly under hyperglycemic conditions. Hyperglycemia-induced

activation of PKC, AGE production, sorbitol accumulation and activation of NF-kB (nuclear factor-kB) have been reported to be reversed after inhibiting active oxygen production caused by mitochondria in aortic endothelial cells, suggesting that mitochondria plays an important role in production of active oxygen under high glucose conditions.

3.1.5 Vitamin C

Vitamin C, also known as ascorbic acid (AA) or ascorbate, is a potent water-soluble antioxidant that is essential for many enzymatic activities.
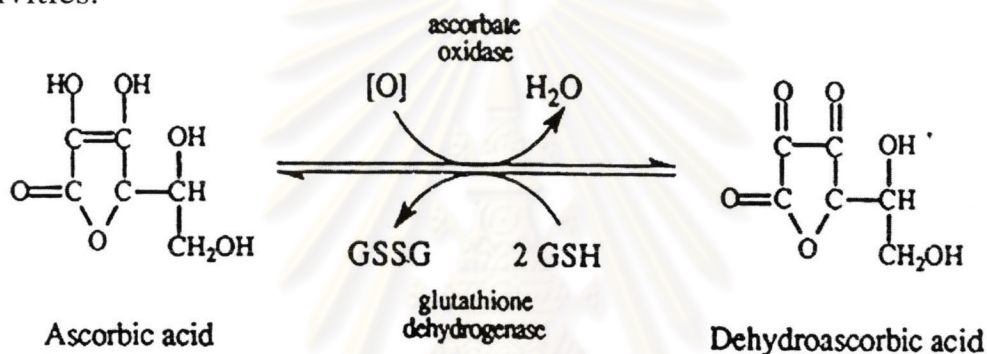

Figure 6. Interconvertibility of ascorbic acid by oxidation and reduction (Tapan KB, 1996)

Vitamin C comprised essentially two compounds, L-ascorbic acid (MW 176), a strong reducing agent, and it is oxidized derivative L-dehydroascorbic acid. Upon interaction with reaction oxygen species, Vitamin C is oxidized to dehydroascorbic via the intermediate ascorbyl free radical. Dehydroascorbate is recycle back to ascorbic acid by dehydroascorbic reductase, or glutathione, or glutaredoxin, or the NADPH –dependent selenoenzyme thioredoxin reductase (Carr AC and Frei B, 1999). Thus, dehydroascorbate occurs in very low level compared with Vitamin C (Stahl W and Sies H, 1997).

The abortion of vitamin C in humans occurs in the buccal mucosa, stomach and small intestine. After absorption, vitamin C rapidly equilibrates intracellular and extracellular compartment. Although no particular organ acts as a storage reservoir for the vitamin, tissue such as the pituitary, adrenal gland, eyes lens and leukocytes. Vitamin C exists in blood and tissue mainly in the reduced form; it's oxidized form is generally less than 10% (Tapan KB et al., 1996).

Vitamin C as antioxidant, two major properties of vitamin C make it an ideal antioxidant. First is the low one-electron reduction potentials of both ascorbate (282 mV) and it's one- electron oxidation product, the ascorbyl radical (-174 mV), which is derived from the one-diol functional group in the molecule (Halliwell B, 1996). These low reduction potentials enable ascorbate and the ascorbyl radical to react with the reduce basically all physiologically relevant radical and oxidants.

The second major property that make vitamin C such as effective antioxidant is the stability and low reactivity of the ascorbic radical formed when ascorbate scarvenges a reactive oxygen or nitrogen specie (Carr AC and Frei B, 1999).

OH•   HOH

RH ⟶ R• ⟶ RH
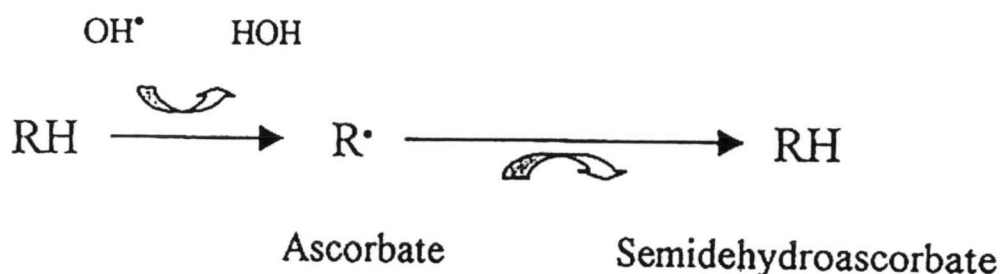
Ascorbate   Semidehydroascorbate

Figure 7. Possible use of ascorbate in reducing damage from
radicals (Brody T, 1994)

Vitamin C is a cofactor for serveral enzymes involved in the biosynthesis of collagen, carnitine, and neurotransmitters.

Procollagen-proline dioxygenase (proline hydroxylase) and procollagen-lysine 5-dioxygenase (lysine hydroxylase), 2 enzymes involved procollagen biosynthesis, require vitamin C for maximal activity posttranslational hydroxylasion of proline and lysine residues by these enzyme is essential for the formation and secretion of stable collagen helixes. A deficiency of vitamin C result in a weakening of caliginous structure, causing tooth loss, joint pains, bone and connective tissue disorder, and poor wound healing. Two deoxygenates involved in the biosynthesis of carnitine also require vitamin C as a cofactor for maximal activity (Carr AC and Frei B, 1999).

In addition, vitamin C is used as a cofactor for catecholamine biosynthesis, in particular the conversion of dopamine to norepinephrine catalyzed by dopamine B- mono-oxygenases involved in peptide amidation and tyrosine metabolism. Vitamin C has also been implicated in the metabolism of cholesterol to bile acids via the enzyme cholesterol 72 mono-oxygenase and in steroid metabolism in the adrenal.

Vitamin C and Diabetes mellitus

There is evidence to suggest that oxidative stress is increased in human with diabetes and in animal model of diabetes (Giugliano D, 1996). Moreover, evidence for oxidative stress in diabetes includes observations of decreased antioxidant plasma concentrations in both diabetes subjects and animal models of diabetes (Kashiba M et al., 2000)

Transport of vitamin C through biological membrane is facilitated by glucose transporters, especially GLUT1 (Mooradian AD, 1987) and hence, chronic hyperglycemia may impose an intracellular deficit of AA through competitive inhibition of membrane transport of AA by the elevated plasma glucose (Dai S, 1995). Though few studies have demonstrated improved blood glucose level upon supplementation of diabetes individuals with vitamin C, dose of between 100 and 600 mg of vitamin C daily have been found to normalize cellular sorbitol levels, which may have implications for decreasing some of the long-term complication of diabetes (Will JC et al., 1996).

Mechanism of vitamin C on endothelial dysfunction

1. Ascorbate and Low density lipoprotein (LDL) Oxidation

Modification of the protein moiety of LDL, either directly by leukocyte-derived oxidants (Carr AC et al., 2000) or indirectly by lipid hydroperoxide breakdown products and malondialdehyde (Esterbauer H et al., 1987), results in a form of LDL that is internalized by macrophages via the scavenger receptor pathway leading to foam cell information (Steinberg D, 1997). Furthermore, there is convincing evidence that in vitro lipid peroxidation LDL is initiated by α-topopheroxyl radicals formed in the lipoprotein on attack by free radicals or other reactive species (Neuzil J et al., 1997). Thus α-topopheroxyl can act as a pro-

oxidant, rather than an antioxidant, in LDL incubated in vivo (Neuzil J et al., 1997).

Experimental data on the effects of vitamin C supplementation of human subjects on ex vivo LDL oxidation are sparse, mainly because ascorbate is removed from LDL during isolation from plasma (Carr AC and Frei B, 1999). However, there is convincing evidence from in vitro studies that physiological concentrations of ascorbate strongly inhibit LDL oxidation by vascular cells and neutrophils (Martin A and Frei B, 1997) as well as in cell- free systems (Carr AC et al., 2000). Ascorbate prevents oxidative modification of LDL primarily by scavenging free radicals and other reactive species in the aqueous milieu. Thus, direct and rapid trapping of these aqueous reactive species by ascorbates prevents them from interacting with and oxidizing LDL.

Ascorbate can also prevents the pro-oxidant activity of α-topopherol by reducing the α-topopheroxyl radical to α-topopherol, thereby acting as a coantioxidant and inhibiting LDL oxidation (Neuzil J et al., 1997).

2. Vitamin C and Leukocyte-endothelial interaction

Cultured endothelial cells exposed to inflammatory cytokines or oxidized LDL exhibit enhanced expression of cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selection (Panes J et al., 1999). These adhesion molecules interact with specific ligands that expressed on the surface of leukocyte, such as the β_1 and β_2 intergrins, and mediate leukocyte rolling, firm attachment to the endothelium, and subsequent migration into the subendothelial space (Panes J et al., 1999).

Several in vivo studies using intravital microscopy in hamster have shown an important role of ascorbate in inhibiting leukocyte endothelial

cell interaction include by cigarette smoke (Lehr HA et al., 1994) and or oxidized LDL (Lehr HA et al., 1995). Lehr HA et al (1995) demonstrated that the induction of leukocyte adhesion to the vascular wall elicited by cigarette smoke is due to the formation of oxidatively modified lipids with platelet-activating factor-like activity. Administration of ascorbate prevented the accumulation of these platelet activating factor-like lipid and the subsequent leukocyte-endothelial cell interaction. Weber C et al (1996) and Adams MK et al (1997) have found that cigarette smokers have decreased plasma level of ascorbate, and monocytes isolated from smokers exhibit increased adhesion to cultured endothelial cells compared with monocyte isolated from nonsmoker. Supplementation of smokers with 2 g/day of vitamin C for 10 days elevated plasma ascorbate levels almost 2- fold and significantly reduced monocyte adhesion to cultured endothelial cell. This finding indicate of that vitamin C supplementation can increased plasma ascorbic acid and deceased monocyte adhesion to cultured endothelial cell.

3. Vitamin C and Endothelial Nitric Oxide

Endothelium-derived NO is a pivotal molecule in the regulation of vascular tone and homeostasis (Furehgott RF, 1996). In addition to stimulating vascular smooth muscle cell relaxation, endothelium-derived NO has been shown to inhibit including smooth muscle cell proliferation, platelet aggregation and leukocyte- endothelial cell interactions (Furehgott RF, 1996). Endothelial vasodilator dysfunction has been observed in patients with coronary artery disease or subjects with coronary risk factors (Keancy JF et al., 1995). Most of these conditions are associated with increased oxidative stress, particularly increased production of superoxide radicals, which can inactivate endothelium-derived NO (Gryglewski RJ et al., 1986). In addition oxidized LDL has been shown to inhibit the

synthesis of endothelium-derived NO or attenuate its biological activity (Chin JH et al., 1992).

There are a number of potential mechanisms underlying the salubrious effects of ascorbate on endothelial function. First, ascorbate may be decreasing the level of superoxide radicals and oxidized LDL (Jackson TS et al., 1998) both of which react with and inactivate NO (Gryglewski RJ et al., 1986; Chin JH et al., 1992).

IV. Diabetic eye complication

Diabetic eye disease refers to a group of sight threatening eye problems that people with diabetes may develop as a complication of disease that include diabetic retinopathy and non retinal ophthalmic abnormalities of diabetes

4.1 Normal eye works

Light first hits the cornea of the eye, which allows light to enter the eye through the iris. The iris controls the amount of light that enters the eye by changing the size of the pupil. As light passes through the pupil, it enters a clear lens, like the lens of a camera, which focuses the light onto the back of the eye. The focused light passes through a clear gel called "vitreous" until it reaches the back of the eye. The back of the eye is known as the retina. The retina changes light signals into electric signals, sent through the optic nerve to the brain, which translates these signals into images we see. The central part of the retina is known as the "macula" and is responsible for sharp, central vision. The rest of the retina, known as the periphery, is important for peripheral vision. Like other parts of the body, the retina needs blood to function adequately. Blood flows to the retina through small blood vessels. Picture showed eye anatomy below this paragraph.
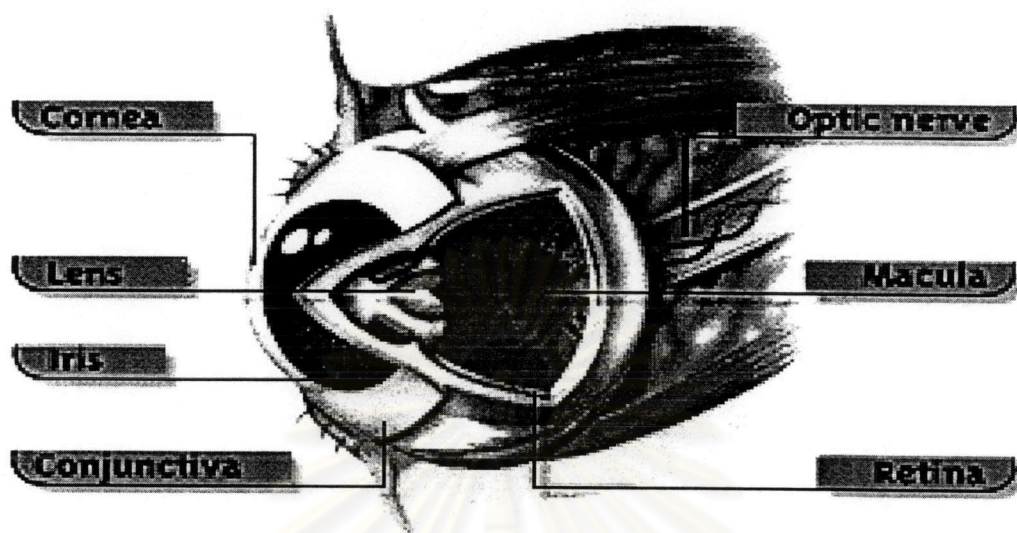
Figure 8. Demonstration of the normal eye anatomy which this picture taken from www.elvex.com/ facts11.htm

4.2 Diabetic eye diseases

4.2.1 Diabetic retinopathy

Diabetes is the leading cause of blindness in young patients. In the eye, diabetic retinopathy is a major threat to sight. Central visual loss of variable severity may result from involvement of macula while proliferative retinopathy and advanced diabetic eye disease can cause total blindness (Klein R et al., 1984). The pathologic features of diabetes arise through two basis and partly interrelated mechanisms thickening of basement membrane (Kinoshita JH, 1986; Engerman RL et al., 1993) and ischemia. Ashton N et al. (1963) demonstrated that obstruction of precapillary arterioles by pathologically thickened basement membrane resulted in capillary closure and the subsequent retinal ischemia that characterizes diabetic retinopathy.

Diabetic retinopathy can be classified into four groups:

a. Non-proliferative retinopathy

Retinopathy before the formation of new vessels is not associated with visual loss. Lesions include microaneurysms, hard exudates, intraretinal hemorrhages, cotton-wood spots (soft exudates).

b. Proliferative retinopathy

New-vessel formation (neovascularization) does not itself cause symptoms, but associated vitreous or preretinal hemorrhage may give rise to visual loss or floaters. Lesions include new vessels and fibrous proliferation on the retinal vessels and optic disc.

c. Advanced diabetic eye disease

Advanced diabetic eye disease is defined as that causing permanent or temporary loss of vision through vitreous or preretinal hemorrhage, retinal detachment, neovascular glaucoma or cataract.

d. Maculopathy

Retinopathy of any degree may involve the macula and cause central visual loss including loss of visual acuity. Lesion include hard exudates, microvascular abnormalities and retinal thickening.

4.2.2 Non retinal ophthalmic abnormalities of diabetes

a. Cornea

The corneal epithelial basement membrane is thickened in diabetics. This also is true of the basement membrane of other intraocular epithelial cells such as the nonpigmented ciliary body epithelium. The thickened basement membrane of the corneal epithelium is significant in that it predisposes these patients to recurrent corneal erosions and prolongs the healing of any epithelial defect. Azar DT et al., (1992) have produced evidence that the numbers of epithelial adhesion plaques are

reduced in the corneal epithelial cells of diabetic patients (Wilson DJ and Green WR, 1997).

b. Iris neovascularization

Iris neovascularization is commonly present in patients with diabetic retinopathy. This neovascularization typically takes the form of nonprogressive neocascularization of the pupillary border with mild ectropion uvea. In some cases, and particularly after vitrectomy iris neovascularization may be more severe. In these case, peripheral anterior synechia formation with subsequent neovascular glaucoma may develop.

The histologic features of iris neovascularization are the presence of fine vessels on the anterior surface of the iris and ectropion uvea. With time, the iris surface vessels may become covered with a basement membrane structure resembling Descement's membrane. This membrane can extend across the trabecular meshwork creating a "pseudoangle."

In patients with chronic hyperglycemia, the iris pigment epithelium becomes laden with glycogen. This results in the histologic appearance of lacy vascuolization (Wilson DJ and Green WR, 1997).

4.3 Effect of Diabetes on Ocular Blood Flow

The human eye is supplied by the separate vascular systems: the retinal blood vessels and the uveal blood vessels. The uveal vessels include the vascular beds of the iris, the ciliary body, and the choroid. The inner layers of the retina are nourished by the retinal vessels, whereas the outer retinal layers including the photoreceptors are nourished by the choroid (Alum A, 1992). In monkeys, 65% of oxygen consumed by the retina is delivered by the choroid (Alum A et al., 1973). There are considerable differences between the fine structure of the retinal and the choroidal vasculature.

With in the basement membrane there are a large number of intramural pericytes. Pericytes as well as endothelial cells are assumed to have an important role in the control of retinal blood flow. The observation that retina perfusion abnormalities are detectable in diabetic patients with no clinical sign of retinopathy (Arend O et al., 1991; Grunwald JE et al., 1996)

The exact nature of ocular blood flow abnormalities in the different stages of diabetic retinopathy is still a matter of controversy. Several haemodynamic abnormalities have been demonstrated early in the course of IDDM including increased basal blood flow in various organs, such as the kidney, retina. Several studies have focused on flow velocities in perimacular capillaries using a scanning laser opthalmoscope. Generally, a reduction in flow velocities was observed in diabetic patients (Wolf S et al., 1991; Arend O et al., 1991). This reduced retinal capillary flow velocity is possibly associated with impaired rheological properties of blood (Chung TW et al., 1993). Several reports indicate that blood rheological factors are impaired in patients with diabetes. Increased plasma viscosity or increased erythrocyte rigidity has been observed in Type 1 is as well in Type 2 diabetes (Chung TW et al., 1993; McMillan DE, 1976). These factors have been hypothesized to contribute to altered retinal blood flow in patients with diabetes (Chung TW et al., 1993). In STZ–induced diabetic rats haematocrit in arterial blood samples was no different from normal but regional haematocrit was altered in ocular tissues. Based on measurements of mean retinal circulation from fluorescein angiograms Cunha Vaz JG, (1978) reported increased retinal blood flow in early stages of retinopathy and decreased retinal blood flow in proliferative retinopathy. Change in retinal blood flow and its regulation have been demonstrated in diabetes (Grunwald JE et al., 1986; Grunwald JE et al., 1996) and the measurement of these retinal blood flow

change can provide a sensitive assessment of physiological change that reflect vascular cell metabolic dysfunction and subsequent development of microvascular abnormalities. Results from clinical studies have shown that retinal blood flow abnormalities are present before the development of clinical diabetic retinopathy (Bursell SE et al., 1996; Kawagishi T et al., 1995) and that the development and progression of diabetic retinopathy is reflected by these retinal blood flow changes (Clermont AC et al., 1997).

A major complication of diabetes mellitus is diabetic retinopathy. Clinical, preproliferative diabetic retinopathy is characterized by tortuousity of vessels, micro- and macro-aneurysms, and vascular nonperfusion, which results in areas of ischemic retina (Yanoff M et al., 1969). One process that could contribute to the vaso-occlusive processes is adhesion of leukocytes to vascular endothelial cells. In an experimental rat model of diabetes, Schroder S et al. (1991) observed increased adherence and diapedesis of moncytes and polymorphonuclear leukocytes (PMNs). Increased numbers of PMNs are present in the activated state in subjects with diabetes and the PMN have been shown to be more rigid in diabetic cats and humans than in nondiabetic subjects (Kantar A et al., 1991). Therefore, once adherent, PMNs could potentially obstruct narrow capillary lumens.

In regard to the literature view from above, it might be said that vitamin C, as an antioxidant, is likely to ameliorate diabetic-induced endothelial dysfunction. As which it might help to prevent the leukocyte-endothelial cell interaction that mostly enhanced the vaso-occlusion and consequently brought about hypoxic condition in diabetic eye. Together, with the idea of vitamin C could reduce the abnormality of diabetic-induced endothelial-dependent vasomotion, Therefore, this present study

is desired to evaluate the possible effects of vitamin C supplementation on diabetic iridopathy characterization by decreased iris blood-flow perfusion and increase number of leukocyte-endothelial cell interaction.