Chapter-2  

Reviews of Literature

Diabetes is recognized as a devastating disease worldwide. Oxidative Stress is one of the significant factors associated with development and progression of diabetes. It is marked by rise in concentration of free radicals with simultaneous drop in redox defense scavengers. (Iwashima Y et al, 1990; Hunt J V et al, 1990; Baynes J W, 1991; Gulteridge J M C, 1993; Wierusz-Wysocka B et al, 1995). Recent studies have suggested that oxidative stress in cells or neurons is associated with elevated levels of glucose. This is attributed to increased levels of anti-oxidant enzymes in the cell as a result of acute glucose elevation. Furthermore, a number of signaling pathways within a cell are either activated or modified by oxidative stress resulting ultimately in insulin resistance along with other complications associated with type 1 and type 2 diabetes.

2.1 Oxidative Stress in Prediabetics

Several studies have illustrated the presence of oxidative stress in prediabetic stage; for example, children have increased oxidative stress and SOD levels at the onset of type 1 diabetes (Dominguez C et al, 1998). Furthermore, subjects with increased glucose intolerance have increased levels of Vitamin A, isoprostanes, TBARS and inflammatory cytokines coupled with reduced levels of AOS (Niskanen LK et al, 1995; Vijailingams S et al, 1996; Tavridou A et al, 1997; Gopoul NK et al, 2001). These increased levels are attributed to increased oxidative stress.

A large pool of data has been evaluated to access the effect of insulin and selected lipid fractions on oxidative stress. It is well established that obesity, characterized by hyperglycemia and dyslipidemia, is associated with oxidative stress. (Ciccone M et al, 1999). Furthermore, studies conducted on oxidative stress have also revealed that variation in insulin sensitivity is related to lipid hydroperoxide levels and reduced levels of catalase and vitamin E in healthy populations (Facchini FS et al, 2000). The study proposed that oxidative stress may be the early event in the long history of diabetes. Moreover, the role of oxidative stress in the development of diabetes is also suggested by an experiment showing the appearance of fasting hyperglycemia within
days after administration of a pro-oxidant to insulin resistant obese Zucker rats (*Laight DW et al, 2000*). In addition, studies performed by *Domínguez C et al.* (*Domínguez C et al, 1998; Domínguez C et al, 1999*) on newly diagnosed children and young diabetic patients with no clinical complications of diabetes revealed presence of increased oxidative stress. This suggests that increased oxidative stress contribute towards the development of diabetes.

### 2.2 Genetics and Diabetes

Diabetes is broadly divided into type 1 and type 2 diabetes. Both type of diabetes run in families suggesting a strong underlying genetic component. However, the etiology of type 1 and type 2 diabetes displays a different genetic component. The probability of onset and developmental risk of the type 2 diabetes is 2 to 6 times higher among the subjects having positive family history of the disease in contrast to individuals with no family history of the disease (*Harrison TA et al, 2003*).

The relative risk of acquiring the disease of genetic origin is referred to as lambda value (*Taylor A, 2006*). This value provides an estimate of the risk of disease in the subjects who are relatives of individuals with diabetic complications compared to those of general population. Therefore it is used in describing the size of familial genetic component. In the case of type 2 diabetic patients the lambda value has been determined to be 3.5 (*Taylor A, 2006*). Furthermore, in case of type 2 diabetic patients, the risk of development of diabetes in offspring is 40%, if only one parent is diagnosed with the disease. This risk increases to 70% when both the parents of offspring have type 2 diabetes (*Tilburg van J et al, 2001*). Moreover, there exists a very high rate of occurrence of type 2 diabetes in monozygotic twins.

Studies have been carried out in India and abroad to better understand the relationship between genetics and diabetes. These studies revealed that Indians have genetic predisposition to diabetes, which get easily unmasked in conditions where adverse environmental conditions, habits and culture etc are prevalent. Furthermore, a strong familial aggregation was observed among Asian Indians with high prevalence among first-degree relatives coupled with vertical transmission through two or more generations (*Mohan V, 2004*). Various studies have investigated the prevalence of diabetes among the offspring of two type 2 diabetic parents in India. These studies
have demonstrated that 55-60 per cent of offspring had diabetes or impaired glucose tolerance (Mohan V et al, 2003). This was considerably higher than those reported for prevalence of diabetes in offspring of American or European diabetic parents (Meigs JB et al, 2000; Sargeant LA et al, 2000).

Before the onset of fully developed type 2 diabetes, individuals at risk for type 2 diabetes show impaired insulin action (Warram JH et al, 1990) and impaired insulin secretion (Pimenta W et al, 1995). Evidence now suggests that both these defects precede type 2 diabetes (Weyer C et al, 1999), and are inherited (Bogardus C et al, 1989; Schumacher MC et al, 1992; Sakul H et al, 1997; Elbein SC et al, 1999). Furthermore, data from multiple laboratories support a genetic basis for both insulin sensitivity and insulin secretion.

2.3 Plasma Antioxidant Potential

Free radical induced oxidative damage is involved in various diseases including cardiovascular disorders (CVD), neural disorders such as Alzheimer and Parkinson’s disease, diabetes and cancer.

A number of methods have been developed for measuring the total antioxidant capacity (TAC) in order to quantify free radicals along with their actions. These methods for measuring antioxidant capacity are mostly based on either quenching of stable free radicals such as 1,4-diphenyl-2-picrylhydrazyl (DPPH) (Aquino R et al, 2001) and 2,2,5-azobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) (Alzoreky N et al, 2001) by antioxidants, or on inhibition of lipid peroxidation (Banerjee D et al, 2003). These methods include (a) FRAP assay (ferric reducing ability of plasma assay) (Benzie & Strain 1996) and (b) Fluorimetric measurement of h-phycoerythrin (in the ORAC, Oxygen Radical Absorbance capacity assay) (Cao G & Prior RL, 2002) etc. These assays are widely used in biochemical analysis of clinical samples due to their sensitivity and ease of sample preparation (William PA et al, 1993). However most of them are time consuming and utilize expensive chemicals and/or instrumentation.

The FRAP assay among these methods offers a putative index of antioxidants along with reducing potential of biological fluids. FRAP assay is simple, fast and convenient and reproduce good results. This method measures the reducing power of
most important antioxidants such as vitamin C, vitamin E, uric acid and bilirubin. However this method is not useful for evaluating the reducing power of glutathione and thiol groups.

Controversial reports have been obtained regarding the plasma antioxidant potential in diabetic patients (Ashour M et al, 1999; Palanduz S et al, 2001; Turk HM et al, 2002; Cigremis Y et al, 2003; Memisogullari R et al, 2003). One of the significant study dealing with the measurement of oxidative damage, plasma antioxidant capacity and glucemic control in elderly non insulin dependent diabetic mellitus (NIDDM) patients resulted in reduced total reactive antioxidant potential (TRAP) of plasma and increased basal oxidation products in erythrocytes of diabetic patients (Aguirre F et al, 1998).

Increased plasma oxidative stress, which is as a result of excessive free radicals, is associated with decreased availability of antioxidants. (Kristal BS et al, 1997; Sukalski KA et al, 1993). A study carried out in acute and diabetic rats predicted significantly low plasma FRAP values in chronic diabetic rats as compared with their respective controls. An insignificant decrease was also observed in acute diabetic rats. (Cakatay U & Kayali R, 2006).

In another concurrent study, the levels of glucose, malondialdehyde (MDA) the by-product of lipid peroxides, and total anti-oxidant capacity (TAC) were estimated in plasma of control and experimental groups of rats. A significant increase in the levels of plasma glucose, and MDA with a concomitant decrease in the levels of TAC was observed in diabetic rats (Milani E et al, 2005).

### 2.4 Erythrocyte Reduced Glutathione

Glutathione (GSH) is one of the most important cellular anti-oxidant and cell protectant. It is synthesized from glutamate, cysteine and glycine. The synthesis is catalyzed sequentially by two cytosolic enzymes i.e. $\gamma$-Glutamylcysteine synthetase and GSH synthetase and is regulated by activity of $\gamma$-Glutamylcysteine synthetase, availability of cysteine and GSH feedback inhibition.

GSH plays an important role in anti-oxidant defense, nutrient metabolism and regulation of cellular events including gene expression, DNA and protein synthesis,
apoptosis, signal transduction, cytokine production, immune response and protein glutathionylation. Glutathione deficiency contributes towards oxidative stress which plays a key role in ageing and pathogenesis of different diseases including Kwashiorker, Seizure, Alzheimers disease, Parkinson’s disease, Liver disease, Cystic fibrosis, sickle cell anemia, HIV, AIDS, Cancer, Heart attack, smoke and Diabetes (Wu G et al, 2004). Animal and human studies have demonstrated that adequate protein nutrition is crucial for the maintenance of GSH homeostasis.

![Structure of Glutathione](image)

**Figure 7** Structure of Glutathione

Erythrocyte is a typical cell where pro-oxidant/antioxidant balance can be disturbed depending on the rate of glycolysis and pentose phosphate pathways (Nelson DA, Davey FR, 2001). In fact, erythrocyte oxidative stress is implicated in the pathogenesis of diabetes mellitus (Dumanswala U et al, 2001; Maxwell SR, 1995) and deficiency of antioxidant defense by the erythrocyte GSH pathway is thought to be one of the factors responsible for development of complications in diabetes mellitus (Dincer Y et al, 2002). Thus, the cell is well endowed with GSH, which is one of the endogenous antioxidants involved in cellular response to oxidative stress (Richards R et al, 2000; Maritim A et al, 2003).

The levels of erythrocyte GSH decreases in patients suffering from diabetes mellitus (Jam SK, McVie R, 1994). This deficiency is attributed to impairment in GSH biosynthesis (Jam SK, McVie R, 1994) and in part, contributes towards symptoms associated with diabetes. Typically glutathione cycle operates inside the cell to
dispose of hydrogen peroxide generated in the cell supplementing the function of catalase. During this cycle, reduced glutathione (GSH) gets converted into oxidized form GSSG. This is catalyzed by glutathione peroxidase. In the second step, oxidized form GSSG is converted back into reduced glutathione (GSH) by the enzyme glutathione reductase. This enzyme requires the cofactor NADPH. The NADPH is supplied by oxidation of glucose through pentose phosphate pathway in the erythrocytes. This pathway is normally promoted by insulin. However, under diabetes mellitus conditions, the efficacy of this pathway is hampered resulting in decreased formation of NADPH which results in altered level of glutathione in these subjects.

The mechanism underlying the relationship between prediabetics belonging to type 2 diabetic families and GSH levels relating to other antioxidants are still a matter of controversy. Some of the studies including that carried by Ezekiel U et al, 2006 have shown significant changes of erythrocyte glutathione content in the course of Diabetes mellitus including the prediabetes stage and cardiovascular disease comorbidity. The erythrocyte GSH levels in prediabetes and diabetes mellitus was significantly lower compared to the control group. The substantially reduced level of GSH in the prediabetes group indicated that both the oxidative stress in diabetes mellitus and the initial phases of response by the erythrocytes to oxidative stress commence prior to the establishment of diabetes (Ezekiel U et al, 2006). Furthermore, a recent report showed the relationship between oxidative stress and pathogenesis of diabetes. This was attributed to an imbalance in the oxidant/antioxidant ratio in first-degree relatives of type 2 diabetic patients (Sathiapriya V et al, 2007). They reported that erythrocyte GSH was depleted in subjects who were non-diabetic first degree relatives of type 2 diabetic patients.

2.5 L - Cysteine influx in erythrocytes

The amino acid L-Cysteine has been termed as the “semi-essential” amino acid. It is the only amino acid that has a free thiol (-SH) functional group. This amino acid is required by erythrocytes for synthesizing glutathione hormone (Griffith OW, 1992). L-cysteine also plays an important role in maintenance of proper intracellular or extracellular redox status.
Since the availability of cysteine is the main limiting factor in the intracellular synthesis of GSH, N-acetyl cysteine (NAC), a precursor of cysteine, is given as a dietary supplement to augment the GSH synthesis in cells. NAC is easily absorbed by the intestine, and gets converted to circulating cysteine by de-acetylation. It helps raise abnormally low GSH levels back to normal. This is the basis for using it as an antidote to acetaminophen's liver toxicity (Corcoran GB & Wong BK, 1986; Hoyumpa AM, 1996).

Studies conducted by Mattia De G et al, 1998 assessed in vivo effects of antioxidant on expression of vascular cell adhesion molecule (VCAM)-1 in healthy individuals as well as in NIDDM patients without complications, before and after one month of consumption of oral N-acetyl-L-Cysteine. Treatment with N-Acetyl-L-Cysteine (NAC) decreased plasma VCAM-1 and intra-erythrocyte GSSG but increased the concentration of GSH and GSH: GSSG ratio in NIDDM patients in contrast to their concentrations in healthy individuals. NAC is a free radical scavenger and a precursor of cysteine has been suggested to increase glutathione synthesis. This study highlighted that treatment of antioxidant counterbalance endothelial activation and may slow down progression of vascular damage in type 2 diabetes mellitus (Mattia De G et al, 1998).

Several lines of evidence suggest that diabetic conditions affect metabolism of all sulfur-containing amino acids such as methionine, homocysteine, and L-cysteine, in human subjects (Herrmann W et al, 2005) and also in diabetic animal models including streptozotocin-treated rats (Glanville NT et al, 1984; Gursu MF et al, 2002) and Zucker fatty rats (Wijekoon EP et al, 2004). Plasma L-cysteine levels was also found to be elevated in diabetic patients with diabetic nephropathy renal complications (Herrmann W et al, 2005) and in the streptozotocin treated rats (Glanville NT et al, 1984).

2.6 Lipid Peroxidation and Malondialdehyde (MDA)

Lipid peroxidation is a free radical chain reaction. It is accelerated by reactive oxygen species (Boff J & Min DB, 2002). Cell membranes are phospholipid bilayers with extrinsic proteins and are the direct target of lipid oxidation (Girotti A, 1998). As lipid oxidation of cell membranes increases, the polarity of lipid phase surface charge
and formation of protein oligomers increase with simultaneous decrease in molecular mobility of lipids, number of SH groups, and resistance to thermal denaturation. A high level of lipid oxidation products can be detected in cell degradation products after cell injury or disease. Increased levels of lipid oxidation products are found in diabetes, atherosclerosis, liver disease, apoplexy and inflammation (Lee M et al, 2003).

The peroxidation of membrane lipids results in one or more of the following effects on membrane:

- increased membrane rigidity
- decreased activity of membrane-bound enzymes
- altered activity of membrane receptors.
- altered permeability

Lipid peroxidation, owing to free-radical activity, plays an important role in the development of complications of diabetes. Increased levels of lipid peroxidation, as a consequence of free radical activity, have been reported in both type 1 and type 2 diabetes with vascular complications (Jennings PE et al, 1991; Griesmacher A et al, 1995). Furthermore, one of the recent studies by Whiting PH et al, 2008 suggested that chronic hyperglycemia can influence the generation of free radicals. This may ultimately lead to lipid peroxidation and depletion of antioxidants resulting in enhanced oxidative stress in subjects with type 2 diabetes.

The primary product of lipid peroxidation is MDA. It causes tissue membrane damage by reaction of oxygen with polyunsaturated fatty acids (Kehrer JP, 1993). The erythrocyte membrane is prone to lipid peroxidation under oxidative stress conditions that involves cleavage of polyunsaturated fatty acids at their double bonds leading to the formation of malondialdehyde (MDA) (Kawamoto R et al, 2005).

As shown in Scheme 1, formation of toxic aldehydes such as MDA involves oxidation of complex lipids caused by oxygen-derived free radicals (OFR). These radicals are formed by lipoxygenases as a response to cell injury, typically from H₂O₂, or melatonin on radical complex.
The major targets of these damaging species are the long-chain polyunsaturated fatty acids of cellular phospholipids, which are particularly prone to attack because of the arrangement of double and single bonds. The resultant lipid peroxide frequently decomposes to a radical (Spiteller G, 1998), which reacts with most biological molecules, including proteins and lipids. Further decomposition of these lipid peroxides produces toxic aldehydes, in particular 4-hydroxynonenal (mainly from linoleic acid), malondialdehyde (mainly from arachidonic acid) (Esterbauer H et al, 1990) and acrolein (Uchida K et al, 1998). The toxicity of MDA arises from its high reactivity, particularly towards proteins and DNA. Under normal circumstances the extent of lipid oxidation is largely controlled by antioxidant concentration in the surrounding medium, which is usually sufficiently high to prevent propagation of oxidative free radical reactions by OFR in blood. However, in tissue, there is a greater likelihood that localized deficiencies of antioxidants would allow lipid oxidation to occur. This led to a huge interest in dietary antioxidants and their protective role in cardiovascular diseases. (Rimm EB, 1993).

A number of studies revealed increased concentration of MDA in plasma of subjects with diabetes mellitus. Furthermore, MDA is also present in the atherosclerotic plaque deposits promoted by diabetes. (Kume S et al, 1995). Moreover, some of the recent findings illustrate a positive correlation between amount of MDA and duration

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**Scheme 1** Peroxidation of Lipids into Malondialdehyde

![Scheme 1](image-url)
of diabetes. This clearly suggests that oxidative stress progressively increases during the course of the diabetes. Other indices of lipid peroxidation and antioxidant defense system have also been shown to be related with duration of diabetes (Hsu WT et al, 2006). In addition, one of the recent studies demonstrated an elevated concentration of MDA in the first degree relatives of subjects with type 2 diabetes. This shows the role played by oxidative stress towards development of diabetes (Sathiapriya V et al, 2007).

2.7 Superoxide Dismutase and diabetes

Superoxide Dismutase (SOD) represents one of the major Reactive Oxygen Species (ROS) dependant enzymes. As shown in Figure 8, SOD converts superoxide anion radicals produced in the body to hydrogen peroxide. This reduces the likelihood of superoxide anion interacting with nitric oxide to form reactive peroxynitrite.

$$O_2^- + O_2^- \xrightarrow{\text{SOD}} O_2 + H_2O_2$$

**Figure 8** Conversion of superoxide anion into hydrogen peroxide by superoxide dismutase

Isoforms of SOD are present at different locations within the cell. For example, CuZn-SOD (Copper Zinc-SOD) occurs in both cytoplasm and nucleus, Mn-SOD (Manganese-SOD) is confined to the mitochondria, but can be released into extracellular space.

Increased interest in SOD, in recent years is triggered by the role that SOD plays in ageing and pathogenesis including diabetes. The effect of diabetes on the activity of SOD is erratic, with no discernable pattern. For example, some studies reported no change in SOD activity (Kesavulu MM et al, 1985; Sekeroglu MR et al, 2002) while others reported increased activity (Marritim AC et al, 2003; Sailja YR et al,
In addition, there are also reports of decreased SOD activity in diabetic patients. *(Sundaram RK *et al*, 1996).*

Studies carried out by *Soliman GZA*, 2008 identified increased levels of MDA and SOD along with decreased level of glutathione in diabetic patients, irrespective of gender. This shows that diabetic patients were exposed to an increased oxidative stress via lipid peroxidation. Moreover, decreased level of glutathione suggests decreased scavenging capacity of glutathione-dependent anti-oxidant defensive system against elevated lipid peroxidation processes in these patients. In addition, results from this study also suggested that increase in lipid peroxidation and the decline in antioxidant defenses may appear early in type 2 diabetic patients, prior to the development of secondary complications. This plays an important role in the initiation and progression of diabetic complications. Finally, studies performed by *Turk HM et al.*, 2002 showed increased activity of superoxide dismutase activity and decreased activity of catalase suggesting that these alterations may be attributed to the compensatory mechanisms of the antioxidant system in type 2 diabetes patients.

### 2.8 Catalase and Diabetes

Catalase is one of the important intracellular reactive oxygen species scavenging enzyme. As shown in *Figure 9*, this enzyme is involved in the reduction of hydrogen peroxide into water and oxygen, and thus protects mammalian cells against oxidative damage. Hydrogen peroxide has been reported to damage pancreatic beta cells *(Murata M *et al*, 1998; *Tiedge M et al*, 1998; *Jorns A et al*, 1999).* This inhibits insulin signaling *(Hausen LL *et al*, 1999)* resulting in abnormal or reduced insulin secretion. Catalase protects the pancreatic beta cells from damage by hydrogen peroxide *(Tiedge M *et al*, 1997; *Tiedge M et al*, 1998).*

Studies carried out by Hainen C *et al* in 2005 showed that reactive oxygen species and nitric oxide are proposed mediators of β-cell dysfunction in type 1 diabetes. They produced transgenic mouse with increased cell expression of manganese superoxide dismutase (MnSOD) and Catalase. Expression of these antioxidants increased the cell ROS scavenging and improved the cell survival after treatment with different sources of oxidative stress. MnSOD or Catalase offered conferred protection against streptozotocin (STZ) induced cell injury. Furthermore, co-expression of MnSOD and
catalase provided synergistic protection against peroxynitrite and STZ. Hence, their study suggested that antioxidants benefit beta cell survival against ROS.

According to the literature, the activity of catalase, like SODs, is modulated by a number of stimuli and is indeed regulated to compensate for the biological requirements imposed by increased oxidative stress.

$$2\text{H}_2\text{O}_2 \xrightarrow{\text{Catalase}} 2\text{H}_2\text{O} + \text{O}_2$$

**Figure 9** Conversion of hydrogen peroxide into water and oxygen by catalase enzyme

There is significant difference in the activity of antioxidant enzymes such as catalase between diabetic and non-diabetic patients (Jandric-Balem M *et al.*, 2003) under normal conditions. A number of studies have been done till date wherein catalase activity in plasma, erythrocyte, leukocyte and tissues were reported in diabetic humans and rats in contrast to their non-diabetic counterparts. For example, studies carried out by Strother RM *et al.*, 1992 showed decreased hepatic and increased cardiac catalase activity in diabetic rats when compared with the non-diabetic ones. Similarly, Turk HM *et al.*, 2002 while studying the plasma catalase activity reported significant decreased in type 2 diabetic patients when compared with normal ones. In another piece of work, studies carried out by Kesavulu MM *et al.*, 2001 showed increased erythrocyte catalase activity in type 2 diabetic patients in contrast to control subjects. In another study, Muchova J *et al.*, 1999 reported that catalase activity showed no difference between normal and type 2 diabetic patients. In addition, in a recent study Qujeq D & Rezvani T, 2007 measured erythrocyte catalase activity in streptozotocin induced diabetic rats which showed increased catalase activity. The increase in the activity of erythrocyte catalase is attributed to the oxidative damage of membrane protein and lipid as a result of increased oxygen free radicals in the body. Cumulative results from these studies suggested that catalase activity differs markedly in different cell types. Furthermore, all these studies converge towards the protective role which catalase plays in removal of free radicals.
2.9 Nitric oxide and Diabetes

Nitric oxide is a vital biological molecule. It is the product of five electron oxidation of amino acid L-Arginine mediated by one of three isoforms of nitric oxide synthase. It plays a significant role in diverse biological processes such as host defense, homeostatic and development function, cardiovascular regulation, signal transduction, neurotransmission and wound healing. Any pathology of Nitric oxide production in the body can result in numerous disorders and diseases. Because of this, Nitric oxide is also called a vital-poison, the right amount of Nitric oxide production is essential for life, but too much or too little can be deadly poisonous. In most life-threatening diseases like hypertension, atherosclerosis, and diabetes, the basal endothelial production of nitric oxide is lower than healthy system (Forte P et al, 1997). Nitric oxide exposed in human plasma can deplete the concentration of ascorbic acid and initiate lipid peroxidation (Halliwell B, 1996).

Nitric oxide is also strongly associated with endothelial function. Endothelial dysfunction defined as the impaired ability of vascular endothelium to stimulate vasodilatation plays a key role in the development of atherosclerosis and in various pathological conditions such as hypercholesterolemia, hypertension, type 2 diabetes, hyperhomocystinemia and chronic renal failure (Kurowska EM, 2004). The major cause of the endothelial dysfunction is decreased bioavailability of nitric oxide (NO), a potent biological vasodilator produced in vascular endothelium from L-arginine by the endothelial NO synthase (eNOS). In vascular diseases, the bioavailability of NO can be impaired by various mechanisms, including decreased NO production by eNOS, and/or enhanced NO breakdown due to increased oxidative stress.

The relationship between nitric oxide (NO) and resistance to insulin-mediated glucose disposal is controversial. For example, there is evidence that endothelial NO synthesis and insulin sensitivity are positively correlated in healthy volunteers (Petrie JAR et al, 1996). This physiological effect could well be mediated by the apparent ability of insulin to increase NO release, accounting for the vasodilatory effects of insulin (Baron AD et al, 1994; Baron AD et al, 1995). The insulin-mediated vasodilatory response is decreased in obese individuals and and patients with type 2 diabetes (Laakso M et al, 1990; Laakso M et al, 1992). Furthermore, NO production may be normal or enhanced in patients and/or in animal models with type 2 diabetes.
(Sobrevia L & Mann GE, 1997; Pieper GM et al., 1998; Catalano M et al, 1997). However, not all studies have confirmed this finding, and it is possible that elevated NO concentrations occur only in patients with type 2 diabetes in early stages, at a time when vascular complications are absent (Munzel T et al, 1997; Pieper GM, 1998).

Moreover, recent study carried out on non-diabetic siblings of patients with type 2 diabetes measured plasma concentration of nitric oxide and cyclic-GMP (Piatti PM et al, 2000). The basal NO level was evaluated by measurement of its stable end products (Nitrite and Nitrate levels). In contrast to healthy individuals, levels of plasma NO levels were found to be significantly higher than its messenger cyclic GMP in subjects with family history of diabetes irrespective of their degree of glucose tolerance. This study suggested that alteration in NO/Cyclic GMP pathway may be an early event in type 2 diabetic families with a possible effect on insulin resistance (Piatti PM et al, 2000).

2.10 Plasma membrane redox system and Ascorbate recycling

Ascorbic acid or Vitamin C is a primary antioxidant in cells and plasma, only humans, higher primates and guinea pigs cannot make ascorbic acid and thus require it through diet, for all other animals ASC is not a vitamin. Studies carried out on
erythrocytes indicate that ascorbate can interact directly with plasma membrane as an antioxidant. It can also donate electrons to transplasma membrane electron transport activity in erythrocytes (Schipfer W et al, 1985; May JM et al, 1995). The tendency of ascorbate to participate in one electron interaction also fits with its ability to transfer electron into and across plasma membrane. The transplasma membrane has been typically measured by tracking the reduction of ferricyanide to ferrocyanide (Mishra RK & Passow H, 1969). Ferricyanide is used to induce oxidative stress across the membrane and to quantify ascorbate recycling. Ferricyanide reduction has been most studied in human erythrocytes. Erythrocytes, being the most abundant cells in the blood, have been reported to play a crucial role in recycling ASC in blood plasma (Mendiratta S et al, 1998). These cells lack intracellular membranes and organelles and thus provide a simple compartment system for study. Erythrocytes reduce the oxidant ferricyanide to ferrocyanide by using electrons from ascorbate, which in turn gets converted into intracellular ascorbate free radical and dehydroascorbic acid (DHA). Although ferricyanide generates both AFR and DHA within the erythrocytes (May JM et al, 1995; May JM et al, 1996), it is uncertain whether the DHA generated results from AFR oxidation or AFR dismutation.

2.11 AFR reductase and Ascorbate recycling

Recycling of ascorbic acid from its oxidized forms plays an important role in maintaining the tissue level of this vitamin. The recycling process is intracellular and occurs from both one and two electron oxidized form of ascorbate, i.e. the AFR radical and DHA respectively (Rose RC & Bode AM, 1993; Wilson JX, 2002). Generation of extracellular ascorbate from AFR takes place in the presence of AFR reductase. The cell surface AFR reductase uses electron from NADH. Furthermore, the cells also have the rapid mechanism for reducing DHA to ascorbate directly by using GSH or GSH and NADH dependent enzymes. Studies have shown that extracellular recycling of ascorbate by white and red blood cells would be especially important in areas of inflammation or atherosclerosis in the vascular bed (Jialal I et al, 1990). There are very few studies that explain ascorbate recycling by erythrocytes.
Figure 10- Illustration of plasma membrane redox system
under condition of altered cellular homeostasis. In one of the recent study, ascorbic acid recycling by erythrocytes has been reported to be increased by smoking (Lykkesfeldt J et al, 2003) as smoking induces oxidative stress and the increase in ascorbate recycling by erythrocytes. This has been explained as a secondary compensatory response in the human antioxidant defense (Lykkesfeldt J et al, 2003).

The redox state of ascorbic acid is very important given the role it plays in transplasma membrane transport activity and also being one of the most important anti-oxidant. A number of studies have provided us with evidence of defective handling of Vitamin C in diabetes (Yue DK et al 1990; Cunningham JJ et al, 1991). Localized decrease in levels of ascorbate might occur in insulin-sensitive tissues under diabetic conditions.

Furthermore, the ascorbate content of mononuclear leukocytes is found to be decreased in patients with insulin-dependent diabetes mellitus even on consumption of adequate amounts of dietary vitamin C (Cunningham JJ et al, 1991). Moreover, plasma concentrations of reduced vitamin C (ascorbate) are decreased and those of oxidized vitamin C (dehydroascorbic acid, DHAA) are elevated in some type 2 diabetic patients (Will JC, Byers TB, 1996; Seghieri G et al1994; Franconi F et al, 1996).

This may reflect oxidative stress, which contributes to the development of complications in diabetes. These studies probably help us to explain the role plasma membrane redox system.

### 2.11 Sodium Hydrogen Antiport

The sodium/hydrogen exchanger (NHE) also called as sodium/hydrogen antiport plays a key house keeping role in all cells by controlling cell volume, maintaining intracellular pH (pHi) and regulating response to stimulus and cell proliferation (Noel J & Pouyssegar J, 1995).

Furthermore, the sodium/hydrogen antiport system plays an important role in renal sodium absorption. It mediates the electroneutral exchange of Na\(^+\) for H\(^+\) ions and in turn regulates the homeostasis of sodium ions. There are atleast five different forms.
of Na/H antiport. The first one is referred to as NHE1 and is expressed in most cell types. NHE1 is extremely sensitive to pHi changes. It is essentially inactive at physiological pHi but gets rapidly activated by small intracellular hydrogen concentration increases (Counillon L & Pouyssegur J, 1995). Moreover, it is also sensitive to amiloride and is activated by growth factors (Noel J & Pouyssegur J, 1995). This electroneutral ion exchange system is known to be present in the plasma membrane of most mammalian cells (Noel J & Pouyssegur J, 1995).

Abnormal functioning of NHE1 is linked to pathology of several diseases, including hypertension, diabetes, congenital secretory diarrhea, and tissue damage caused by ischemia/reperfusion and oncogenic transformation (Odowski J & Grinstein S, 2004). Furthermore, abnormalities in intracellular pH regulation have been proposed to be important in type 2 diabetes and the associated cardiomyopathy and hypertension (Yang J et al, 2002). Moreover, NHE1 activity has been implicated in vascular smooth muscle cell proliferation as related to atherosclerosis and diabetes (Hannan KM & Little PJ, 1998).

In vitro studies on human erythrocytes carried out by Matteucci E et al, 2001 measured the NHE activity in both type 1 and type 2 diabetic patients. The results have shown an increased activity in both the groups. This study may have important implications. Furthermore, studies carried out by Trevisan R et al, 1999 have shown familial concordance for Na/H antiport activity in long term cultured fibroblasts obtained from type 1 diabetic sibling. This suggests that at least some of the phenotypical characteristics of these cells are likely to be genetically determined and to be at least in part, independent of in vivo metabolic control.