INTRODUCTION
&
LITERATURE REVIEW
1. Diabetes Mellitus

Diabetes mellitus is a complex debilitating disorder characterized by alterations in not only carbohydrate but also in lipid and protein metabolism. It results from a deficiency of insulin or decreased target cell responsiveness to insulin (Berhanu & Olefsky, 1985).

As a function of time, these metabolic derangements lead to the development of chronic degenerative pathological states, especially in non-insulin dependent tissues like the eyes, kidneys, nerves and blood vessels, resulting in the well known chronic clinical complications of diabetes including cataract, renal failure, peripheral and autonomic neuropathy, occlusive peripheral vascular and coronary artery diseases and ulcerations of the feet (Khan, 1994). The hallmark of this disease is an inability to control blood glucose level (Taylor & Agius, 1988). Diabetes mellitus is commonly found all over the world and currently affects about 135 million people and may reach as high as 300 million within 30 years (Vaaler, 1997).

One typical feature of diabetes is the hyperglycemic state. The role of pancreas in regulating blood glucose and carbohydrate metabolism was first recognized by von Mering and Minkowsky who, in 1889, demonstrated that total pancreatectomy in dogs leads to a syndrome of hyperglycemia, glycosuria, ketosis and death. Banting and Best (1921) obtained pancreatic extracts that contained biologically active insulin and then showed its effectiveness in treating diabetes. This major discovery led to several other investigations which subsequently led to the crystallization, purification, structural determination, and commercial preparation of insulin for clinical use.

The Etiology and Pathophysiology of Diabetes

Although diabetes mellitus has been recognized for centuries, the concepts regarding its etiology and pathogenesis still continue to undergo major changes. Diabetes has been clinically considered to be a disease with glucose overproduction by liver and under-utilization by insulin requiring tissues such as muscle, adipose tissue, and heart (Taylor & Agius, 1988). The cells of those tissues, which have an insulin-dependent glucose transport system, are relatively
unaffected by the high blood glucose concentration since the specific transport system for glucose into the cells in a diabetic subject is not active in the absence of insulin. This is not so, however, for the insulin-independent cells in which glucose entry is largely governed by the concentration gradient between the exterior and interior of the cell. Consequently, over-utilization of glucose can possibly occur in these tissues. Thus in diabetes, there appears to be diversion of glucose from insulin-dependent pathways to those not requiring the hormone (Sochor et al, 1985, Baquer et al, 1998, Baquer, 1998).

In uncontrolled or poorly controlled diabetes, there is increased glycosylation of a number of proteins, including haemoglobin and the protein α-crystallin of lens (Brownlee & Cerami, 1981). In long term diabetes, the glycosylated form of haemoglobin (haemoglobin A1c) has altered affinity to oxygen and this may be a factor in tissue anoxia, while glycosylation of crystallin may lead to cataract formation. A glucoseamine-protein complex is also formed in long standing diabetes resulting in biochemical and morphological alterations to the capillary system. There is some evidence that increased glycosylation of collagen is related to basement membrane thickening of the kidney (Osterby, 1992).

Classification

Diabetes is broadly classified into two types (National diabetes data group, 1979):

A. Type I diabetes, which in most instances, has its onset during early young ages and hence the term juvenile-onset diabetes. Approximately 10% of all diabetics fall in this category, also known as insulin dependent diabetes mellitus (IDDM). This form of the disease is characterized by insulinopenia, an abrupt onset of symptoms, ketosis, and dependence on exogenous insulin to sustain life. The disease arises due to insulin deficiency resulting from pancreatic β-cell damage. Thus, in this form of the disease, target tissue insulin resistance is not a major feature and if at all it occurs, it is most likely owing to the metabolic derangements resulting from the diabetic state itself rather than from intrinsic target tissues in insulin action (Berhanu & Olefsky, 1985). There is increasing evidence that Type I diabetes is etiologically distinct from other types of diabetes mellitus. Studies in identical twins and families have demonstrated that the
## Table B

### Broad Classification of Diabetes mellitus and Impaired Glucose Intolerance

<table>
<thead>
<tr>
<th>CLASS</th>
<th>FORMER TERMINOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Insulin Dependent Diabetes mellitus</td>
<td>Juvenile onset diabetes (JOD), Kitosis</td>
</tr>
<tr>
<td>prone Diabetes</td>
<td></td>
</tr>
<tr>
<td>IDDM (Type I diabetes)</td>
<td></td>
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<tr>
<td>2. Noninsulin Dependent Diabetes mellitus</td>
<td>Adult/maturity onset diabetes (MOD),</td>
</tr>
<tr>
<td>Ketosis-resistant</td>
<td>Diabetes</td>
</tr>
<tr>
<td>NIDDM (Type II diabetes)</td>
<td></td>
</tr>
<tr>
<td>a) Obese, and</td>
<td></td>
</tr>
<tr>
<td>b) Nonobese</td>
<td></td>
</tr>
<tr>
<td>3. Malnutrition Related Diabetes mellitus</td>
<td>Tropical Diabetes, Pancreatic Diabetes</td>
</tr>
<tr>
<td>a) Fibrocalculous Pancreatic diabetes</td>
<td></td>
</tr>
<tr>
<td>b) Protein deficient pancreatic diabetes</td>
<td></td>
</tr>
<tr>
<td>4. Gestational Diabetes mellitus (GDM)</td>
<td>Gestational Diabetes</td>
</tr>
<tr>
<td>5. Other types of Diabetes</td>
<td>Secondary Diabetes</td>
</tr>
<tr>
<td>a) Hormonal</td>
<td></td>
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<tr>
<td>b) Drug or chemical induced</td>
<td></td>
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<tr>
<td>c) Certain genetic syndromes</td>
<td></td>
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<tr>
<td>d) Insulin or its receptor abnormalities</td>
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<tr>
<td>6. Impaired Glucose Tolerance (IGT)</td>
<td>Asymptomatic Diabetes, Chemical</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Subclinical Diabetes, Borderline Diabetes</td>
</tr>
<tr>
<td>a) Nonobese IGT</td>
<td>Latent Diabetes</td>
</tr>
<tr>
<td>b) Obese IGT</td>
<td></td>
</tr>
<tr>
<td>c) IGT associated with some syndromes</td>
<td></td>
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<tr>
<td>7. Miscellaneous Types</td>
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</table>
familial aggregation of each major type of diabetes is consistent. Immunological phenomena, particularly the organ specific islet antibodies and leucocyte migration inhibition to pancreatic subcellular antigenes are confined to Type I diabetes (Cudworth & Bodansky, 1982).

**B. Type II diabetes** or non-insulin dependent diabetes mellitus (NIDDM), which comprises approximately 90% of the diabetic population, is usually diagnosed after thirty-five years and hence the term adult-onset diabetes mellitus. These adults are not ketosis prone and are not dependent entirely on exogenous insulin to sustain life. Target tissue insulin resistance is a characteristic feature of NIDDM. Furthermore, the majority of patients with NIDDM have obesity, characterized by hyperinsulinemia and target tissue insulin resistance. Type II diabetes is a heterogeneous condition which as a syndrome is exceedingly common and particularly so in ethnic groups which have undergone rapid environmental change, e.g. Pima Indians and Nauruans. Moreover, the evolution of a metabolic genotype, which is inappropriate to western dietary patterns may be one factor contributing towards obesity and glucose intolerance (Cudworth & Bodansky, 1982). Other types of diabetes are given in Table (Table B).

**The Complications of Diabetes**

More than 75 years have passed since Banting and Best (1922) made the breakthrough in the treatment of IDDM. This discovery of insulin remains the most important factor that has saved many lives and avoided sufferings. However, the introduction of insulin unmasked the second consequence of diabetes, the late diabetic complications. These complications are a heterogeneous group of clinical disorders, which affect many organs of the body like the vascular tissue, retina, kidney, peripheral nerve, skin and lens (Brownlee & Cerami, 1981; Taylor & Augius, 1988) (Figure 1).

During diabetes, there is an increased concentration of glucose in the blood that leads to hyperglycemia. This is due to decrease in the uptake of glucose, especially by insulin dependent tissues as a result of inability of secretion of insulin, defects in insulin receptor or production of defective insulin. Chronic hyperglycemia results in glycation of a number of proteins, which contributes to diabetic complications (Lyons, 1993). Glycation of enzymes and proteins causes changes in their activity, solubility and susceptibility to degradation (Abdel-Waheb et al, 1997).
General Pathway by which Increased Oxidative Stress may Contribute to Development of Complications in Diabetes modified from Baynes (1991).

**FORMATION OF PRECURSORS**

- Autoxidation
- Metal Catalyzed
- Enzymatic reactions, electron transport, Oxidases

**ACTION OF INHIBITORS AND SCAVENGERS**

- \( \text{ROOH} \)
- \( \text{H}_2\text{O}_2 \)
- \( \text{O}_2^{-} \)
- \( \text{M}^{n+} \)
- \( \text{RO}^{\cdot} \)
- \( \cdot \text{OH} \)

**TARGETS**

- Lipids
- Proteins
- Nucleic acids
- Glycoconjugates

**DAMAGE**

- Oxidation
- Fragmentation
- Crosslinking
- Fluorescence
At high concentration of glucose, glycation of hemoglobin occurs by a non-enzymatic reaction of glucose and the amino-terminal of the β-chain of the heme protein to form defective hemoglobin called hemoglobin A1c. Clinically this leads to tissue hypoxia due to decreased oxygenation of cells (Schmid-Schonbein & Volgar, 1976).

It has been proposed that protein glycation greatly contributes to the medical complications caused by diabetes, e.g., coronary heart disease, retinopathy, nephropathy, cataracts, and neuropathy (Vlassara, 1992). Increased glycation of lens proteins may contribute to the development of diabetic cataract. Collagen, laminin, vitronectin, and other matrix proteins can also be glycated and undergo alterations in biological properties (Brownlee, 1992) such as self-assembly and binding of other matrix molecules. Glycated proteins and lipoproteins can also be recognized by receptors present on macrophages, which are intimately involved in the formation of atherosclerotic plaques (Lyons, 1993). Type II diabetic patients sometimes develop a condition called hyperglycemic-hyperosmolar coma. This is particularly common in the elderly and can even occur in individuals under severe metabolic stress who were not recognized as having diabetes beforehand (Cruz-Caudillo & Sabatini, 1995).

The extent of tissue browning measured by collagen-associated fluorescence in skin biopsies has been correlated with the incidence and severity of diabetic complications (Monier et al., 1986). Due attention has been given to the reducing properties of monosaccharides in relation to diabetes. In addition to direct glycosylation, sugars can enolize and thereby reduce molecular oxygen under physiological conditions, yielding alphaketoaldehydes, hydrogen peroxide, and free radical intermediates (Thornalley et al., 1984; Wolf & Dean, 1987). The occurrence of this process in vivo could contribute to the elevated levels of plasma peroxides in diabetic subjects, particularly those with complications, and may contribute to protein modification.

This has also been confirmed by reactions performed with excess glucose in vitro (Wolf & Dean, 1987). Mild to moderate hypertriglyceridemia with increased VLDL is more common in diabetes, even when blood glucose is well regulated. These changes are found with both relatively under-insulinized IDDM and NIDDM patients. In general, the hyperglyceridemia becomes worse with deteriorating glycemic control (Alberti & Press, 1982).
Degenerative changes in heart and kidney are almost universal complications of diabetes mellitus. Cardiomyopathy and neuropathy share a common etiology, i.e., the altered hormonal and biochemical milieu resulting from the lack of insulin. Insulin-dependent diabetes often leads to coronary atherosclerosis related to hyperglyceridemia and altered lipoprotein profiles, resulting in vascular insufficiency, ischemia and marked increased risk of myocardial infarction. A second pattern of diabetic heart disease, diabetic cardiomyopathy, is less well defined, presenting as contractile dysfunction in individuals with no evidence of macrovascular disease and leading to cardiac pump failure (parinandi et al, 1990).

The diabetic kidney exhibits a characteristic pattern of changes in the glomerulus producing initial hyperfiltration, but eventually leading to renal insufficiency or complete kidney failure. The primary pathology involves marked thickening of the glomerular basement membrane and functional alteration of the mesangial cells in disposing of filtration residues (Cohen et al, 1995). Membrane alterations caused by free radical induced lipid peroxidation may be a common mechanism in the development of both diabetic cardiomyopathy and renal failure (Parinandi et al, 1990).

Opacity of the ocular lens, cataract, occurs earlier and more frequently in diabetic patients. In many experimental animals and Type I diabetes, the development of cataract is particularly rapid. It is also noted that the proteins of human cataractous lenses contained a greater number of disulfide bonds than did normal lenses, which reflects markedly increased sulfhydryl oxidation (Brownlee & Cerami, 1981).

Untreated and newly diagnosed diabetics exhibit decreased sensor and motor nerve conduction velocities. The most frequent symptoms of diabetic peripheral neuropathy are numbness and paraesthesias. These are thought to be the clinical expressions of changes in nerve electrophysiology and reflect altered structural and biochemical properties of the axon and the myelin sheath (Brownlee & Cerami, 1981). Abnormal growth hormone secretion has been observed in many diabetic patients. It has been proposed that this may in various ways contribute to the development of microangiopathy (Holly et al, 1988).
Experimental Diabetes

Diabetes can be induced in experimental animals using diabetogenic chemicals like streptozotocin, alloxan, chlorozotocin and vacor. However, streptozotocin and alloxan are the most widely and commonly used pancreatic β-cell cytotoxic agents. The diabetes induced is typically analogous to the clinical conditions of IDDM characterized by hyperglycemia, glucosuria, polydipsia, polyuria, body weight loss, ketosis, acidosis, and hyperlipidemia (Emudianughe et al, 1988). It has been known for sometime that structural alterations in the beta cells of the pancreas occur within 48 hours of the administration of alloxan and streptozotocin and last for up to four months, progressing finally to total degeneration of β-cells (Arison et al, 1967). Alloxan causes a decrease in glycogen level within 24 to 72 hours, an effect partially reversible by insulin. Streptozotocin is more specific than alloxan, less likely to cause ketosis, and less prone to inter-animal variability in terms of effective dose (Watkins & Sanders, 1995).

Alloxan generally produces greater cytotoxicity owing to its conversion to anionic radicals (Nukatsuka et al, 1989). Pancreatic islet cells treated with alloxan exhibit multiple cellular necrosis, marked degranulation, and extensive vasiculation of the endoplasmic reticulum and golgi complex, as well as enlarged mitochondria with disrupted cristae and ruptures (Abdel-Rahman et al, 1992). The toxicity of alloxan to pancreatic β-cells is attributable to the generation of toxic oxygen radicals due to its reduction to dialuric acid by two-electron reduction process and then rapid autoxidation by molecular oxygen to generate superoxide anion radical (Grankvist et al, 1978). The selective toxicity of β-cells of pancreas may be because these cells are more effective than other cells in capturing and reducing alloxan resulting in a high production of active oxygen radicals or perhaps the cells may have low protection against free radicals due to low endogenous antioxidant enzyme levels (Grankvist et al, 1981).

2. Insulin: Mechanism of Action

Insulin plays a central role in the control of a number of metabolic processes. Its diverse
Figure 2. The three levels of Insulin and possible sites of Vanadium action; Modified from C. Ronald Khan (1994).
biological action include: stimulation of membrane transport of glucose, amino acids, nucleotide precursors, and certain ions; increased storage of glycogen and triglycerides, inhibition of hepatic lipolysis and gluconeogenesis, and stimulation of protein, RNA, and DNA synthesis. Although the basic mechanisms, by which insulin exerts such diverse cellular effects remain largely unknown, important advances have been made in understanding the characteristics of insulin interaction with its target cells and also in identifying some of the alterations of these interactions in pathophysiological states such as obesity and Type II diabetes mellitus (Paulos & Olefsky, 1985).

**Prereceptor phase:** The prereceptor stage is the most characterized in the overall insulin action scheme which encompasses the series of events from insulin synthesis and secretion to its transport to the target tissue site of action. In the pancreatic β cell, insulin is produced as preproinsulin, which is rapidly converted to proinsulin that in turn converts to insulin and connecting peptide (C-peptide) by specific proteolytic steps within the beta cell secretory granules. After a brief circulation time in the vascular compartment, it interacts with the cells of its target tissues and exerts its biological effects.

**Receptor binding phase:** The multiple biological actions of insulin at the cellular level are initiated by binding of the hormone to its specific high affinity glycoprotein receptors on the surface of target cells. Magnitudes of biological responses to insulin can be regulated not only by the concentrations of circulating insulin but also by the number and affinity of cell surface insulin receptors. The insulin-receptor interaction has been extensively studied in intact cells and membrane preparations (Jacobs & Cuatrecasas, 1981; Czech, 1981). The insulin receptor is a tetramer of four subunits with stoichiometry of α2β2 (Jacobs, 1985). The insulin binding site is essentially on the α-subunit, in view of the results of affinity crosslinking and photoaffinity labeling studies (Berhanu & Olefsky, 1982). See figure 2.

**Postreceptor phase:** Comprises all the complex series of steps distal to insulin binding by which the interaction of insulin with its receptor is transformed to specific cellular biological responses. Binding of insulin induces a conformational change that relieves the tonic inhibition
on the tyrosine kinase activity of the β-subunit, leading to the autophosphorylation of the insulin receptor and the action of the intrinsic tyrosine kinase activity (Kasuga et al, 1983), catalyzing the phosphorylation of endogenous substrates, which may be effector proteins capable of sustaining a phosphorylation cascade.

**Insulin Resistance**

Insulin is a hormone produced by the pancreas that allows glucose to enter cells in the body where it can be changed to energy. Insulin has two major target organs: the skeletal muscle and the liver. Glucose stimulates the pancreas to secrete insulin, and insulin forces glucose out of the blood into muscle tissues, resulting in lower sugar levels. Insulin also decreases glucose production from the liver, which lowers blood sugar and increases the storage of glucose as glycogen in the liver (Garber et al, 1996).

Insulin resistance is a phenomenon increasingly believed to underline a number of common medical disorders, including Type II diabetes and obesity (DeFronzo, 1992). It is a condition in which the tissues of the body fail to respond normally to insulin. Although insulin is produced, the body is unable to use to convert food into energy. As a result, the bodies of people with insulin resistance begin secreting abnormally high amount of insulin to compensate for this defect. Eventually, however, their system can no longer stimulate glucose transport properly in muscle and fat and suppress hepatic glucose production (Polonsky et al, 1996). Glucose, that does not reach the cells, remains in the blood stream and blood sugar levels inevitably rise, leading to Type II diabetes and other diseases. Over time, excessive blood sugar levels can severely damage the heart, blood vessels, kidneys, eyes, and nerves.

3. **Vanadium Compounds and their Insulin Mimetic Actions**

**Historical Perspective**

Vanadium is a trace element required for normal growth and development of animals and also for the growth and survival of mammalian cell culture (Macara, 1980). Great interest in the role of vanadate in biology emerged after the observation that it was a potent inhibitor of the enzyme Na/K-ATPase. Cantley and co-workers (1978) discovered that the commercially
Figure 3. Different coordination compounds of vanadium
available ATP, inhibited the enzyme sporadically which they subsequently based on ultraviolet absorbance and thin-layer chromatographic migration showed that it was because of the impurity sodium orthovanadate (Na$_3$VO$_4$). This discovery led to the intensive research on the potential biological role of vanadate in the biochemical and physiological function of cells and tissues as well as in the intact organism (Dafnis & Sabatini, 1994). There is much evidence in the literature that vanadate plays an essential nutritional role in certain plants and animals. Deficiency of vanadium in chicks and rats is associated with slow development and impaired fertility (Marca, 1980; Shwarz & Milne, 1971).

The biochemistry and function of vanadate with its plausible role in health as well as in diseases remain one of the most fascinating stories in biology. In vivo, vanadate enhances protein tyrosine phosphorylation and is known to mimic a number of metabolic actions of insulin (Shechter, 1990) leading to its successful use as a therapeutic agent in various rodent models of diabetes (Brichard & Henquin, 1995) and to preliminary trials in human subjects with Type I and Type II subjects (Cohen et al, 1995; Goldfine et al, 1995).

Vanadate solutions possess insulin-like effects on glucose metabolism. A new insight into vanadium research came to the forefront when Heliger et al (1985) found that oral administration of sodium orthovanadate to streptozotocin (STZ) treated diabetic rats normalized the elevated blood glucose level and depressed cardiac performance. Ever since there has been a growing interest in the potential biological importance of vanadium and its compounds, with special preference to the treatment of diabetes.

**Insulin Mimetic Actions of Vanadium Compounds**

Vanadium compounds are known to exert insulin-like actions in target tissues and hence have an effect on carbohydrate metabolism (Sochor et al, 1992). This group of workers showed that vanadate treatment increased the activities of glycolytic enzymes like hexokinase, phosphofructokinase, and pyruvate kinase in diabetic rat heart with concomitant increase in fructose 2,6-bisphosphate level. Vanadate added to the drinking water of STZ induced diabetic rats improved cardiac performance and normalized the increased blood glucose level with partial improvement of body weight. The major insulin like effect of vanadate is enhanced
glucose transport in varieties of tissues, skeletal muscle (Okumara & Shimazu, 1992), brain (Meyerovitsh et al, 1989), adipocytes (Shechter & Karlish, 1980), and liver (Brichard et al, 1993).

Tolman et al (1979) reported that the addition of metavanadate or vanadylsulphate to preparations of adipocytes, diaphragm, and hepatocytes from rats showed stimulation of glycogen synthesis in liver and diaphragm, stimulation of glucose oxidation and transport in adipocytes and inhibition of hepatic gluconeogenesis, similar to that obtained with insulin. These investigators suggested that the action of vanadate is at a step beyond or in addition to that type of effect by insulin. This opinion was further verified by Dubiac and Kleinzeller (1980) and by Shechter and Karlish (1980) whose findings indicated that effects depend on the ability of vanadate to inhibit the activities of some phosphatases.

These valuable results triggered further searches and investigations with outcomes which deciphered that, vanadium salts and complexes have actions resembling to that of insulin in its signaling cascade with some exceptions, e.g., lack of effect on muscle protein metabolism (Ramasarma, 1996). Innumerable effects on related systems were reported and these may have as yet undelineated physiological significance. In addition, oral administration of vanadyl (V⁴⁺) or organic vanadyl complexes (Yuen et al, 1992; Cam et al, 1993) produced marked glucose lowering effects in STZ-diabetic rats. The insulin synergic effect of vanadate showed restoration of the expression of genes for key enzymes in the glucose and ketone body metabolism in the liver of diabetic rats (Valera et al, 1993), and liver mRNA levels of glycolytic enzymes (glucokinase, L-pyruvate kinase), which were lowered during diabetes (Table A).

Furthermore, impaired glycogen synthase, glycogen phosphorylase activity, and glycogen content in liver were reversed to normal values (Sekar et al, 1990; Rossetti and Laughlin, 1989), the elevated levels of phosphoehanolpyruvate carboxykinase (PEPCK) and the glucose transporter in liver (GLUT2) and in muscle (GLUT4) during diabetes were also reversed by treatment with vanadate (Valera et al, 1993). Vanadate normalized the increased activities of some selected enzymes of amino acid metabolism; alanine aminotransferase, aspartate aminotransferase, glutamate dehydrogenase, and arginase in the cytosolic and mitochondrial fractions of alloxan diabetic rat kidney (Salimuddin et al, 1996).
### Table A

Insulin-like actions mediated by orthovanadate in tissues and cell cultures

<table>
<thead>
<tr>
<th>Activity</th>
<th>Effect Observed</th>
<th>Target Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexose transport</td>
<td>Stimulated</td>
<td>Rat Skeletal muscle &amp; adipocytes Brain, liver</td>
</tr>
<tr>
<td>Lipogenesis</td>
<td>Stimulated</td>
<td>Rat adipocytes</td>
</tr>
<tr>
<td>Glucose Oxidation</td>
<td>Stimulated</td>
<td>Rat adipocytes</td>
</tr>
<tr>
<td>Lipolysis</td>
<td>Inhibited</td>
<td>Rat adipocytes</td>
</tr>
<tr>
<td>Glycogen Synthase</td>
<td>Stimulated</td>
<td>Rat skeletal muscle, adipocytes</td>
</tr>
<tr>
<td>Mitogenic activity</td>
<td>Augmented</td>
<td>Various cultured cells</td>
</tr>
<tr>
<td>Translocation of IGF-II factor</td>
<td>Stimulated</td>
<td>Cardiac muscle cells</td>
</tr>
<tr>
<td>K⁺ uptake</td>
<td>Stimulated</td>
<td>Cardiac muscle cells</td>
</tr>
<tr>
<td>Ca²⁺-Mg²⁺-ATPase</td>
<td>Inhibited</td>
<td>Membrane of rat Adipocytes</td>
</tr>
<tr>
<td>Ca²⁺ influx</td>
<td>Stimulated</td>
<td>Adipose tissue</td>
</tr>
<tr>
<td>Intracellular pH</td>
<td>Elevated</td>
<td>A-431 cells</td>
</tr>
<tr>
<td>Cyt-PTK</td>
<td>Stimulated</td>
<td>Rat adipocytes</td>
</tr>
<tr>
<td>Glycogenesis</td>
<td>Stimulated</td>
<td>Rat hepatocytes, Skeletal muscle and adipocytes</td>
</tr>
<tr>
<td>Gluconeogenesis</td>
<td>Inhibited</td>
<td>Rat Liver</td>
</tr>
<tr>
<td>Glycolysis</td>
<td>Stimulated</td>
<td>Rat Liver</td>
</tr>
<tr>
<td>Glycogenolysis</td>
<td>Inhibited</td>
<td>Rat Liver</td>
</tr>
<tr>
<td>mRNA level of glycolytic enzymes</td>
<td>Augmented</td>
<td>Rat Liver</td>
</tr>
<tr>
<td>Protein tyrosine phosphatases</td>
<td>Inhibited</td>
<td>Rat Liver</td>
</tr>
<tr>
<td>Blood glucose level</td>
<td>Lowered</td>
<td>Rat blood</td>
</tr>
</tbody>
</table>

Modified from Shechter (1990) and Sekar (1996).
Oral administration of sodium orthovanadate to alloxan diabetic rats for three weeks showed an effect on some enzymes of the polyol pathway (Saxena et al, 1992). An enhancement in aldose reductase in medulla and cortex and sorbitol dehydrogenase in cortex was observed during alloxan diabetes. Vanadate treatment counteracted hyperglycemia, normalized the elevated enzymatic activities, decreased the elevated blood glucose level, markedly checked the increase in kidney weight with concomitant prevention of accumulation of sorbitol in the medulla. These results show that vanadate causes marked improvement in renal hypertrophy and has antidiabetogenic effect on polyol pathway (Saxena et al, 1992).

Khandelwal and Pugazhenthi (1995), in their study of the in vivo effects of vanadate on hepatic glycogen metabolizing and lipogenic enzymes in IDDM and NIDDM rats, showed that vanadate normalized blood glucose levels in all animals; glucose-6-phosphate dehydrogenase, glycogen synthase, glycogen phosphorylase and malic enzyme were decreased and vanadate treatment restored all to near normal levels. The insulin resistant models were characterized by hyperinsulinemia, hypertriglyceridemia, increase in the activities of lipogenic enzymes and marginal changes in glycogen metabolizing enzymes (Sekar et al, 1996, Baquer et al, 1998).

A combination of vanadate and hydrogen peroxide, a weak insulin mimetic agent, showed a strong additive effect and potentially activated the insulin receptor with corresponding biological effects in rat adipocytes (Fantus et al, 1989), which is believed to be due to the in situ formation of peroxovanadium compounds that are hundred fold more potent than vanadate in manifesting insulin-like biological effects (Sekar et al, 1996).

**Mechanism of Action of Vanadium**

Vanadate is one of several agents that elicit insulin like responses in target tissue cells and may provide insight into the mechanism of insulin action. However, since vanadate has many potential effects on target cells (Table A), the mechanism by which it mediates its insulin-like effect in vivo is still obscure (Mooney et al, 1989). Unlike insulin, vanadate does not seem to stimulate the autophosphorylation of insulin receptor kinase or other intracellular proteins either directly or by virtue of its known inhibitory effects on protein phosphotyrosine phosphatase.
(Swarup et al, 1982). Results of many studies support a model in which vanadate activates glucose metabolism by either utilizing an alternative (insulin-independent) cascade or bypassing the early events of the insulin-dependent cascade (Shechter, 1990). Either of these possibilities is of clinical importance because early insulin events may become defective, as a result of severe hyperinsulinemia, and may contribute to insulin resistance.

Alternative pathways by which vanadate stimulates glucose metabolism, such as, by increasing intracellular Ca\(^{2+}\) levels and/or regulating intracellular and intravesicular pH, have been opined by Shechter's group (1990). It is now established that because of its low molecular weight and its structural analogy to phosphate, vanadate can permeate the plasma membrane with ease. This occurs either by passive diffusion or by means of the anion-carrier channels (Cantley et al, 1978). Vanadate (V\(^{5+}\)) is intracellularly reduced to vanadyl (V\(^{4+}\)) by glutathione which is an effectual inhibitor of Na/K-ATPase. Recently, it has been shown that this enzyme was not inhibited by vanadate over a concentration range of 0.1-0.7mM (Nechay, 1984).

\[ H^+ + VO_3^- + 2GSH \leftrightarrow VO^{2+} + G_2S_2 + OH^- + e^- + H_2O \]

Vanadate binds to one high and to one low affinity sites of the enzyme molecule and interferes with the activity by slowing down the E\(_2\rightarrow\)E\(_1\) conformational changes (E\(_1\) is the active form and E\(_2\) the inactive form of the enzyme Na/K-ATPase). Vanadate exerts its inhibitory action on this enzyme from the cytoplasmic side, thus, it must first enter the cell where it combines with the phosphorous site of the enzyme to stabilize the pump in the E\(_2\) configuration (Cantley et al, 1978). Vanadate is a potent inhibitor of cellular protein tyrosine phosphatases (PTPases).

It was earlier believed to act intracellularly by blocking these PTPases, which dephosphorylate the insulin receptor and therefore activate it in an insulin independent manner. But, this may not be true since many workers reported no significant increase in the phosphotyrosine content of the insulin receptor of vanadate treated cells or tissues (D'onofrio et al, 1994; Mooney, 1989). This notion was further substantiated by the finding that the stimulatory effect of vanadate on hexose transport and lipogenesis were not blocked by quercetin (Sekar et al, 1996).
Vanadate mimics several other insulin-like effects in rat adipocytes via a strautosporine-sensitive soluble protein tyrosine kinases (CytPTK). This action is very specific to vanadate since neither insulin, isopretrenol, dibutylcAMP, okadeic acid, hydrogenperoxide nor phorbol esters altered CytPTK. Other possible sites of vanadium actions including receptor and post receptor level actions have been also suggested (Figure 2). Insulin-like actions were obtained even in cell systems without insulin receptors indicating that vanadate can exert its effect at the post receptor levels too (Green, 1986). The most significant end result of vanadate treatment of cells is to improve and retain the phosphorylation status of proteins which also include enzymes such as glycogen-bound protein phosphatases (PP1-G), in the down stream signal cascade of insulin (Ramasarma, 1996). In general, the insulin mimic property and mechanism of action of vanadate still needs further elaboration and remains an area to work on.

Toxicity of Vanadium and its Compounds

Albeit the insulin-like and other pharmacological effects of vanadium, the toxicity of this element should be studied before its mass production and distribution as a useful drug. The industrial toxicological profile of vanadium has been established (Kawai et al, 1989). Inhalation and exposure to vanadium causes conjunctivitis, Pharyngitis, rhinitis, chronic cough, and tightness of the chest (Nechay, 1984) resulting in pulmonary oedema, broncho pneumonia and asthma. Several workers had observed toxic responses in animals treated with vanadium compounds such as severe diarrhoea, decrease in weight gain, and deaths due to dehydration (Shechter 1990; Ramasarma, 1996).

Consumption of excess metavanadate causes nausea, vomiting, giddiness, and high incidence of bradycardia and coronary insufficiency (Sekar et al, 1996). In the intact animal, vasoconstriction is the most consistently observed pharmacological toxic effect of vanadate. There are obscure species differences in its renal, cardiac, and occular actions. Administration of vanadate may cause diuresis or antiuresis, it may have a positive or negative ionotopic effect in the heart (Nechay, 1984).

Hepatotoxicity of vanadium was also observed in isolated perfused hepatocytes (Younes et al, 1991). In isolated perfused rat livers, significant lipid peroxidation was seen (Saxena, 1994).
The major goal of vanadium research is the use of chelating agents to reduce its toxicity and improve insulin potency. Inspite of the recent advances, the understanding of vanadium effects remains incomplete because multiple mechanisms may be involved in its action.

4. Creatine Kinase Isoenzymes

General

Creatine kinase (CK) (EC 2.7.3.2) constitutes a family of enzymes catalyzing the reversible transfer of high energy phosphates from ATP to Creatine (Cr), with subsequent generation of ADP and phosphocreatine (PCr). Creatine kinase isoenzymes play a key role in cellular energy production and its transduction (Pyne et al, 1991) connecting sites of energy production to that of utilization.

\[ \text{CK} \]

\[ \text{MgATP}^2 + \text{Cr} \rightleftharpoons \text{MgADP} + \text{PCr} + n\text{H}^+ \]

All processes involved in growth movement and metabolism of cells require an input of energy. In living cells, production, transport, conversion, and utilization of energy are fundamental processes that are facilitated via metabolic pathways involving a large number of tightly regulated enzyme catalyzed reactions. The creatine kinase/phosphocreatine (CK/PCr) system is implicated to play a complex and multifaceted role in cellular energy homeostasis (Wallimann et al, 1992).

Tissue Distribution of Isoenzymes

The CKs comprise a multi-gene family and in vertebrate tissues, three cytosolic isoforms and two mitochondrial isoforms are expressed in tissue specific manner (Pyne et al, 1991). The cytosolic isoforms form dimeric molecules MM, BB, and MB (M-standing for muscle, B-standing for Brain, and MB a hybrid of the muscle and brain type subunits) with an approximate Mr of 80,000 - 86,000. Whereas MM-CK is very specific for differentiated sarcomeric muscles and heart; BB-CK is found mainly in brain, smooth muscle tissues, myoblasts, as well as in many embryonic cell types (Wirz et al, 1990). MB-CK is found in adult
mammalian heart as well as in differentiated skeletal muscles during the developmental transition from BB-CK to MM-CK. Surprisingly enough, during muscle cell differentiation in vitro and in vivo, a developmental transition from BB-CK via MB-CK hybrid to the MM-CK homodimer was observed (Trask et al, 1988). Sub-cellular fractionation like, isolation of myofibrils, sarcoplasmic reticulum, plasma membranes, etc., as well as direct in situ immunolocalization studies revealed a cellular and sub-cellular compartmentation of the cytoplasmic CK isoenzymes (Bessman & Carpenter, 1985). In muscle a small but significant fraction of MM-CK is specifically associated with the myofibrillar M-band where it directly rephosphorylates ADP generated by the actin-activated myosin ATPase (Ventura-Clapier et al, 1994).

The two mitochondrial creatine kinase (mi-CK) isoenzymes called straited muscle specific (mi,-CK) and so called, ubiquitous (mi,-CK) are also expressed in a tissue specific manner in only tissues where cytosolic isoforms are expressed. Separate genes encode the two mi-CK isoenzymes (Hass & Strauss, 1990). Mitochondrial CK enzymes are localized in the outer surface of the inner mitochondrial membrane and form, in contrast to the cytosolic CK isoenzymes, octameric as well as dimeric molecules which, by protein chemical analyses were found to be distinct isoenzymes differing in molecular weight, NH2-Terminal amino acid sequences, and isoelectric points (Schlegel et al, 1988). The ATP binding site of mi-CK from chicken heart has been studied (James et al, 1990) by modifying the purified enzyme with a 14C-labelled ATP analog, C1RATP, in which the reactive label was covalently bonded to the γ-phosphate group of ATP.

Scholte (1973) showed with evidence that, besides the cytosol and mitochondria, CK enzymes are localized in the myofibrils of both rat heart and masseter muscle. Two isoforms of CK, brain type BB-CK and mitochondrial mi-CK were identified in spermatozoa from rooster, man, and sea urchin (Van Dorsen et al, 1997; Iyengar et al, 1982). While the main CK isoform in smooth muscle is BB-CK, small amounts of MB-CK and traces of MM-CK were also found (Iyengar et al, 1982). Recently mi-CK, mostly in its octameric form, was detected in mitochondrial fractions of intestinal and vascular smooth muscles of guinea pig (Ishida et al, 1991) and rat (Scholte, 1973).
High amounts of BB-CK and mi-CK activity have been measured in the photoreceptor cells of the retina (Wallimann et al., 1986a). The electrocytes of the electric fish are known to have the MM-CK (Gysin et al., 1986). In uterus, PCr level increases significantly before labor, when large metabolic demands are made on the contracting myometrium indicating that CK is also critically involved in the energetics of uterine contraction. Interestingly, B-CK gene transcription in uterus is oestrogen-induced (Reiss & Kaye, 1981). BB-CK is the major cytosolic CK isoenzyme in brain and is abundantly found in the entire brain. In cerebral cortex, especially in the molecular layer, higher levels of CK and PCr levels were measured than in white matter (Maker et al., 1973).

The cerebellum, striatum and pyramidal tracts also contain higher CK activities (Manos et al., 1991). Creatine kinase activities associated with brain mitochondria have been reported (Wyss et al., 1990). The collecting tubule, Henle's loop and epithelial cells in the cortex contain BB-CK (Ikeda, 1988). This defined cellular localization of BB-CK in the kidney raises the possibility that CK may have a role in renal tubular functions, e.g. by supporting ATP to the membrane bound ion exchangers and ion pumps present in these cells (Wallimann et al., 1992).

Mitochondrial CK isoenzymes were isolated from human liver and characterized (Kanemitsu et al., 1982). When analyzed by cellulose acetate electrophoresis, pig and rabbit heart mitochondrial CK was shown to exist as less cathodic (dimeric form) and a higher cathodic form (octameric form) of molecular weight 350,000 (Marcillar et al., 1987). The latter form was readily convertible to the former by incubation at alkaline pH or when the enzyme forms a reactive or abortive complex with its substrates. Thus, the octameric form was proposed to be the actual form associated with heart mitochondria in vivo based on membrane interaction properties and the molecule shape seems well suited to support metabolite channeling (Kaldis & Wallimann, 1995).

The Function of CK and the Phosphocreatine Shuttle

Varieties of different phosphogenes, e.g., phosphoarginine, phospholombricine, phosphoglycocyamine are found in lower animal phyla and plants (Ellington, 1989). However, phosphocreatine is the sole phosphogene in vertebrates. Creatine synthesized in liver and
kidney, but not muscle, is transported through the blood and taken up by tissues with high demands via an active transport system (Wallimann et al., 1992). The CK catalyzes the reversible transfer of the γ-phosphate group of ATP to Cr to yield PCr and ADP and replenishes high energy phosphate pools to ensure effective energy transport from the sites of production (mitochondria, glycolysis), to the sites of utilization (Kinases, ATPases).

In skeletal muscle, PCr concentrations could reach 20-35mM or even more depending on the species and the fiber type (Burt et al., 1976; Ackerman et al., 1980) whereas in other excitable tissues like brain, electric organ, smooth-muscle and kidney, PCr is in the range of 5-10mM (Iyengar, 1984). The finding of an isoenzyme of CK attached to M-line region of the myofibril revealed peripheral receptor for the mitochondrially generated PCr. This established a molecular basis for PCr/Cr shuttle of energy transport in heart and skeletal muscle and provided an explanation for the inability to demonstrate experimentally a direct relation between muscle activity, [ATP] and [ADP] (Ikeda, 1988).

The suggested main functions of the CK/PCr system are:

i) The pioneering main function of the CK, widely accepted and cited in many text books, is that of temporal energy buffer.

ii) Its use as an energy transport system or spatial "energy-buffer" elaborated by many workers (Bessman & Geiger, 1981; Jacobus & Lehninger, 1972; Ventura-Clapier et al., 1987), where PCr serves as an energy carrier.

iii) The CK/PCr system prevents a rise in intracellular [Free ADP], thus avoiding an inactivation of cellular ATPases and a net loss of adenine nucleotides (Iyengar, 1984).

iv) It functions in buffering of protons. Since the CK reaction utilizes ADP and H⁺(protons) which are both products of ATP hydrolysis, an intimate functional coupling with ATPases prevents the local or global acidification of cells. This buffering action is especially indispensable in the early phase of severe exercise, before glycogenolysis is activated.
v) The CK/PCr system maintains an appropriate ATP/ADP ratios at sub-cellular sites where CK is coupled to ATP consuming enzymes or processes.

From kinetic studies (Jacobus & Lehninger, 1972; Saks et al, 1976), CK was found to have a low $K_m$ for ADP, it increases the thermodynamic efficiency of ATP hydrolysis and can be considered as a low threshold ADP sensor. Based on a recent data on the structure, localization and function of mi-CK, an integrated "PCr circuit" model was proposed (Walliman et al, 1992). According to this model, mi-CK, which is bound to the outer face of the inner mitochondrial membrane catalyzes the transfer of the $\gamma$-phosphoryl group of ATP synthesized by mitochondrial oxidative phosphorylation to creatine (Cr). Phosphocreatine (PCr) then diffuses out of the mitochondria to sites within the cell where energy is consumed and continuously regenerates ATP from ADP. The diffusion of Cr back to the mitochondria completes the circuit.

In very clear terms, the CK/PCr/Cr system is shunted in between sites of ATP production and ATP consumption. There were controversies on the "buffering role" and "transport role" of CK/PCr system but according to Wyss et al (1992) both buffer and transport functions of the system may be operational at the same time, with the relative contributions of the two models depending on the metabolic demands of a tissue.

**Functional Coupling of mi-CK with Oxidative Phosphorylation**

Jacobus and Lehninger (1972) showed that, in addition to high energy phosphate storage function previously suggested, the CK/PCr system has an effect on the rate of oxidative respiration of heart mitochondria, as well as to channel high energy phosphate to the contractile system via the mitochondria and sarcoplasmic isoenzymes of creatine kinase. Jacobus and Diffley (1986) further elaborated this concept and suggested that, when the production of PCr is coupled to electron transport via mi-CK, the net reaction and rate of oxygen consumption are regulated by changes in [PCr], [Cr] or [Pi] alone or in combination.

Studies in energy transport in heart cells by Saks et al (1980) revealed that, mi-CK due to the specific localization of mi-CK on the inner mitochondrial membrane, ATP generated in the
Figure 4: The PCr circuit model and its coupling to glycolysis and oxidative phosphorylation
mitochondrial matrix is more effectively used for PCr synthesis than ATP present in the surrounding medium. The work by Moreadith and Jacobus (1982) has clearly evidenced that myosin ATPase preferentially hydrolyzes ATP regenerated by the CK reaction than ATP supplied exogenously.

**Functional Coupling of mi-CK with Adenine Nucleotide Translocase**

The influence of mitochondrial CK in subcellular high energy systems has been investigated using rat heart mitochondria (Jacobus & Diffley, 1986; Saks et al, 1980; Moreadith & Jacobus, 1982), mitolpasts and intact heart and skeletal muscle tissue (Soboll et al, 1994). In all cases, it has been shown that there is a special interaction between the ADP generated by mi-CK and its uptake by the adenine nucleotide translocase (ANT). Creatine uptake into mitochondria is inhibited by carboxyatractyloside which indicates the possible role of the mitochondrial ANT in Cr uptake.

**Coupling of Cytosolic CK with Glycolysis**

The functional coupling of CK with glycogenolysis and glycolysis is supported by several lines of evidence. Similar to CK, glycolytic enzymes are known to be localized on the thin filaments of sarcoplasmic reticulum (I-band) and plasma membrane and in addition, the amount of cytosolic MM-CK correlates with the glycolytic potential of muscle (Wallimann et al, 1984; Wegmann et al, 1992); e.g. the highest [PCr] and CK activity are found in those skeletal muscles with the greatest anaerobic potential (Newsholme et al, 1978).

The ATP produced by glycolysis upon stimulation of a fast-twitch glycolytic muscle was found not to accumulate as such, but is immediately and efficiently transphosphorylated by CK into PCr to replenish the PCr pools and maintain a high PCr/Cr ratio in the cell (Wallimann et al, 1992). This was also shown by studies with a reconstituted glycolytic system of muscle, indicating that Cr is efficiently phosphorylated to PCr via CK coupled to anaerobic glycolysis (Scopes, 1973) which was further supported by the finding that a depletion of [PCr] together with lowered ATP levels occurred in an animal model with defective glycolysis.
In the heart cells, which naturally depend on an oxidative metabolism, energy derived from glycolysis contributes the maintenance of high-energy phosphate levels and contractile function (Doorey & Barry, 1983) and prevent malfunctioning of myosin ATPase (Ventura-Clapier et al, 1994). Further evidences for the coupling of glycolysis with CK were demonstrated by NMR experiments which revealed that at elevated levels of free ADP, the CK reaction and anaerobic glycolysis are coupled via protons (H⁺) as a common intermediate (Hochachka & Mommsen, 1983). Aerobic glycolysis is the primary pathway of ATP synthesis in brain (Wallimann et al, 1992). A readily available source of ATP synthesis in brain is the CK/PCr system which was justified by high CK activity measured in brain (Norwood et al, 1983).

Creatine Kinase, its Regulation and Signalling Pathways

The CK system is subject to complex regulation due to its association to sub-cellular structures and also with respect to enzyme kinetics (Matsumoto et al, 1995; Jacobs & Kuby, 1970). Since intracellular ATP/ADP ratios regulate a variety of cellular processes and since the CK system is involved in regulation of this ratios (Newsholme et al, 1978; Rossi et al, 1990), it is of interest to consider that CK itself may also be a subject of regulation. An example is, regulation by post translational modification of the BB-CK in chicken brain (Quest et al, 1990) via phosphorylation, which induces a 50% decrease in the K_m value of the enzyme for PCr. CK enzymes seem to be phosphorylated by protein Kinase (PKC) and are connected to signal transduction pathways. The phosphorylation of CK by PKC resulted in an increase in its ability to catalyze the transfer of high-energy phosphate to ADP and a specific inhibitor of PKC (H-7) abolished the increase in enzyme activity of CK (Chida et al, 1990).

Kinetic Properties of Creatine kinase

There has been a hypothesis put forward that the mechanism of the reaction catalyzed by CK is of the random rapid-equilibrium type (Morrison & James, 1965; Cleland, 1963). This involves the rapid binding of substrates and release of products and quasi-equilibrium formation of the intermediate steps, with rate limiting step being the transition state conversion of the central ternary enzyme-substrate complex to the enzyme-product complex (Cleland, 1963).
According to the study by Jacobs and Kuby (1970), the steady state kinetics of the calf brain CK seemed to be adequately expressed by the same random quasi-equilibrium type of mechanism for a case without independent binding of the substrate. They also showed that, for the forward reaction of CK, there was a significant difference in the derived parameters; especially in $V_{max}$ (reverse reaction) with a large increase in $K_m$ (PCr) with a large decrease at pH 7.4 as compared to the data obtained at pH 8.8.

Jacobus and Saks (1982) made a complete kinetic analysis of the forward CK reaction to define the mechanism for its rate enhancement when coupled to oxidative phosphorylation. Two experimental systems were employed. Firstly, ATP was produced by oxidative phosphorylation and secondly, heart mitochondria were pretreated with rotenone and oligomycin thereby, regenerating ATP by phosphoenolpyruvate/pyruvate kinase system.

Product inhibition studies showed that oxidative phosphorylation did not affect the binding of PCr to the enzyme. PCr interacted completely with both ATP and Cr. Human mi-CK isoenzymes from normal human heart and liver tissues have also been characterized (Kanemitsu et al, 1982) and the apparent $K_m$ values for PCr were found to be 0.63 and 0.24mM for heart and liver mi-CK respectively and 2.9mM for MM-CK. The liver mi-CK was more stable to heat and has a higher apparent affinity to PCr than the heart mi-CK.

Studies by Scholte (1973) on apparent $K_m$ values for PCr of rat heart mi-CK and cytosolic CK gave values of 1mM and 1.8mM respectively. The kinetic properties of partially purified human heart and skeletal muscle CK isoenzymes were investigated (Savabi & Kirsch, 1991) utilizing the backward reaction of CK where $K_m$ values for heart and skeletal muscle MM-CK (3.7mM) were greater than the MB-CK value (2.1mM) which intern was greater than the mi-CK values (1.8mM) at variable [PCr] and fixed [ADP]. Such results show that, kinetic analysis of CK isoenzymes can not differenciate the tissue source of elevated blood CK isoenzymes after myocardial infarction, muscular dystrophy, malignancy or due to other inflamations.

There are some reports where kinetic parameters have been determined for CK isoenzymes (Table C) in different species and tissues. Morrison and James (1965) showed that MgATP is a non-competetive inhibitor whereas, Cr is a competetive inhibitor of the reverse CK reaction,
where PCr is the substrate. In an attempt to identify inhibitory effects of some compounds on CK isoenzymes, ureate and cystine (Jacobs et al, 1978) isolated and characterized from human serum were found to have a non-competitive type of inhibitory effects on the forward reaction.

A decrease in heart CK activity with a marked loss of the BB isoenzyme during diabetes mellitus (Savabi & Kirsch, 1991) and in food restriction conditions (Kirsch & Savabi, 1992) have been observed. In the same reports, total CK activities were significantly decreased in atria, ventricles and skeletal muscle. Moreover, in the diabetic rat heart, ATP and Cr levels were decreased by 27% and 42% respectively. PCr and Cr levels were not affected by food restriction; however, ATP was decreased by 23%. Quest et al (1990) reported that, phosphorylation of chicken BB-CK reduces the $K_m$ of the enzyme to PCr by about 50% ($K_m$ values for PCr was 1.6$mM$ and 0.8$mM$ for non-phosphorylated and phosphorylated enzyme respectively).

Kaldis and Wallimann (1995) determined kinetic constants for the dimeric and octameric mi-CK in the direction of PCr and ATP synthesis and found that $K_m$ values are 2 to 3 times higher for octameric than for the dimeric mi-CK. Accordingly, they found that $K_m$(Cr) was 3.69$mM$ and 10.1$mM$, $K_m$(PCr) was 1.12$mM$ and 1.7$mM$ for octameric and dimeric mi-CK respectively. From studies of energy transport in heart cells (Saks et al, 1980), calculated $K_m$ values for bound mi-CK at pH 7.4 and 30 °C; $K_m$(Cr) was 5$mM$ and $K_m$(ATP) was 0.35$mM$. The apparent $K_m$ values of human heart and liver mi-CK for PCr were calculated to be 0.63$mM$ and 0.24$mM$ respectively and 2.9$mM$ for MM-CK (Kanematsu et al, 1982). Sholte (1973) reported $K_m$ values for rat heart CK reaction, $K_m$(Cr) was 4.7, $K_m$(PCr) was 1 and for masseter muscle, $K_m$(Cr) was 18 and $K_m$(PCr) was 3.1 in $mM$.

Generally, kinetic constant values of the CK isoenzymes greatly depend on the species studied, the calculation methods used, the preparation of the enzyme, purification method, temperature, pH as well as the type of buffer used (Ogunro et al, 1977). In some cases, the storage, method of assay of enzymes and the isoenzymes investigated also matters in the determination of kinetic constants. The pH optima of the CK isoenzyme assay ranged from 6.0 - 7.0 for the reverse reaction i.e. in the direction of ATP synthesis and 7.5 - 9.0 in the forward direction i.e. in the direction of PCr synthesis (Ogunro et al, 1977; Tombes & Shapiro, 1987). This range
works for both the cytosolic and mitochondrial CK isoenzymes. All purified mi-CK isoenzymes investigated so far have a lower $K_m$ value for PCr than for Cr and the same relationship has been found for the cytosolic isoenzymes also (Bittl et al, 1987).

**Creatine Kinase Isoenzymes and their Clinical Significance**

Clinically the CK isoenzymes have been utilized for the diagnosis of many diseases. Namely: myocardial infarction, muscular dystrophy (Duchenne type), cardiac myopathy, tumour, nerve ending problems and Alzheimer's disease. Elevated serum CK activity is widely recognized as a manifestation of various human muscular dystrophies (Goto et al, 1969). It is an established fact that vitamin E deficiency produces a type of muscular dystrophy but the etiology and pathogenesis of the disease is still vague; a rise in serum CK activity attributable entirely to MM-CK appears to be a sensitive indicator of the onset of the disease (Armstrong et al, 1977).

The detection of CK isoenzymes (MB-CK) in the serum of patients has been extensively used for the early diagnosis of myocardial infarction (Sax et al, 1979; Roberts, 1998). MB-CK activities exceeding 2.6U/L with normal values for total CK or accounting for over 2.6% of the total activity when the latter is increased suggest recent myocardial infarction (Sax et al, 1979). One feature of diabetic cardiomyopathy is the appearance of contractile dysfunction as the work load increases, which is assumed to be as a result of an impaired CK/PCr system (Matsumoto et al, 1995), accompanied by reduced CK flux by about 31%.

Significantly lower levels of PCr have been found in sedentary diabetic rats than sedentary controls, which have been improved by physical training (Mokhtar et al, 1993). Moreover, alloxan diabetes has been shown to cause a decrease in CK enzyme activity and mRNA level in the rat heart causing impaired heart contractile performance (Popovich et al, 1989; Popovich et al, 1991).

Although most of the CK activity is present in skeletal muscle and myocardium, some activity in other organs has been reported (Tsung, 1976; Wallimann et al, 1994). Destruction of these organs by ischemic necrosis, inflammation, traumatic injury, or surgery liberates soluble enzymes into the circulation (Swei & Tsung, 1981). BB-CK is found in serum of patients with various
types of cancer and it can be used as a typical tumor marker particularly for the adenocarcinoma of the prostate (Silverman et al, 1979).

Recently it was realized that long term zidovudine (AZT) therapy, used for the treatment of acquired immunodeficiency syndrome (AIDS), can cause a toxic mitochondrial myopathy with increased levels of CK in serum of up to 15% (Dalakas et al, 1990). The BB-CK isoenzyme is an intracellular enzyme released in various neurologic conditions including central nervous system (CNS) infections (Lustar et al, 1994). Significantly elevated levels of BB-CK activity in the cerebrospinal fluid (CSF) were found in children with bacterial meningitis as compared to children with aseptic meningitis or normal CSF findings (Nussinovitch et al, 1996). Acrylamide intoxication caused suppression of BB-CK in brain of mice and rats (Matsuoka et al, 1996; Khriyama et al, 1994). A 80% decline in CK activity was observed in patients with Alzheimer's disease (Hensley et al, 1995).

5. Diabetes and Lipid Peroxidation

The basic chemistry of the propagation of lipid peroxidation reactions has been understood since long time back, but the mechanism of initiation of this process in biological membrane systems is still uncertain. Lipid peroxidation can occur as a result of tissue injury in many disease states and may sometimes contribute significantly to worsening the tissue injury and cell death (Marx, 1987). According to the reports of Kakar et al (1995), diabetes is associated with increased levels of lipid peroxides in pancreas, heart and blood. A significant elevation in thiobarbituric acid reactive species (TBARS) in liver and kidney but a decrease in heart in alloxan diabetic rats has been reported by Prince et al (1998). In another report, plasma and myocardial levels of TBARS were significantly increased in streptozotocin induced diabetic rats (Nonoda et al, 1993).

The occurrence of lipid peroxidation in biological membranes causes impairment of membrane functioning, decreased fluidity, inactivation of membrane bound receptors and enzymes, and increased nonspecific permeability to ions such as Ca$^{2+}$. In addition, lipid hydroperoxides decompose upon exposure to iron or copper ions. Simple chelates of these metal ions (e.g. with
phosphate esters), heme, and some iron proteins including hemoglobin and myoglobin (Halliwell & Gutteridge, 1989).

Oxidative damage affects fatty acids especially the unsaturated fatty acid constituents of triglycerides and cholesteryl esters. As with glucose, reactive aldehydes, including glyoxal, malondialdehyde (MDA), and 4-hydroxynonenal (4-HNE) are formed, and these may react with lysine residues in proteins. The resulting adducts termed lipoxidation products in apoprotein B of LDL have recently been identified (Requena et al., 1996). The role of these products in the pathogenesis of atherosclerosis or diabetic complications is yet to be verified.

Rosenberg et al. (1979) were the first to study collagen glycation in animals with and without diabetes. They found a significant increase in glycation and peroxidation of aortic collagen in diabetic compared to non-diabetic rats. Vascular endothelial cells, which play an active role in the physiological process of vessel tone regulation and vascular permeability, form a border separating deeper layers of the blood vessel wall and cellular interstitial space from the blood and circulating cells. Damage or dysfunction of endothelial cells may reduce their effectiveness as selective permeability barrier to plasma components including cholesterol rich lipid substances (Hennig & Chow, 1988), which could lead to atherosclerosis.

Experimental evidence indicates that free radical mediated lipid peroxidation can induce cell injury causing deaths due to diabetes mellitus, hyperlipidemia, hypertension or obesity with complications (Hennig & Chow, 1988). IDDM often leads to coronary atherosclerosis related to hyperglyceridemia and altered lipoprotein profiles resulting in vascular insufficiency, ischemia, and a markedly increased risk of myocardial infarction (Parinandi et al., 1990).

**Mechanism of Lipid Peroxidation**

One of the oldest description of lipid peroxidation was given by de Saussure in the 1820s who used a simple mercury manometer to study the uptake of oxygen by walnut oil on water surface. The process of lipid peroxidation is primarily initiated by the formation of radicals and proceeds via a radicalic chain reaction (Gutteridge & Halliwell, 1990). Peroxidation is initiated by the attack of any chemical species that has sufficient reactivity to abstract a hydrogen atom
Figure 5


Initiation

Removal of H. (at any place)

Molecular rearrangement

Oxidation

Major reaction

Attack on Membrane proteins, cross linking of two or more radicals meet

Attack on membrane Proteins, reaction of two peroxyl radicals to cause singlet oxygen formation (\(^1\text{O}_2\))

H Abstraction

From adjacent Membrane lipid

\[ \text{CHO}_2^- + \text{CHO}_2^- \rightarrow C = O + -C \rightarrow \text{OH} + \text{O}_2 \]

Lipid hydroperoxide
from a methylene carbon in the side chain of fatty acids (Figure 5). This forms a radical on the
carbon atom to which the hydrogen was originally attached. This carbon-centered radical can
undergo rearrangement followed by reaction with oxygen to give a peroxyl radical. Such
radicals may combine with each other or they can attack membrane proteins or plasma proteins,
but they can possibly abstract hydrogen from adjacent fatty acid chains in a membrane thereby
propagating the chain reaction of lipid peroxidation.

A single initiation event therefore, can result in the conversion of hundreds of fatty acid side
chains into lipid hydroperoxides. The extent of propagation depends upon many factors,
including the lipid/protein ratio in a membrane, the fatty acid composition, oxygen
concentration, and the presence within the membrane of chain-braking antioxidants (Gutteridge
& Halliwell, 1990). The most important chain-breaking antioxidant in human membranes is
alphatocopherol. There is evidence that the alphatocopherol radical can be converted back to
tocopherol by reduction with ascorbic acid at the surface of biological membranes (Jenkins et
al, 1996).

6. Free Radicals and Antioxidant Status During Diabetes

Modifications of extant plasma proteins, structural proteins, and other macromolecules are
enhanced during diabetes because of increased glycation, secondary to increased glucose
concentration (hyperglycemia) and perhaps because of increased oxidative stress. The
combined effects of protein modification by glycation and oxidation may contribute to the
development of accelerated atherosclerosis in diabetes, macrovascular complications and early
cataract (Lyons & Jenkins, 1997). Oxygen free radicals generated from elevated levels of
glucose by autoxidation, are implicated in the pathogenesis of diabetes mellitus. These oxygen
radicals initiate membrane lipid peroxidation resulting in cell damage and tissue injury (Freeman
& Grapo, 1982).

Degenerative changes in the heart and kidney are almost universal complications of IDDM.
Cardiomyopathy and nephropathy share a common etiology in diabetes, i.e., the altered
hormonal and chemical milieu resulting from the lack of insulin. One possible mechanism is
cellular damage from cytotoxic oxygen free radical species (Parinand et al, 1990). Oxygen free
radicals are generated through various metabolic pathways in all cells and must be rapidly and efficiently scavenged if cellular damage is to be prevented. Indeed, there is evidence that diabetes alters free radical metabolism in blood and tissues but these alterations are quite heterogeneous, and a clear picture of how they may be involved in organ-specific complications has yet to emerge (Halliwell & Gutteridge, 1984; Oberley, 1988).

Increased levels of non-enzymatic antioxidants such as glutathione have been noted previously in the liver of STZ-diabetic rats (Oberley, 1988). In addition, increased activities of the protective enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the tissues of human diabetics as well as experimental models were reported (Oberley, 1988). Diabetic rats, twelve weeks after administration of streptozotocin, exhibited elevated activities of catalase and glutathione reductase (GR) in the heart and of copper-zinc superoxide dismutase (Cu-ZnSOD) and GR in pancreas (Wohaib & Godin, 1987). Moreover, in this same study, diabetic rat kidney showed increased activities of only GPx, whereas, the activities of catalase and Cu-ZnSOD were decreased. The same types of results were obtained in diabetic rat liver in another study in which diabetes was induced by alloxan treatment (Saxena et al, 1993, Gupta & Baquer, 1998).

It is now becoming increasingly apparent that oxidation is a common mechanism underlying many pathological and mutagenic conditions at the cellular level and contributes to the aging process (Cerutti, 1985). Reactive species capable of mediating oxidative reactions include partially reduced oxygen, transition metals, and free radicals. The sources of oxygen species may be endogenous or exogenous to the organism (Scherer & Deamer, 1986).

Endogenously, active oxygen species are generated during normal metabolism by the electron transport chain (Freeman et al, 1981), by oxidases (including the flavonoid enzymes and cyt-p-450), and as part of the neutrophil inflammatory response (Weiss et al, 1985; Zimmerman & Cerutti, 1984). Exogenous sources of oxidants include several chemical toxins and xenobiotics (including alloxan and streptozotocin), and exposure to hyperoxia and ionizing radiation (Pryor et al, 1983).
7. Antidiabetic Properties of *Trigonella foenum graecum* L.

A number of plant species are very widely used in folk medicine. It is well known that, plants next to microorganisms, are the largest pools of modern drugs. This has led many scientists especially, natural product chemists, biochemists, and pharmacologists, to give impetus to their research in the discovery of new and potential drugs of plant origin. In line with this, extracts of plants of medical importance capable of decreasing blood sugar level have been known. Of the many plants used in traditional medicine, fenugreek (*Trigonella foenum graecum* L.) is known for its hypoglycemic effect since a long time ago (Mishinsky et al, 1967; Sharma, 1986).

In an attempt to study the antidiabetic properties of fenugreek, Ribes *et al* (1986) defatted the seeds that gave a product rich in fibers, saponins, and proteins. This fraction when administered to diabetic dogs together with insulin resulted in a clear decrease of hyperglycemia and glycosuria accompanied by a reduction of the high plasma glucagon and somatostatin levels. They also suggested that the active substances are contained in the testa and endosperm.

Moorthy *et al* (1989) made some studies on the isolation and effect of orally active hypoglycemic principles from the seeds of fenugreek. In addition to the seeds, the leaves were also investigated in which the aqueous extract of the leaves, given both orally and intraperitoneally showed a hypoglycemic effect in normoglycemic and alloxan induced hyperglycemic rats (Abdel-Barry *et al*, 1997; Murthy, 1995).

In another placebo-controlled study (Bordia *et al*, 1997), fenugreek powder given at a dose of 2.5g twice a day for three months to patients of coronary artery disease (CAD) combined with NIDDM, significantly decreased the blood lipids (total cholesterol and triglycerides) without affecting the HDL and the blood sugar(fasting and post prandial). However, in their finding, fenugreek administration did not affect platelet aggregation, fibrinolytic activity and fibrinogen.

Petit *et al* (1995) demonstrated that, fenugreek seed extracts contain steroid saponins (furostanol type), which when administered chronically mixed with food (12.5mg/day/300g body weight) to normal and to streptozotocin induced diabetic rats significantly increased food intake and motivation to eat in normal rats; while modifying the cercadian rhythm of feeding behavior, it also stabilized the food consumption in diabetic rats, which was accompanied by a progressive weight gain and decrease in plasma cholesterol levels.
Fenugreek powder, its methanol extract, and the residue remaining after extraction have significant hypoglycemic effect when fed simultaneously with glucose to NIDDM model rats. Chemical analysis showed that the major constituent of the soluble dietary fiber (SDF) is galactomannan (Ali et al, 1995). The effect of fenugreek seed powder on glyoxylase I, creatine kinase and some gluconeogenic enzymes have been investigated on alloxan induced diabetic rats (Raju et al, 1999; Genet et al, 1999 and Gupta et al, 1999). Fenugreek seed powder when administered orally (5% w/w) in the diet of diabetic rats significantly lowered the rise in blood glucose level and the disturbed enzyme activities were also significantly reversed to normal values.