CHAPTER II
REVIEW LITERATURE

I. DEFINITION OF DIABETES MELLITUS

Diabetes mellitus is a disorder of carbohydrate, fat, protein metabolism which best characterized as a state of chronic hyperglycemia. Diabetes occur when the blood glucose is too high as a result of a deficiency of available, effective insulin. This insulin deficiency can be absolute when the pancreas does not produce enough insulin or reduction in the biological effectiveness of insulin (Natirass and Santiago, 1984).

As a result of this dificiency, the cells lack of fuel, and the body suffers from a lack of energy. People with diabetes complain of weakness and tiredness. When the cells are stared of their fuel, the body recognized that not enough food has been eaten and triggered a. sense of extreme hunger, called polyphagia. The glucose level in the blood rises because it is not used. At sometimes, out of desperation, the body turns to stored fuel-glycogen, fat and protein to try to meet its energy needs. The level of glucose in the blood continues to rise, as does the blood level of fats (Natirass and Santiago, 1984). The large excess of unused glucose circulates through the kidney, which normally rescues useful glucose from the filtered fluid to keep it from being lost in the urine. There is a level of blood glucose, however known as the renal threshold, above which the kidneys can not keep up with the job of retrieving glucose, and it escapes into the urine. As the blood glucose rises, excess glucose appears in the urine. The body knows when the urine is to loaded with glucose and is too

concentrated and tries to dilute it by allowing more and more fluid to flow through the kidneys. Hence, the person with high blood glucose levels and glycosuria experiences frequent urination of large amounts of fluid, known as polyuria (Beason, 1989). With the fluid loss, the body senses of dehydration, and the thirst center is triggered, making the individual drink more fluid. This vicious cycle of glucose and water loss, and the attempts to correct this loss, lead to the classic symptoms of diabetes. All of these are due to the body's inability to use glucose properly as the body fuel (Beason, 1989).

II. INSULIN-DEPENDENT DIABETES MELLITUS (IDDM)

The definition feature of IDDM is insulin deficiency. The most common cause of IDDM is the autoimmune destruction of β-cells, which presents most commonly in childhood and adolescence, however, it can be recognized and may become symptomatic for the first time at any age (Atkinson MA et al., 1994). Insulin dependence implies that the administration of insulin is essential to prevent spontaneous ketosis, coma, and death(Srikanta S et al., 1983). IDDM is also the result from interaction between environtmental factors and an inherited predisposition to the disease. The most important of inheritance of susceptibility to IDDM appears to reside in the HLA major histocompatibility complex (Atkinson MA et al., 1994). The environtmental factors that might lead to IDDM, including viral infections, mycobacterial infection, and chemical toxin in foods(Atkinson MA et al., 1994). Nevertheless, IDDM appears to be heterogeneous in terms of the genetic, environtmental, and autoimmune factors that precipitate the disease.

1. THE COMPLICATION OF IDDM

The pathophysiological changes precipitated by hyperglycemia dyslipidemia, and free radical generation are tissue damages which lead to chronic diabetic complication (Figure 1). Microangiopathy is a major complication of both IDDM and NIDDM patients. In addition, IDDM patients have an increasing risk of developing microangiopathy as time progresses (Atkinson MA et al., 1994).

Pathophysiological changes of IDDM

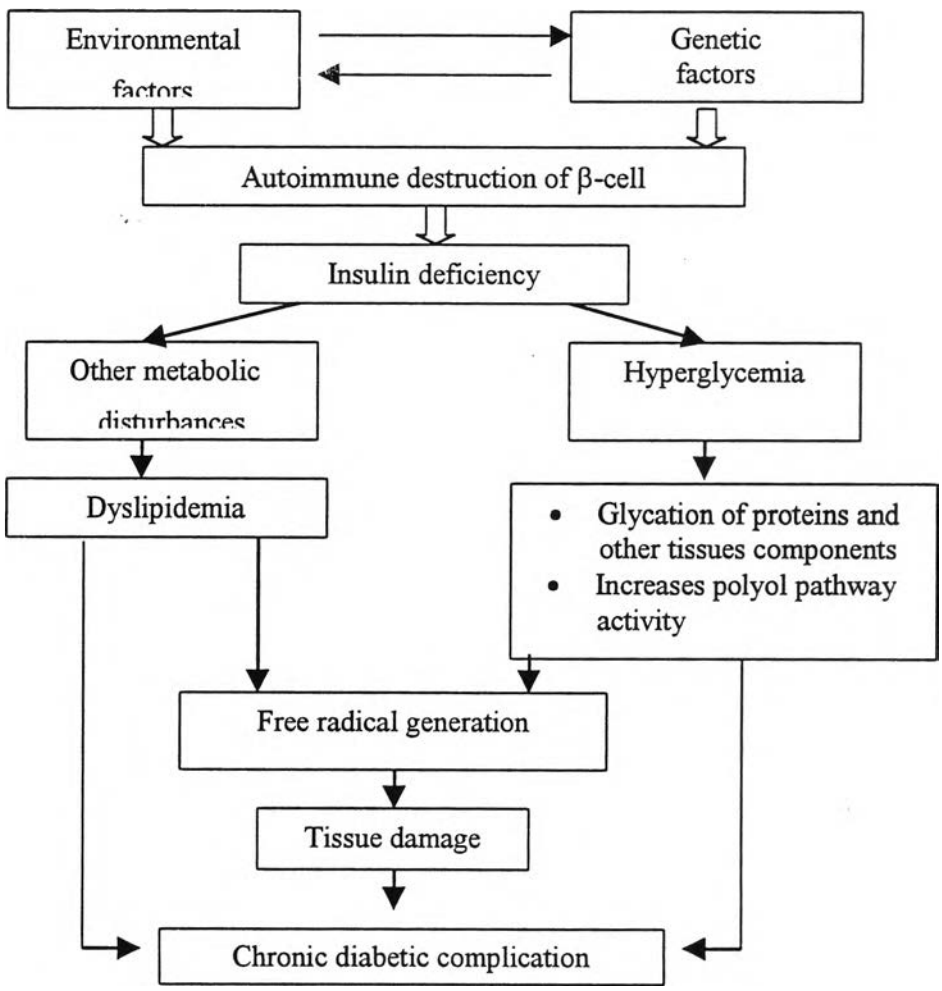

Figure 1. Process of pathophysiological changes of IDDM (Text book of Diabetes V1; 1997)

2. VASCULAR COMPLICATION AND INSULIN DEPENDENT DIABETES MELLITUS (IDDM)

Endothelial dysfunction plays a crucial role in the pathogenesis of diabetic vascular disease. The mechanisms underlying the development of microvascular and macrovascular angiopathy in diabetes mellitus are complex and incompletely understood. Diabetic microangiopathy is characterized both morphological and functional alterations of microvessels (Barnell AH, 1991). Morphologically, diabetes produces thickening of vascular basement membrane, enhanced endothelial adhesion of leukocytes, platelets, and increased endothelial cell surface area(Had cock S et al., 1991; Malik RA et al., 1993)

2.1 ALTERATIONS IN THE VASCULAR WALL-BASEMENT MEMBRANE

Thickness of the capillary basement membrane is widely recognized as the ultrastructural hallmark of microvascular damage in diabetes mellitus (Schneider SK et al., 1988). This manefestation of diabetic microangiopathy appears to be due, at least in part, to substantial alterations in the production and distribution of its functional components. For example, collagen type IV levels are increased by approximately 1.5 to 3-fold in glomerular basement membranes of diabetic humans and animal (Boyd RB et al., 1990).

Basement membrane is composed of collagen-like glycoprotein material, which is especially rich in the amino acid; glycine, hydroxyproline, and hydroxylysine. The sugar composition of basement membrane are

devided in two distinct types of carbohydrate units (Spiro RG, 1976). The first type is complex asparagine-liked heteropolysaccharide that contain galactose, mannose, sucrose. The second type is comprised of a glucose-galactose dissaccharide unit attached to hydroxylysine (Spiro GR, 1976). The thickening of basement membrane is resulted from an increase in the proportion of hydroxylysine and its glycosidically linked dissacharide units in diabetic basement membranes and related to an increase in the activity of hydroxylysine (Spiro GR, 1973). Basement membranes of diabetes also contain large amounts of albumin, fibronection, IgG. Excessive nonenzymatic glycosylation of matrix components is also a prominent feature of the diabetic basement membrane (Boyd RB et al., 1990). To support this hypothesis Cagliero E et al., (1988) suggested that DNA glycation is thought to result in increased synthesis of mRNAs for specific extracellular matrix components, thereby promoting increased thickness of capillary basement membranes. Additional, excessive glycosylation of basement membrane components may increase the resistance of collagen to degradation by collagenase, result in reduced cross-linking, alter the binding of fibronectin to basement membrane components (Schneider SK, 1995; Lein YH 1984). Moreover, it has been suggested that diabetes induces proliferative responses in endothelial cells that contribute to basement membrane thickening (Porta M et al., 1987). As a result of increased cell turnover, basal laminae accumulate and thicken in diabetes. Although the mechanism involved in increase turnover of endothelial cells in diabetes is unclear, it has been suggested that endothelial cell injury and/or elevated capillary pressure or shear rate may contribute to this proliferative response (Tooke JE, 1989; Zatz R et al., 1986). Therefore, from these observations suggest that while hyperglycemia induces a number

of important biochemical changes which ultimately result in basement membrane thickening, the extent and nature of these changes may be modified by hemodynamics changes.

2.2 ALTERATIONS IN THE VASCULAR WALL-ENDOTHELIUM

MORPHOLOGICAL CHANGES OF THE ENDOTHELIUM IN IDDM

The endothelial cell forms the lining of blood vessels wall sepalating the lumen from the vascular smooth muscle. Morphological investigations of the vascular endothelium in IDDM have largly been confined to the microcirculation.

Changes in morphology of the vasculature in diabetes have been characterized in both human and animal models. In alloxan-diabetic rabbits, the alterations of endothelial cells, including adhesion of leukocytes and platelets and fibrin deposition occur as early as 2 weeks after the onset of diabetes(Hadcock S et al., 1991). Moreover, Moore SA and Colleague (1985) found the widespread endothelial cell necrosis and abnormal morphology of the vascular smooth muscle in cerebral arterioles of STZ-induced diabetic rats. Furthermore, diabetic neuropathy has also been shown increase numbers of endothelial nuclei (Malik RA et al., 1993), endothelial cell area (Malik RA, 1989) and increase capillary permeability (Lin SJ, 1993).

III. ENDOTHELIUM-DEPENDENT VASCULAR FUNCTION.

1. ENDOTHELIUM-DERIVED RELAXING FACTOR (NITRIC OXIDE) SYNTHESIS

Endothelium plays an important role in the regulation of vascular tone, including cerebral vascular through the production and release of endothelium-derived relaxing factor (EDRF) (Furchgott RE et al., 1980). Endothelium produces and releases EDRF under basal conditions and in response to a number of receptor-mediated agonists, such as acetylcholine. (Moncada S et al., 1991).

Nitric oxide (NO) was found to have major similarities to EDRF. NO is synthesized from the amino acid L-arginine by an enzyme, the NO-synthase (NOS) which has been localized in the endothelium but not the vascular smooth muscle. After release by endothelium, NO acts on vascular smooth muscle by stimulating a cytosolic enzyme, soluble guanylate cyclase. Activation of this enzyme accelerates the formation of a cyclic necleotide, cyclic 3′, 5′ guanosine-monophosphate (cGMP) which causes relaxation (Figure 2) (Moncada S et al., 1991).

The endothelial cell secretes NO not only towards the underlying vascular smooth muscle but also into the blood vessel lumen. Under physiological conditions, the presence of oxyhemoglobin in the erythrocytes immediately neutralizes the NO which only has a physiological role at the interface between the endothelial cells and the blood content (Palmer RMJ et al., 1987). In particular, NO inhibits the adhesion of platelets and

leukocytes to the endothelium. The production of NO largely explains endothelium-dependent relaxations in many isolated arteries. It's contribution to vasodilation in vivo has been demonstrated, using inhibitors of NO synthesis, not only in the animals but also in the human (Moncada S et al., 1991).

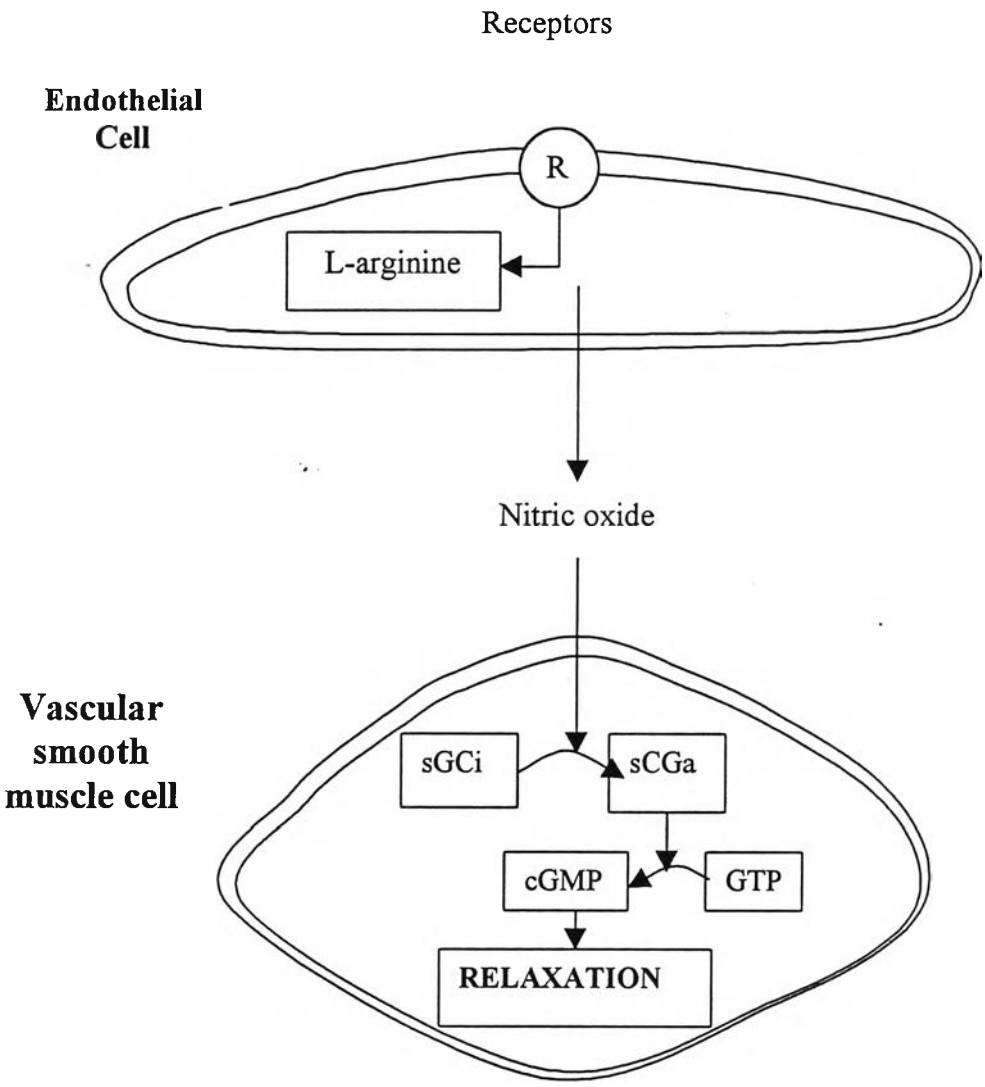

Figure 2. Production and mechanism of action of nitric oxide (NO) released by endothelial cells. Endothelial receptor (R) stimulation activated NO synthesis from L-arginine.

2. OTHER RELAXING FACTORS

In addition to EDRF (NO), endothelial cells can produce other relaxing factors, including prostacyclin,endothelium-dependent hyperpolarizing factor (EDHF). Prostacyclin can be considered as an endothelium-derived relaxing substance given its vasodilator activity and its primarily endothelial origin. Prostacyclin causes relaxation of vascular smooth muscle by activating adenylase cyclase and increasing the production of another cyclic nucleotide, cyclic 3′, 5′-adenosine monophosphate (cAMP). The relaxant effect of prostacyclin is essentially additive to that of NO (Luscher TF et al., 1987).

3. ENDOTHELIAL DYSFUNCTION IN DIABETES MELLITUS

Several studies have demonstrated that the large and resistance arteries of diabetic animals are presence of endothelial dysfunction, which mostly characterized by the impairement of endothelium-dependent vasodilation (Oyama Y et al., 1986; Mayhan WG et al., 1991; Miyama N et al., 1992; Tesfamarium B et al., 1989) and increased leukocyte adhesion to endothelial cells (Yang X-D et al., 1996; Schroden SW et al., 1991)

Impairement of endothelium-dependent vasodilation is a common feature in both large (Oyama Y et al., 1986) and resistance arteries (Mayhan WG et al., 1991) of chemically induced experimental diabetic animals. In two genetic model of IDDM, similar impaired relaxation has been demonstrated in aorta; and mesenteric arteries of diabetes-prone BB/W and

BB/E rats (Miyama N et al., 1992). In alloxan diabetic rabbit, decreased endothelium-dependent vasodilation to acetylcholine and adenosine diphosphate of the isolated abdominal aorta have been demonstrated after 6 weeks of diabetes in these animals (Tesfamariam B et al., 1989).

Furthermore, several studies that have shown impaired endothelium-dependent vasodilation whereas normal dilation responses to nitrovasodilator. This dilator dilate vascular smooth muscle by activating guanylate cyclase but, unlike NO, do not require the presence of the endothelium. Thus, the intrinsic property to activate vascular smooth muscle guanylate cyclase appears not to be altered by experimental diabetes. Impaired endothelium-dependent vasodilation of arteries has been demonstrated in IDDM patients. There is evidence that the basal synthesis of NO may be abnormal in the diabetic forearm. In the human forearm, local infusion of L-NMMA causes a near doubling of arteriolar resistance. Calver A et al., (1992) have demonstrated that there is an abnormality of basal-nitric oxide-mediated dilation in the forearm arterial bed of patients with IDDM patients. In summary, collectively, from the above reviewed suggest that impaired endothelium-vasodilation were occurred in both diabetic human and experimental diabetic animals.

3.1. MECHANISMS OF ENDOTHELIAL DYSFUNCTION IN DIABETES MELLITUS.

The endothelial dysfunction in diabetes mellitus can be explainned by: 1) a decrease in NO release/synthesis 2) increase inactivation of NO by several mechanisms such as increased levels of oxygen-derived free radical and increased advanced glycosylation end products (AGEs) 3) the release of an endothelium-derived constructing factor arising from cyclooxygenase pathway (Cohen RA, 1993; Guido RY et al., 1997; Pieper GM, 1997).

3.1.1. ROLE OF OXIDATIVE STRESS AND ENDOTHELIAL DYSFUNCTION IN DIABETES MELLITUS

As we have reviewed earlier that the vascular endothelial function is impaired in diabetic blood vessels. Although, the mechanisms to contribute diabetic vascular dysfunction are not clearly understood. However, evidence has accumulated indicating that the generation of oxygen-derived free radical (oxidative stress) may play an important role in the pathology of diabetic vascular complication (Baynes JW, 1992). Tesfamariam and Cohen (1992) have shown that oxidative stress induced by hyperglycemia is implicated as a source of altered endothelium-vasodilation in diabetes. Furthermore, other have reported that the transient endothelium-dependent vasodilation in the aorta of streptozotocin-induced diabetic rats was due to accumulation of superoxide anion(O_2^-) (Hattori Y, 1991) which comfirms previous report that oxygen-derived free radical inactivate endothelium-

derived relaxing factors (Gryglewski RJ, 1986) and selectively attenuate endothelium-dependent vasodilation (Pieper GM, 1988).

There are many biochemical ways by which hyperglycemia may increase the generation of oxygen-derived free radicals, including glucose autoxidation, polyol pathway, prostanoid synthesis, and protein glycation (Giugliano D, 1995). Moreover, disturbances of antioxidant defence systems in diabetes are shown including alteration in antioxidant enzymes (Strain JJ, 1991), impaired glutathione metabolism (Mclennan SV, 1991), and decreased ascorbic acid levels (Young IS et al., 1992).

3.2 FREE RADICAL PRODUCTIONS IN DIABETES MELLITUS

A. POLYOL PATHWAY MECHANISM

Aldose reductase (AR) and sorbitol dehydrogenase are the enzymes that constitute the polyol pathway. 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. Hyperglycemia stimulates this polyol pathway, leading to an increase in sorbitol flux and fructose synthesis through enhanced aldose reductase activity (Kashiwagi A et al., 1994). In addition, it has been proposed that polyol pathway activity may increase oxidative stress on the cell by virture of the fact that aldose reductase activity requires and may deplete cellular nicotine-adenine dinucleotide phosphate (NADPH), which inturn is required for the antioxidant activity of glutathione reductase (Gonzales AM et al., 1986; Gonzales RG et al., 1984). A shortfall incellular

NADPH could also be deleterious to endothelial cell function as it is an absolute requirement for the cell glutathione redox cycle, an important pathway for cytoprotection against free radical damage (Brolin SE et al, 1988). The depletion of NADPH cell stores by AR may inhibit the activity of other NADPH–requiring enzymes. This possibility is supported by the finding that human umbilical vein endothelial cell exposed to 33 mmol/l glucose show a 42% decrease in NADPH concentration and a 34% reduction of glutathione release into medium (Kashiwagi A et al., 1994). Furthermore, Cameron NE and Cotter MA (1992) had demonstrated that aldose reductase inhibitors (ARIs) can prevent the development of impaired NO-mediated endothelium-dependent relaxation in large vessels from diabetic rats. Therefore, the conversion of glucose to sorbitol in polyol pathway could affect vascular function is given in Figure 3. The cofactor for aldose reductase, NADPH, is diminished by elevated polyol pathway flux, thus imparing the GSH redox cycle, which is an important cellular-protection mechanism against oxygen free radicals. The latter are markedly increased in diabetes, and if not scavenged, they will damage vascular endothelium and neutralize NO, there by reducing vascular relaxation.

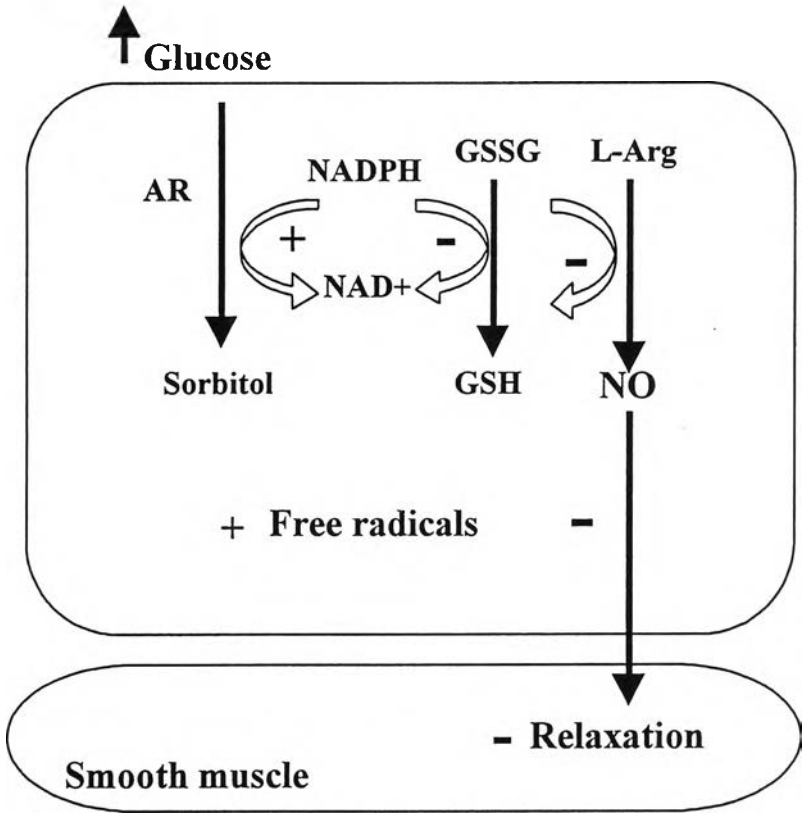

Figure 3. The relation between the metabolism of glucose by the polyol pathway with nitric oxide (NO). Use of NADPH requiring enzymes such as glutathione reductase and nitric oxide synthase. Free radicals could arise in endothelial cells exposed to elevate glucose by diminished reduced glutathione (GSH) or NO levels.

B. NON ENZYMATIC GLYCOSYLATION MECHANISM

Nonenzymatic glycosylation (glycation) is a modification of macromolecules (proteins and DNA), occurring initially with the formation of a Schiff base intermediate between sugars in the open chain form and amine groups on protein or DNA. The early glycated molecules (Amadori

product) subsequently degrade into α-ketoaldehyde compounds such as 3-deoxyglucosone. The secondary compounds are more reactive than the parent monosaccharide and can react with proteins or DNA to form covalent adducts and cross-links called advanced glycation end products (AGEs). AGEs are closely related to products of oxidative protein modification because of their potency of autoxidation (Hunt et al 1990, Mullarkey et al., 1990). The autoxidation of glucose, catalyzed by trace amounts of free transition metal ions such as irons or copper is a potential source of reactive oxygen species in diabetes. AGEs formation also produces free radicals (Baynes JW.,1991), and some of these reactions are catalyzed by transition metals. AGEs are known to induce variable endothelial cell responses. For example, it has been reported that AGEs induce increase endothelial cell permeability and modification of surface cell coagulation properties (Esposio et al., 1989), transendothelial monocyte chemotaxis (Kirstein et al., 1990), defective endothelium dependent relaxing by quenching nitric oxide (Bucala et al., 1991). In addition, Takata and co-worker (1988) have reported that transendothelial monocyte chemotaxis might be induced when AGEs are recognized by their receptors on the cell surface of monocyte/macrophages. Additional, in blood-vessel walls, AGEs crosslinking can cause damage in several ways. For example, in vitro study, human LDL binds covalently to collagen modified by advanced glycation, in direct proportion to the AGEs content (Brownlee M et al., 1985). This indicate that LDL binds specifically to AGEs, and suggests that excessive crosslinking by hyperglycemia-induced AGEs may accelerate atherosclerosis in diabetes mellitus (Bucala R et al.,1993)

AGEs on matrix proteins can also crosslink adjacent matrix components. Crosslinking by AGEs has detrimental effects on other important properties of matrix proteins, leading to disorder in the interaction between type IV collagen, laminin, heparan sulphate, proteoglycan and entactin (Tarsio JF et al., 1987). In chronic diabetes, the basement membrane content of anionic proteoglycan is markedly decreased in several tissues including the renal glomerulus (Tsilibary EC et al., 1988). There is evidence that this change may resulted in an increase in basement membrane synthesis lead to increase basement membrane thickening.

Furthermore, specific AGEs receptors are found on macrophages, endothelial cells and glomerular mesanglial cells. The receptor is composed of two subunits, and postreceptor signalling may involved generation of oxygen-derived free radicals (Yam SD et al., 1994) which inturn activate NF-κB, a transcription factor that regulates many genes including tissue factor and endothelin-1. AGEs binding to endothelial cells results in increase oxidative stress that can be blocked by antibodies directed against either of the AGE-receptor components or against AGE themselves (Yan SD et al., 1994).

C. VASOCONSTRICTOR PROSTANOIDS MECHANISM

Numerous studies have been demonstrated the effect of diabetes mellitus on abnormal prostaglandin metabolism in various vessels. In the vascular wall, prostacyclin (PGI_2) produced mainly by endothelial cells acts both as a potent vasodilator and as an inhibitor of platelet adhesion and aggregation (Moncada et al., 1996). On the other hand, thromboxane A_2

(TXA_2) released from activated-platelets is a potent vasoconstrictor and also a stimulator of platelet aggregation. Homeostasis of the vasculature is maintained by the balance of these vasoactive prostaglandins (Moncada S, 1982). The imbalance of PGI_2 and TXA_2 production may also cause diabetic microangiopathy. Furthermore, as described the above, hyperglycemia may lead to a deficiency in the glutathione redox cycle which plays an important role in the scavenging of superoxide(O_2^-) and hydrogen peroxide (H_2O_2). In vitro study, impaired endothelium-dependent vasodilation to acetylcholine had been shown in aorta from diabetic rabbits (Tesfamariam B et al., 1989). This impairment was restored by treatment with various cyclooxygenase inhibitors, indicating that contractile prostanoids could counteract nitric oxide-mediated vasodilation (Tesfamariam B et al., 1989). In addition, PGH_2/TXA_2-receptor blockade also prevents the abnormal endothelium-dependent vasodilation of diabetic aorta and aorta exposed to elevated glucose, suggesting that PGH_2, the precursor of prostaglandin or other prostaglandins could account for the impairment. Moreover, Tesfamariam B and Cohen RA (1992) demonstrated that the antioxidant, superoxide dismutase has effect to inhibit vasoconstriction of the rabbit aorta caused by exogenous PGH_2 (Figure 4.). From the above review, indicate that hyperglycemia induces the increased oxygen-derived free radicals through the generation of PGH_2.

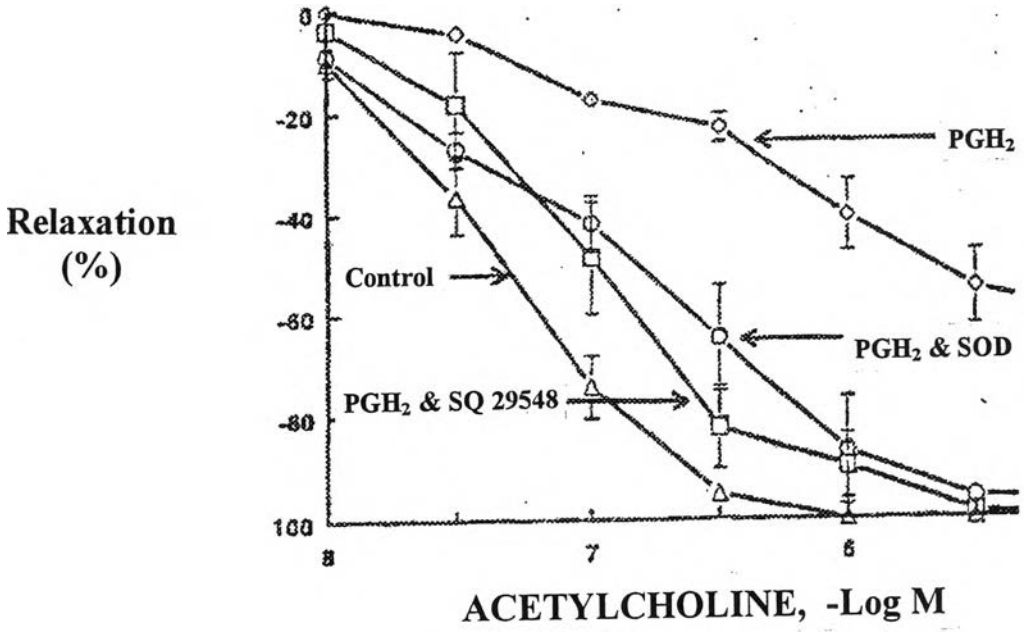

Figure 4. Plot of effect of superoxide dismutase (SOD) and SQ29548 on the inhibitory action of prostaglandin H_2 (PGH_2) on acetylcholine-induced relaxation. The relaxation to acetylcholine was inhibited by preteating with PGH_2. This inhibitory effect of PGH_2 was prevented by either SOD or SQ29548, suggesting an interactiion of PGH with superoxide anion (Circulation 1993;87(suppl V):V67-V76).

3.1.3 DYSLIPIDEMIA IN DIABETES MELLITUS

Although hyperglycemia obviously presents as the primary mediator of diabetic endothelial dysfunction, the similarity between the abnormalities of endothelium-dependent relaxation in diabetes and hypercholesterolemia might suggest a role of abnormal lipid. The most common dyslipidemia in

patients with poorly controlled IDDM is combinded elevated triglyceride and cholesterol levels with reduced high-density lipoprotein (HDL) cholesterol. Hypertriglyceridemia caused by increased level of VLDL occurs commonly in poorly control diabetes patients. Moreover, persistant hypertriglyceridemia and low HDL levels also are associated with poor glycemic control. It is well demonstrated that diabetes mellitus in man and in experimental animals is associated with elevated plasma lipid level, in particular triglyceride (Nikkila EA, 1974).

Besides hypertriglyceridemia, hypercholesterolmia is also suggested a role for abnormal lipids in diabetes mellitus. High or borderline elevation of total cholesterol is evident in a high percentage of diabetic patients, and of these many will have raised low-density lipoprotein (LDL) cholesterol (Gibbons GE, 1986; Harris MI, 1991). As well as having increased levels of LDL_s, patients with diabetes demonstrate an increase in the susceptibility of LDL to oxidation (Babiy AV et al., 1992) and increased circulating levels of oxidized LDL_s (Bucala R et al., 1993). Oxidized LDL cholesterol has been implicated in abnormalities of endothelial function observed in the arteries from non-diabetic hyperlipidemic humans (Bassaller C et al., 1987) and animal (Shimokawa K et al., 1989). Furthermore, superoxide anion (O_2^-) has been implicated in the oxidation of LDL (Yuichi O et al., 1993). The increased production of O_2^- by the endothelium in hypercholesterolemia is further enhance LDL modification and thus lipid accunulation within the vascular wall (Heim K et al., 1991). The previous observation in porcine coronary arteries demonstrated that acute hypercholesterolemia reduced the endothelium-dependent vasodilation to acetylcholine (Shimokawa K et al., 1989). LDL-cholesterol has been implicated in endothelial dysfunction in

hypercholesterolemia as it inhibit endothelium dependent vasodilation through the direct inactivation of NO (Jacobs M et al., 1998), but endothelial cells can also modify LDL to oxidized LDL (Chang KC et al., 1993) which is more atherogenic than native LDL (Morel DW et al., 1984) and which causes irreversible inhibition of endothelium-dependent vasodilation in animal arteries (Jacobs M et al., 1990).

Interestingly, the dyslipidemia seen in many diabetic patients, including high triglycerides, low HDL-cholesterol, is associated with low lipoprotein lipase (LPL) activity (Ginsberg HN, 1991). In insulin-deficient animals, increased LPL activity can improved dyslipidemia (Tsutsumi K et al., 1995). LPL is rate-limiting for removal of triglycerides from the circulation and critical for the generation of HDL particles. Higest expression is found in heart, adipose tissue, and skeleton muscle (Seip RL, 1995). In adipose tissue and muscle, the enzyme is transported from parenchymal cells across endothelial cells by unknown-mechanisms and binds to glycosaminoglycans at the luminal site of the capillary endothelium. From this site, it hydrolyzes triglycerides, relasing free fatty acids for uptake by tissues (Isutsumi K et al., 1995). Therefore, underlying defects in LPL activity could exacerbate dyslipidemia in diabetes and promote vascular damage.

3.1.4 VASCULAR LEUKOCYTE-ENDOTHELIAL INTERACTION IN DIABETES MELLITUS

As we have metioned firstly that both impaired endothelial-dependent vasodilation and increased leukocyte adhesion on endothelial cell are major factors for endothelial dysfunction. Enhanced adhesion of monocytes to

endothelial cells has been documented in IDDM patients. The sequence of events that allows the traveling of leukocytes to sites of host defense is designated the multistep paradigms of leukocyte recruitment. It involves important events encompass the transvascular movement of leukocytes: 1) margination and captering of free-flowing leukocytes, 2) leukocyte rolling, 3) activation and firm adhesion, 4) spreading; transendothelial diapedesis and chemotactic migration of the leukocytes (Figure 5). Different mechanisms appear to mediate leukocyte rolling and adhesion, the former is dependent on selectins expressed on endothelium (P-selectin) and leukocytes (L-selectins), where as the latter is dependent on the integrins (CD11/CD18) found on leukocytes and their ligands (ICAM-1, VCAM-1) on endothelial cell (Yang X-D et al., 1996).

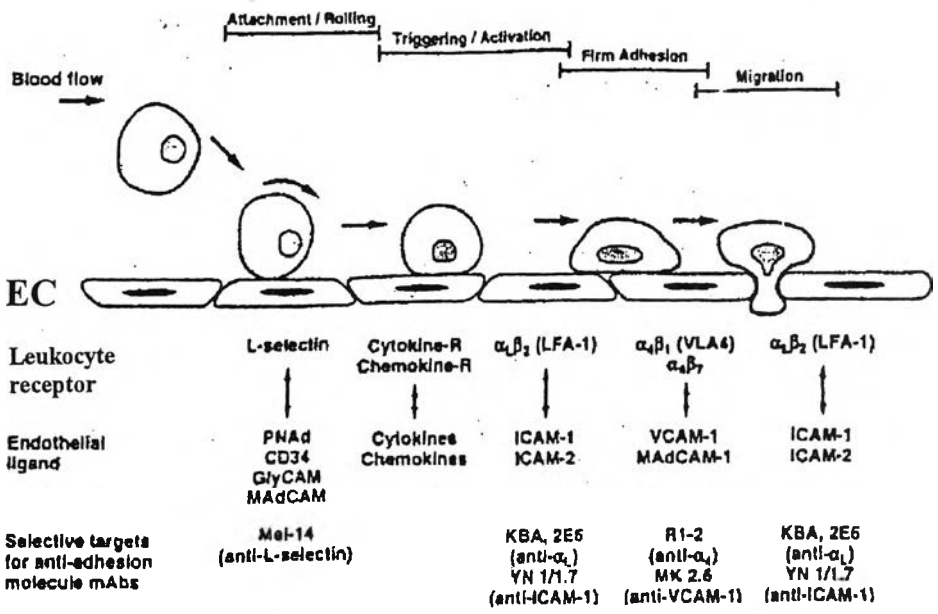

Figure 5. Sequential multistep model of leukocyte/endothelial adhesion. Extravasculation of leukocytes from blood into the tissues mediated by cascade of adhesive interactions between leukocytes and endothelial cells.

There is now a large body of evidences that enhanced leukocyte-endothelium interaction were demonstrated in diabetes mellitus (Yang X-D et al., 1996). Hadcocks SM and Co-worker, (1991) have shown that in vivo leukocytes accumulation in the endothelium were increased in rabbits with alloxan diabetes. There is also evidence that capillary occlusions by monocytes and granulocytes preceded destruction of capillary bed in diabetic retinopathy of rats (Schroder SW et al., 1991).

Elevated concentrations of circulating intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in IDDM patients reflects endothelial activation and stimulation of leukocytes in a chronic diabetes mellitus (Baumgartner-Parzer SM et al., 1995).

Increasing evidence indicated that hyperglycemia accelerates oxidation processes and caused endothelial damage by oxidized LDL (Lopes-Virella MF et al., 1992) and increased formation of free radicals by glucose autoxidation, which stimulate cytokine release and consequently induce expression of adhesion molecule on the endothelial cell surface (Pigott R et al., 1992). Pevious study showed an increased expression of VCAM-1 and also ICAM-1 on retinal and choroidal tissue of diabetic patients with retinopathy (McLeod DS et al., 1995). Similarly, VCAM-1 was found immunohistochemically in renal biopsies of patients with diabetic nephropathy and was elevated in its circulating form in serum of microalbuminuric patients (Schmidt AM et al., 1996). Furthermore, Fasching P and Co-worker (1996) found a correlation between LDL cholesterol and circulating VCAM-1 in diabetic patients which supported the in vitro observation by khan BV et al (1995) and Schmidt AM (1996) that oxidized particles induce expression and release of adhesion molecule

(AM) from prestimulated endothelial cells. Recent in vitro study has also shown that high glucose concentration and advance glycosylation end products (AGEs) can induce the expression of adhesion molecules on cultured endothelial cells (Baumgartner-Parzer SM et al., 1995).

A. OXIDIZED LDL-INDUCED LEUKOCYTE ADHESION

Elevated plasma levels of cell adhesion molecules have been linked to the development of atherosclerosis in diabetic patients. Oxidative stress is one important factor for enhanced expression of cell adhesion in diabetic patients. Indeed, evidence exists that modified LDL increases cytokine-activated VCAM-1 gene expression in human endothelial cells (Khan V et al., 1995).

Looking for leukocyte attractant and adhesion-promoting factors, Alderson LM and co-worker (1986) have demonstrated that exposure of cultured endothelial cells to human LDL or to VLDL from cholesterol-fed animals enhanced their adhesivity for monocytes. This effect was described to the peroxidative damage of membrane phospholipids and/or cholesterol transfer into endothelial cell membrane-bilayers, leading to alterations in the cholesterol/phospholipid molar ratio and hence to pronounced changes in biophysical membrane properties, and oxidized LDL more increased adhesion. In addition, study in vivo model of oxidized LDL-induced leukocyte adhesion were also demonstrated in dorsal skin fold model (Lehr HA et al., 1991).

B. HYPERGLYCEMIA AND LEUKOCYTE ADHESION

In human diabetes, enhanced expression of ICAM-1 has been observed in the vascular endothelium of pancreatic islets and in the retina along with upregulation of P-selectin. In vitro study had shown that high concentration of glucose (25 mM) induced a significant increase in monocyte binding to human aortic endothelial cells. Vlassara H (1992) has suggested the deleterious effect of high glucose on cell function and cell-cell interaction is additionally mediated by AGEs that accumulate in the plasma and vascular tissue of diabetes. AGEs are highly reactive substances resulting from prolonged exposure of proteins to high glucose concentration. AGEs promotes monocyte migration which are mediated by interaction with a specific receptor, RAGE, a newly identified member of the immunoglobulin superfamily expressed on mononuclear phagocytes as well as vascular smooth muscle and endothelial cells (Vlassa H. 1992; Brett J et al., 1994). Furthermore, in endothelial cell culture, AGEs by interacting with endothelial RAGE, enhanced the expression of cell associated VCAM-1 and released soluble VCAM-1 antigen into cell supernatant by induction of cellular oxidant stress and activation of nuclear factor-κB (NF-κB) (Schmidt AM et al., 1995). Taken together, from the above review it can suggested that both oxidized LDL and hyperglycemia response for enhancing the adhesion of leukocytes to endothelial cells.

IV. ANTIOXIDANT EFFECTS OF VITAMIN C AND DIABETES MELLITUS

1. FREE RADICALS AND REACTIVE OXYGEN METABOLITES

The difinition of free radical can be defined as very reactive molecules possessing an unpaired electron in its outer orbital. Such unpaired electrons make these species very unstable and therefore quite reactive. Common examples of free radicals include the hydroxyl radical (OH^-), superoxide anion (O_2^-), transition metals such as iron and copper, and peroxynitrite ($ONOO^-$). Free radicals can be formed in three ways: 1) by the hemolytic cleavage of a covalent bond of a normal molecule, 2) by the loss of a single electron from a normal molecule, 3) by the addition of a single electron to a normal molecule (Halliwell B, 1996). Reduction of oxygen by the transfer to it of a single electron will produce the superoxide-free radical anion (O_2^-).

$$O_2 + e^- \longrightarrow O_2^-$$

A two-electron reduction of oxygen would yield hydrogen peroxide (H_2O_2):

$$O_2 + 2e^- + 2H^+ \longrightarrow H_2O_2$$

Hydrogen peroxide is often generated in biological systems via the production of superoxide: two superoxide molecules can react together to form hydrogen peroxide and oxygen (Reilly PM et al., 1991)

$$2O_2^{-}+2H^{+} \longrightarrow H_2O_2 + O_2$$

Thus, superoxide radical anion (O_2^{-}) appear to play a central role, since other reactive intermediates are formed from it (Figure 6).

Superoxide is formed by one-electron reduction of oxygen mediated by enzyme such as NADPH oxidase or xanthine oxidase or by the respiratory chain. The half-life of O_2^{-} in tissues depends on the presence of the enzyme superoxide dismutase. Reactive oxygen species are also produced in the organism as a part of the primary immune defense. Phagocytic cells such as neutrophils, monocytes, or macrophages defend against foreign organisms by generating O^{-}_2 and nitric oxide. Both compounds can combine (Figure 6) to form peroxynitrite ($ONOO^{-}$), a reactive species which capable for inducing lipid peroxidation in lipoproteins (Stahl W et al., 1996).

Hydrogen peroxide (H_2O_2) is not a free radical but falls into the category of reactive oxygen species. H_2O_2 is an important compound in free radical biochemistry because it can easily-break down. It is efficiently converted to water by the enzyme catalase or by glutathione peroxidase (Bendich A, 1990).

The hydroxyl radical (OH^{-}) is the most reactive oxygen species with an estimated half-life of a few nanoseconds. It is formed in vivo upon high energy irradiation by hemolytic cleavage of water, or from hydrogen peroxide in a metal-catalyzed process (Figure 6)

Peroxyl radical ($LOO^{\bullet}$) can be generated in the process of lipid peroxidation, which is initiated by the abstraction of a hydrogen atom from polyunsaturated fatty acid (Stahl W et al., 1996).

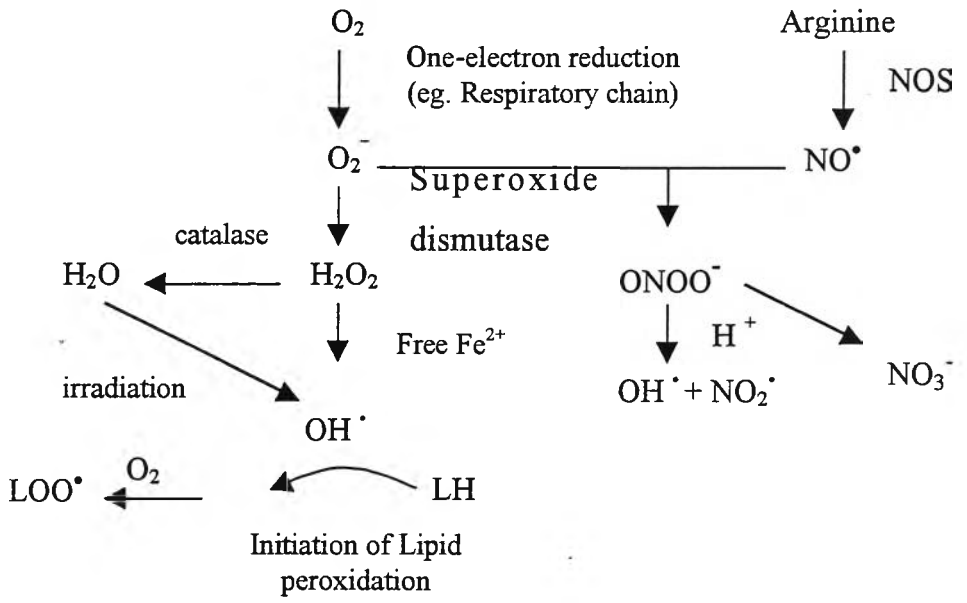

Figure 6. Pathway for the formation of reactive oxygen species (Diabetes 1997; 46(suppl2):s14-s18).

2. ANTIOXIDANT DEFENSE : VITAMIN C

Because some free radical production in cells is inevitable and because they can be very damaging, defences against the deleterious action of free radicals have evolved.

Antioxidant defense system are operative, including enzymatic and nonenzymatic antioxidants. Enzymes directly involved in the detoxification of reactive oxygen species are superoxide dismutase (SOD), catalase, and

glutathione peroxidase (Reilly PM et al., 1991). Nonenzymatic endogenous antioxidant mechanisms also exist within the normal cell, including vitamin E (α-tocopherol), vitamin C (ascorbic acid), and β-caroteine (Diplock AT, 1995).

Diabetic patients have a well documented defect in antioxidant protection. Several reports indicated that not only increased oxygen derived free radical production but also reduced degradation of both superoxide anion and H_2O_2 in the hyperglycemic condition may deleteriorate endothelial cell function, which is one of early events in the course of diabetic vascular dysfunction (Kashiwagi A et al., 1996).

Impaired generation of naturally occuring antioxidants (Vitamin E, vitamin C, and GSH) are also result in increase oxidative injury by failure of protective mechanisms. The oxygen-derived free radicals play a major role for the pathogenesis of diabetic vascular complications. Inaddition, several studieshave shown that antioxidants such as vitamin E, superoxide dismutase (SOD), catalase, GSH and vitamin C (Karpen CW et al., 1982; Wolff SP et al., 1987) are all decreased in diabetic tissues and blood (Young IS et al., 1992).

Vitamin C or ascorbic acid is a water-soluble antioxidant and free radical scavenger. The best know functions of ascorbic acid are due to its properties as an electrondonor. Given its low redox potential, ascorbic acid is a broad-spectrum radical scavenger that is effective against peroxyl-and hydroxyl-radicals. Superoxide, singlet oxygen, and peroxynitrite (Vatassery GT, 1996).

3. EFFECTS OF VITAMIN C ON LIPID METABOLISM

VITAMIN C AND CHOLESTEROL METABOLISM

Besides its antioxidant role, vitamin C acts on many biologically diverse systems, including biosynthesis of collagen (Levine M, 1986) carnitine, (Levine M, 1986), and lipid metabolism (Dai S et al., 1995). There is disagreement about the effects of vitamin C on cholesterol metabolism. Some investigators have described hypocholesterolemic and altheroma-reducing effects of vitamin C in rats (Dai S et al., 1995), but others did not find this effect (Freyschus A et al., 1997).

In cholesterol metabolism, cholesterol is transformed to bile acid in the liver and the rate of this process very probably depends on the ascorbic acid concentration in the liver cells, thus there is a relatively close linear correlation between the rate of bile acid synthesis and the ascorbic acid concentration in the liver (Ginter E, 1976). Cholesterol transformation to bile acid is a multistage process occuring in the liver cell microsomes, supernatant fraction, and mitochondria (Ginter E, 1973).

The first step in cholesterol transformation to bile acids is the production of 7α-hydroxycholesterol and this reaction is rate limiting for cholesterol catabolism (Figure 7). Ginter E(1973) has shown that there is indeed a decreased rate of transformation of cholesterol to bile acids, in chroincally ascorbate-deficient guinea pigs. The mechanism responseible for the elevation of cholesterol in the ascorbic acid-deficiency animals is involve an impaired conversion of cholesterol to bile acids (Ginter E and Nemec R, 1972). Inaddition, Bjorkheim I and Kallner (1976) reported that the extent of conversion of endogenous cholesterol into 7α-

hydroxycholesterol as determined by mass fragmentography was more than 10 times lower in incubations with microsomal fraction from vitamin C-deficient guinea pigs than in those from guinea pigs treated with vitamin C . On the other side, 26-hydroxylation was not significantly affected by the ascorbic acid status of animals (Figure 7). Therefore, it can suggested that marginal vitamin C deficeincy interferes with the biosynthesis of bile acid solely at the stage of 7α-hydroxylation of the cholesterol nucleus.

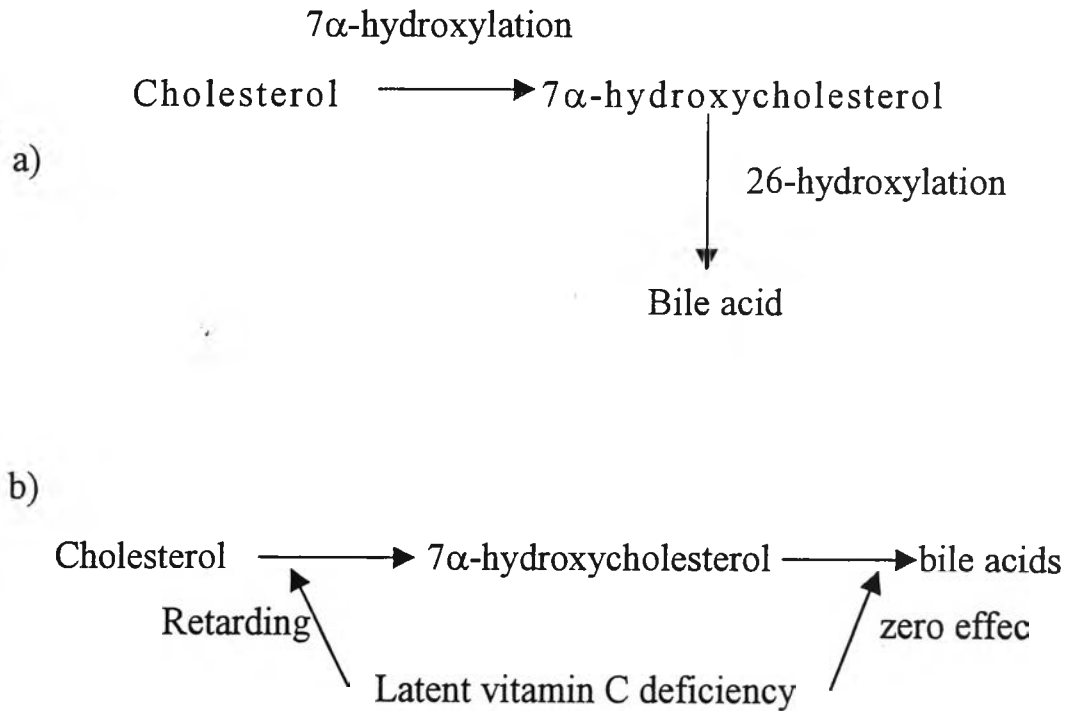

Figure 7. Cholesterol metabolism and vitamin C deficiency (Science 1973;176 :702).

4. VITAMIN C (ASCORBIC ACID) METABOLISM AND DIABETES MELLITUS

Vitamin C refers to all compounds that exhibit the biological activity of ascorbic acid, including both ascorbic acid and its oxidized form, dehydroascorbic acid (DHAA) (Washko PW et al., 1992). Removal of one electron from ascorbic acid yields-semidehydroascorbic acid (ascorbate radical). This form of the vitamin is a free radical; it contains an unpaired-electron. The remove of a second electron yields dehydroascorbic acid, conversion of ascorbic acid to dehydroascorbic acid, conversion of ascorbic acid to dehydroascorbic acid, via the removal of two electron. Dehydroascorbic acid, this enzyme requires glutathione (GSH) as a source of reducing power. Both ascorbic acid and dehydroascorbic acid have biological activity.

The latter compound may break down to form diketogulonic acid (Figure 8). Diketogulonic acid is an orange compound that has no biological activity (Levine M, 1986).

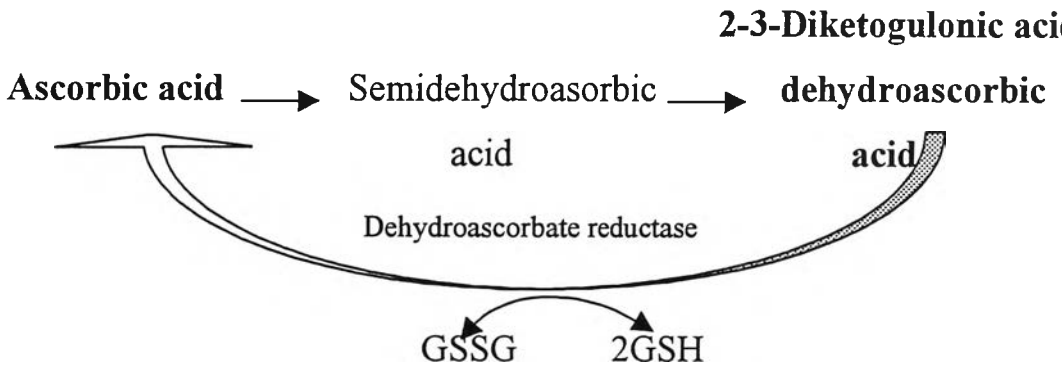

Figure 8. Metabolism pathway of Vitamin C (N Eng J Med 1986;314:892-902) .

Abnormalities of collagen synthesis (Schnide S et al., 1981) and increased oxidative stress (Baynes JW, 1991) have been implicated in the pathogenesis of diabetic microangiopathy. Decreased plasma ascorbic acid concentrations and increased turnover of ascorbic acid to the oxidized metabolite dehycdroascorbic acid (DHAA) have been reported in diabetes subjects, particullary in patients with poor glycemic control (Yee DK et al., 1990).

The low plasma ascorbic acid level in diabetes is apparently due to high turnover of ascorbic acid in the body. The high turnover is possible due to increased oxidation of ascorbic acid to DHAA, because the rate of oxidation of ascorbic acid is greatly increase in tissue mitochondrial fractions of diabetic rats. Recent study has observed reduction of plasma and tissue ascorbic acid concentration in STZ-diabetic rats (Lindsay RM et al., 1998).

Moreover, Ng Keekwong FC and co-worker (1997) have shown that chronic exposure of cells to high glucose levels could lead to a reduction in V_{max} of DHAA uptake. Furthermore , the recent study by Ng LL et al have been shown the similar result that DHAA uptake-rates in diabetic cells were impaired when compared with control cells (Ng LL et al., 1998). This impaired uptake would reduce the regeneration of ascorbic acid from DHAA within the cells (Figure 9). Now it is clear that ascorbic acid is transported across cellular membranes by two distinct mechanisms. Ascorbic acid it self is transported by a sodium-dependent saturable transporter which has not been isolated (Welch RV et al., 1995). Ascorbic

acid outside cells can be oxidized to dehydroascorbic acid (DHAA), which is transported by a different mechanism. Once within cells, DHAA is immediately reduced to ascorbic acid by both chemical and protein mediated processes. DHAA is shown to be transported by facilitated difussion which uses the member of the family of mamalian hexose transporters (especially GLUT-1)(NG LL et al., 1998).

In vivo study, exposure to hyperglycemia exacerbate the impaired DHAA uptake, leading to loss of DHAA through its hydrolysis in aqueous solution. This impairment may be resulted from DHAA and glucose compete for the same uptake mechanism (Ng Keek wong FC et al. 1997). Therefore, it can suggested that impaired DHAA uptake in hyperglycemic condition, leads to impaired regeneration of ascorbic acid and depletion of antioxidant defences.

5. EFFECTS OF VITAMIN C ON ENDOTHELIAL DYSFUNCTION

The development of endothelial cell dysfunction is characterized by an impairment in vasodilation and increased adhesiveness of the endothelial cell lining. Several lines of evidence indicate that endothelial cell dysfunction is associated with alterations in the cell redox state.

The potentiated diabetic micro-and macroangiopathy is related to the induction of oxidative stress. Similarly, hyperlipidemia also increases the generation of superoxide anions and thereby promotes the oxidation of low

density lipoprotein (OxLDL) cholesterol within the vessel wall. Disturbances in endothelial cell function decrease nitric oxide production / action and thus reduce the vasodilation of the vessel wall (Gibbons GH et al., 1996). The mechanisms responsible for endothelial dysfunction in patients with diabetes mellitus are not completely under stood. As we have mentioned above that the possible causes of endothelial dysfunction are : decreased synthesis or release of nitric oxide by endothelial cells, increased inactivation of endothelium-derived nitric oxide by oxygen-derived free radicals particularly superoxide anion (Gryglewski RJ et al., 1986). Interestingly, now, the antioxidants have become a center of interest for their role on prevention of diabetic endothelial dysfunction.

Vitamin C or ascorbic acid is the most important water soluble antioxidant in human plasma (Frei B et al., 1989). The recent study, has shown the effect of vitamin C on NIDDM patients and found that acute administration of the antioxidant, vitmain C, significantly improved impaired endothelim-dependent vasodilation to methacholine, where as the endothelium-independent vasodilator response to nitropusside, an exogenous nitric oxide donor, and to verapanil, a direct vascular smooth-muscle relaxation were not significantly altered by administration of vitamin C (Ting HH et al., 1996). The investigators suggested that this improvement in endothelium-dependent vasodilation in diabetic subject is probably mediated by the ability of vitamin C to scavenge excess superoxide anions and, thereby, decrease nitric oxide inactivation. Moreover, numerous clinical studies have consistently demonstrated beneficial effects of vitamin C treatment on endothelium-dependent vasodilation in individuals with coronary artery disease and coronary risk factors (Carr AC, 1999).

5.1 VITAMIN C (ASCORBIC ACID) METABOLISM IN PLASMA AND ENDOTHELIAL CELLS

Ascorbic acid circulates in plasma at concentration of 30-60 μM in unsupplemented individival (Evans RM et al., 1982). Plasma ascorbic acid concentrations are maintained by intestinal-absorption, renal absorption (Mellors AJ et al., 1977), slow release from circulating erythrocytes (Mendiratta S et al., 1999). Intracellular ascorbic acid concentrations are 6-8 mM in human white blood cells (Washko P et al., 1989). Typical of most nucleated cells, human umbilical vein endothelial cells in culture take up ascorbic acid against a concentration gradient by a high-affinity, energy-dependent process (EK A et al., 1995).

5.2 MECHANISMS OF VITAMIN C ON ENDOTHELIAL DYSFUNCTION

PREVENTIVE EFFECT OF VITAMIN C ON ENDOTHELIAL DYSFUNCTION INDUCED BY OXIDIZED LDL.

There are several ways of ascorbic acid can prevent endothelial dysfunction due to LDL.First, Ascorbic acid prevents oxidative modification of LDL primarily by scarvenging free radicals and other reactive oxygen species in the aqueous milieu (Frei B et al., 1989). The initial step for ascorbic acid prevent interaction of these oxygen species to oxidized LDL

by direct and rapid trapping of these aqeous oxygen species. Ascorbyl radicals formed in this process are reduced back to ascorbic acid by dismutation, chemical reduction (eg, by glutathione) (May JM et al., 1998). Dismutation also produces DHAA, which in turn can be reduced back to ascorbic acid by glutathione, thioredoxin reductase, and glutaredoxin (Park JB et al., 1996). Second, it has been shown in vitro that ascorbic acid can recycle α-tocopherol in LDL, which inturn help to prevent LDL oxidation (Diaz MN et al., 1997). Without ascorbic acid, the α-tocopheroxyl radical can act as a pro-oxidant role and continue or even enhance the chain reaction of lipid peroxidation in LDL (Thomas SR et al., 1995). Ascorbic acid can prevent this pro-oxidant activity of α-tocopherol by reducing the α-tocopheroxyl radical to α-tocopherol. Thus ascorbic acid acts as a "coantioxidant" and inhibiting LDL oxidation (Neuzil J et al., 1997). It is known that oxidized LDL is directly toxic to endothelial cells, and induced leukocytes adhesion to endothelium. Therefore the effects of ascorbic acid to prevent LDL oxidation could well contribute to the ability of it to prevent or improve endothelial dysfunction in developing atherosclerosis.

5.2.1 VITAMIN C AND LEUKOCYTE-ENDOTHELIAL INTERACTION

Adhesion of leukocytes to the endothelium is an important initial step in atherosclerosis. Several studies, have shown the potential effect of inflammatory cytokines and oxidized LDL to increased expression of adhesion molecules, leading to leukocyte adhesion on endothelium (Poston RN et al., 1996; Davies MJ et al., 1993). Experiments with phospholipase or lipoxygenase inhibitors or with SOD have suggested that the process of

cell-mediated LDL oxidation involves the generation of reactive oxygen species, in particular, of superoxide anion (Rankin SM et al., 1991). Moreover, the electron microscopic evidence showed that oxidized LDL-induced leukocyte adhesion which is not presented only to the microcirculation (Lehr HA et al., 1994) but also affects large blood vessel (Lehr HA et al., 1995). The recent study, under intravital microscopy in the dorsal skinfold chamber model in hamster demonstrated that oxidized LDL-induced leukocyte adhesion was almost entirely prevented by pretreatment with dietary or intravenous vitamin C (Lehr HA et al 1995). In another study, supplementation of smokers with 2g/day of vitamain C for 10 days elevated plasma ascorbic acid levels almost 2-fold and significantly reduced monocyte adhesion to cultured endothelial cells (Weber C et al., 1996). This finding indicated that upregulation of ligands on monocytes is inhibited by vitamin C.

5.2.2 VITAMIN C AND ENDOTHELIAL NITRIC OXIDE (EDNO)

Vitamin C is the most important water-soluble antioxidant in human plasma (Frei B et al., 1989). Several clinical studies have consistenly show the potential effects of vitamin C treatment to prevent impairment of endothelial-derived vasodilation. The effect of vitamain C on EDNO-mediated arterial relaxation has been examined in human subjects. Ting HH et al (1997) found that impaired EDNO-mediated forearm blood flow responses to methacholine were improved by a concomitant infusion of ascorbic acid in patients with diabetes or hypercholesterolemia. In addition, Heitzer T and co-worker (1996) found that an acute arterial infusion of

ascorbic acid normalized EDNO-mediated forearm blood flow responses to acetylcholine. An improvement in EDNO-mediated brachial artery dilation has also been reported with in patients with heart failure (Horning B, 1998).

These studies have suggested that ascorbic acid improves EDNO-mediated responses through its antioxidant effect. (Ting HH et al., 1997; Heitzer T et al., 1996; Horning B, 1998). There are several of potential mechanisms of ascorbic acid on endothelial function.

A. ASCORBIC ACID IMPROVED ENDOTHELIAL DYSFUNCTION THROUGH SCAVENGING OF SUPEROXIDE.

Endothelial cells generate superoxide and H_2O_2 as a result of both cytoplasmic (prostaglandins, cytochrome P_{450}, protein kinase C) and mitochondrial metabolism. Further, superoxide reacts with NO, leading to inactivate NO and producing peroxynitrite, which is damaging to cells. By scavenging superoxide, ascorbic acid could decrease NO consumption (Bendich A et al., 1986).

B. ASCORBIC ACID PREVENTION OF ENDOTHELIL DYSFUNCTION INDUCED BY OXIDIZED LDL

Ascorbic acid might prevent endothelial dysfunction induced by LDL in several ways; 1). It can decrease oxidation of LDL by scavenging reactive oxygen species directly. In plasma, ascorbic acid is one of the most important antioxidants, sparing other antioxidants by forming the first line

of defense against free radicals (Frei B et al., 1989), 2) it has been shown in vitro that ascorbic acid can recycle α-tocopherol (vitamin E) in LDL which inturn help to prevent LDL oxidation (Kaneko T et al., 1993), 3) intracellular ascorbic acid can decrease the ability of endothelial cells to modify LDL. Endothelial cells in culture are known to oxidatively modify LDL by metal ion-dependent mechanism (Martin A et al., 1997; Steinbrecher UP et al., 1984). Martin and Frei (1997) found that ascorbic acid loading of culture human aortic endothelial cells decreases their capacity to modify LDL and that this protection parallels the intracellukar ascorbate concentration.

C. ASCORBIC ACID-INDUCED RELEASE OF NO FROM S-NITROSOTHIOLS IN PLASMA

Ascorbic acid could increase dilivery of NO from plasma to vascular wall. Ascorbic acid may directly enhance endothelium-dependent vasodilation by sparing intracellular thiols, which inturn stabilize EDNO through the formation of biologically active α-nitrosothiols (Figure 9). NO in plasma can be carried as an S-nitrosothiol on albumin and free cystein (Keaney JF et al., 1993). The plasma S-nitrosothiols concentration have been reported to range with 0.45 μM to as high as 7 μM (Stamler JS et al., 1992), with 82% in the form of S-nitrosoalbumin. Ascorbic acid can release NO from both low-molecular weight S-nitrosothiols and S-nitrosoalbumin (Figure 9). Although most NO released into the blood vessel lumen will be quickly scavenged by hemoglobin in erythrocytes, it is possible that some could reach vascular smooth muscle cells. Further, hemoglobin itself can carry measurable amounts of NO as S-nitrosothiols (Jia L et al., 1996).

Since S-nitrosothiols can transfer NO to other thiols in transnitrosation reactions, it has been suggested by Jia L et al., (1996) that such transfer from S-nitrosohemoglobin to S-nitrosocystein could move NO out of erythrocyts and into plasma.

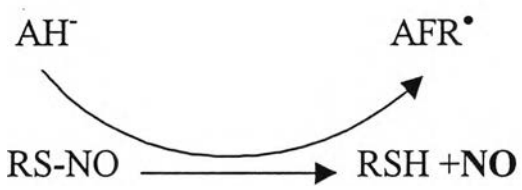

Figure 9. Ascorbic acid reduction of S-nitrosothiols.
Abbreviations : AH^- : ascorbic acid, AFR : ascorbic acid free radical, NO:nitric oxide, RS-NO : S-nitrosothiol, RSH: reduced thiol (Free Radical Biology& Medicine 2000;28(9) :1421-1429).

In conclusion, from the overall reviewed, it can concluded that the increased oxygen-derived free radical mediated by hyperglycemia, metabolic changes and decrease antioxidant defenses, particular vitamin C are important factors for developing the endothelial dysfunction. Vitamin c supplementation can prevent endothelial dysfunction by several mechanisms, including scavenge free radical especially superoxide anion , decrease in LDL oxidation, and release of NO from circulating or tissue S-nitrosothiols.