Review of Literature
Review of Literature

A) Insulin Resistance

Following the discovery of insulin by Banting and Best in 1922, it was widely assumed that human diabetes was due exclusively to a deficiency in the secretion of hormone. However, ten years later Himsworth (1936) noted variations in the responses of diabetic patients to insulin and proposed the notion that insulin sensitivity, not deficiency was defining biochemical defect in many diabetic patients. This idea was not considered seriously until the development of radioimmunoassay by Berson and Yalow (1960) who showed that subjects with adult onset (type 2) diabetes tended to exhibit higher than average levels of circulating insulin. Later studies supported these findings and provided a mechanistic basis for the idea that insulin resistance plays a major role in diabetes (Kahn et al, 1976; Olefsky et al, 1973; Reaven, 1988; Kolterman et al, 1980). We now know that the pathophysiology of type 2 diabetes involves defects in three organ systems that conspire together to produce abnormal glucose and lipid metabolism (DeFronzo, 1987). While there is some uncertainty regarding the primary lesion, metabolic defects in the liver and the peripheral target tissues, such as fat and muscle and pancreatic beta cells; these all contribute to the syndrome. Insulin resistance which has been defined as a state of reduced responsiveness to normal circulating concentrations of insulin is now recognized as a characteristic trait of type 2 diabetes and contributes to abnormalities in all these tissues. Various prospective epidemiological studies across several population groups indicate that type 2 diabetes progresses over a continuum of worsening insulin action, beginning with the peripheral insulin resistance and ending with a loss of insulin secretion (Fig.A). In most patients, insulin resistance can be detected long before the deterioration of glucose tolerance. Insulin resistance is a state
often associated with aging and a sedentary life style, as well as a genetic predisposition. The state seems to be fueled by, or perhaps to the certain extent, result of, obesity. The ensuing dysregulation of carbohydrate and lipid metabolism that occurs as a consequence of insulin resistance further exacerbates its progression. Beta cells of the pancreas normally compensate for the insulin resistant state by increasing basal and postprandial insulin secretion. At some point, the beta cells can no longer compensate, thus failing to respond appropriately to glucose. This ultimately leads to deterioration of glucose homeostasis and the development of glucose intolerance. Approximately 5-10% of glucose intolerant patients in a given year progress to frank diabetes that continues to worsen as insulin resistant increases. Adipose cells generate more fatty acids, the liver produces more glucose in an unregulated fashion, and the beta cells undergo complete failure resulting in the late stages of the disease where high doses of exogenous insulin may be required.

Fig.A: Metabolic staging of type 2 diabetes.
Even in the absence of diabetes, insulin resistance is a key feature of other human disease states. Impaired insulin action coupled with hyperinsulinemia leads to a variety of abnormalities, including elevated triglycerides, low levels of HDL, enhanced secretion of VLDL, disorders of coagulation, increased vascular resistance, changes in steroid hormone levels, attenuation of peripheral blood flow, and weight gain. Thus, insulin resistance is often associated with central obesity, hypertension, polysystic ovarian syndrome, dyslipidemia and atherosclerosis. This constellation of syndromes is often referred to as syndrome X or insulin resistance syndrome (Reaven, 1993). Whether impaired insulin action is directly responsible for all the symptoms in these patients remains unclear. However, the broad prevalence of insulin resistance and its association with profound metabolic abnormalities are widely accepted.

**Fig.B:** Syndrome X, its contributors and their interdependence.
CLINICAL CHARACTERISTICS, COMPLICATIONS AND PATHOPHYSIOLOGY OF INSULIN RESISTANCE:

As mentioned above there are several characteristics observed in insulin resistance such as increase in central obesity, hypertension, hyperinsulinemia, abnormal lipid profile etc. These features can be observed clinically as mentioned in the following table.

Table 1: Clinical and metabolic characteristics of the insulin resistant group and their percent differences from the control group

<table>
<thead>
<tr>
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<th>Mean ± SEM in insulin resistant population</th>
<th>Percent difference from control (non-insulin resistant population)</th>
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<tbody>
<tr>
<td>Waist: hip ratio</td>
<td>0.932 ± 0.012</td>
<td>+15%</td>
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<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.53 ± 0.05</td>
<td>+5%</td>
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<tr>
<td>2-h glucose (mmol/l)</td>
<td>7.80 ± 0.11</td>
<td>+20%</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>129 ± 3</td>
<td>+36%</td>
</tr>
<tr>
<td>2-h insulin (pmol/l)</td>
<td>787 ± 18</td>
<td>+37%</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.01 ± 0.03</td>
<td>+50%</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.33 ± 0.03</td>
<td>+10%</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.14 ± 0.01</td>
<td>-8%</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>123 ± 0.4</td>
<td>+4%</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>74 ± 0.2</td>
<td>+4%</td>
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The comparison with the control group is made after adjusting for age, sex, ethnicity, and body mass index (BMI), and calculated at the mean population age (43 years) and BMI (27.9 kg/m²) for a male Mexican-American subject.

Insulin resistance may take place as a consequence of physiology of necessity. During prolonged fasting state, a decrease in insulin sensitivity may occur to mobilize other energy substrates from peripheral tissues so that metabolic function can be maintained (Baba and Neugebauer, 1993). Similarly, insulin resistance is seen in patients with insulinoma, who experience recurrent episodes of hypoglycemia. Insulin resistance in such patients protects them from excessive lowering of plasma glucose levels (Nankervis et al, 1985; Pontiroli et al, 1992). Insulin resistance is one of the
Insulin resistance

common characteristics in individuals with obesity (Reaven, 1988; Defronzo et al, 1985; Groop et al, 1989; Kolterman et al, 1981; Landsberg and Krieger, 1985; Bonadonna et al, 1990; DeFronzo and Ferrannini, 1991) and non-insulin dependent diabetes mellitus (NIDDM) (Groop et al, 1989; Kolterman et al, 1981; Landsberg and Krieger, 1989). Besides these, there are several other clinical states where insulin resistance can be observed (Table 2).

Table 2: Clinical presentation of insulin resistant states

<table>
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<th>Condition</th>
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<tr>
<td>Obesity (Especially upper body obesity): &gt; 130-140% of ideal body weight</td>
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<tr>
<td>Age</td>
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<tr>
<td>Non-insulin-dependent diabetes mellitus</td>
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<tr>
<td>Insulin dependent diabetes (IDDM), requiring large daily amounts of insulin (e.g. 200U/day)</td>
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<tr>
<td>Gestational diabetes (glucose intolerance with onset during pregnancy)</td>
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<tr>
<td>Non-diabetic, non-obese hypertriglyceridemia</td>
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<tr>
<td>Acanthosis nigricans</td>
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<tr>
<td>Polycystic ovary syndrome</td>
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<tr>
<td>Lipodystrophy (partial or generalized)</td>
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<tr>
<td>Leprechaunism</td>
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<tr>
<td>Pineal hyperplasia syndrome</td>
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<tr>
<td>Hormonal disorders (excess counterinsulin hormones, e.g. pheochromocytoma, acromegali, Cushing’s syndrome)</td>
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<tr>
<td>Anti-insulin-antibodies (Prereceptor resistance)</td>
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<tr>
<td>Absent or dysfunctional insulin receptor (Type A syndrome)</td>
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<tr>
<td>Anti-insulin-receptor antibodies (Type B syndrome)</td>
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<tr>
<td>Abnormal or mutated insulin molecule</td>
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<tr>
<td>Incomplete conversion of proinsulin to insulin</td>
</tr>
<tr>
<td>Drug induced (e.g. thiazide diuretics, glucocorticoids)</td>
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<td>Smoking</td>
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In subjects who retain insulin secretory function, particularly non-diabetics, a relatively linear relation is found between measures of insulin resistance and plasma
insulin concentration, i.e. more the insulin resistance, greater is the magnitude of hyperinsulinemia. Insulin resistance and hyperinsulinemia are more severe and closely associated with hypertension in obese patients than in non-obese patients. Hyperinsulinemia is also a consequence of insulin resistance that stimulates the sympathetic nervous system increasing sympathetically mediated thermogenesis and reestablishing the energy balance. The increase in sympathetic nervous system activity, however, also contributes to hypertension by stimulating the heart, the vasculature and the kidneys (Landsberg and Krieger, 1989; Rowe et al, 1981). Hyperinsulinemia has been identified as a primary risk factor for coronary artery disease (CAD). Variety of direct effects of insulin at cellular level and the role played by it in the regulation of lipoprotein metabolism makes insulin one of the primary risk factors of CAD (Austin, 1989). As mentioned earlier, the resistance to insulin stimulated glucose uptake and compensatory hyperinsulinemia are responsible for hypertriglyceridemia in patients with high blood pressure. It has now been emphasized that the combination of a high plasma triglyceride and low high density lipoprotein (HDL) cholesterol concentration are the consequences of insulin resistance responsible for the increasing risk of CAD (Castelli, 1986). Fig. C summarizes the sequel of clinical and pathophysiological events leading to the insulin resistance.
Fig.C: Pathogenesis and sequence of events leading to development of glucose intolerance, insulin resistance, and impaired insulin secretion in non-insulin dependent diabetes mellitus. (+) shows positive feedback loops, that results in self perpetuation of primary defects.

Endothelial dysfunction in metabolically important capillary beds has also been suggested as the principal explanation for the development of the insulin resistance syndrome in parallel with large artery atherosclerosis (Pinkney et al, 1997). The endothelium is a monolayer of flat cells strategically located between circulating blood and vascular smooth muscle. They are the source of substances that profoundly affect the vascular tone, growth, platelet function, coagulation and monocyte function. The endothelium releases both contracting and relaxing factors. The contracting
Insulin resistance and vascular tissue:

Both insulin resistance and possibly hyperinsulinemia have been suggested as risk factors for the development of cardiovascular complications in diabetes. In hyperglycemia the increased amounts of glucose can also be transported intracellularly and metabolized to increase flux through the sorbitol pathway, change the redox potential or alter signal transduction pathways, such as the activation of the diacylglycerol (DAG) and protein kinase C (PKC) levels (Fig. D). The activation of DAG-kinase pathway causes increased expression of transforming growth factor-β (TGF-β) which has been implicated in the development of mesangial expansion and basement membrane thickening in diabetes. The increased PKC and cytosolic phospholipase A₂ (cPLA₂) activities result in increases of arachidonic acid release and prostaglandin E₂ (PGE₂) production and decreases in Na⁺-K⁺ ATPase activity. PKC activation can also regulate vascular permeability and neovascularization via the expression of growth factors such as vascular endothelial growth factor (VEGF). Similarly, the PKC activation also causes increase in the expressions of plasminogen activator inhibitor-1 (PAI-1) and endothelin-1 (ET-1) leading to increased contractility and coagulation respectively. Thus, activation of the PKC pathway can, in vascular
Insulin resistance cells, regulate permeability, contractility, extracellular matrix, cell growth, angiogenesis, cytokine actions, and leukocyte adhesions, all of which are abnormal in diabetes (King, 1999).

**HYPERGLYCEMIA**

- Oxidants
- DAG=PKCβ+δ
- Glycated products

- TGF-β
- Collagen fibronectin
- Na+K+ATPase
- Permeability
- Basement matrix thickening
- Vascular hypertrophy

- cPLA2
- PEG2
- PKC
- Angiogenesis

- VEGF
- c-fos
- ANP
- TGF
- Cardiac myopathy
- Atherosclerosis and Cardiac dysfunction

- PAI-1
- Et-1
- Contractility

**HYPERTENSION AND CONGESTIVE CARDIAC FAILURE**

*Fig.D: Schematic diagram of the adverse effects of hyperglycemia in vascular cells.*

In vascular cells insulin produces two types of actions: anti-atherogenic and atherogenic. An example of insulin's anti-atherogenic action is the ability to increase nitrous acid production that can cause vasodilatation and retard migration and growth of arterial smooth muscle cells. On the other hand, insulin is known to promote proliferation of aortic or arterial smooth muscle cell cultures. But in hyperinsulinemia
or insulin resistance, hypertrophic action of insulin on vascular smooth muscles is predominantly observed leading to atherosclerosis and other cardiovascular complications. Vascular cells contain a significant number of high affinity insulin receptors that are structurally similar to those in other tissues and can activate two different signal transduction pathways: PI-3 kinase and MAP-kinase cascades (King et al, 1991; Follo et al, 1997). In phosphatidylinositol PI-3 kinase pathway, insulin receptor substrate (IRS)-1 and 2 are required while, mitogen activated protein (MAP)-kinase does not need these substrates. It is shown that activation of PI3 kinase pathway by insulin mediates metabolic effects, such as glucose transport whereas, activation of MAP kinase pathways are responsible for chronic effects like growth (Bruning et al, 1997). Therefore, it is postulated that in insulin resistant state, the pathway leading from insulin receptors to the activation of PI-3 kinase could be blunted in vascular tissues resulting in lowered nitric oxide (NO) production from the vascular wall and endothelial nitric oxide (eNOS) gene expression. (King, 1999).

MOLECULAR MECHANISMS OF INSULIN RESISTANCE:

The insulin resistance at molecular level appears to occur at the level of insulin receptor, receptor kinase activation, glucose carriers (GLUT) and or gene expression.

The Insulin Receptor:

Insulin action is initiated through the binding to and activation of its cell surface receptor that consists of two α subunits and two β subunits that are disulfide linked into an α2β2 heteromeric complex. Insulin binds to the extracellular α subunits, transmitting a signal across the plasma membrane that activates the intracellular tyrosine kinase domain of the β subunit. The receptor then undergoes a series of
intramolecular transphosphorylation reactions in which one β subunit phosphorylates its adjacent partner on specific tyrosine residues. Some evidences suggest that different tyrosine residues account for distinct functions. e.g. phosphorylation of COOH-terminal tyrosines mediates the mitogenic actions of insulin. The phosphorylated tyrosines in the juxtamembrane domain may participate in substrate binding, whereas, those found within the kinase domain regulate the catalytic activity of the insulin receptor β subunit. Some forms of insulin resistance may involve the receptor itself. Alterations in insulin receptor expression, binding, phosphorylation state, and/or kinase activity could account for many insulin-resistance phenotypes. In addition, it is possible that the selected blockade of distinct phosphorylation sites selectively inhibits certain actions of insulin. In this regard, individuals have been identified with rare genetic effects in insulin receptor that influence expression, ligand binding, and tyrosine kinase activity. These patients demonstrate severe insulin resistance, manifest as clinically diverse syndromes including Type A syndrome, leprechaunism, Rabson-Mendenhall syndrome, and lipoatropic diabetes (Taylor and Arioglu, 1998; Krook and O’Rahilly, 1996).
Insulin receptor

Fig.E: Schematic illustration of major signaling pathways of insulin action. The phosphorylated insulin receptor binds and phosphorylates IRS proteins and Shc that bind differentially to various downstream signaling proteins. PI-3 kinase is critical for metabolic actions of insulin, such as glucose transport, glycogen synthesis, and protein synthesis, whereas Grb-2/SOS complex, which activates the MAP kinase cascade, is critical in mitogenic response. PI-3 kinase probably modulates mitogenic response as well.

Kinases and their role in insulin resistance:

Insulin receptor is a ligand-activated tyrosine protein kinase. Binding of insulin to the alpha subunits of the heterotetrameric insulin receptor leads to the rapid intramolecular autophosphorylation of several tyrosine residues in the beta subunits. In the intact cells, the insulin receptor is also phosphorylated on the serine and threonine residues presumably by protein kinase C or cyclic AMP dependent protein
kinase. Such phosphorylation inhibits tyrosine kinase activity of the insulin receptor. The tyrosine kinase activity is required for the signal transduction. The activated receptor kinase initiates a cascade of events first by phosphorylating a protein called insulin receptor substrate-1 (IRS-1). Phosphorylated IRS-1 serves as a docking protein for the other proteins that contain so called Src homology 2 (SH2) domains. One of such SH2 domain proteins is phosphoinositide (PI) 3-kinase. PI 3 kinase catalyzes the addition of phosphates to phosphoinositides on the 3-position of the D-myo-inositol ring and these compounds are apparently involved in the signal transduction. The Ras oncoprotein is one of the most potent mitogens. Ras has been linked to the insulin action pathway because it is known to activate the cascade of the mitogen activated protein (MAP) kinases. MAP kinases are among the many of such kinases that are known to be activated by insulin. Insulin also activates of serine/threonine phosphorylation cascades. Serine kinases have a dual function in the insulin signaling pathway i.e. further transduction of the insulin signal and activation of glycogen synthase or MAP kinase activation (Davis and Grannel, 1996).

**MAP-kinase activation:**

Insulin binds to the α subunits and causes activation of β subunit tyrosine kinase, resulting in receptor autophosphorylation and phosphorylation of Shc. Shc is associated with Grb-2. Grb-2, in turn, associates with the Ras guanylnucleotide exchange factor Son of Sevenless (SOS), resulting in an increase in GTP-bound Ras. Ras in GTP-bound state can interact with Raf that functions as an upstream kinase for mitogen activated protein kinase kinase (MEK) and results in MAP kinase activation. Role of this MAP kinase cascade in insulin resistance is still unclear. Chang et al (1995) measured the activation of MAP kinase in the muscles of the lean and obese...
mice. They found that insulin could cause the activation of MAP kinase only in the lean mice and not in the obese mice. It was concluded that the lack of MAP kinase response to insulin can be one of the elements of insulin resistance in the obese animals. However, as mentioned earlier, many workers feel that MAP kinase cascade is not involved in the action of insulin on glucose metabolism. Lazar et al (1995) have reported that Inhibition of MAP kinase activation does not reduce insulin stimulated glucose transport or glycogen synthesis. Moreover, in insulin resistant subjects with obesity or type 2 diabetes, there is profound insulin resistance in PI-3 kinase pathway of insulin receptor signaling, whereas, MAP-kinase pathway is reported to be normal (Cusi et al, 2000).

PI-3 kinase activation:

At present, only one signaling molecule is unequivocally essential for insulin-stimulated GLUT4 translocation that is the type 1A PI-3 kinase. Multiple studies using various pharmacological inhibitors, microinjection of blocking antibodies, and expression of dominant interfering and constitutively active mutants are all consistent with a necessary role of PI-3 kinase activity in insulin-stimulated glucose uptake and GLUT4 translocation (Czech and Corvera, 1999). Several studies have suggested that the interaction of IRS with PI-3 kinase is necessary for the appropriate activation and/or targeting of the enzyme to a critical intracellular site. However, the targets of PI-3 kinase action are controversial. Two classes of serine/threonine kinases are known to act downstream of PI-3 kinase, namely the serine/threonine kinase Akt, also known as protein kinase B (PKB), and the atypical protein kinase C isoforms ζ and λ. Stable expression of constitutively active, membrane bound form of Akt in 3T3L1 adipocyte results in increased glucose transport and persistent localization of GLUT4
to the plasma membrane (Kohn et al, 1996; Kohn et al, 1998). Conversely, expression of a dominant-interfering Akt mutant inhibits insulin stimulated GLUT4 translocation (Cong et al, 1997; Wang et al, 1999). Similarly PKCζ is also activated by the formation of polyphosphoinositides that accumulate in the insulin treated cells. Expression of PKCζ and PKCλ are also reported to induce GLUT4 translocation (Kotani et al, 1998; Kitamura et al, 1998). Several investigators have examined the role of Akt and PI-3 kinase in the regulation of peripheral insulin sensitivity. There appears to be relative decrease in insulin stimulated association of IRS proteins with PI-3 kinase and activation of Akt in insulin resistant skeletal muscle (Cusi et al, 2000; Krook et al, 1998). PI-3 kinase activity is reported to be lower in the obese animals as compared to the lean animals (Heydrick et al, 1993). Evidence suggests that PI-3 kinase impairment develops much before the emergence of overt insulin resistance. In adipocytes from young obese mice that are not insulin resistant, insulin receptor autophosphorylation and pp60 phosphorylation are found to occur normally, but there is a marked defect in IRS-1 tyrosine phosphorylation. In older obese mice however, all the three protein groups are less phosphorylated than in lean controls (Le Marchand-Brustel, 1999).

The capacity of platelet-derived growth factor (PDGF) to alter the ability of insulin to phosphorylate IRS-1 on tyrosine residues in cultured adipocytes is recently demonstrated. Short treatment with PDGF causes IRS-1 serine/threonine phosphorylation through a PI-3 kinase dependent pathway and this prevents phosphorylation of tyrosine residues on IRS-1 (Rocort et al, 1997). Insulin like Growth Factor (IGF)-1 is as efficient as insulin in promoting glucose uptake. Its action is mediated through a PI3-kinase dependent pathway since, like insulin stimulated glucose uptake, it is completely inhibited by PI-3 kinase inhibitor
wortmannin (Poggi et al, 1979). It is reported that IGF-1 signaling is defective in obese mice as compared to lean animals and that this is due to post receptor defects only (Jullien et al, 1996).

**Glucose transporters and their role in insulin resistance:**

Facilitative glucose uptake occurs through a family of highly related integral membrane proteins that share significant sequence similarity. Although several lines of evidence suggest the presence of additional glucose transporters, only four members of this gene family have been established functionally. GLUT5, that was originally thought to be a glucose transporter ultimately proved to be a fructose transporter (Burant et al, 1992). Of the four glucose transporters isoforms, GLUT4 is highly expressed in adipose tissue and striated muscle with lower levels of GLUT1 isoform (Charron et al, 1999). In the basal state GLUT4 cycles slowly between the plasma membrane and one or more intracellular compartments with the vast majority of transporter residing in vesicular compartments within the cell interior (Rea and James, 1997; Kandror and Pilch, 1996). Activation of insulin receptor triggers a large increase in the rate of GLUT4 vesicle exocytosis and a smaller decrease in the rate of internalization by endocytosis (Satoh et al, 1993; Jhun et al, 1992). The stimulation of exocytosis by insulin is probably the major step for the GLUT4 translocation because complete inhibition of GLUT4 endocytosis only modestly increases plasma membrane associated GLUT4 protein without affecting the extent of insulin stimulated GLUT4 translocation (Shibata et al, 1995; Kao et al, 1998; Ceresa et al, 1998).
In contrast to GLUT4, GLUT1 is localized both to the plasma membrane and intracellular storage sites in the basal state but displays only modest insulin stimulated redistribution to the plasma membrane. Thus, the overall insulin dependent shift in the cellular dynamics of GLUT4 vesicle trafficking results in a net increase of GLUT4 on the cell surface, thereby increasing the rate of glucose uptake (Pessin et al, 1999). As mentioned in earlier sections, PI-3 kinase dependent signal transduction pathway is involved in the translocation of GLUT4 to the plasma membrane. In addition, several studies have demonstrated the presence of PI-3 kinase independent mechanisms of GLUT4 translocation like osmotic shock, GTPγS, and exercise/contraction in skeletal
muscle induce GLUT4 translocation in the complete absence of PI-3 kinase activity (Chen et al, 1997; Baldini et al, 1991; Elmendorf et al, 1998; Yeh et al, 1995). It is well established that integral membrane proteins traffic to and from the plasma membrane through an endosomal recycling membrane system. Immunoelectron microscopy has demonstrated that the GLUT4 protein is predominantly localized in small vesicles and tubulovesicular structures with relatively smaller amounts found in the trans-Golgi network, clathrin-coated vesicles, and endosomes (Rodnick et al, 1992; Slot et al, 1991a; Slot et al, 1991b). However, several studies have observed the presence of a separate population of GLUT4 containing vesicles different from the constitutively recycling endosomal system. For example, GLUT4 containing vesicles are enriched in the vesicle SNAP [soluble N-ethylmaleimide-sensitive factor attachment protein] receptor (v-SNARE) protein and VAMP2 (vesicle attachment membrane protein 2), but not the related VAMP3/cellubrevin isoform, which is present in the constitutively recycling endosomal population (Martin et al, 1998; Martin et al, 1996; Sevilla et al, 1997). The presence of distinct vesicle population may also account for the ability of insulin to stimulate 2-4 fold plasma membrane increases of several recycling proteins (i.e. GLUT1, transferring, and IGF-2/mannose-6-phosphate receptors), whereas plasma membrane GLUT4 content can increase 10-20 fold. Thus it appears that this unique subpopulation of GLUT4 containing vesicles is primarily responsible for the majority of insulin stimulated translocation (Pessin et al, 1999). Although these SNARE-VAMP interactions are essential, none of these core proteins are reported to be direct targets of insulin action. Similarly although several important SNARE accessory proteins such as Munc18, Synip, and NSF are also required for the control of GLUT4 docking and fusion events, the molecular mechanisms by which insulin regulates their function has yet to be elucidated. So
even though significant progress has been made in our understanding of GLUT4 compartmentalization and SNARE protein interactions critical to insulin stimulated GLUT4 translocation, there is no evidence that the known components are defective or display aberrant function in insulin resistant states (Pessin et al, 2000). However, as we have only identified some of the core SNARE machinery, future studies will be necessary to focus on isolated additional regulatory proteins in the GLUT4 vesicle physiology.

Role of gene expression in insulin resistance:

Alterations in the various enzymes and proteins mentioned above are mediated through a change in rate of mRNA synthesis from specific genes. Various mutations have been detected in the insulin receptor gene in patients with genetic syndromes of extreme insulin resistance. A mutation in the structural gene coding for the transacting factor that impairs its ability to bind DNA or that affects the rate of transacting factors may be involved e.g. unrestricted gluconeogenesis is the primary source of the excessive overproduction of glucose in NIDDM. Because phosphoenol pyruvate carboxykinase (PEPCK) catalyzes the rate-limiting step in gluconeogenesis, it is possible that faulty regulation of the PEPCK gene could be involved in insulin resistance (Granner and O'Brien, 1992).

A family of nuclear receptors has been identified that regulates gene transcription and is activated by fatty acids. These are peroxisome proliferator-activated receptors (PPAR). Three different genes of PPARs have been identified (α, β and γ). PPARα is activated by fibrates and regulates lipoprotein expression and peroxisome proliferation, whereas, PPARβ and PPARγ regulate lipid synthesis and carbohydrate metabolism. Decreased expression of PPARα is now considered as the
mechanism responsible for diabetes mellitus and insulin resistance. In fact, PPAR is a target for antidiabetic drugs (thiazolidinediones) that increases sensitivity of insulin (Torra et al, 1999).

**Insulin Resistance and obesity**

Insulin is a critical regulator of virtually all aspects of adipocyte biology, and adipocytes are one of the most highly insulin-responsive cell types. Insulin promotes adipocyte triglyceride stores by a number of mechanisms, including fostering the differentiation of preadipocytes to adipocytes and, in mature adipocytes, stimulating glucose transport and triglyceride synthesis (lipogenesis), as well as inhibiting lipolysis (Kahn and Flier, 2000). Insulin also increases the uptake of fatty acids derived from circulating lipoproteins by stimulating lipoprotein lipase activity in adipose tissue. Insulin’s metabolic effects are mediated by a broad array of tissue-specific actions that involve rapid changes in protein phosphorylation and function, as well as changes in gene expression. Insulin action in adipocytes also involves changes in gene transcription. The transcription factor ADD-1/SREBP-1c (adipocyte determination and differentiation factor-1/sterol regulatory element-binding protein-1c) may play a critical role in the actions of insulin to regulate adipocyte gene expression (Kim et al, 1998; Shimomura et al, 1999; Foretz et al, 1999), by inducing genes involved in lipogenesis and repressing those involved in fatty acid oxidation. The relationship between obesity and insulin resistance is seen across all ethnic groups and is evident across the full range of body weights. Large epidemiologic studies reveal that the risk for diabetes, and presumably insulin resistance, rises as body fat content (measured by body mass index [BMI]) increases from the very lean to the very obese, implying that the “dose” of body fat has an effect on insulin
sensitivity across a broad range (Colditz et al, 1990). Although this relationship is seen with measures of adiposity such as BMI, which reflect general adiposity, it is critical to realize that all sites of adiposity are not equal in this regard. Central (intra-abdominal) depots of fat are much more strongly linked to insulin resistance, type 2 diabetes, and cardiovascular disease than are peripheral (gluteal/subcutaneous) fat depots (Kissebah and Krakower, 1994). This fact about fat and insulin sensitivity has not been adequately explained. It is possible that an unknown common factor, either genetic or environmental, produces both insulin resistance and the central pattern of regional adiposity, and that central obesity does not actually cause insulin resistance. Alternatively, some biochemical feature of intra-abdominal adipocytes may directly influence systemic insulin sensitivity. A leading hypothesis in this regard is that intra-abdominal adipocytes are more lipolytically active, in part due to their complement of adrenergic receptors. This would increase intraportal FFA levels and flux, which might inhibit insulin clearance and promote insulin resistance by mechanisms that are still uncertain (Kahn and Flier, 2000).

Adipocytes express and secrete numerous peptide hormones and cytokines, including TNF-α; plasminogen-activator inhibitor-1, which helps maintain hemostasis; angiotensinogen, whose proteolytic product regulates vascular tone; and leptin, which plays a central role in regulating energy balance. Adipose tissue can also produce active steroid hormones, including estrogen and cortisol (Bujalska et al, 1997; Deslypere et al, 1985). Leptin, the product of the ob gene, exerts pleiotropic effects, including profound effects on satiety, energy expenditure, and neuroendocrine function. Severe insulin resistance is a well known feature of deficiency of leptin or its receptor in the ob/ob or db/db mouse strains, and these models were among the first to be investigated for the pathogenesis of insulin resistance in the early 1970s.
The result of leptin replacement in *ob/ob* mice on diabetes and insulin resistance is dramatic. Leptin treatment causes both glucose and insulin levels to fall within hours of administration, before changes in either food intake or body weight occur, and prolonged leptin has effects on glucose and insulin that exceed those seen in pair-fed *ob/ob* mice (Halaas et al, 1995). Leptin has a clear insulin-sensitizing effect acutely and also after chronic administration to normal rodents (Halaas et al, 1995; Campfield et al, 1995; Pelleymounter et al, 1995). Some evidence suggests that in tissues including muscle and β cells, leptin promotes lipid oxidation and inhibits lipid synthesis, which would promote insulin sensitivity (Muoio et al, 1997; Shimabukuro et al, 1997). So, one of the future courses for research in the field of insulin resistance might be targeted towards development of leptin sensitizers.

**DRUG TREATMENT IN PATIENTS WITH INSULIN RESISTANCE:**

Although with advent of research, the molecular mechanisms of insulin resistance are becoming better understood and there is development of new therapeutic agents like insulin sensitizers (thizolidinediones), in clinical practice, as of today, a patient with insulin resistance is looked upon as hypertensive or having diabetes mellitus. Accordingly he is taking either antihypertensives or antidiabetic drugs (Sulfonylureas, biguanides or insulin) or both. It is thus essential to look into the effects of these agents on insulin sensitivity. In recent years some scattered studies have been conducted to evaluate the effect of various antihypertensives and antidiabetics on insulin sensitivity.

The antihypertensive drugs most often used clinically have been reported not to affect glucose metabolism and insulin sensitivity favorably. Thiazide diuretics promote the development of glucose intolerance (Bengtsson et al, 1984; Murphy et al,
1982; Struthers et al, 1985) and have been shown to increase the insulin resistance (Pollare et al, 1989a). Beta blockers are reported to worsen glucose tolerance, especially in combination with diuretics (Bengtsson et al, 1984). They are also found to worsen hypertriglyceridemia (Pollare et al, 1989b). However, there are exceptions too. Alpha-adrenoceptor blocker prazocin has been reported to improve insulin sensitivity (Satia et al, 1997). The newer antihypertensives used clinically viz. the ACE inhibitors and calcium channel blockers are better than the beta blockers and diuretics as far as their effect on insulin sensitivity is concerned. ACE inhibitors do not raise serum insulin levels and captopril has been reported to reduce insulin resistance (Satia et al, 1997; Parulekar et al, 1996; Mehta et al, 1999). Calcium channel blockers also improve insulin sensitivity (Satia et al, 1997; Srinivasan et al, 1997; Gokhale et al, 1998).

Hyperglycemia rarely occurs in isolation, and other conditions pertaining to the insulin resistance syndrome are usually in evidence. Thus, it is important that new antidiabetic agents should not impede and preferably directly benefit the cardiovascular risk profile of these patients.

NEWER THERAPEUTIC AGENTS AS INSULIN SENSITIZERS:

There are several groups of pharmacological agents with immense potential for therapeutic use in insulin resistance associated with the above conditions. They can be classified depending on the mechanism of action.

I. Insulin sensitizing agents:

*Thiazolidinedione* derivatives have emerged out as a new class of antidiabetics. They include rosiglitazone, ciglitazone, pioglitazone, troglitazone and
englitazone. The hypoglycemic action of these agents has been reported in various animal models of NIDDM and insulin resistance including obese Zuckar rats, ob/ob mice, and KKA mice (Hoffman et al, 1991; Fujita et al, 1983). Thiazolidinediones increase glucose oxidation (via stimulation of pyruvate dehydrogenase) in adipose tissue and muscle, increase glycogen and lipid synthesis from glucose and decrease glycogenolysis (Whitehouse et al, 1974; Steiner and Lein, 1987). Treatment of insulin resistant obese KKA mice with pioglitazone corrects the deficiencies in the glucose transport and GLUT 4 mRNA and protein abundance in both skeletal muscle and adipose tissue, and this increase in transporter number and function have a strict dependence on the presence of circulating insulin (Bell et al, 1990). Because thiazolidinediones enhance certain actions of insulin via the peroxisome proliferator-activated receptor γ (PPAR-γ) other types of PPAR-γ agonists are being sought (Spiegelman, 1998).

Dichloroacetate has been shown to increase oxidative glucose metabolism via stimulation of pyruvate dehydrogenase in the in vitro studies. It also decreases free fatty acid oxidation resulting in inhibition of hepatic gluconeogenesis (Stacpoole and Green, 1992).

Vanadate has been reported to mimic most of the metabolic effects of insulin. In STZ-treated rats vanadate increased glucose uptake and oxidation in muscle (Shechter, 1990). The impaired glycogen synthase activity and glycogen reserves returned to normal in diabetic rats (Heyliger et al, 1985).

II. Inhibitors of fatty acid oxidation:

Increased free fatty acid (FFA) levels lead to insulin resistance in skeletal muscle (Randle et al, 1965). This may be a result of decrease in glucose utilization by
inhibiting glucose oxidation via inhibition of pyruvate dehydrogenase and reduction in glucose uptake by inhibiting hexokinase (Foley, 1992). A decrease in FFA oxidation in patients with NIDDM should therefore have a favorable effect not only on hepatic glucose overproduction, but also on peripheral glucose disposal. Carnitine palmitoyl translocase-I (CPT-I) is responsible for the transport of activated long chain fatty acids across the mitochondrial membrane in hepatocytes. Drugs that inhibit this enzyme could thus prevent the intramitochondrial oxidation of FFA to acetyl CoA, ketone bodies and nicotinamide adenine dinucleotide (NADH). CPT-I inhibitors include clomoxir, etomoxir, TDGA. TDGA has been reported to decrease glucose levels in IDDM and NIDDM subjects. Etomoxir has been reported to produce a 33% increase in insulin mediated glucose uptake (Hubinger, 1992). It also produces a decrease in hepatic glucose production in obese NIDDM patients (Ratheiser et al, 1991). However, there have been incidences of cardiac hypertrophy in long term animal toxicology studies with both TDGA and etomoxir (Lee et al, 1982).

III. Beta₃ adrenoceptor agonists:

Beta 3 adrenergic receptors are expressed almost exclusively in fat. Treatment with β 3 receptor agonist CL316,243 results in enhanced sensitivity of both whole body glucose uptake and suppression of hepatic glucose production in insulin resistant obese rodents and humans (deSouza et al, 1997; Weyer et al, 1998). These effects are accompanied by increased glucose uptake in adipose tissue with no effect in multiple muscle groups studied. Thus, increasing glucose uptake selectively in fat with β₃ adrenergic receptor agonists may improve whole body glucose uptake, with the effects in fat indirectly resulting in increased insulin sensitivity in liver. Alternatively, β₃ agonists may work by changing the release of some adipocyte product that
influences systemic insulin sensitivity. BRL37344, an active metabolite of BRL35135, is a potent $\beta_3$ adrenoceptor agonist. It produces a dose dependent increase in energy expenditure, weight loss, post glucose load hyperinsulinemia and an improvement in glucose tolerance in otherwise healthy obese patients. BRL 35135 is reported to increase insulin sensitivity in obese patients with NIDDM (Cawthorn et al, 1992). Long term clinical trials with beta adrenoceptor agonists is the need of the hour. These agents could be effective in the treatment of diabetic as well as non-diabetic obese patients.

IV. Inhibitors of gluconeogenesis:

Hepatic insulin resistance causes gluconeogenesis and thus the inhibition of enzyme pyruvate carboxylase leading to inhibition of gluconeogenesis could prove beneficial. Phenylalkanoic acid derivatives inhibit gluconeogenesis in hepatocytes at concentrations that do not inhibit cell metabolism (Bressler and Johnson, 1992). Though there have been no human studies with these agents, a little success has been achieved in animal models.

V. Inhibitors of lipolysis:

Adenosine inhibits lipolysis through its action on adipocytes. This action is probably mediated via the alpha receptor subtype (Stiles, 1992). GR79236 is an analogue of adenosine and specific receptor for alpha receptor subtype. This compound inhibits lipolysis in human abdominal wall adipocytes and decreases plasma non-esterified free fatty acids (Strong et al, 1993). However, this is the only evidence for the association of these changes with significant reduction in plasma
glucose levels. However, these agents have a short duration of action and the hypoglycemic response is variable.

VI. Aldose reductase inhibitors:

The use of aldose reductase inhibitors to enhance insulin sensitivity in diabetes mellitus has been reported. These agents probably act by restoring and preserving intracellular reduced glutathione levels, thereby enhancing formation of insulin receptor mixed disulphide bonds (York, 1988). Tolrestat, Statil, Sorbinil are a few aldose reductase inhibitors.

VII. Alpha-glucosidase inhibitors:

Another class of drugs that is reported to be effective in the treatment of hyperinsulinemia is alpha-glucosidase inhibitors. These agents reduce gastrointestinal breakdown and absorption of carbohydrates. They lower plasma glucose concentration and tend to cause weight loss. Acarbose belongs to this class of drugs and is reported to lower insulin levels and gastrointestinal peptide (GIP) levels (Hoffman and Spengler, 1994). This however would potentiate glucose mediated insulin secretion. In addition, acarbose has other effects on gastrointestinal hormones. Alpha-glucosidase inhibitors might be of value in obese diabetic patients but they cannot be useful in normal weight diabetics because of their effects on nutrition.

VIII. Alpha-2 antagonists and imidazolines:

Insulin secretion is normally subjected to tonic suppression via α-2 adrenoceptors. The possibility of relieving this suppression with selective α-2 antagonists such as midaglizole and MK-912 has been considered (Kashiwagi et al, 1994).
1986). While this approach has been shown to raise insulin concentrations and improve glycemic control in type 2 patients, it has proved difficult to achieve potency with sufficient selectivity to avoid pressor responses (Ortiz-Alonso et al, 1991).

Imidazoline compounds like efaroxan can stimulate insulin secretion independently of an α-2 blockade. There is evidence that this may occur via a closure of K⁺-ATP channels and possibly other K⁺ channels as well as effects at more distal steps in the control of exocytosis (Morgan et al, 1994; Hirose et al, 1997).

**Gene therapy for reducing insulin resistance in type-2 diabetes:**

There are two approaches for the treatment of insulin resistance in type-2 diabetes:

1. Transferring genes that are important in insulin transduction pathways and

2. Delivering hormones or soluble factors that can decrease insulin resistance. The goal for gene therapy for type-2 diabetes is to increase peripheral glucose uptake, primarily in muscle and fat, and to decrease hepatic glucose output.

Streptozotocin-treated diabetic mice show decreased glucokinase expression and activity, resulting in inefficient conversion of glucose to glucose-6-phosphate, inhibition of glycolysis, and stimulation of gluconeogenesis. The best candidate for gene transfer experiments aimed at reducing insulin resistance is the hormone leptin. Leptin, the ob gene product secreted from adipocytes and gastric mucosal cells, is a major regulator of body weight by modulating food intake and energy metabolism. Transfer of leptin receptor Ob-Rb to islets from ZDF rats, in which the receptor is mutated, resulted in BCL-2-mediated protection of the islets from the toxic effects mediated by triglyceride accumulation. Thus treatment with leptin may protect pancreatic islets against apoptosis induced by obesity and insulin resistance. Patients with obesity and type-2 diabetes often exhibit elevated plasma leptin levels and leptin
resistance, rather than leptin deficiency. Leptin gene therapy using adenoviral vectors in ob/ob and lean mice resulted in dramatic reductions in both, food intake and body weight, as well as normalization of serum insulin levels and glucose tolerance. Overall, gene therapy for diabetes will most likely require the transfer of multiple genes. It is still unclear which combination of genes and cells will be required to achieve long term physiologic regulation of blood glucose without graft rejection or recurrent autoimmunity (Leibowitz and Levine, 1999).

CONCLUSIONS:

With the newer approaches to the therapeutic benefit in insulin resistance related disorders, a lot remains to be studied and scrutinized. Although it is a long way in the research the day does not seem to be far when suitable pharmacological agents with least untoward effects will be available for the treatment of insulin resistance.
B) Diabetic Cardiomyopathy

With the discovery of insulin, mortality from acute complications such as diabetic ketoacidosis has been particularly eliminated and the quality of life of many patients with diabetes has improved. However, the prolongation of survival of patients with diabetes is accompanied by the development of chronic degenerative complications that include retinopathy, renal failure, and cardiovascular disease. Clinical studies have confirmed that the incidence of heart disease is much greater in diabetics, and is a leading cause of death in these patients (Kannel and McGee, 1979; Palumbo et al, 1981). The cardiac disease includes a lower stroke volume, cardiac index, ejection fraction and a higher left ventricular end diastolic pressure (Hamby et al, 1974; Regan et al, 1977; D’Elia et al, 1979). Factors that have been implicated in the development of cardiovascular dysfunction during diabetes include atherosclerosis of coronary arteries (Young et al, 1994), microangiopathy and autonomic neuropathy (Ledet et al, 1979). However, it has also become apparent that these factors, although important, are not exclusive determinants of the cardiac problems associated with diabetes. Indeed, a significant number of patients with diabetes do not develop atherosclerosis continue to suffer from cardiomegali, left ventricular dysfunction, and clinically overt congestive heart failure (Hamby et al, 1974; Regan et al, 1977; D’Elia et al, 1979; Ahmed et al, 1975). This suggests that a specific cardiac muscle disease, i.e. diabetic cardiomyopathy, may also occur during diabetes (Fein and Sonnenblick, 1985; Galderisi et al, 1991) and could be a causal factor in producing the increase in mortality and morbidity of diabetes.

In animals, insulin dependent diabetes (type 1) is produced by injection of chemical agents like alloxan or streptozotocin (STZ) to adult rats. These toxins selectively induce beta cell necrosis in the pancreas and provide relatively permanent
Diabetic cardiomyopathy. These animals also develop myocardial abnormalities with time. Stroke volume, stroke work, cardiac output, peak left ventricular pressure, and rate of rise and fall of ventricular pressure (±dP/dt) are all depressed, whereas left ventricular compliance is increased in cardiac muscle preparations from these diabetic animals (Regan et al, 1974; Miller, 1979; Vadlamudi et al, 1982; Fein et al, 1985; Rodrigues and McNeill, 1986; Okayama et al, 1994). Non-insulin dependent diabetes (type 2) is produced in rats by injecting single injection of STZ to 0-5 day old pups. Although diabetic cardiomyopathy develops slowly in these rats, its progression is similar to the development of other NIDDM-linked defects. The first signs of impaired contractile function are noted 8 months after administration of STZ. However, by 12 to 14 months the abnormality develops into an overt cardiomyopathy, characterized by decrease in ±dP/dt and impairment in maximal systolic pressure, cardiac work, and cardiac output (Schaffer et al, 1985).

Metabolic Changes in Diabetic Cardiomyopathy

Fatty acids

In the early stages of diabetes, alterations in both fuel supply and utilization by the heart tissue may be the initiating factor for the development of diabetic cardiomyopathy. Mitochondrial generation of ATP in the heart is through the oxidation of various substrates that include glucose, free fatty acids (FFA), lactate and ketone bodies (van der Vusse et al, 1992). The breakdown of glucose or glycogen to pyruvate (glycolysis) provides some energy. However, it is the subsequent entry of pyruvate into the mitochondria and its conversion into acetyl coenzyme A (CoA) that provides the majority of energy obtained from glucose. Acetyl CoA also can be derived from amino acids and FFA. In fact, the heart muscle of rat is known to
account for the largest consumption of FFA with respect to body weight (Neely and Morgan, 1968). The heart has a limited potential to synthesize FFA. Hence, FFA are supplied to cardiac cells from several sources: (a) Lipolysis of endogenous triglyceride within the cardioadipocyte or cardiomyocytes, (b) lipolysis of adipose tissue triglyceride with subsequent entry of FFA into the blood where they are carried to the heart, and (c) lipolysis of circulating triglycerides in chylomicrons and very low density lipoproteins (VLDL) by coronary endothelial bound lipoprotein lipase (LPL). Vascular endothelial bound LPL is the rate limiting enzyme that determines the clearance of plasma triglyceride and partially regulates the FFA supply to the tissues, hence, it is also called as heparin releasable functional LPL (Eckel, 1989). In diabetes, energy production in the heart is almost entirely via β oxidation of FFA because of inadequate glucose transport and oxidation. This may have deleterious effects on myocardial function. In an insulin deficient state, adipose tissue lipolysis is enhanced resulting in elevated circulating FFA (Rodrigues et al, 1992). In addition, an increased activity of myocardial enzymes that catalyze the synthesis of triglyceride, together with the rise in CoA levels, promotes the production of triglyceride during diabetes (Murthy et al, 1983). Subsequent hydrolysis of this augmented triglyceride store could also lead to high tissue FFA levels (Kenno and Severson, 1985; Chattopadhyay et al, 1990). These processes serve to guarantee FFA supply to the diabetic heart to compensate for diminished contribution of glucose as an energy source.

Consequences of excessive utilization of fatty acids

1. An increased susceptibility to arrhythmias (Opie, 1970; Willebrands et al, 1973; Fields et al, 1986)

2. Esterification to complex lipids and hence higher tissue levels of triglyceride
3. An increased requirement of oxygen for catabolism

4. Reduction in both, basal and insulin stimulated glucose transport and metabolism (Randle et al, 1965)

5. Modification of the structure of sarcolemmal and other sub cellular membranes thereby altering membrane fluidity and molecular dynamics (Katz and Messineo, 1981)

6. Inhibition of critical enzyme systems such as Ca\(^{2+}\)-ATPase of sarcoplasmic reticulum, and Na\(^+\), K\(^+\)-ATPase, Na\(^+\)/Ca\(^{2+}\) exchange and Ca\(^{2+}\) pump in myocardial sarcolemma (Adams et al, 1979; Kramer and Weglicki, 1985; Dhalla et al, 1991)

7. Inhibition of the adenine nucleotide translocator in isolated mitochondria leading to a reduction in the myocardial levels of ATP (Vaartjes et al, 1972)

**Carbohydrates**

The major metabolic action of insulin is its stimulation of glucose oxidation. Insulin achieves this effect by controlling the transport of glucose and does so by inducing a rapid, reversible translocation of glucose transporter proteins from intracellular pool to the plasma membrane (Suzuki and Kono, 1980). Activation of glucose transport causes enhanced glycolytic rates and glycogen deposition. In absence of insulin, the major restriction to glucose utilization by the heart is the slow rate of glucose transport across the sarcolemmal membrane into the myocardium, probably as a result of cellular depletion of glucose transporters (Koboyashi and Olefsky, 1979). Reduction in insulin responsive glucose transporter GLUT4 is reported in rat heart (Eckel and Reinauer, 1990). It has been demonstrated that the
activity of glucose transporter in vitro is influenced by the composition and structure of cell membranes (Zuniga-Guarjardo et al, 1991). Since the diabetic state is associated with hypertriglyceridemia and a considerable alteration in fatty acid profile of the membranes, it could explain the decrease in glucose transporters and a reduction in insulin stimulated cardiac glucose utilization (Bieger et al, 1984). In support of this concept, cardiomyocytes cultured in the presence of palmitate exhibit a largely reduced insulin responsiveness that is partially restored by the inhibition of fatty acid oxidation (Eckel et al, 1991). Glucose oxidation in diabetic heart is markedly impaired, not only as a result of impaired glucose transport into the myocyte but also by a reduced rate of phosphorylation of glucose within the cell. The reduced phosphorylation, in turn, probably results from increased metabolism of FFA (Das, 1993). Excessive oxidation of FFA is partly responsible for the insulin resistance and depression of glucose uptake and oxidation (Randle et al, 1965). An increased availability of FFA could increase the TCA cycle activity and thus citrate concentration. The citrate formed may inhibit phosphofructokinase, thereby decreasing the rate of glycolysis. This in turn, may result in an impairment of glucose uptake and oxidation. The reduction of substrate flow through the glycolytic pathway results in an eventual increase in the tissue levels of glucose-6-phosphate that ther. activates glycogen synthase and inhibits phosphorylase. These changes in enzyme activity appear to account for glycogen accumulation, as the small amount of glucose that is taken up is diverted to glycogen (Chen and Ianuzzo, 1982). Another explanation for the reduced oxidation of glucose by the diabetic heart is that the activity of pyruvate dehydrogenase complex is also depressed may be as a result of an increased fatty acid oxidation that causes increased acetyl CoA/CoA ratio. The end
result is an impaired pyruvate oxidation. This leads to inhibition of glucose oxidation by fatty acids (Wall and Lopaschuk, 1989).

**Subcellular Remodeling in Diabetic Cardiomyopathy**

As mentioned earlier chronic diabetes induced in rats is associated with heart dysfunction, including reduced heart rate, depressed peak ventricular pressure as well as depressed rates of contraction and relaxation in the left ventricle. It is believed that heart dysfunction in diabetes is due to subcellular remodeling of the myocardium along with the metabolic changes taking place in the heart. Animal studies have shown decreased activities of the Ca$^{2+}$-ATPase of cardiac myofibrils, actomyosin and myosin (Fein et al, 1981; Pierce and Dhall, 1981; Dillmann, 1980). Since the ability of diabetic heart to generate contractile force is dependent upon the magnitude of myofibrillar ATPase activation by Ca$^{2+}$, the depressed ATPase activities of contractile proteins can be seen to play an important role in the development of heart dysfunction due to diabetes. It has been identified that the depressed ATPase activity of contractile proteins can be seen to play an important role in the development of heart dysfunction in diabetes. The depressed ATPase activity of myofibrils is due to alteration in myosin isozyme composition and regulatory proteins as well as to the phosphorylation of regulatory proteins in the diabetic heart (Pierce and Dhall, 1985; Liu et al, 1997; Liu et al, 1996). Diabetes is associated with a shift in myosin isozyme content from V$_1$ to V$_3$ in the heart (Afzal et al, 1989; Dillmann, 1982; Rupp et al, 1989). The analysis of $\alpha$-and $\beta$-myosin heavy chain (MHC) mRNA and protein expression indicated the predominance of $\alpha$-MHC and $\beta$-MHC in control and diabetic hearts respectively. Different Ca$^{2+}$ antagonists, metabolic interventions and exercise are found to improve the contractile function of the diabetic heart, increase myosin Ca$^{2+}$-ATPase activity.
and prevent the shift in myosin isozymes (Afzal et al, 1989; Tahiliani and McNeill, 1986). Since the phosphorylation of myosin light chain (MLC) by myosin light chain kinase (MLCK) has been shown to play a modulatory role in the generation of contractile force in vertebrate striated muscle, MLC phosphorylation in cardiac muscle fibers showed a leftward shift in the force-pCa relationship and decreased cooperation (Sweeney and Stull, 1986). It has been reported that the protein contents of MLC and MLCK as well as MLC phosphorylation are decreased significantly in the diabetic heart and the change in reversible upon insulin treatment (Liu et al, 1997).

The troponin-tropomyosin (TnTm) complex is made of three troponin (Tn) subunits (TnC: the Ca$^{2+}$ binding unit, TnI: the ATPase inhibitory unit, TnT: the tropomyosin binding unit) and tropomyosin. Phosphorylation of TnI and TnT by protein kinase C (PKC) results in decrease in the actomyosin ATPase activity and a decrease in actin-myosin interaction. Phosphorylation of TnI and TnT by protein kinase A (PKA) has been associated with a reduced sensitivity of the myofibrillar Mg$^{2+}$-ATPase to Ca$^{2+}$. Thus, changes in the regulatory effects of the TnTm complex on actin and myosin can be seen to explain the depressed myofibrillar ATPase activities in the diabetic heart. In fact, the depressed actomyosin ATPase activity in the hearts of diabetic animals is partially reversed when myosin from diabetic rats interacts with the TnTm protein complex isolated from control hearts (Malhotra et al, 1995). Some studies have suggested that the increased phosphorylation of TnI in the diabetic hearts is a result of changes in the subcellular distribution of PKC isozymes (Malhotra et al, 1997). Some studies focused on the relationship between the cardiac TnT isoforms and the force-pCa characteristics of the diabetic heart and have observed a significant shift from TnT$_1$ to TnT$_2$ and TnT$_3$ (Akella et al, 1995). These findings have raised the possibility that changes in the Ca$^{2+}$ sensitivity of myofibrils
Diabetic cardiomyopathy

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in the diabetic myocardium are coupled with TnT alterations. Thus, it is evident that contractile as well as regulatory protein are remodeled in diabetic cardiomyopathy and that these changes are reflected by decreased myofibrillar ATPase activity and heart dysfunction.

Similarly, remodeling of sarcoplasmic reticulum and sarcolemmal membranes takes place in the heart during the development of chronic diabetes (Dhalla et al., 1985; Schaffer, 1991; Golfman et al., 1996). Depression in the sarcoplasmic reticulum Ca$^{2+}$ pump activity is reported (Ganguly et al., 1983; Lopaschuk et al., 1983) that is considered to account for inability of the diabetic heart to relax fully. The occurrence of Ca$^{2+}$ handling abnormalities in the cardiac cells has also been attributed to defects in the sarcolemmal Na$^{+}$-Ca$^{2+}$ exchange and Ca$^{2+}$ pump activities in diabetic hearts (Heyliger et al., 1987; Makino et al., 1987). Sarcolemmal Na$^{+}$-K$^{+}$ ATPase and Ca$^{2+}$ binding activities are decreased (Pierce and Dhalla, 1983; Pierce et al., 1983), whereas sarcolemmal Ca$^{2+}$/Mg$^{2+}$ ecto-ATPase activities are increased (Borda et al., 1988; Dhalla et al., 1986) in diabetic cardiomyopathy. The changes observed in the cell membrane of the diabetic heart favor the occurrence of intracellular Ca$^{2+}$ overload in cardiomyocytes and elevated levels of Na$^{+}$ and Ca$^{2+}$ content are reported in diabetes (Regan et al., 1981; Afzal et al., 1988). The intracellular Ca$^{2+}$ overload had been shown to result in functional abnormalities in the heart (Dhalla et al., 1978; Dhalla et al., 1982). Additionally, verapamil, a Ca$^{2+}$ antagonist, is found to produce beneficial effects in diabetic cardiomyopathy by attenuating changes in heart function, ultrastructure, subcellular activities and Ca$^{2+}$ content in diabetic animals (Afzal et al., 1988). Verapamil has also been shown to prevent an increase in cardiac PKC activity in diabetic rats (Tanaka et al., 1991). Thus, it is evident that remodeling of both sarcolemmal and sarcoplasmic reticular membranes may result in Ca$^{2+}$ handling
abnormalities in cardiomyocytes and subsequent heart dysfunction during the development of diabetic cardiomyopathy.

5-Hydroxytryptamine (5-HT) and Cardiac Function

5-HT is known to regulate cardiovascular function through central (McCall and Clement, 1994) as well as peripheral mechanisms (Sharma et al, 1999; Lee and Wu, 1999). 5-HT is reported to be essential for heart development in the embryonic stage (Nebigil et al, 2000). Inactivation of 5-HT$_{2B}$ gene leads to embryonic and neonatal death caused by heart defects. 5-HT$_{2B}$ mutant embryos exhibit a lack of trabeculae in the heart and a specific reduction in the expression levels of a tyrosine kinase receptor, ErbB-2, leading to midgestation lethality. All surviving mutant newborn mice display a severe ventricular hypoplasia caused by impaired proliferative capacity of myocytes. In the adult mutant mice, cardiac histopathological changes including myocyte disarray and ventricular dilation are consistently observed. This shows the importance of 5-HT in differentiation and proliferation of developing and adult heart. The vascular endothelium is the interface between the blood and the interstitium and it fulfils the essential function of regulating the exchange of fluid, solutes and cells between these two compartments. 5-HT is reported to stimulate a biphasic change in cytosolic Ca$^{2+}$ of cultured rat heart endothelial cells (Lee and Wu, 1999), an initial transient increase that primarily reflects the release of Ca$^{2+}$ from internal stores, followed by a slow rise in Ca$^{2+}$. 5-HT also induces an increase in endothelial permeability that parallels the rise in Ca$^{2+}$ and is blocked by 5-HT$_2$ receptor antagonist cyproheptadine. Further, this 5-HT stimulated rise in Ca$^{2+}$ is inhibited by phospholipase C (PLC) inhibitors indicating the effect is via 5-HT$_2$ receptor and mediated through PLC signaling cascade. As mentioned in earlier
section, in diabetic cardiomyopathy myocardial glucose transport is reported to be defective. A reduction in the capacity of myocardial glucose transport can be detrimental to the diabetic patient during periods of increased myocardial work or ischemia that is common with diabetes when the demand for glucose uptake is increased (James et al, 1989). Glucose is carried across the plasma membrane by a family of glucose transporters including GLUT 1 and GLUT 4. Myocardial GLUT 4 and GLUT 1 are also shown to be decreased in diabetes. It is reported that GLUT 1 is recruited to plasma membrane by various types of glucose transport stimuli, and 5-HT is one of those (Fischer et al, 1996). 5-HT plays critical role in the regulation of blood pressure. Activation of 5-HT2 receptors results in pressor response associated with large increases in sympathetic activity. Circulating levels of 5-HT are increased in the diabetic as well as patients with coronary artery disease as compared to the normal subjects. This increase in 5-HT levels may be due to release from platelets as well as cardiac mast cells. Atherosclerosis is one of the diseases coexisting with diabetes mellitus. Blood platelets are involved in the development of atherosclerosis (Wolf, 1978; Lewis and Kottke, 1977). It is demonstrated that platelets are activated and aggregate at the sites of coronary artery stenosis and endothelial injury (Bush et al, 1984; Ashton et al, 1986). Activated platelets release 5-HT in substantial quantities causing vasoconstriction (Golino et al, 1989). Thus, 5-HT clearly has important vascular action and may be it is involved in atherogenesis that is one of the symptoms in diabetes related cardiovascular complications (Vikenes et al, 1999).
Ventricular performance (independent of vascular defects)

Diabetic cardiomyopathy

Review of literature

Fig. G: Schematic diagram showing molecular mechanisms of diabetic cardiomyopathy.
C) Diabetic Nephropathy

The association of proteinuria with diabetes mellitus was first recognized in eighteenth century but it was Kimmelstiel and Wilson (1936) in 1936 who defined the condition by describing the lesions of nodular glomerulosclerosis and the association with proteinuria and hypertension in type 2 diabetes. These features represent a late stage in the progression of the condition. Subsequent work, mainly on type 1 diabetes led to the definition of several distinct phases in the evolution of the disease (Mogensen, 1999; Foggensteiner et al, 2001). So, diabetic nephropathy is defined as a clinical syndrome characterized by the development of persistent proteinuria, systemic hypertension, and declining renal function in subjects with diabetes mellitus. It is the leading cause of end-stage renal disease in developed countries and leads to a heavy burden of dialysis and transplantation. The risk of premature death in patients with diabetic nephropathy is increased by the factor of 40-100, and other complications such as retinopathy and neuropathy cluster in these patients (Borch-Johnsen et al, 1985). Early studies suggested that the cumulative death rate of diabetic subjects with nephropathy was around 70% at 10 years (Borch-Johnsen et al, 1985). Diabetic nephropathy may develop in 30%-40% patients with diabetes mellitus (Andersen et al, 1983), however, recent studies suggest that the incidence in this group is declining (Bojestig et al, 1994). Although type 1 (insulin dependent) and type 2 (non-insulin dependent) diabetes are etiologically and epidemiologically different conditions affecting different segments of population, no major difference has been identified between the nephropathies seen in these conditions, either pathophysiologically or in terms of management. However, the patients with type 2 diabetes tend to be older and more hypertensive, thus more likely to have concomitant hypertensive and renovascular disease (Foggensteiner et al, 2001).
In animals, streptozotocin (STZ)-induced model of type 1 diabetes mellitus in the rat has been the most widely studied model to demonstrate the hemodynamic and structural alterations of the diabetic state on the kidney. Although STZ-induced model of type 2 diabetes is relatively less studied for diabetes related changes in kidney, scattered reports are available in this direction. Progression of diabetic nephropathy can be described in the following stages:

i. Renal hemodynamic alterations (hyperfiltration) and structural changes

ii. Microalbuminuria

iii. Overt nephropathy (proteinuria)

Renal Hemodynamic Alterations

Glomerular hyperfiltration is a common diagnosis of diabetes mellitus and is often accompanied by renal hypertrophy and increased renal plasma flow. Unilateral renal artery stenosis protects the affected kidney from the development of diabetic glomerulosclerosis, while nephrectomy leads to increased risk of glomerulosclerosis in the remaining kidney. Intraglomerular hypertension is consequent upon hyperfiltration that leads to mesangial expansion and basement membrane thickening. These features can be the hallmarks of diabetic glomerulosclerosis. A wide variety of growth factors and cytokines have been implicated in the etiology of glomerular hyperfiltration. An early abnormality seen in diabetes is increased levels of insulin-like growth factor-1 (IGF-1) (Flyvberg et al, 1988). IGF-1 may have a role in renal hypertrophy by virtue of its mitogenic activity on mesangial and vascular muscle cells. Inhibition of IGF-1 with octreotide reverses renal hypertrophy in experimental animals (Flyvberg et al, 1989). TGF-β may also play a part in the development of glomerular hyperfiltration by promoting synthesis of extracellular matrix proteins and
modulating cell growth. Increases in TGF-β mRNA are seen in renal tissue exposed to hyperglycemia (Wolf et al, 1992), and inhibition of TGF-β with antibodies reverses cellular hypertrophy and inhibits collagen biosynthesis (Sharma et al, 1996). Atrial natriuretic peptide (ANP) may also have a role in the development of diabetic nephropathy. Infusion of ANP in non-diabetic rats induces similar glomerular changes to those seen in diabetic rats (Dunn et al, 1986), whereas, inhibition of ANP reverses the hyperfiltration observed in rodent diabetes (Zhang et al, 1994). Furthermore, longitudinal observation of urinary ANP levels in humans has shown a good correlation with changes in albumin excretion (Jungmann et al, 1993). Alteration in blood rheology may also influence the onset of nephropathy. Reduction in fibrinolysis and increased factor VIII levels have been noted in diabetic nephropathy (Fuller et al, 1979). Raised levels of thromboxane A2 have also been noted in diabetes along with reduction in prostaglandin I2 (Dollery et al, 1979). Nitric oxide (NO) appears to be an attractive mediator of glomerular hyperfiltration as it has a predominant action of decreasing preglomerular vascular tone (Deng and Baylis, 1993) and increased NO metabolites in the urine of STZ diabetic rats has been described (Bank and Aynedjian, 1993; Tolins et al, 1993). Inhibition of intrarenal NOS with L-arginine analogues leads to increased afferent arteriolar resistance and a decrease in hydraulic glomerular capillary ultrafiltration coefficient (Kf) (Deng and Baylis, 1993). The most intensively studied factor that may underlie the altered renal hemodynamics in STZ diabetic rats is angiotensin II (AII) as this peptide has potent constrictor effects preferentially on the efferent arteriole. Relative efferent arteriolar constriction may be postulated to be due to increased intraglomerular AII production. It is demonstrated that ACE staining is increased in the glomerular endothelial cells of diabetic rats (Anderson et al, 1993; suggesting increased local production of AII. It may also be postulated that the
afferent arteriole from STZ diabetic rats has reduced responsiveness accounting for afferent vasodilatation. However, conflicting reports are available about the state of renin-angiotensin system in diabetic kidney. It has been shown that in the early course of STZ diabetes in rats, there is a reduction in the density of glomerular AII receptors (Bellermann et al, 1984; Wilkes, 1987). Another group of investigators found an increased density of glomerular AII receptors but impaired contractile responsiveness of diabetic glomeruli to AII (Kikkawa et al, 1986).

**Glomerular Structural Changes**

Diabetic nephropathy in humans presents several structural changes that are characterized by early hypertrophy of both glomerular and tubuloepithelial elements, thickening of the glomerular basement membrane, progressive accumulation of extracellular matrix components in the glomerular mesangium, and less well recognized lesions such as tubulointerstitial fibrosis and renal arteriosclerosis (Mauer et al, 1984; Osterby et al, 1988; Steffes et al, 1992; Ziyadeh et al, 1989).

**Glomerular hypertrophy**

Progressive increase in kidney weight and glomerular volume in moderately hyperglycemic STZ diabetic rats during a period of six months of diabetes has been reported (Rasch, 1979). In severely hyperglycemic rats without insulin treatment the glomerular volume is reported to be continuously increased during the first eight months of diabetes (Hirose et al, 1982).

**Mesangial matrix expansion**

Progressive expansion of the glomerular mesangial matrix is considered to be the most important lesion for the development of chronic renal failure in the diabetic population (Steffes et al, 1989). Diffuse intracapillary sclerosis correlates closely with
the progressive decline in the glomerular capillary surface area available for filtration, hence it is the structural counterpart of reduced glomerular filtration rate (GFR) (Steffes et al, 1989). Mesangial expansion is predominantly due to an overabundance of normal structural components of the mesangial extracellular matrix including collagen Type IV, laminin, and fibronectin. It is reported that whole glomeruli from STZ diabetic rats demonstrate a significant increase in the steady state mRNA levels encoding the $\alpha_1$ chain of Type IV collagen, fibronectin, and laminin B1 and B2 (Fukui et al, 1992). Yamamoto et al (1993) have showed that glomerular staining for fibronectin and tenascin is also increased in long term STZ diabetic rats. The phenotypic expression of mesangial extracellular matrix such as the appearance of interstitial collagen Type III may also be altered in STZ diabetic rats (Abrass et al, 1988). Appreciable induction of gene expression for interstitial collagen types I and III is also demonstrated (Scheinman et al, 1978). These fibrillar collagens are distinctly absent in non-diabetic rats.

**Glomerular basement membrane thickening and increased permeability**

The exact biochemical and ultrastructural basis for the thickening of glomerular basement membrane and the increased permeability for macromolecules across the filtration barrier of the glomerular capillary wall remains to be completely understood. It has been suggested that increased synthesis and/or decreased degradation of collagens and laminins are responsible for increased glomerular basement membrane thickness (Ziyadeh, 1993). The thickness of basement membrane is reversible by strict blood glucose control with insulin (Ziyadeh, 1995).

Diabetic glomerulopathy is associated with changes in the amount and composition of extracellular matrix proteins, such as heparan sulfate proteoglycan (HSPG). HSPG is a major determinant of permselectivity in the glomerular basement.
membrane by virtue of its negative charge. Renal biopsy studies show a significant reduction in HSPG in the glomerular basement membrane of patients with diabetic nephropathy (Tamsma et al, 1994). This may, in part, explain the loss of selectivity of the glomerular basement membrane in the condition that leads to proteinuria. Reduced biosynthesis of HSPG has been demonstrated in humans with diabetic nephropathy with the use of [3H]glucosamine (Deckert et al, 1991) and in diabetic rats by mRNA analysis (Fukui et al, 1992). From these data it has been postulated that deficient regulation of HSPG biosynthesis results in a lower content of HSPG in the glomerular basement membrane. Diabetic subjects with defects in this biosynthetic pathway may be more susceptible to the development of nephropathy. Interestingly, treatment of proteinuric insulin dependent diabetic patients with heparin appears to attenuate protein excretion, although the mechanism of this effect is uncertain (Myrup et al, 1995). A further pathological change seen in the diabetic kidney is deposition of advanced glycosylation end products (AGEs). These originate from glucose derived Schiff base and Amadori products and undergo series of complex rearrangements to form stable protein bound complexes. They accumulate on long-lived tissue proteins, such as collagen, and have been implicated in diabetic complications (Brownlee et al, 1988). Administration of AGEs to non-diabetic animals can induce similar vascular changes to those seen in diabetes (Vlassara et al, 1994) and, in particular, can induced genes for growth factors, such as transforming growth factor-β (TGF-β), and extracellular matrix protein deposition (Vlassara et al, 1988).

Microalbuminuria

The normal urinary protein excretion rate is up to 300 mg/24 hr, of which about 10% is albumin, equivalent to an albumin excretion rate of 20 μg/min. Albumin excretion rates of 20-200 μg/min, equivalent to a urine albumin creatinine ratio
Diabetic nephropathy

Review of literature

(ACR) of 10-25 mg/mmol, are defined as microalbuminuria (also called as incipient nephropathy) as these levels are not detectable by conventional urine dipstick analysis. The onset of microalbuminuria is highly significant since its presence predicts the development of overt renal disease in both type 1 and type 2 diabetes (Mogensen, 1984; Viberti et al, 1982). Furthermore, microalbuminuria is associated with an increased risk of cardiovascular and microvascular complications as well as increase in mortality, especially in type 2 diabetes (Dinneen and Gerstein, 1997). Renal histology at this stage reveals typical glomerulosclerosis. Once microalbuminuria is established the trend is one of increasing proteinuria until overt nephropathy develops.

Table 3: Associations with microalbuminuria

- Development of overt nephropathy and end stage renal disease
- Increased cardiovascular risk
- Blood pressure changes:
  - Loss of nocturnal dip in blood pressure
  - Rise in blood pressure (mean 3 mmHg per year)
- Other microvascular complications of diabetes:
  - Proliferative diabetic retinopathy
  - Macular edema
  - Neuropathy
- Dyslipidemia
- Insulin resistance
Overt Nephropathy

Proteinuria is generally regarded as a marker for the degree of glomerular damage. The levels of proteinuria correlates well with the prognosis of renal function, and interventions that retard the progression of diabetic renal disease also reduce proteinuria. However, it is still unknown whether the flux of protein across the glomerular basement membrane is causally implicated in the evolution of diabetic renal disease or simply reflects glomerular damage (Remuzzi and Bertani, 1990). Albumin excretion rates above 200 μg/min or 300 mg/day (equivalent to an ACR of >25 mg/mmol) are dipstick positive and defined as overt nephropathy. This is usually associated with a relentless loss of glomerular filtration rate (by 1-24 ml/min per year) until end stage renal failure necessitates dialysis or renal transplantation. The rate of progression of microalbuminuria and overt nephropathy is heavily influenced by blood pressure control, glycemic control and the use of angiotensin converting enzyme (ACE) inhibitors.

Table 4: Definitions of diabetic renal disease

<table>
<thead>
<tr>
<th></th>
<th>Microalbuminuria</th>
<th>Clinical nephropathy</th>
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<tbody>
<tr>
<td></td>
<td>Normal (incipient nephropathy)</td>
<td>'overt' nephropathy</td>
</tr>
<tr>
<td>24 Hr urinary albumin (mg/day)</td>
<td>&lt; 30 30-300 &gt;300</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>Urine albumin excretion rate (μg/min)</td>
<td>&lt; 20 20-200 &gt;200</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>Urine albumin/creatinine ratio (mg/mmol)</td>
<td>&lt; 2.5 M 10-25 &gt; 25</td>
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Genetic Factors

A number of observations suggest that there may be an important hereditary component in the etiopathogenesis of diabetic nephropathy. Familial clustering of diabetic nephropathy has been observed by some workers (Borch-Johnsen et al, 1992; Seaquist et al, 1989) suggesting a fourfold increase in risk of development of nephropathy in type 1 diabetic subjects whose type 1 diabetic siblings have nephropathy. A similar phenomenon has been observed among Pima Indians and African-Americans with type 2 diabetes (Pettitt et al, 1990). These patterns of incidence suggest that nonmetabolic factors are influential in the development of nephropathy. Parental history of hypertension and coronary heart disease are found more commonly in patients with diabetic nephropathy than in diabetic subjects who do not develop this complication (Earle et al, 1990; Viberti et al, 1985). Genetic loci involved in susceptibility to these conditions are, therefore, possible determinants of inherited susceptibility to nephropathy. Genes controlling the expression of the rennin-angiotensin system (RAS) have received particular attention in this respect. Disturbance of the RAS is observed in diabetic nephropathy with increased levels of plasma rennin, prorenin, ACE and angiotensin II. An insertion (I)/deletion (D) polymorphism in the ACE gene has been identifies that is strongly associated with raised circulating ACE levels and with increased risk of coronary artery disease in non-diabetic individuals. Some studies have found the DD genotype to be associated with an increased risk of diabetic nephropathy and a rapid decline of GFR in both type 1 and type 2 diabetes (Yoshida et al, 1996). The clinical implications are yet to be explored. Other genetic loci that may be involved include the sodium-lithium exchanger and the sodium-hydrogen antiporter genes (Foggensteiner et al, 2001).
Diabetic nephropathy Review of literature

Diabetes and Renal Dopamine Function

The biological effects of dopamine are mediated through five genetically distinct dopamine receptors: D₁, D₂, D₃, D₄, D₅ (Sibley and Monsma, 1992). These receptors are classified into two major families as D₁-like (includes D₁ and D₅, whose rat homologues are D₁A and D₁B) and D₂-like (includes D₂, D₃, and D₄) dopamine receptors based on the stimulation and inhibition of adenylyl cyclase respectively (Sibley and Monsma, 1992). Dopamine receptors are located at various regions within the kidney of both experimental animals and humans including the renal vasculature, sympathetic nerve terminals innervating different sites, juxtaglomerular apparatus, and renal tubules (Lokhandwala and Amenta, 1991; Willems et al, 1985; Lokhandwala, 1988; Felder et al, 1984). The D₁-like receptors are present on the smooth muscle of renal arteries and juxtaglomerular apparatus and on the renal tubules (Lokhandwala and Amenta, 1991; Felder et al, 1984; Huo and Healy, 1989). The D₂-like receptors are expressed in the intimal layer of the renal vasculature, glomeruli, sympathetic nerve terminals and the renal tubules (Lokhandwala and Amenta, 1991; Felder et al, 1984; Missale et al, 1985).

Physiological Role of Dopamine in the Kidney

The natriuretic and diuretic effects of dopamine were first demonstrated in 1964 (McDonald et al, 1964). It is now known that dopamine exerts pronounced cardiovascular and renal actions by activating both D₁-like and D₂-like dopamine receptors located at various sites within the cardiac, vascular, and renal regions (Lokhandwala and Amenta, 1991; Felder et al, 1984; Lokhandwala, 1988). At higher doses dopamine also activates β and α adrenoceptors (van Veldhiusen, 1992). Several studies have shown that selective agonists at D₁-like receptors cause hypotension,
increase in blood flow to certain organs, diuresis, and natriuresis, whereas $D_2$-like receptor agonists produce hypotension, bradycardia, a decrease in afterload, and vasodilatation in certain vascular beds (Jose et al, 1992; Lokhandwala and Chen, 1994). $D_1$-like receptors are reported to cause an increase in renal blood flow (RBF) and glomerular filtration rate, as well as increase in urinary excretion of water and sodium (Jose et al, 1992; Hegde et al, 1989a). A positive correlation has been reported between sodium intake/ urinary excretion and renal dopamine production/ urinary excretion in both experimental animals and humans (Ball et al, 1978; Romero-Vacchione et al, 1995). Several studies have shown the role of dopamine in the regulation of sodium excretion during acute volume expansion and during acute increase in sodium intake (Chen and Lokhandwala, 1991; Hegde et al, 1989b; Oates et al, 1979). The increased sodium excretion seen in animals placed on a high sodium diet is accompanied by an increase in urinary dopamine excretion (Vyas et al, 1992a; Bertorello et al, 1988). These results suggest that endogenously formed kidney dopamine plays a pivotal role in maintaining body sodium homeostasis during increases in sodium intake.

**Dopamine Receptor Linked Cellular Signaling**

Regulation of sodium transport across the proximal tubules occurs through the involvement of two important proteins: $\text{Na}^+, \text{H}^+$-exchanger, located on the brush border membrane, and $\text{Na}^+, \text{K}^+$-ATPase, located on the basolateral membrane of the proximal tubule (Gesek and Schoolwerth, 1990; Felder et al, 1990; Chen et al, 1993). These proteins have been identified as the target for the action of dopamine. Dopamine inhibits the activity of these proteins. $D_1$-like and $D_2$-like both the receptors are co-expressed in the kidney proximal tubules (Lokhandwala and Amenta,
The activation of D₁-like receptors by dopamine produces inhibition in the Na⁺, K⁺-ATPase activity in proximal tubules and other parts of the nephron, such as medullary thick ascending limb (mTAL) and cortical collecting duct (CCD) (Chen and Lokhandwala, 1993; Satoh et al, 1993). The activation of D₁-like receptors by dopamine and D₁-like agonists also produces inhibition of Na⁺, H⁺-exchanger activity in proximal tubules of nephron (Gesek and Schoolwerth, 1990; Felder et al, 1990). On the other hand D₂-like receptor activation leads to stimulation of Na⁺, K⁺-ATPase activity in proximal tubules (Hussain et al, 1997). The stimulation of Na⁺, K⁺-ATPase activity by the D₂-like receptor agonists involves pertussis toxin sensitive, G protein-linked inhibition of cAMP. It is suggested that activation of D₂-like receptors causes antidiuresis and antinatriuresis (Siragey et al, 1990). But the activation of D₂-like receptor does not affect the activity of Na⁺, H⁺-exchanger in the proximal tubule (Felder et al, 1990).

The process from activation of D₁-like receptor to the inhibition of Na⁺, K⁺-ATPase and Na⁺, H⁺-exchanger activity involves multiple cellular signaling pathways that are yet to be fully understood. A positive correlation between dopamine infusion and urinary cAMP excretion implicated adenylyl cyclase as one of the second messengers in the action of dopamine (Vlanchoyannis et al, 1976). Moreover, the inhibition of Na⁺, H⁺-exchanger by dopamine is shown to be linked to cAMP-dependent as well as cAMP-independent mechanisms (Felder et al, 1993). Further studies have revealed that activation of D₁-like receptors causes the stimulation of phospholipase C (PLC) with resulting generation of inositol triphosphate (IP₃) and diacylglycerol (DAG). DAG stimulates phosphokinase C (PKC) which in turn induces inhibition of Na⁺, K⁺-ATPase activity (Gopalkrishnan et al, 1995).
Fig. H: Signaling through $D_1$-like dopamine receptor.


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Fig. I: Signaling through $D_2$-like dopamine receptor.
So, two pathways, adenylyl cyclase and PLC are proposed to be linked to the D₁-like receptors through the coupling of G₅ and G₉₁₁ proteins in the proximal tubules (Felder et al, 1989b; Felder et al, 1989c; Vyas et al, 1992b). The role of the phospholipase A (PLA) pathway has also been reported in the cellular signaling systems involved in dopamine-induced inhibition of Na⁺, K⁺-ATPase activity in the proximal tubules (Hussian and Lokhandwala, 1986). According to the proposed mechanism, during dopamine activation of D₁-like receptors, PKC leads to the activation of PLA₂ that in turn releases arachidonic acid from membrane lipids. Arachidonic acid is further metabolized by cytochrome P450 producing various metabolites. It is also reported that one of the metabolites of arachidonic acid, 20-HETE, inhibits Na, K-ATPase activity via a PKC dependent pathway (Nowicki et al, 1997).

**Insulin Resistance and Renal Dopamine Function**

Insulin is one of the several factors that affect the renal function. DeFronzo et al (1975) first demonstrated that insulin exerts an antidiuretic effect independent of glycemic status in healthy human. A similar effect was later reported in dog and rat (DeFronzo et al, 1976; Kirchner, 1988). These results indicate that insulin may directly control renal sodium handling. The antinatriuretic effect of insulin is thought to originate in part in proximal tubules as insulin is shown to increase fluid and sodium absorption in in vitro microperfused rabbit proximal convoluted tubules (PCT) (Baum, 1987). The stimulatory action of insulin is, at least in part, accounted for by an alteration of Na⁺, K⁺-ATPase activity. Insulin enhances the transport activity of Na⁺, K⁺-ATPase independently of the apical sodium entry in isolated rat PCT.
Diabetic nephropathy

(Feraille et al, 1994; Feraille et al, 1992). Insulin increases apparent sodium affinity of \( \text{Na}^+ \), \( \text{K}^+ \)-ATPase. However, this effect of insulin on \( \text{Na}^+ \), \( \text{K}^+ \)-ATPase in rat PCT is independent of protein kinase C (PKC). In fact, insulin modulates \( \text{Na}^+ \), \( \text{K}^+ \)-ATPase activity through a tyrosine phosphorylation process, raising a possibility of a direct tyrosine phosphorylation of the \( \text{Na}^+ \), \( \text{K}^+ \)-ATPase (Feraille et al, 1997). It is now confirmed that insulin phosphorylates \( \text{Na}^+ \), \( \text{K}^+ \)-ATPase at Tyr-10 in isolated rat PCT and insulin increases the proportion of tyrosine phosphorylated \( \text{Na}^+ \), \( \text{K}^+ \)-ATPase units by 10 to 25% (Feraille et al, 1999). In addition insulin may also stimulate directly the apical sodium entry through the \( \text{Na}^+ \), \( \text{H}^+ \)-exchanger, which is shown in suspensions of rat PCT (Gesek and Schoolwerth, 1991).

Thus, it is clear that dopamine and insulin both are important modulators of renal function mediating their action through \( \text{Na}^+ \), \( \text{K}^+ \)-ATPase and \( \text{Na}^+ \), \( \text{H}^+ \)-exchanger pump proteins. Dopamine induces natriuresis by inhibiting, whereas, insulin acts as an antinatriuretic agent by stimulating these pump proteins. Though insulin and dopamine, both act thorough \( \text{Na}^+ \), \( \text{K}^+ \)-ATPase, action of dopamine is PKC dependent while that of insulin is PKC independent. So it is not clear that whether there is a link between actions of these two at the signal transductional level, if any.

However, few reports are available to indicate a possible link between renal actions of dopamine and insulin at physiological level. Renal dopamine production is reduced in both, type 1 (insulin dependent) and type 2 (non-insulin dependent) diabetes mellitus leading to sodium retention which further aggravates hypertension associated with diabetes mellitus (Segers et al, 1995; Segers et al, 1996). It is reported that renal dopamine receptor function is defective in insulin resistant obese Zucker rats. There is a decrease in D1-like receptor binding sites and diminished activation of G proteins resulting in reduced inhibition of both, \( \text{Na}^+ \), \( \text{K}^+ \)-ATPase and \( \text{Na}^+ \), \( \text{H}^+ \)-
exchanger activity by dopamine in these animals (Hussain et al, 1999; Hussain et al, 2002). Thus, it is possible that renal dopamine function is dependent on insulin sensitivity.
D) Pharmacology of Sarpogrelate hydrochloride (MCI-9042)

Thrombosis is a leading cause of morbidity and mortality in the western world. The three classes of antithrombotic agents include: platelet antiaggregatory agents, anticoagulants and thrombolytic drugs. 5-HT is well reported to interact with platelets, vascular endothelium and vascular smooth muscle (van Zwieten et al, 1990; Hillis and Lange, 1991). During platelet aggregation, 5-HT and other substances such as adenosine di phosphate, thromboxane A2 are released accelerating aggregation. This action of 5-HT is mediated via 5-HT2A receptors. Hence, attempts were made to develop 5-HT2A antagonist agent as a platelet antiaggregating drug, sarpogrelate being one example of such a drug.

Sarpogrelate

Chemically sarpogrelate is succinic acid (±)-1-(dimethylaminomethyl)-2-[2-[2-(3-methoxyphenyl) ethyl] phenoxy] ethyl monoester hydrochloride (±)-2-[2-(3-carboxypropionyloxy)-2-dimethylaminoproxy]-3'-bibenzyl hydrochloride.

Sarpogrelate HCl is a specific 5-HT2A receptor antagonist. It has been evaluated as antiplatelet aggregating agent with its important benefits, such as high absorption and good availability as well as remarkable lack of toxicity (Tobesseda and Mealy, 1994).
5-HT$_{2A}$ receptors are widely distributed in peripheral tissues (Bradley et al., 1968). The pharmacological effects mediated through these receptors include contractile responses in many vascular smooth muscle preparations (e.g. rabbit aorta, rat caudal artery, dog gastroplenic vein), contractile response in bronchial, uterine and urinary smooth muscles and part of the contractile effect of 5-HT on guinea pig ileum. In addition, platelet aggregation and increased capillary permeability can be included as 5-HT$_{2A}$ receptor mediated actions. Some neuroendocrine functions such as release of β endorphins, corticosterone and leutinizing hormone in rats and prolactin release in rhesus monkeys, appear to be mediated by 5-HT receptors (Koenig et al, 1987; Lenahan et al, 1987; Heninger et al, 1987). 5-HT-induced release of adrenaline from the adrenal medulla in the dogs also appears to be mediated through 5-HT$_{2A}$ receptors (Humphrey and Feniuk, 1987).

The 5-HT$_{2A}$ receptors are linked to phosphotidylinositol turnover. This has been demonstrated in rat cortex, aortic smooth muscle and human platelets (Conn and Sanders-Bush, 1984; Conn and Sanders-Bush, 1985; Roth et al, 1984; De Chaffoy et al, 1985; Doyle et al, 1986). The receptors are coupled to phospholipase C, and inositol phospholipid hydrolysis and [Ca$^{2+}$]$_i$ mobilization are involved in the post receptor events.

Sarpogrelate inhibits 5-HT-induced intracellular free [Ca$^{2+}$]$_i$ elevation of rat platelets (Shimada et al, 1991). It also inhibits 5-HT-induced increase in [Ca$^{2+}$], and thereby antagonizes the contractile action of 5-HT in rat mesangial cells (Kanamori, 1994). Sarpogrelate can block acetylcholine and histamine induced contractile responses in isolated guinea pig ileum (Hori et al, 1991).

5-HT is involved in the control of vasoconstriction and platelet aggregation. 5-HT released from platelets stimulates collagen fibrils, which induces further
aggregation of platelets and contraction of vessels via 5-HT$_{2A}$ receptors. This accelerates arterial thrombus formation. Sarpogrelate is found to inhibit thrombus formation by inhibiting platelet aggregation and vascular contraction (Takada et al, 1997). It is reported to reduce the mortality due to serotonin plus collagen induced acute pulmonary thromboembolic disease in rats and prolonged bleeding time without altering blood coagulation or fibrinolysis system (Hara et al, 1991). Long term treatment with sarpogrelate is effective in chronic arterial obliterans (Moro et al, 1997; Nakamura et al, 2001). Sarpogrelate has been reported to be effective in arteriosclerotic obliterans (Myojin, 1996). Sarpogrelate also inhibits 5-HT or collagen induced platelet-aggregation in diabetes mellitus (Pietraszek et al, 1993). It is an effective agent in the treatment of diabetic nephropathy and neuropathy (Ishimura et al, 1997). Sarpogrelate antagonizes mitogenic effects of 5-HT in renal mesangial cells of rat by inhibiting DNA synthesis. This is effect is claimed to be helpful in the treatment of glomerulonephritis associated with mesangial cell proliferation (Eto et al, 1997). Usefulness of sarpogrelate treatment is reported in Raynaud’s disease and Buerger’s disease (Kumagai et al, 1998; Rydzewski et al, 1996).

Other than antiplatelet aggregation activity, sarpogrelate is reported to have beneficial effects in cardiovascular complications also. It is reported to be a useful drug for patients with implanted heart valve prostheses and subsequent high serum lactate dehydrogenase because it works as an antiplatelet drug and mechanical hemolysis (Usui et al, 2000). Sarpogrelate suppresses respiratory failure and right ventricular failure with pulmonary hypertension in patients with systemic sclerosis (Kato et al, 2000). In cardiac tissues 5-HT is rich in vascular platelets, mast cells, sympathetic nerve endings and the receptors are present in platelets and cardiomyocytes. Sarpogrelate is reported to reduce the myocardial infarct size by
inhibiting 5-HT release followed by enhancement of PKCe translocation and opening of the mitochondrial KATP channel in ischemic myocytes (Shimizu et al, 2002). Maintenance of proper coronary circulation is very important in patients suffering from angina pectoris and myocardial infarction. These diseases can be a result of coronary artery disease that may be due to abnormal coronary circulation. 5-HT reduces the coronary blood flow as a product of aggregating platelets. Sarpogrelate has been reported to increase the collateral coronary blood flow in humans. It improves microcirculation by antagonizing the vasoconstrictive products of the aggregating platelets in coronary artery disease (Satomura et al, 2002). In patients with angina pectoris with well developed collaterals sarpogrelate is reported to improve exercise capacity and attenuate myocardial ischemia during exercise (Kinugawa et al, 2002).

Neointimal hyperproliferation and platelet activation/aggregation are two major cardiovascular abnormalities commonly observed in blood vessels after an insult such as cellular injury, mechanical or physiological stress or overload due to peripheral resistance (Schwartz et al, 1986; Schwartz and Reidy, 1987). 5-HT, angiotensin-II (AT-II), endothelin and platelet derived growth factors are primarily involved in cardiovascular remodeling (Nemecek et al, 1986; Saward and Zahradka, 1996). These agents act specific cell surface receptors to trigger various intracellular transduction cascades involved in cellular hyperplasia. Smooth muscle cell and endothelial cell proliferation in response to vascular injury is mediated by 5-HT released from adhering platelets (Pakala et al, 1997). Hence sarpogrelate inhibits 5-HT-induced vascular smooth muscle cell proliferation (Sharma et al, 1999).

Cardiac hypertrophy is one of the major complications in cardiac diseases, such as hypertension and cardiac failure. Some putative causes of cardiac hypertrophy
are attributed to the response to the increased afterload of an elevated systemic pressure (Kaplan, 1997) or direct and indirect effects of hypertrophic agents such as AT-II, endothelin-I (ET-I) (Tojo et al, 1996; Harada et al, 1997; Ikeda et al, 1998). In such patients with hypertension or heart failure humoral factors in plasma like ET-I are elevated (Schichiri et al, 1993; Wei et al, 1994). These hypertrophic agents can facilitate cardiac hypertrophy leading to cardiac dysfunction and ischemia. Sarpogrelate is reported to decrease AT-II and ET-I induced increases of [3H]-leucine uptake into myocytes or nonmyocytes. Thus it has partial inhibitory effect on ET-I induced cardiomyocyte hypertrophy (Ikeda et al, 2000).

5-HT neurons are considered as one of the pivotal factors of the tissue inflammation that leads to pain behavior. Sarpogrelate, by its virtue of 5-ET antagonistic action is reported to provoke antinociceptive effect. It inhibits apoptosis and neuronal degeneration after chronic construction injury when applied locally (Nakanishi and Ishikawa, 2001). This action of sarpogrelate is peripheral (Obata et al, 2000).

**Pharmacokinetics:**

After oral administration to rats, sarpogrelate is rapidly and almost completely absorbed from the gastrointestinal tract (Komatsu et al, 1991). In dogs, after oral dosing, peak plasma concentrations are reached in 15-45 min and then decreased. The absorption rate and bioavailability are 84% and 57% respectively. Excretion is mainly via feces (71-74%) and urine (26-31%) and occurred within 12 hrs after oral or intravenous administration (Komatsu et al, 1991). During chronic administration to male rats (20mg/kg/day, orally) sarpogrelate tissue levels are reported to increase gradually until the 7th or 14th day and then remain constant thereafter (Komatsu et al, 1991).
Sarpogrelate is currently in clinical practice for the treatment of atherosclerosis obliterans in several countries including China, Korea and Japan.

**Brand Name:** Anplag (Mistubishi Chem Ind.)
E) Pharmacology of Ondansetron hydrochloride (GR-38032F)

Ondansetron hydrochloride is used as an effective antiemetic drug in chemotherapy induced emesis.

Chemically ondansetron is 1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl-1-l-imidazole-1-yl)methyl]-4H-carbazole-4-one.

Ondansetron is a highly selective 5-HT3 receptor antagonist. These types of 5-HT receptors are present centrally as well as peripherally. In the nervous system, these receptors are present either on vegal nerve terminals or centrally in the chemoreceptor trigger zone (CTZ). The peripheral vegal receptors appear to be associated with afferent fibers that serve sensory receptors in the gastric wall and project to the tractus and nucleus soliterious in the brainstem. Chemotherapy-induced emesis results from the neural stimulus of the vagal and sympathetic fibers via the spinal cord to distinct areas of the brain that control vomiting (Cubeddu, 1992). Chemotherapy at high doses induces vomiting by releasing large amounts of 5-HT which stimulates the 5-HT3 receptors on vagal abdominal afferent fibers in the gastrointestinal tract (Ettinger, 1995). Thus, by blocking the 5-HT3 receptors,
ondansetron produces antiemetic effect for which it is the drug of choice in chemotherapy induced emesis.

5-HT₃ receptor activation triggers a rapid depolarization because of transient inward current response on the opening of cation-selective channels (Peters et al., 1991; Reiser, 1991; Wallis and Elliott, 1991). The response desensitizes and resensitizes rapidly (Yakel et al., 1991). The channel opened by 5-HT permits the passage of Na⁺ and K⁺ (Peters et al., 1991). The major consequence of cellular depolarization is a rapid increase in cytosolic Ca²⁺ concentration because an influx of Ca²⁺ from the extracellular environment. Subsequent events triggered by the increase in cytosolic Ca²⁺ include neurotransmitter release from both peripheral (Fozard, 1984; Wallis, 1989; Saria et al., 1990) and central (Blandina et al., 1989; Paudice and Raiteri, 1991) and increase in cGMP because of activation of nitric oxide formation from L-arginine (Tohda and Nomura, 1990; Reiser, 1991; Tohda et al., 1991). Ondansetron possesses a distinct ability to reverse K⁺ influx of tumor cells exposed to estramustine phosphate due the effect of cellular Na⁺, K⁺ ATPase and Na⁺, K⁺, Cl⁻ cotransport activity (Behnam-Motlagh et al., 2001).

In intact animals, activation of 5-HT₃ receptors profoundly influences principal body systems. Major effects on the cardiovascular system are seen on the heart, which may be inhibited or stimulated by a combination of local and reflex effects (Saxena and Villalon, 1991), and on blood vessels where reflex activation results in vasodilatation (Blauw et al., 1988; Orwin and Fozard, 1986). Voltage dependent Na⁺ and K⁺ channels are important determinants of the human electrocardiogram (ECG). The Na⁺ channel is responsible for the upstroke of the cardiac action potential, propagation of the cardiac impulse and contributes to the plateau of the cardiac action potential (Wilson et al., 1985; Fozzard and Hanck, 1996).
Voltage dependent $K^+$ channels are important for the repolarizing current $I_K$. Two types of voltage dependent $K^+$ channels produce rapid and slow type of $I_K$ currents (Sanguinetti et al., 1995; Barhanin et al., 1996). Thus $Na^+$ channel is determinant of QRS complex of the ECG and $K^+$ channels are responsible for QT interval. Ondansetron is reported to widen the QRS complex and prolong the QT interval by inhibiting $Na^+$ channel and both types of $K^+$ channels (Kuryshev et al., 2000). Ondansetron also reduces blood pressure in obese Zucker rats (Gerges et al., 2002).

Ondansetron, a selective 5-HT$_3$ receptor antagonist exhibits surmountable blocking activity at 5-HT$_4$ receptors in the micromolar range (Bockaert et al., 1992).

Acute hyperglycemia causes delay of solid gastric emptying in diabetic rats. Ondansetron is reported to enhance gastric emptying in normal as well as diabetic rats and thereby reverses the impairment of STZ-induced diabetic gastroparesis. Diabetic neuropathy is another complication associated with diabetes mellitus that may result in diabetic diarrhea. Ondansetron is found to be effective in the treatment of diabetic diarrhea (Bossi et al., 1994).

Ondansetron is reported to possess anticonvulsant activity along with improving the cognitive function in rats (Balakrishnan et al., 2000). However, it does not have beneficial effect on cognition in Alzheimer’s dementia (Dysken et al., 2002). Ondansetron also has a role to play in addiction and withdrawal. 5-HT3 receptor antagonists are proposed to serve as potential anti-addictive and anti-psychotic therapies. Ondansetron is reported to inhibit self administration of cocaine in rats. It is hypothesized that ondansetron may be a useful treatment for cocaine addicts who have undergone previous sensitization periods (Davidson et al., 2002). Ondansetron is also shown to produce mild analgesia in ureteral colic (Ergene et al., 2001).
Ondansetron lowers the incidences of pruritis that is associated with intrathecal injection of fentanyl (Gurkan and Toker, 2002).

In the pancreas 5-HT is primarily present in beta cells of islets of Langerhans (Ekholm et al, 1971; Cetin, 1992) and in serotonergic pancreatic nerve fibers (Gershon et al, 1994; Kirchgessner and Gershon, 1990). However, it is also present in enterochromaffin cells in exocrine pancreas. Here, 5-HT is reported to inhibit fluid secretion from pancreatic duct cells. Ondansetron is reported to inhibit 5-HT action stimulating the secretions by these ducts (Suzuki et al, 2001).

**Pharmacokinetics:**

Ondansetron is intended for intravenous administration. However, oral administration is also used. Oral bioavailability of ondansetron is 60-70%. It undergoes first pass metabolism. Volume of distribution is 1.9-2.6 l/kg, indicating that much of the drug is taken up by body tissues. Ondansetron is moderately (70%) bound to plasma proteins and crosses membranes readily. It is excreted in urine (approximately 65%) and feces (35%) after extensive hepatic metabolism involving hydroxylation and conjugation reactions. The plasma half life of ondansetron is about 3.5 hrs.

Ondansetron is a well established as an antiemetic agent in chemotherapy induced emesis and is present all over the world.

**Brand Name:** Zofran (GlaxoSmithkline)
**F) Pharmacology of Fenoldopam mesylate (SKF 82526-J)**

Fenoldopam mesylate is the first selective dopamine 1 receptor agonist that has been approved for clinical use. Administered parenterally, it acts predominantly as a vasodilator in peripheral arteries and as a diuretic in the kidneys. It has been approved by the U. S. Food and Drug Administration for the in-hospital, short term (up to 48hr) management of severe hypertension.

![Fenoldopam](image)

Chemically fenoldopam is a 6-chloro-2, 3, 4, 5-tetrahydro-1-(p-hydroxyphenyl)-1 H-3-benzazepine-7,8-diol methanesulfonate.

All the dopamine receptors are members of the superfamily of G-protein coupled receptors (Dohlman et al, 1991). The peripheral DA1 receptor is defined as the receptor that mediates renal arterial vasodilatation and natriuresis during the intravenous and intraarterial administration of dopamine in anesthetized dogs (Goldberg, 1977). Vascular DA1 receptors are located on the smooth muscle of most arterial beds, particularly in the renal and splanchnic arteries, with lesser density in coronary or cerebral arteries (Idem, 1984). Activation of DA1 receptors increases intracellular cyclic adenosine monophosphate (cAMP)-dependent protein kinase A activity, thus promoting the relaxation of smooth muscles (Lokhandwala and Barrett, 1982). Activation of DA1 receptors on renal tubular cells decreases sodium transport by cAMP-dependent and cAMP-independent mechanisms. Increasing cAMP...
production in proximal tubular cells and medullary part of the thick ascending limb of
the loop of Henle inhibits the Na$^+$, H$^+$ exchanger (Felder et al, 1990) and Na$^+$, K$^+$

Fenoldopam is indicated for management of severe hypertension, when rapid
but quickly reversible reduction of blood pressure is required. This includes malignant
hypertension with deteriorating end organ function. In the earlier clinical studies,
fenoldopam was given orally to patients with mild to moderate hypertension in dose
range of 25 to 100mg resulted in variable and short lived reduction in blood pressure
(Ventura et al, 1984; Carey et al, 1984; Gluck et al, 1987). In some studies increase in:
plasma renin activity along with increased serum aldosterone concentrations and
urinary flow was reported (Harvey et al, 1986; Gluck et al, 1987). However, poor and
variable bioavailability forced to shift the administration route to parenteral than oral.

In one study, parenteral administration of fenoldopam over 15 min period,
resulted in dose dependent decrease in blood pressure, an increase in heart rate and an
increase in plasma catecholamine concentration (Murphy et al, 1987). In a
continuation to this study, second study using i.v. fenoldopam is reported to decrease
in blood pressure along with increased urinary flow, and urinary sodium excretion
without change in urinary potassium excretion. This was accompanied with increased
plasma renin activity, renal blood flow and the glomerular filtration rate (Murphy et
al, 1987).

Fenoldopam has also been used in preoperative and perioperative management
of hypertension. Preoperative hypertension is associated with an increased risk of
myocardial ischemia during anesthesia. And this risk is reduced by antihypertensive
treatment. Post operative hypertension is associated with complications such as
bleeding, cerebrovascular accident and myocardial infarction (Stone et al, 1988).
Rapid establishment of blood pressure control is necessary in such conditions. Various studies, which used fenoldopam and compared it with other drugs like esmolol, nicardipine, nitroprusside and nifedipine have established that fenoldopam can be a drug of choice for the short term control of perioperative hypertension (Tempe et al, 1999; Squara et al, 1994; Goldberg et al, 1993; Hill et al, 1993; Gombotz et al, 1998).

Adverse effects of fenoldopam as antihypertensive drug include headache, flushing, dizziness and tachycardia or bradycardia. These adverse effects are attributed to its vasodilator action (Brogden and Markham, 1997). Fenoldopam also causes an increase in intraocular pressure. This increase is partly due to diminished drainage of aqueous humor (Piltz et al, 1998).

Due to the actions of dopamine in renal physiology, fenoldopam has been used as a renal protective agent in clinical situations known to lead to impaired renal function. In rats with acute nephrotoxicity induced by antibiotics, the administration of fenoldopam has been reported to produce beneficial effects on renal hemodynamics, function, and histology (Brooks et al, 1990; Brooks et al, 1991; Nichols et al, 1992). In dogs, fenoldopam also protects against the acute renal vasoconstriction that may be induced by radio-contrast medium (Bakris et al, 1999). In mildly hypertensive recipients of kidney transplants, the administration of fenoldopam for three weeks reportedly resulted in a significant increase in renal plasma flow (Jorkasky et al, 1992).

**Pharmacokinetics:**

Less than 6% of an orally administered dose of fenoldopam is absorbed (Flaharty et al, 1991), because of the extensive presystemic formation of sulfate,
methyl, and glucuronide conjugates (Cryonak et al, 1987). The mean elimination half life of intravenously administered fenoldopam, estimated on the basis of the decline in the plasma concentration in hypertensive patients after the cessation of a 2 hour long infusion is 9.8 min (Weber et al, 1988). During longer infusions (up to 48hrs), the elimination half life may be shorter (Taylor et al, 1999a; Taylor et al, 1999b). After an infusion has begun, steady state plasma concentrations are reached within 30-60 min (Taylor et al, 1999a). The mean rate of clearance from the body has been estimated at 30.3ml/kg of body weight/min. In plasma, 85-90% of fenoldopam is bound to proteins and its volume of distribution is approximately 600ml/kg (Weber et al, 1988). There is a predictable relation between the dose and the plasma concentration of fenoldopam, and there is a linear relation between the reduction in blood pressure and the rate of infusion of fenoldopam (Weber et al, 1988).

**Brand Name:** Corlopam (SKF).