CHAPTER II

BACKGROUND INFORMATIONS

Glucose is a very common sugar that is an extremely diverse molecule. Not only is it the primary energy source for most organisms, but it can also be toxic to cells if allowed to accumulate in high concentrations. The molecular formula of glucose is $C_6H_{12}O_6$. Glycolysis is the first stage in the release of energy from the glucose molecule. It involves in the breaking down of glucose into two smaller molecules or pyruvic acid. Glycolysis occurs in the cytoplasm involving in a great many enzymes.

During glycolysis, important intermediate substances were produced such as reduced nicotinamide adenine dinucleotide (NADH) and pyruvic acid. Pyruvic acid passes through the mitochondrial membrane and entry in Krebs cycle or tricarboxylic acid cycle (TCA cycle) and the electron transport chain. In the hyperglycemic stage NADH accumulated in the cells and entry to the polyol pathway leading to the cell damage (Williamson et al., 1993).

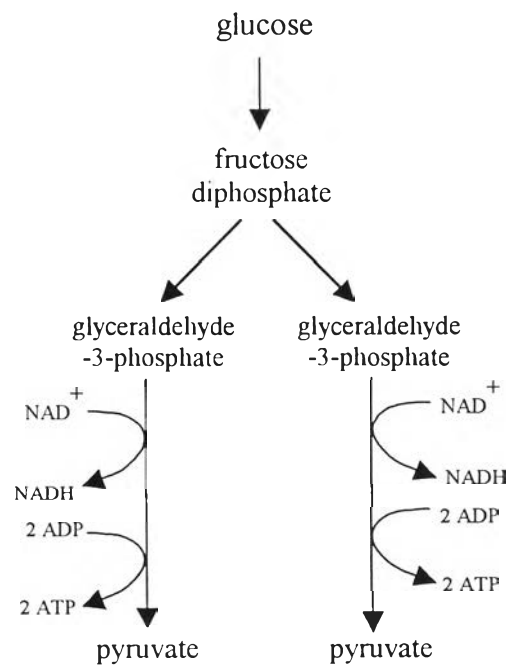

Figure 2-1 The pathway of glycolysis

Electron transport and oxidative phosphorylation

During TCA cycle the two carbons of acetate were completely oxidized to two CO_2 molecules. Some of the energy released during the oxidative process was stored as electrons on 3 NADH and 1$FADH_2$. The NADH and $FADH_2$ must be oxidized to NAD^+ and FAD in order to keep the TCA cycle operating. During the oxidative process the energy released is used to generate high energy phosphate compounds (ATP). The oxidation of NADH and $FADH_2$ and the generation of ATP is accomplished by the electron transport/oxidative phosphorylation pathway (Figure 2-4).

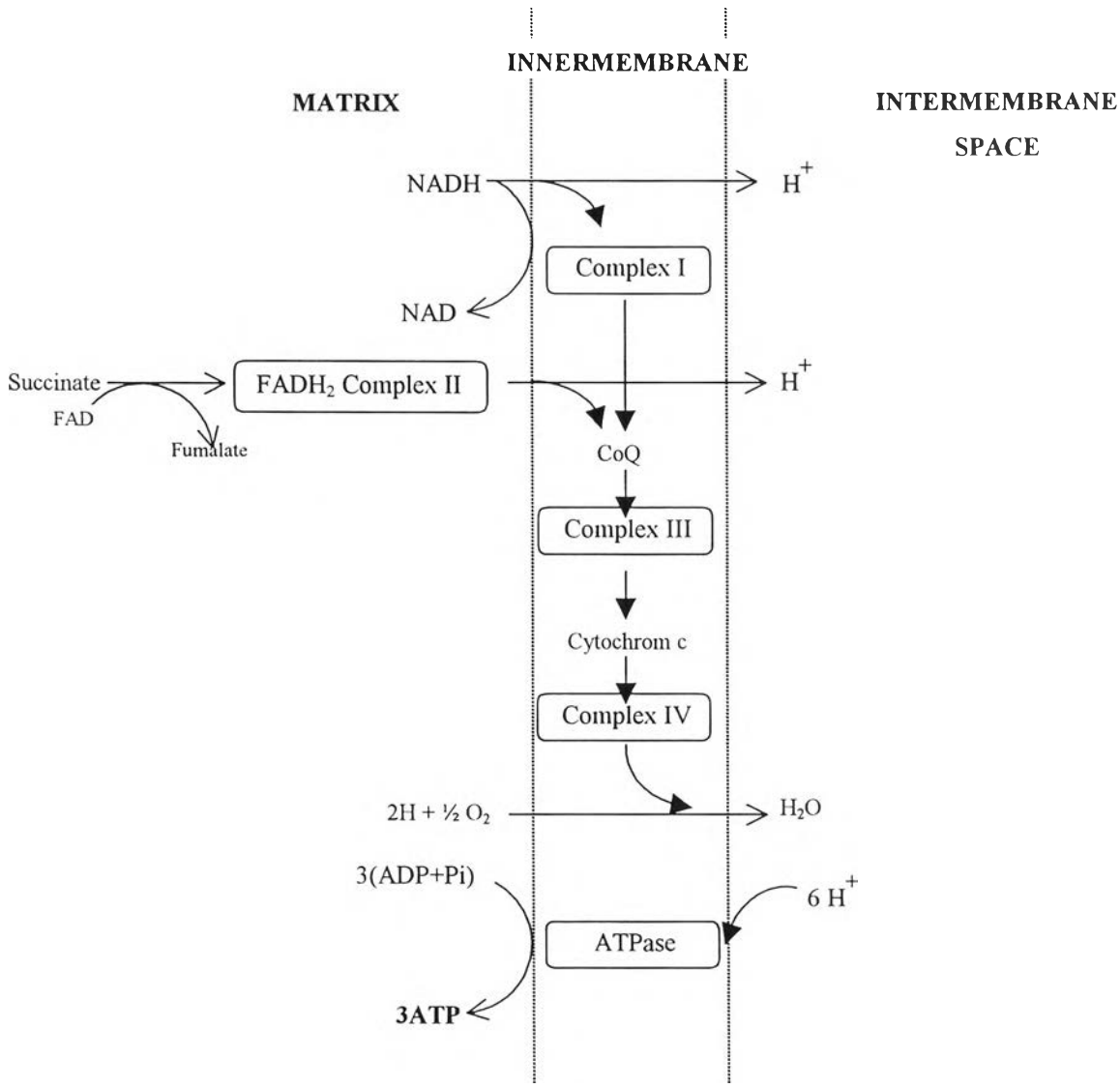

Figure 2-2 Electron transport chain

(Modified from Terry, 1997; Huskey, 1999)

System components of electron transport chain

Electron transport / oxidative phosphorylation pathway is composed of five large protein complexes, Complex I, II, III, IV, and V. All five complexes are intrinsic membrane protein complexes embedded in the inner mitochondrial membrane. The relative ratios of complexes I, II, III, IV, and V have been estimated to be 1:2:3:6:6. Complex I, II, III and IV collectively contain a series of electron carriers that pass electrons down an energy gradient. The gradient starts with the high energy electrons carried NADH and $FADH_2$. At the final step, four electrons are passed to O_2 to form two H_2O. Complex I, III, and IV are also proton pumps /proton translocators. As electrons flow through these complexes down the energy gradient, the energy released is used to pump protons (H^+) across the inner mitochondrial membrane. Protons are translocated (pumped) from the matrix to the intermembrane space. These complexes are oriented in the membrane to accomplish this task. The complexes contain the enzymes necessary to catalyze the electron transfer reactions, the molecules that undergo reversible oxidation/reduction, and the transport proteins that translocate protons across the inner mitochondrial membrane. Complex V, also called the F_0/F_1 complex, uses the energy stored in the proton gradient established by electron transport to generate ATP from ADP and PO_4^{-3}.

Complex I: or NADH dehydrogenase is one of the largest macromolecular assemblies in the cell and transfers electrons from NADH in the matrix space to ubiquinone (CoQ) dissolved in the membrane lipids. The products are ubiquinol which remains within the inner membrane, and NAD which is re-used for further rounds of mitochondrial substrate oxidation within the matrix space. Complex 1 contains FMN and at least four different kinds of non-heme iron. It can be inhibited by the natural product *rotenone.*

Complex II: or succinate dehydrogenase transfers electrons from succinate to ubiquinone. It is one of the eight enzymes that catalyse the Krebs cycle, as well as being physically part of the electron transport chain. The products are fumarate which is released into the matrix space, and ubiquinol which remains in the membrane. Complex II contains FAD and another four varieties of non-heme iron.

In addition, several other major flavoproteins such as acyl-CoA dehydrogenase (the first step in fatty acyl CoA oxidation) and minor pathways such as glycerol phosphate oxidase also feed electrons directly or indirectly into ubiquinone.

Complex III: or cytochrome c reductase transfers electrons from ubquinol within the membrane to cytochrome c, located in the inter-membrane space. By this point the respiratory chain has traversed the full thickness of the mitochondrial inner membrane, but in the next stage it will double back on itself to finish where it started in the matrix space. Complex III can be inhibited by the antibiotic antimycin A. It contains non-heme iron, two kinds of cytochrome b, and a high molecular weight version of cytochrome c, called cytochrome c1.

Complex IV: or cytochrome oxidase transfers electrons from cytochrome c in the inter-membrane space to oxygen, forming water in the matrix space. This means that electrons must re-traverse the inner membrane. The functional unit in complex IV contains two molecules of heme and two atoms of copper, so in total it can hold up to four electrons, and accomplish the complete reduction of one oxygen molecule to water in a single step. It is important to complete this step because partial reduction of oxygen generates dangerous molecules such as superoxide radicals and hydrogen peroxide which are highly toxic to cells.

F_1/F_0 ATPase: This enzyme involves in ATP synthesis. It composes of the multi-protein complex, that covers the inner surface of the mitochondrial inner membrane with a dense array of spherical particles, raised above the membrane on stalks. It was first identified as a *coupling factor*, and used to restore phosphorylation to inner membrane vesicles that had lost their ability to make ATP. It can be split into two components called F_1 and F_0. F_1 is the spherical particle and most of the stalk. F_0 is the base plate, plugged right through the inner membrane like the respiratory complexes described above. When isolated this assembly is unable to make ATP because it has been disconnected from its power supply.

Glucose transporters (Gluts)

Glucose is an essential molecule for the cells, but too much glucose can be harmful. So the level of glucose in the cell is important. Cells have glucose transporters that regulate the flux of glucose into and out of the cell. A malfunction of these glucose transporters effects the level of glucose in the cells making a trouble to the cell. Mammalian cells employ at least two types of transporters, Na^+-dependent cotransporters and facilitative transporters. Transport of glucose across the plasma membrane of mammalian cells is facilitated by the family of Glut proteins. The Glut proteins all contain approximately 500 amino acids. They all share a similar structure. These proteins are characterized by 12 predicted transmembrane helices with the amino and carboxyl termini situated on the cytoplasmic phase (Olson et al., 1996) (Figure 2-3).

blood cells and the endothelial cells of blood vessels. Mesangial cells are also found Glut1 which is important glucose transporter involving in the diabetic nephropathy (Rasch, 1979; Heilig et al., 1997; Inoki et al., 1999). The proposed three dimensional model of Glu proteins suggests the clustering of amphipathic residues together to form an aqueous pore or barrel-like structure through which the glucose can traverse (Figure 2-4). The interior surface of the aqueous pore would be made up of polar side chains, and it is expected that these polar groups provide hydrogen-binding sites for glucose and other similar molecules (Mueckler, 1994).

The transporter has two ligand binding sites, one on the inside and another on the outside of the transporter. The exofacial binding site is responsible for glucose entry into the transporter while the endofacial binding site is required for the efflux of glucose. This allows the transporters to control the flow of glucose through the pore, depending on the intracellular and extracellular concentrations (Mueckler, 1994).

The Glut family

Two families of glucose transporters have been identified as the facilitated-diffusion glucose transporters (Glut family) and the Na^+-dependent glucose transporters (SGLT family). The majority of glucose transporters belong to a family of proteins, called Glut. There are at least twelve glucose transporters identified,

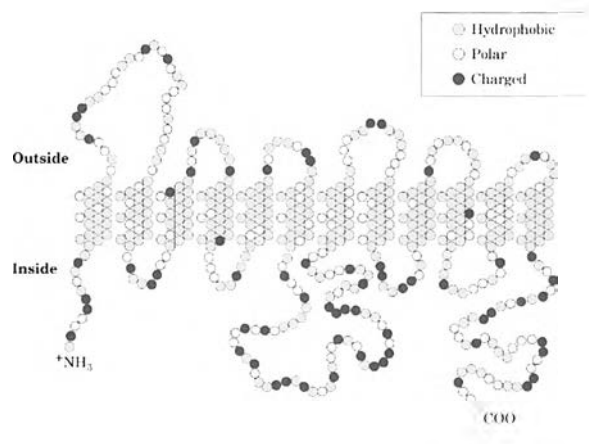

Figure 2-3 The two dimension of glucose transporter

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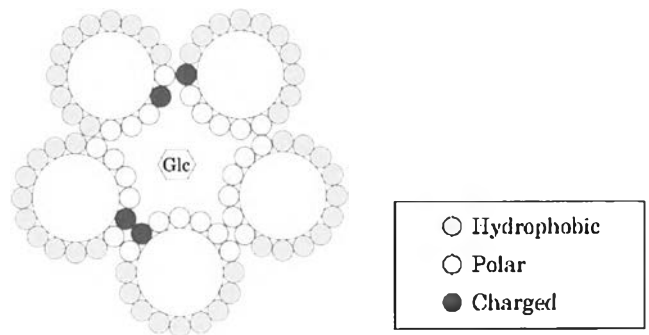


Figure 2-4 The three dimension of glucose transporter

(Available from the University of Texas http://www.courses.cm.utexas.edu/.../ ch12_struct-GluT1.jpg)

Glut 1 to Glut 12 and SGLT1. Kidneys have Glut 1, Glut 2, Glut 5, Glut 9. Glut 5 and Glut 9 have been noted as a transporter for fructose. Glut1, Glut3 and Glut4 also transport dehydroascorbic acid, the oxidized form of vitamin C (Hediger et al., 2004). Glut1 is a high-affinity glucose transporter (K_m = 1-2 mM) (Table 1-1), which is expressed in erythrocytes and several other tissues. Glut1 plays a crucial role in the transfer of glucose across the blood brain-barrier. In the rat kidney, Glut1 is expressed in mesangial cells and in the basolateral membranes of cells forming the proximal straight tubule, where it is probably involved in transepithelial glucose transport and delivery of glucose for energy production through the glycolytic pathway.

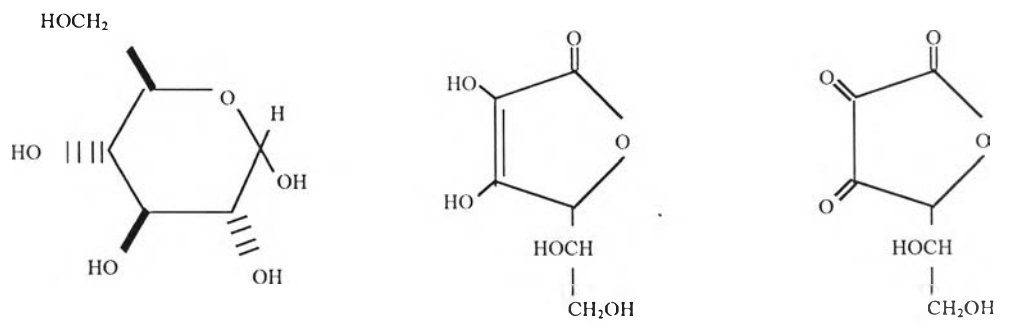
Table 1-1 The different types of glucose transporters (Mueckler et al., 1994; Olson et al., 1996).

Protein	Tissue Distribution	Proposed	K_m
SGLT1	kidney, intestine	Na+-dependent active transport; concentration across apical epithelial membranes	-
GLUT1	multiple fetal and adult tissues, most abundant in human erythrocytes, endothelia and immortalized cell lines	Basal glucose and increased supply for growing/dividing cells; transport across blood brain barrier and other barrier tissues	16.9-26.2 mM
GLUT2	hepatocytes, pancreatic β cells, intestine and kidney	high-capacity low-affinity transport; transepithelial transport (basolateral membrane)	40 mM
GLUT3	widely distributed in human tissues, found only in brain in other species	Basal transport in many human cells; uptake from cerebral fluid into brain parenchymal cells	10.6 mM
GLUT4	skeletal muscle, heart, adipocytes	Rapid increase in transport in response to elevated blood insulin; important in whole-body glucose disposal	1.8-4.8 mM
GLUT5	intestine, adipose, muscle, brain and kidney	Intestinal absorption of fructose and other hexoses	-
GLUT6	hepatocytes and other gluconeogenic tissues	Mediates flux across endoplasmic reticulum membrane	-

Dehydroascorbate (DHA) transport

Ascrobic acid or vitamin C is a water-soluble antioxidant so it can work both inside and outside the cells to scavenge free radicals. L-ascorbic acid has a primary role to neutralize aqueous peroxyl radicals before these destructive substances have a chance to damage the lipids, including the cell membrane. It works along with α-tochopherol (vitamin E), a fat-soluble antioxidant, and the enzyme glutathione peroxidase to stop free radical chain reactions (lipid peroxidation).

Some animals, including primates and guinea pigs, cannot produce their own supply of vitamin C. They need to obtain ascorbic acid in a diet. After ascorbic acid is intaken, it will be oxidized to the oxidized form, dehydroascorbate (DHA). Ascorbic acid and DHA occurs by separated mechanisms. Ascorbate can be oxidized outside the cell changing to DHA. DHA has a structure very similar to that of glucose (Figure 2-5), this allows glucose transporter to act as DHA transporters. Once transported, DHA was immediately reduced intracellularly to ascorbate. Ascorbate can be transported into the cell directly through a sodium-dependent carrier-mediated active transport. Glut 1, Glut 3 and Glut 4, facilitated glucose transporter isoforms, are the specific glucose transporter which mediate DHA transport and subsequent accumulation of ascorbate (Rumsey et al., 1997). DHA transport is at least 10-fold faster than ascorbate transport and was sodium-independent. (Welch et al., 1995).



Glocose **Ascorbate** **Dehydroascorbate**

Figure 2-5 Molecular structure of glucose, ascorbic acid and dehydroascorbate

Streptozotocin (STZ) induces type I diabetes mellitus

Streptozotocin or streptozocin is a nitrosourea derived from the mold *Streptomyces griseus*. It induces severe insulin-defficient diabetes in rodents. A single intravenous or intraperitoneal injection of STZ with a dose of 50-100 mg/kg is commonly used to induce type I diabetes in experimental model. STZ causes β-cell necrosis, and diabetes appears within 1-2 days. The diabetic animals typically fall in insulin level to 10-30 % of normal, leading to hyperglycemia (20-30 mmole/L), polyuria, polydipsia and weight loss. At a dosage of 50 mg/kg, severe ketosis does not develop and the rats survive for some weeks without insulin replacement. Higher dosages induce more insulin deficiency. The rats develop spontaneous ketosis and will be dead if insulin is not supplemented (Lally and Bone, 2003). Multiple low doses of STZ (5 mg/kw) can induce diabetes in mice (Like and Rossini, 1976). The syndrome is similar to the human type I diabetes, as the infiltration with mononuclear cells of the islets (insulitis), consistent with cell-mediated immunity rather than a direct toxic effect of STZ. This model has been used to study the immune process, which may modulate the effect of β-cell toxic agents, but does not mimic the type I diabetes model.

The mechanisms of STZ inducing experimental diabetes in animals are at least three mechanisms, including attacking mitochondria, breaking DNA and generate free radicals (Fig 2-6). STZ is transported into pancreatic β-cells through glucose transporter in the cell membranes and attacks mitochondria. Mitochondrial ATP generation is inhibited and the resulting in a marked depletion of intracellular ATP in β-cells (Nukatsuka et al., 1990). The decrease in ATP resulting in a high concentration of intracellular ADP causes its degradation providing hypoxanthine, a substrate of xanthine oxidase (XOD) whose activity is intrinsically very high in β-cells. Then, XOD-catalyzing reaction is proceeded as proved by increased formation of uric acid and O2- radicals are produced, but β-cells are inefficient to scavenge these radicals because of their extremely low activity of superoxide dismutase. On the other hand, STZ directly activates XOD and enhances O2- generation. Consequently, pancreatic β-cells are dually suffered from O2- radicals or

probably hydroxyl radicals derived from the former when exposed to STZ (Kawada, 1992).

The high level of free radicals causes the damage of the β-cell membrane, causing β-cell necrosis. In addition, the free radicals can break DNA strands, leading to the activation of poly (ADP-ribose) synthetase and the depletion of nicotinamide adenine dinucleotide (NAD). Inhibition of poly (ADP-ribose) synthetase with nicotinamide and picolinamide can inhibit NAD depletion and preserve the β-cell function (Ho et al., 1972; Schein et al., 1973).

Furthermore, STZ has not only a direct toxic effect on the pancreatic β-cells but also indirect effect of an immune reaction by acting as a hapten *in vivo*. The STZ-specific immune reactions can exert the immune system, leading to enhance the toxic effect of STZ on the pancreatic β-cells (Klinkhammer et al., 1988).

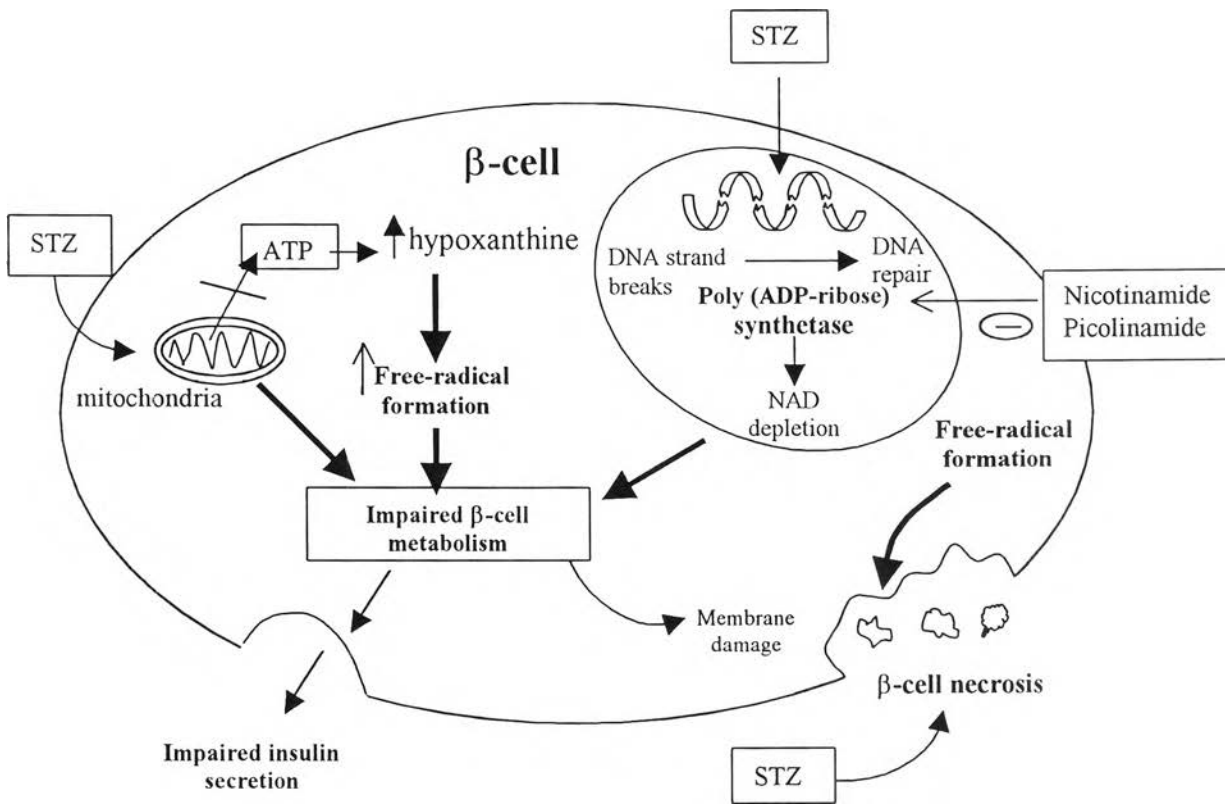


Figure 2-6 Suggested mechanisms of streptozotocin toxicity on β-cell.

Modified from (Lally and Bone, 2003)

Mechanisms of diabetic nephropathy development

Hyperglycemia, hypertension and proteinuria involved in some molecular mediators have been elucidated as the causes of diabetic nephropathy.

1. Hyperglycemia

Diabetes melitus that is poor blood-glucose controled contributes to the development of albuminuria. It has been elucidated that hyperglycemia is involed in the morphological and functional abnormalities in diabetic kidney disease. Extracellularly, glucose reacts non-enzymatically with primary amines of proteins, forming glycated compounds. Glucose, transported into cells by glucose transporters, is partly metabolized to sorbitol via the polyol pathway and to hexosamines. All these biochemical pathways have been implicated in hyperglycaemia-induced kidney damage. Furthermore, excess glucose can directly exert toxic effects by activating intracellular signalling pathways and inducing a number of cytokines injurious to the kidney.

1.1 Glucotoxicity

The high glucose milieu has been confirmed that it directly alter extracellular matrix deposition in the kidney. Studies on mesangial cells as well as tubular epithelial cells have demonstrated that high glucose concentrations induces cell hypertrophy, and increases in the extracellular matrix components, such as collagen, laminin and fibronectin (Ayo et al., 1990]. A further mechanism of the high glucose concentrations leading to the matrix deposition is the decrease in the activity of metalloproteinases, the enzymes responsible for extracellular matrix degradation (McLennan et al., 1994; McLennan et al., 2004). In mesangial cells, high glucose levels induce the transcription and secretion of transforming growth factor-β1 (TGF-β1), one of the most prosclerotic cytokines (Sharma et al., 1995, 1996).

Kidney cells do not have an absolute requirement of insulin for glucose uptake, so that the intracellular glucose level more directly reflects its plasma concentration. The importance of excess glucose entry into mesangial cells is an increase in extracellular matrix production and overexpressing the cellular glucose transporter Glut 1 (Heilig et al., 1997). Increased Glut 1 expression leads to increased

basal glucose uptake, with secondary increased aldose reductase expression and protein kinase C (PKC) activation, which results in increased extracellular matrix production (Henry et al., 1999). Using an antisense Glut 1 to protect mesangial cells from glucose–induced Glut 1 overexpression, high glucose concentrations failed to induce extracellular matrix proteins (Heilig et al., 2001). This finding indicates that factors regulating glucose transporter expression or activity can influence glucose uptake and glucotoxicity. In mesangial cells cultured in vitro, glucose itself upregulates Glut 1 expression (Heilig et al., 1997) and TGF-β1 is able to induce Glut1 upregulation (Inoki et al., 1999).

Glucose is metabolized to fructose-6-phosphate in glycolysis. Fructose-6-phosphate is converted to glucosamine-6-phosphate by the rate-limiting enzyme glucosamine-fructose-6-phosphate amidotransferase in the hexosamine pathway, leading to the formation of N-acetylglucosamine. Activation of the hexosamine pathway is implicated in development of diabetic chronic complication associated with PKC activation and TGF-β1 overexpression (Schieicher et al., 2000; Haneda et al., 2003).

1.2 Non-enzymatic glycation

Chronic hyperglycaemia leads to non-enzymatic protein glycation. Glycation results from exposure of lysine amino-terminal groups of proteins to high glucose concentrations. This leads to increased covalent binding of glucose to proteins resulting in the formation of Schiff base and form stable ketoamines, the Amadori products. These glycated proteins undergo the other reactions such as dehydration, cyclization, oxidation and rearrangement to form advanced glycation end-products (AGEs). The reaction is not reversible, and AGEs gradually accumulate in the tissues (Soulis-Liparota et al., 1995; Heidland et al., 2001). In addition, reactive oxygen intermediates are generated during the AGEs reaction (Chappey, 1997; Nagai, 1997). AGEs accumulate in renal glomeruli and tubules in diabetic animals. The accumulation of AGE in the kidney is paralleled by the development of albuminuria, mesangial expansion and glomerular basement membrane thickening in diabetes mellitus. The molecular cross-linking is also found in blood vessels caused the diabetic vascular complication (Hunt et al., 1991).

It has been elucidated various AGE-specific receptors. AGE-binding proteins or receptor for advanced glycosylation end-products (RAGE) such as AGE-R1, AGE-R2, AGE-R3, lysozyme and the macrophage scavenger receptor transduce the effect of AGE. AGE-specific receptors are present on many cell types, including mesangial, glomerular epithelial and tubular epithelial cells (Tanji et al., 2000). Interaction of AGE-modified proteins with AGE receptors results in the degradation of AGE proteins, but also induces the synthesis and release of cytokines, such as TGF-β1, platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF), and results in enhanced collagen, laminin and fibronectin production (Pugliese et al., 1997). In mesangial and endothelial cells, the AGE-RAGE interaction causes enhanced formation of oxygen radicals with subsequent activation of nuclear factor-kappa B and release of pro-inflammatory cytokines (interleukin-6, tumor necrosis factor-alpha or TNF-α), growth factors (TGF-β 1, IGF-1) and adhesion molecules (vascular cell adhesion molecule-1, intercellular adhesion molecule-1) (Heidland et al., 2001)

1.3 The polyol pathway

Glucose is reduced to sorbitol by the enzyme aldose reductase in the polyol pathway. Sorbitol accumulation in a variety of tissues, including renal tubules and glomeruli, in chronic hyperglycemia. The accumulation of sorbitol disturbs cellular osmoregulation by depletion of myoinositol (Greene, Chakrabarti and Lattimer, 1987) and by changing in the cellular redox potential (Greene, Lattimer and Sima, 1987) resulting in tissue damage in chronic diabetes mellitus. The polyol pathway is involed in the pathogenesis of diabetic nephropathy (Dunlop, 2000) and associated with Glut1 (Henry, 1999) PKC activation and TGF-β1 production (Ishii et al., 1998). Vasscular endothelial cells are also affected by sorbitol accumulation causing the vascular complication in diabetese mellitus (Koh et al., 1986). Hyperglycemic condition increases the production of reactive oxygen species (ROSs), which is implicated in sorbitol accumulation. Excessive flux of glucose in the polyol pathway results in an incerase in the ratio of reduced nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD) resulting in metabolic imbalances,

mimicking the effects of the same redox change induced by hypoxia (Williamson, 1993).

2. Hypertension

Hypertension plays a critical role in the progression of diabetic nephropathy. The development of proteinuria most takes place parallel with a gradual rise in systemic blood pressure. The increase in blood pressure closely relate to the rate of decline in glomerular filtration rate (Mogensen and Christensen, 1985). There are evidences that elevated arterial pressure causes the glomerular lesions and antihypertensive therapy can prevent the renal changes and proteinuria in diabetes mellitus (Zatz et al., 1986; Nelson et al., 1993). In addition, both type 1 and type 2 diabetic patients with normal albumin excretion, who have higher arterial pressure levels, eventually progress to microalbuminuria (Ravid et al., 1998).

3. Proteinuria

Proteinuria is a key feature of diabetic nephropathy and a strong predictor of speed of progression towards end-stage renal failure (Ruggenenti et al., 1997). Proteinuria reflects not only renal impairment and a key pathogenic element of disease progression, but also more generalized vascular damage. Excessive protein overload can induce tubulo-interstitial damage and contributes to the disease progression (Benigni et al., 1995). The excessive tubular reabsorption of proteins and the consequent accumulation of proteins in tubular epithelial cells induce the release of vasoactive and inflammatory mediators, such as, TGF-β1 endothelin 1, osteopontin and macrophage chemotactic protein-1. These factors in turn lead to infiltration of mononuclear cells, causing injury to the tubulo-interstitium, and ultimately renal damage. The changes in renal hemodynamics either primary or in response to nephron loss induce a further proteinuria contributing the progressive renal impairment Remuzzi et al., 1998; Gilbert et al., 1999).

Diabetic microvascular complications

Nitric oxide (NO) is the potent vasodilator molecule, which is produced by endothelial cells. Nitric oxide synthase (eNOS) catalyze the reaction of L-arginine changing to NO. NO also inhibits vascular smooth muscle cell (VSMC) proliferation and migration, mononuclear (MN) and polymorphonuclear (PMN) leukocyte adhesion molecule expression and platelet aggregation. Injured vascular smooth muscle cells or endothelial cells, and activated vascular wall mast cells, fibroblasts, macrophages, and leukocytes, as well as oxidation of norepinephrine (NE) from renal sympathetic nerves, produce increased amounts of reactive oxygen species (ROS). ROS then interact with NO to form the potent cytotoxic peroxynitrite radical ($OONO^-$) that interact with proteins in the kidney leading to the glomerular and tubular dysfunction (Figure 2-7).

Factors for coronary artery disease (CAD) and progressive renal insufficiency adversely affect endothelial cell or vascular smooth muscle cell function by increasing the formation of reactive oxygen species such as superoxide anion and hydrogen peroxide. These ROS result in the decreases in vasodilators and growth inhibitors such as prostacyclin and nitric oxide but increases in the formation of endothelium-derived vasoconstrictors and growth promoter such as angiotensin II, endothelin-1, and plasminogen activator inhibitor (PAI-1). These changes lead to the vascular and renal pathophysiology (Figure 2-8).

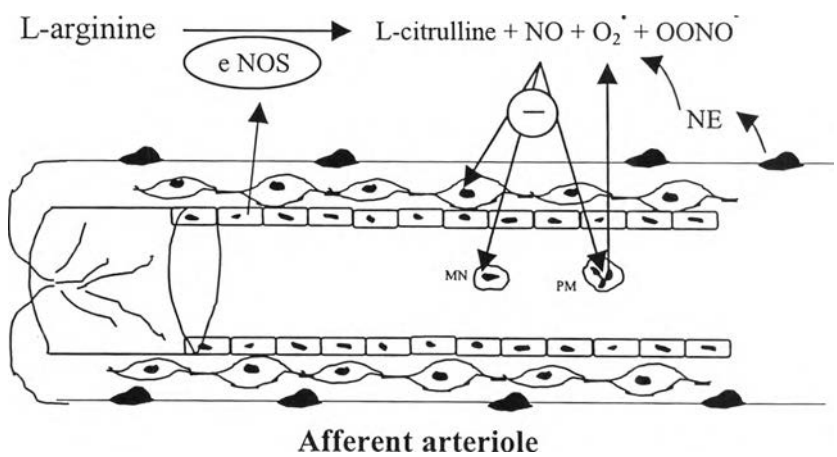


Figure 2-7 ROS reduces the biological effects of NO and induces the renal vascular complications.
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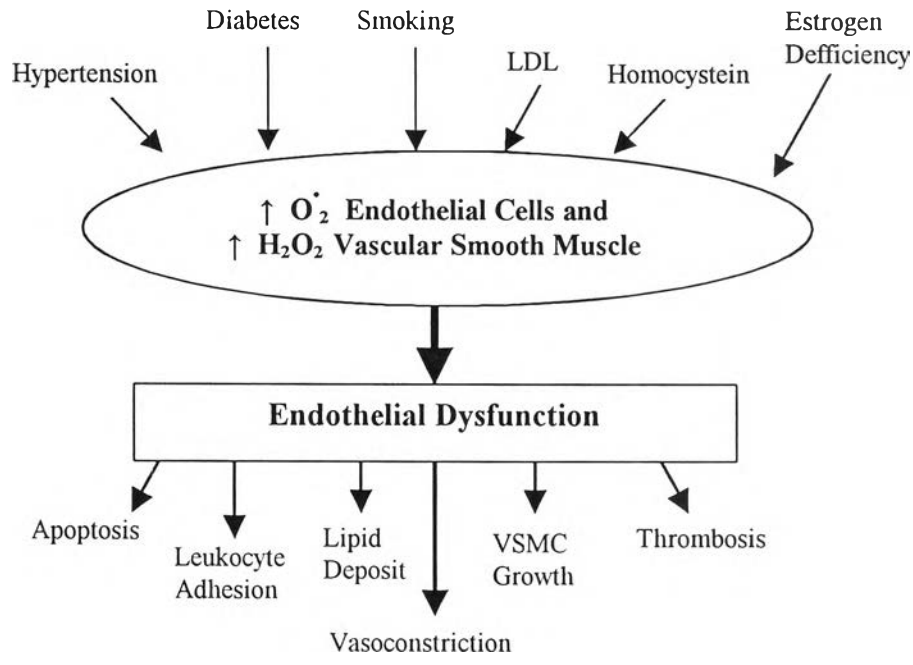

Figure 2-8 Risk factors of the endothelial dysfunction lead to the coronary artery disease and renal pathophysiology. Available from: http://www.hypertensiononline.org [2005,March 20]

Some mechanisms of the progressive coronary and renal injury including increased apoptosis or programmed cell death contribute to vascular wall remodeling and activation of cell adhesion molecules. The adherence of both mononuclear and polymorphonuclear leukocytes to the vascular wall results in infiltration and deposition of oxidized lipids in the vessel wall, which consequently cause vasoconstriction, hypertrophy and hyperplasia of vascular smooth muscle cells and thrombus formation.

Small blood vessel dysfunctions has been noted in patients with diabetes mellitus and in diabetic animals, khown as diabetic microvascular complications, resuling in tissues damages. The diabetic microvascular complications include diabetic diabetic peripheral neuropathy, diabetic retinopathy, and diabetic nephropathy. The diabetic complications are irreversible, including lower extremity amputation, blindness, and end-stage renal disease. Research in experimental animals has demonstrated that PKC overactivation can result in microvascular damage, which

is a common underlying pathophysiology of the diabetic complications (Sheetz et al., 2002). Protein kinase C β (PKC β) is an enzyme that acts as a signal transducer involved in many biochemical processes within the human body, including processes that affect vascular blood flow (Du et al., 2000; Nishikawa et al., 2000; Sheetz et al., 2002; Du et al., 2003). There are at least 12 isoforms of PKC that are located in various tissues throughout the body. Two of the isoforms, specifically β1 and β2, are located within the nerves, eyes, and kidneys and have been hypothesized to play a major role in both the development and progression of DMC (Sheetz et al., 2002). There are many biochemistry abnormalities in diabetes involved in the activation of PKC. Hyperglycemia activates at least four metabolic pathways that contribute to diabetes-induced microvascular damage. In the normal physiological glucose concentrations, the activation of PKC occurs via the cell signal transductions, which is associated with a complex sequence of biochemical interactions through G-protein coupled receptor-mediated pathway [Liu et al., 1998; Alberts, 1994; Inoguchi et al., 1994). Diacylglycerol (DAG) is the intermediate substance of glucose metabolism, which is the important mediator to signal the activation of PKC (Haneda et al., 2001). Other pathways, including glycation, polyol pathway and superoxide overproduction also play the role of diabetic microvascular complications. The activation of PKC β also accounts for the pathophysiology associated with diabetic nephropathy. The diabetic microvascular complications including an initial increase in glomerular filtration rate or creatinine clearance, mesangial matrix expansion, glomerular capillary crowding, and glomerular occlusion result in the decreased renal function (Mogensen et al., 1999; Osterby et al., 1993). The relationship of the involved factors in the microvascular damage, leading to the diabetic complications as shown in the Figure 2-9.

Hyperglycemia stimulates the development of diabetic nephropathy.

Diabetic nephropathy is one of the major causes of end- stage renal disease. The hallmarks of the pathological changes are the thickening of glomerular and tubular basement membrane (Brownlee et al., 1979). Glomerulosclerosis is a conspicuous morphological change in diabetes (Osterby et al., 1992). Glycemic control is an important way to prevent the development of glomerulosclerosis in the

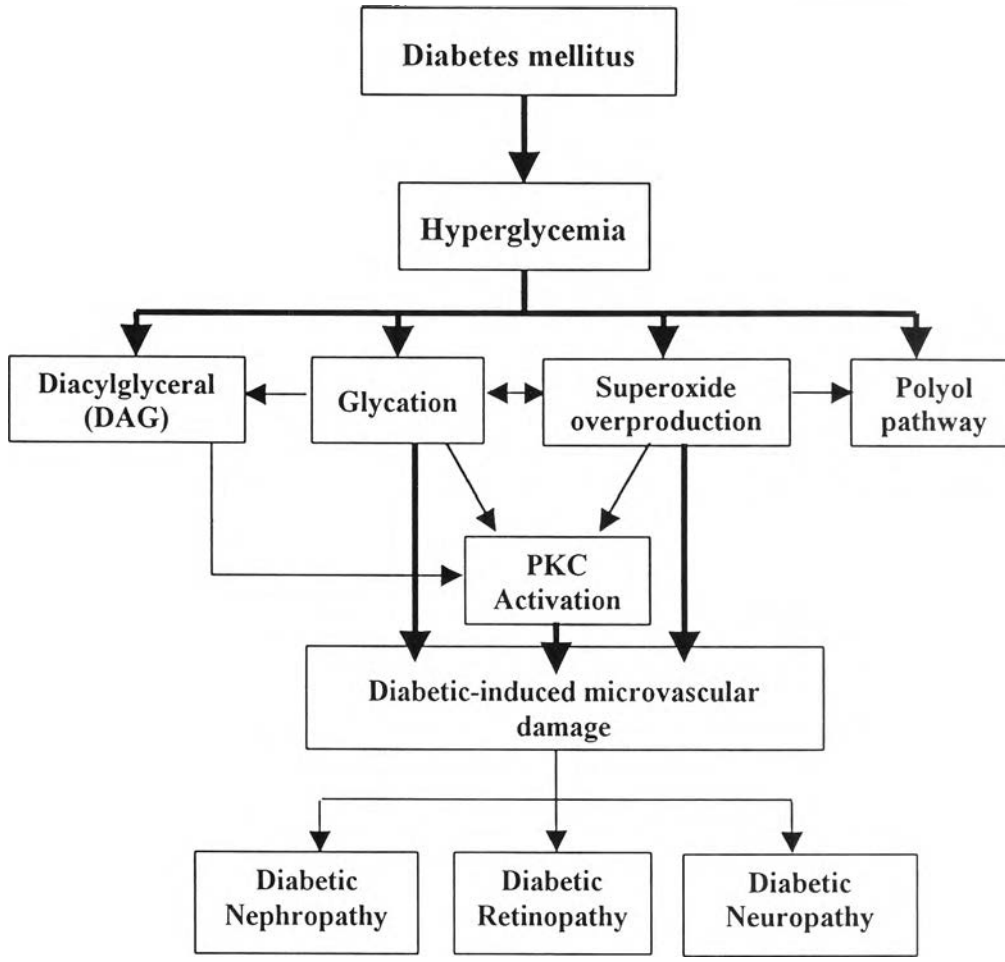

Figure 2-9 The relationship of the involved factors in the microvascular damage in diabetes mellitus. (modified from Fader, 2005)

streptozotocin-induced diabetic rats. The poorly-controlled diabetic rats with constant high plasma glucose levels showed a significant increase in the basement membrane-like materials and mesangial cell mass in comparison with the well-controlled diabetic rats (Rasch et al., 1979; Dahl-Jorgensen et al., 1992).

Transforming growth factor-beta1 (TGF-β 1) is found as a key mediator of the development of diabetic kidney disease (Sharma et al., 1995). Neutralization of TGF-β_1 by anti-TGF-β 1 antibody has been shown to attenuate kidney hypertrophy and the enhanced extracellular matrix gene expression in streptozotocin-induced diabetic mice (Sharma et al., 1996). Type II diabetic patients demonstrated increased renal production of TGF-β 1. The urinary levels of bioassable TGF-β 1 were also significantly increased compared with non-diabetic patients (Sharma et al., 1997). The

long-term administration of neutralizing anti-TGF-β 1 antibody can prevent glomerulosclerosis and renal insufficiency in diabetic *db/db* mice, the genetic model of non-insulin dependent diabetes mellitus (Ziyadeh et al., 2000). It was also noted that high glucose concentration stimulated TGF-β 1 expression in glomerular mesangial cell and proximal tubular cell culture (Sharma et al., 1995). Weigert and co-workers (2000) showed that high glucose induced TGF-β 1 gene expression via two adjacent activating protein-1 (AP-1) binding sites. It enhanced the binding activity of nuclear proteins to the AP-1 binding sites to increase TGF-β 1 promoter activity. In addition, protein kinase C (PKC) and p38 mitogen-activated protein kinase (p38 MAPK), regulating TGF-β, promoter activity were activated by high glucose concentration in the mesangial cell culture (Weigert et al., 2000).

Hyperglycemia does not only generate more reactive oxygen species but also impaires antioxidant mechanisms leading to the nephropathy in diabetes mellitus (Wohaieb et al., 1987; Willems et al., 1998; Sano et al., 1998; Chen et al., 1999; Lehmann et al., 2000; Scivittaro et al., 2000; Ha, 2001; Sozmen et al., 2001; Clarkson et al., 2002). It has been confirmed by in vitro studies that increase in oxidative stress in glomerular mesangial cells is directly induced by high glucose concentration (Ha, 2000; Catherwood, 2002). Recently, a study in rabbit kidney-cortex tubules indicated the increase in intrcellular hydroxyl free radical generation in diabetes mellitus and that might be responsible for a diminished intracellular glutathione redox state (GSH/GSSG) despite elevated glutathione reductase activity (Winiarska et al., 2004). In additon, alloxan-induced diabetic rat kidneys showed the decrease in xanthine oxidase and catalase activities. Insulin treatment could restore the antioxidant enzyme activities (Satav, Dave and Katyare, 2000).

Moreover, the high glucose concentration inhibited (^{3}H)-thymidine incorporation and decreased renal tubular cell growth. However, it increased [^{3}H]-leucine incorporation and protein content (Park, 2001). In addition, TGF-β 1-mRNA and protein overexpression, which are always found and lead to the increase in extracellular matrix synthesis in diabetic nephropathy, are stimulated by high glucose both *in vivo* and *in vitro*. It has been investigated that PKC is involved in the renal injury in diabetes mellitus. In the condition of high glucose ambient, PKC is

translocated and activated following the overexpression of TGF-β 1-mRNA and protein. Mesangial cells and endothelial cells are affected by the high glucose concentration, causing the mesangial expansion and the thickening of glomerular basement membrane in diabetic nephropathy (Mauer et al., 1984; Osterby et al., 1992; Heidland et al., 2001). The high glucose concentration increases the secretion of TGF-β 1 via the PKC-oxidative stress pathway (McLennan et al., 1994; Park, 2001). Inhibition of PKC effectively blocks high glucose-induced fibronectin production. It also inhibits the fibronectin production induced by H_2O_2 (Ha et al., 1999).

Oxidative stress induces diabetic nephropathy

With the evidences from *in vitro* and *in vivo* studies indicated that lipid peroxidation was enhanced both diabetic animals and humans (Zhang et al., 1997; Sano et al., 1998; Clarkson et al., 2002). Decreases in the antioxidant enzymes including catalase, superoxide dismutase and glutathione peroxidase were also found in diabetes. The presence of increased oxidative stress in uncontrolled diabetes manifests the marked alterations in tissue antioxidant status (Wohaieb et al., 1987; Willems et al., 1998; Chen et al., 1999). Under physiological conditions, glucose has been shown to undergo oxidation, producing protein reactive ketoaldehyde, hydrogenperoxide and highly reactive oxidant (Hunt and Wolff, 1991). Hyperlipidemia in diabetics can induce nephropathy via the generation of reactive oxygen species (Kasiske et al., 1990; Eddy et al., 1998; Scheuer et al., 2000; Clarkson et al., 2002). Attachment of these reactive oxygen species to proteins contributes to protein fragmentation and cross-linking in diabetes mellitus (Baynes et al., 1991; Soulis-Liparota et al., 1995; Salahuddeen et al., 1997). Furthermore, increased free radicals can induce apoptosis, which also contributes to the development of diabetic nephropathy (Sharma et al., 1996; Riedle et al., 1997; Ha et al., 1999).

High glucose milieu induces Glut1 and TGF-β 1 overexpression in diabetic glomeruli.

Glut1, which is a major glucose transporter of kidney glomeruli and glomerular mesangial cells, plays a key role in the pathogenesis of glomerulo-sclerosis. It is upregulated in mesangial cells exposed to the condition simulating diabetes and also on diabetic mouse mesangial cells (Zhang et al., 2000). The increased rate of glucose metabolism intracellularly promotes the pathological changes in diabetic nephropathy. It has been demonstrated that elevated extracellular glucose concentration (20 mM) facilitates Glut1 expression in rat mesangial cells and uptake of glucose analog ^{3}H 2-deoxyglucose (^{3}H 2-DOG) compared to the cells chronically adapted to physiological glucose concentration (8 mM). Overexpression of Glut 1 in mesangial cells can increase in aldose reductase, protein kinase Cα and native Glut 1 transcription in both normal glucose and high glucose condition (Kashiwagi et al., 2001). Glucose transport has been shown to be a rate-limiting for extracellular matrix production in the mesangial cells (Koya et al., 1997). Hyperglycemia induces Glut 1 overexpression leading to the increase in glucose uptake of mesangial cells resulting pathological changes of diabetic nephropathy.

It has been elucidated that not only Glut 1 but also TGF-β is upregulated by high glucose concentration in glomerular mesangial cells (Mogyorosi et al., 2000). The high glucose ambient increased Glut 1 expression and glucose transport activity when compared with physiologic glucose concentration. Treatment of rat mesangial cells with TGF-β can regulate Glut 1 mRNA and protein levels and significant increase in glucose uptake. Cultured mesangial cells transducted with the human Glut1 gene and thus over expressing the Glut 1 protein showed the marked increase in glucose uptake and the synthesis of extracellular matrix molecules. Both proteins of TGF-β and Glut 1 can influence the expression of one another. Therefore, the successful interuption of the TGF-β - Glut 1 axis may beneficially affect on the striction of glucose control in the development of diabetic nephropathy (Koya et al., 1998).

Alterations of renal hemodynamics in diabetes mellitus

The elevated glomerular filtration rate (GFR) is a frequent finding in patients with early insulin-dependent diabetes mellitus (IDDM). The evidences of vascular endothelium dysfunction have been found in experimental and human diabetes mellitus. Changes in endothelial vasoactive factors, endothelial and leukocyte adhesion molecules in diabetic patients were demonstrated the involvement to the development of diabetes complications such as nephropathy and retinopathy (Dedov et al., 2001). In addition, it has been suggested that changes in various K^+ channels on smooth muscle, the biosynthesis of cyclooxygenase products such as 6-keto-prostaglandin F1α and thromboxane A2 and endothelium-dependent relaxation in streptozotocin-induced diabetic rats may contribute to the development of vascular complications (Kamata, Ohuchi and Kirisawa, 2000). In vitro study showed that angiotensin II produced renal perfusion pressure in isolated kidneys of streptozotocin-induced diabetic rats. It indicated that role of thromboxane A2 as mediators of the increase in renal vascular resistance produced by Ang II was reduced in diabetes (Cediel et al., 2002). So thromboxane (TX) A2 plays important roles on renal injuries in streptozotocin -induced diabetic rats. The ratio of levels of urinary metabolites of thromboxane A2 and prostacyclin (TX/PGIratio) of diabetics were higher than non-diabetes, suggesting the hypercoagulative states of this disease. That showed positive correlations with the levels of blood glucose reflecting the pathological conditions of diabetes (Hishinuma et al., 1999). Renal micropuncture techniques have shown that single-nephron GFR (SNGFR) is elevated in moderately hyperglycemic diabetic rats. This may be because of the elevated glomerular capillary pressure (Pgc). Several potential mediators of increased SNGFR have been examined, including hyperglycemia, increased glomerular prostaglandin production, and decreased sensitivity of the tubuloglomerular feedback mechanism. (O'Donnell, Kasiske and Keane, 1988).

Type I diabetes mellitus demonstrated a correlation of the increase in glomerular filtration rate or effective renal plasma flow and the increase in HbA1c in the early stage of diabetes mellitus. That reflected the correlation of hyperfiltration or

renal hyperperfusion and hyperglycemia in diabetes. (Soper, Barron and Hyer, 1998). In addition, no reduction in basal vasodilating NO-tone in the circulation was found at the early onset of diabetic rats. The sensitivity to vasodilating effects of acetylcholine at the level of small resistance was not impaired in the diabetic rats. A normal action of NO in the investigated tissues such as in the kidney, heart and muscle was found in the early STZ-diabetic rat (Granstam and Granstam, 2003). However increase in glomerular filtration rate and renal blood flow were found in diabetic rats at the sixth week after the onset of insulin-treated streptozotocin diabetes in Munich-Wistar rats. Simultaneously, an increase in kidney weight and a reduction in renal vascular resistance was found at the early stage of diabetes (Vallon, Wead and Blantz, 1995). In chronic diabetes mellitus, the mechanisms of the elevated GFR in insulin-dependent diabetics are enhanced RPF, increased transglomerular hydrostatic pressure gradient and increased glomerular ultrafiltration coefficient (Christiansen et al., 1981). The major renal pathologic changes of diabetes include thickening of renal extracellular basement membranes and mesangial matrix is closely and inversely related to measures of peripheral capillary wall filtration surface and to clinical features of proteinuria, hypertension, and decreasing glomerular filtration rate. These lesions are markedly advanced by the time of renal dysfunction (Lane, Steffes and Mauer, 1991).

A local inhibition of nitric oxide in the kidney associated with renal hyperfiltration, an increase in glomerular filtration rate, renal plasma flow was reported. (Ito et al., 2001). This evidence is according to the evidence of endothelium dysfunction in diabetes mellitus. However, in early diabetic stage, renal hyperfiltration and hyperperfusion may caused by the increase in nitric oxide production has been noted (Yamada et al., 1995; Mattar et al., 1996).

Oxidative stress induces mitochondrial dysfunction

Oxidative stress was apparent in various tissues of STZ-induced diabetic rats. Lipid peroxide levels were significantly increased in liver, kidney and pancreas homogenates and mitochondrial fractions in STZ-rats compared with control rats. GSH levels in hepatic and pancreatic tissues but unchanged in the kidney of diabetic rats. GSH content in hepatic mitochondrial fraction were decreased (Bastar et al.,

1998). The study of Asayama (1989) demonstrated that mitochondria in diabetic cells also underwent oxidative stress. The lipid peroxide level and mitochondrial glutathione peroxidase activity were increased while mitochondrial catalase activity was decreased in the kidneys in STZ-induced diabetic rat two weeks after the diabetic induction. The increase in mitochondrial glutathione peroxidase and manganese superoxide dismutase activities and the decrease in copper-zinc superoxide dismutase and catalase were demonstrated in diabetic liver. In diabetic heart, increases in mitochondrial glutathione peroxidase and catalase were found (Asayama et al., 1989). Eight weeks of diabetic mellitus, the levels of malondialdehyde (MDA) and carbonyls and the activities of manganese superoxide dismutase (MnSOD) were significantly increased in liver, kidney and pancreas mitochondria. While mitochondrial glutathione peroxidase activity was elevated in kidney and pancreas but decreased in liver mitochondria (Jang et al., 2000)

Metabolic and mitochondrial disturbances occurred in STZ-induced diabetic rats. Three days of diabetic stage (early ketosis) did not alter heart, kidney, or liver mitochondrial respiratory rates with glutamate or succinate even though serum glucose and triglycerides were elevated. Kidney or liver mitochondrial respiratory rates with glutamate or succinate did not change during 5 weeks of diabetes mellitus although the diabetic rats had weight gain depressed. The amount of kidney mitochondrial protein isolated per gram of tissue was increased by 30% in the diabetic rats. Seven weeks of diabetic stage, the rats had elevated liver free fatty acids and glutamate dehydrogenase activity. Serum leucine, isoleucine, and valine were elevated and serum lysine and arginine were depressed (Rogers, Friend and Higgins, 1986). Diabetes mellitus generally results in an increased systemic fatty acid mobilization which can be associated with an increase in mitochondrial and peroxisomal beta-oxidation of fatty acids in tissues. Peroxisomal beta-oxidation of fatty acids are usually accompanied by a concomitant increase in the tissue content of cytoplasmic fatty acid-binding protein (FABP) which functions in the intracellular translocation of fatty acids. It was previously found that in liver clofibrate-induced proliferation of peroxisomes and increase in FABP expression each are dependent on the induction by cytochrome P4504A1-mediated (CYP4A1) formation of dicarboxylic acids. In livers of the diabetic rat, concomitant increases of the activities of CYP4A1 and the peroxisomal key enzyme fatty acyl-CoA oxidase (FACO) and of the FABP

content were observed. In the diabetic heart FACO activity and FABP content also increased, but there was no induction of cytochrome P450 4A1 activity. Conversely, there was a marked induction of CYP4A1 activity in spite of no increase in FACO activity nor FABP content in diabetic kidney (Engels et al., 1999).

Diminished GSH level in cardiac mitochondria in type 1 diabeic mouse model indicated an impaired mitochondrial function and increased oxidative stress. This damage coincides with and may stimulate mitochondrial biogenesis. Quantitative RT-PCR indicated the increase in messenger RNA levels. Morphological study of diabetic hearts showed significantly increased mitochondrial area and number as well as focal regions with severe damage to mitochondria. Diabetic mitochondria also showed reduced respiratory control ratio apparently due to reduced state 3 rate. Further examination revealed increased mitochondrial DNA and a tendency to higher as well as increased mRNA level for mitochondrial transcription factor A and two mitochondrial encoded proteins (Shen et al., 2004). Treatment of brain mitochondria isolated from brain hemispheres of 4-month-old male Fisher rats with peroxynitrite (the product of the reaction between nitric oxide and superoxide) indicated that peroxynitrite caused a concentration-dependent impairment of oxidative phosphorylation and depletion of the endogenous antioxidants alpha-tocopherol and ascorbic acid. Peroxynitrite -induced mitochondrial dysfunction was characterized by 1) decreases in state 3 respiration and oxidative phosphorylation, 2) loss of respiratory control [ratio of ADP-stimulated (state 3) to basal (state 4) respiration], and 3) uncoupling of oxidative phosphorylation. Peroxynitrite induced reduction in respiratory control, oxidative phosphorylation and the oxidation of membrane tocopherol. (Vatassery et al., 2004).

In type II diabetes mellitus, who had the insulin resistance of skeletal muscle, underwent the impaired bioenergetic capacity of skeletal muscle mitochondria. Creatine kinase and citrate synthase activities were measured to represent markers of myocyte and mitochondria content, respectively. The activity of rotenone-sensitive NADH:O(2) oxidoreductase reflected the overall activity of the respiratory chain. The mitochondrial function study showed decreases in NADH:O(2) oxidoreductase activity and citrate synthase activity in type II diabetic patients. Electron microscopy study showed the smaller skeletal muscle mitochondria than normal subjects (Kelley et al., 2002; Schrauwen and Hesselink, 2004). In type I diabetes meelitus, STZ-

induced diabetic rats were markedly decreased in activity of 5'-nucleotidase, K^+-dependent p-nitrophenylphosphatase, ATPase and mitochondrial succinate dehydrogenase in the kidneys indicating the decrease in mitochondrial respiration in complex II at week 24 of diabetic stage. In contrast, activity of beta-hydroxybutyrate dehydrogenase was moderately increased in kidney of diabetic rats indicated the increase in mitochondrial respiration in complex I (Stefek et al., 2002).

Not only the glomerular part of the kidney develops sclerosis but also the renal tubular part (Wang et al., 2001). Renal hypertrophy and the proliferation of renal tubular epithelium were demonstrated in diabetic rats two weeks after the diabetic induction. Eight and twelve weeks after the diabetic induction, Armanni-Ebstein lesions were found in the distal tubules (Dobashi et al., 1991). Renal hypertrophy and the proliferation of renal tubular epithelium were demonstrated in diabetic rats two weeks after the diabetic induction. Simultaneously, superoxide dismutase (CuZn-SOD) in proximal tubular cells was decreased but total CuZn-SOD content of the kidneys did not increase. An intense staining of CuZn-SOD in the cells of distal tubular lesions at 8 and 12 weeks after the diabetic induction suggested an adaptive response to the enhanced oxidative stress in diabetic rats. The enhanced MnSOD staining in the thick ascending limbs of Henle's loops was assumed to response to hypermetabolism associated with the proliferation of renal tubules. This was most marked in the cells, which were rich in mitochondria, again suggesting an adaptive response to enhanced oxidative stress induced by diabetes mellitus (Dobashi et al., 1991).

The studies in renal tubular functions indicated increase in absolute proximal tubular fluid reabsorption (Bark et al., 2001) but decrease in distal tubular reabsorption (Ward et al., 2001; Slomowitz et al., 2002) in diabetes mellitus. The elevations of urine flow rate and urinary excretion of both protein and glucose may represent the reduction of renal tubular reabsorption rather than renal hyperfiltration in diabetic rats (Suanarunsawat et al., 1999). Aldose reductase and polyol pathway as well as advanced glycation end products enhance the hyperglycemia-induced cellular impairment in renal tubular cells (Dunlop, 2000). High level of advanced glycation end products in hyperglycemic condition has direct toxicity for renal mitochondria. It has an inhibitory effect on both the tricarboxylic acid cycle and the electron respiratory chain (Rosca et al., 2002). In addition, the advanced glycation products

activating the proinflammatory gene products, such as interleukin-6 (IL-6), plays a role in damaging the renal tubule (Morcos et al., 2002). However, it has been reported that STZ-induced diabetic rats did not promote brain mitochondrial dysfunction, suggesting that oxidative stress associated with type 1 diabetes is not directly related to mitochondrial dysfunction, but probably is related to extramitochondrial factors (Moreira et al., 2004).

Evidences of L-ascorbic acid beneficial effect on diabetes mellitus

Ascorbic acid (vitamin C) has been demonstrated as an antioxidant in preventing and inhibiting the renal damage induced by cytotoxic agents in rats (Appenroth et al., 1998, Greggi et al., 2000). It has a stimulatory effect on the sulphate incorporation into mesangial cells and matrix proteoglycan leading to the negatively charged extracellular matrix. High glucose is capable to inhibit the effect of ascorbic acid on the sulphation stimulation of proteoglycan molecules (McAuliffe et al., 1997). These may be particularly important in pathophysiology of diabetic nephropathy, a condition where ascorbic acid concentration is already comprised. Serum ascorbic acid is reduced in diabetic patients because of high urinary excretion of ascorbic acid, impaired tubular reabsorption of filtered ascorbic acid and increased ascorbic acid clearance (Seghieri, et al., 1994; Hirsch et al., 1998). Moreover, the maximal uptake rate (V_{max}) is significantly lower in the diabetic nephropathy cells compared to the normal (Ng et al., 1998). Supplementation of ascorbic acid can increase the concentration L-ascorbic acid in the kidneys of diabetic rats (Siman et al., 1997; Lindsay et al., 1998). A marked increase in both plasma and renal cortical ascorbic acid concentration in diabetic rats supplemented with ascorbic acid (10 g/kg. BW/d) is greater than those in untreated rats (Craven et al., 1997). It can reduce kidney weight, and prevent the increase in glomerular volume in diabetic rats. In addition, the increase in glomerular TGF-β and albumin clearance in diabetic rats can be prevented by ascorbic acid treatment (Craven et al., 1997).

The *in vitro* study of the effect of L-ascorbic acid on the nephrotoxic cysteine conjugate dichlorovinyl-L-cysteine (DCVC)-induced sublethal injury of renal proximal tubular cells indicated that L-ascorbic acid phosphate promoted recovery of the renal proximal tubular cell functions. DCVC exposure resulted in 60% RPTC

death and loss from the monolayer and decreased in mitochondrial function (54%), active Na^+ transport (66%), and Na^+-K^+-ATPase activity (77%). Immunocytochemistry and confocal laser scanning microscopy revealed the loss of Na^+-K^+-ATPase protein from the basolateral membrane of RPTC treated with DCVC. DCVC-injured RPTC cultured in the presence of 500 μM L-ascorbic acid phosphate proliferated, recovered all examined physiological functions, and the basolateral membrane expression of Na^+-K^+-ATPase by day 4 following DCVC injury. The study demonstrated that pharmacological concentrations of L-ascorbic acid phosphate did not prevent toxicant-induced cell injury and death but promote complete recovery of mitochondrial function, active Na^+ transport, and proliferation following toxicant-induced injury (Nowak et al., 2000; Nony et al., 2001). In cancer development, the resistance of cells to apoptosis is one of the most crucial steps. Apoptosis was assessed based on caspase-3-like activity, plasma membrane disintegration and finally nuclear fragmentation and chromatin condensation.

Ascorbic acid has been tested for the effect on the apoptotic response of cells to the classical antitumor drug camptothecin or the flavonoid flavone in HT-29 human colon carcinoma cells. Camptothecin and flavone induces the increase in generation of mitochondrial $O_2^{\cdot-}$ leading to the decrease in bcl- X_L. Ascorbic acid dose-dependently inhibited the apoptotic response of cells to camptothecin and flavone by preventing the generation of this reactive oxygen species resulting in specifically blocking the decrease of bcl-X_L. This evidence indicates that ascorbic acid functions as a potent antioxidant in mitochondria and thereby blocks drug-mediated apoptosis induction allowing cancer cells to become insensitive to chemotherapeutics (Wenzel et al., 2004). Supplementation of vitamin C and E has been shown to decrease retinal superoxide production and plasma hydroperoxide in diabetic rats but marginal effects on nephropathy were noted (Kil et al., 2004). In addition, supplementation of ascorbic acid with oats extract could increase ratio of P/O of liver mitochondria in alloxan-induced diabetic rats has been reported (Shamrai et al., 1978).

Transportation of dehydroascorbate and glucose via Glut 1

GLUT1 has been found in different cell types of different animal species such as rat adipocytes, Xenopus laevis oocytes (Rumsey et al., 1997), spermatozoa of rats,

bull and human (Angulo et al., 1998), rat granulosa cells (Kodaman and Behrman, 1999), osteoblast, leukocytes (Wolf, 1996), erythocytes (Klepper et al., 1998; May, 1998) and endothelial cells at the blood-brain barrier (Agus et al., 1997; Klepper et al., 1999). GLUT1 is found to be a trasporter of both glucose and dehydroascorbic acid (DHA) (May, 1998). There are two channels in Glut 1. One of them transversing the structure completely composing of amphipathic residues to form an aqueous pore or barrel-like structure. The interior surface of the aqueous pore would be made up of polar side chains. These polar groups provide hydrogen-binding sites for glucose and other similar molecules. Glut proteins act through conformational changes that expose glucose-binding site alternatively to the external and internal surfaces of the transporter. The transporter has two ligand binding sites both inside and outside of the transporter. The exofacial binding site is responsible for glucose entry into the transporter while the endofacial binding site is required for efflux of glucose (Zuniga et al., 2001).

Ascorbic acid is transported into cells via two mechanisms. Firstly, it can be transported into cells directly through sodium-dependent transporter (Wolf, 1996). Secondly, after ascorbic acid is oxidized and changed to dehydroascorbic acid (DHA) outside the cells, DHA is taken up by those cells via Glut 1. Intracellular DHA is rapidly reduced to ascorbate by GSH. Since DHA has a structure similar to that of glucose, thus, glucose transporter is also act as DHA transporter (Zuniga et al., 2001).