Chapter - 2

REVIEW OF LITERATURE
1. **Energy Homeostasis, Metabolic Syndrome and Insulin Resistance**

Energy homeostasis is a complex multi-component system that involves processing of energy substrates through interrelated metabolic pathways. Much is known about the mediators, pathways and mechanisms of this model but many of its components still elude our understanding. Central to the concept of this model is the role of glucose and fat metabolism. Derangements in the processing of sugar and lipids adversely affect not just energy homeostasis but all the physiological systems. In fact, impaired fasting glucose and central obesity are two necessary inclusion criteria in the definitions of ‘metabolic syndrome’ proposed by WHO\(^1\) and International Diabetes Federation\(^2\).

The definition of metabolic syndrome (also called syndrome X\(^3\) or the deadly quartet\(^4\)) includes obesity, non-insulin-dependent diabetes mellitus (NIDDM or Type-2 DM), atherogenic dyslipidaemia and hypertension resulting in a high risk of cardiovascular disorders\(^5\). These generally appear as a cluster of co-existing disorders with interlinked pathophysiology. Though there is only little consensus among the medical community regarding the validity of the concept\(^6, 7\) of metabolic syndrome, multiple studies have proven that controlling the elevated glucose and lipid levels can significantly reduce the risk of micro-vascular disease (Retinopathy, Nephropathy), Macro-vascular disease (Coronary artery disease, Cerebral and carotid arteriosclerotic vascular disease and Peripheral vascular disease) and several neuropathies\(^8\) underscoring the importance of these conditions.

Currently, the usage of the term ‘Metabolic Syndrome’ has been largely replaced with the term ‘Insulin Resistance Syndrome’\(^9\) because the common etiological factor that relates to all the aforementioned conditions is Insulin resistance. It has been well established that an increase in caloric intake (in obese diabetic patients) and genetic defects (in lean diabetics) markedly decrease tissue insulin sensitivity and can lead to hyperinsulinemic state and insulin resistance\(^11\). Whether the cause is genetic or environmental, studies indicate that a reduction in blood glucose level can reduce the risk of the deadly quartet.

Strategies aimed at reducing the glucose and lipid levels generally target the “substrate processing sites”. A generalized picture of the various components involved in energy homeostasis is given below (Figure 2.1). They include peripheral signals from GI tract (glucose sensing), pancreas (hormonal function), liver (storage and synthesis), adipocytes (storage and hormonal function), skeletal muscles (utilization and storage) and the CNS (central control). An effective anti-hyperglycaemic or anti-hyperlipidaemic drug targets at least one of the sites.
Many biological mediators and environmental factors can control the energy balance on the body. Insulin, adiponectin, leptin etc. are to name a few.

**a) Role of insulin signaling in energy homeostasis**

One of the major hormones that control glucose disposition in peripheral tissues is insulin. Secreted from the beta cells of pancreas, this peptide hormone with 51 amino acids can control glucose as well as lipid metabolism both directly through receptor mediated actions and indirectly through genetic modulation.

Binding of insulin with its receptor, sets in motion a cascade of inter-related and diverging phosphorylation reactions that control a multitude of cellular processes. The biological action of insulin depends on the effector systems expressed in the target cells/organs. In skeletal muscle, upon binding with insulin and activation by auto-phosphorylation, the insulin receptor activates many adaptor proteins with SH2 homology, important one among which is the IRS (insulin receptor substrate). Activation of IRS causes activation of PI3Kinase (phosphatidylinositol 3 (PI3) kinase) and protein kinase C and subsequent events lead to translocation of GLUT4 transporters to cytoplasmic membranes for glucose uptake. An IRS independent pathway - CAP/Cbl/TC-10 pathway is also implicated in insulin mediated GLUT 4 vesicle trafficking\(^{11,12}\).

In adipose tissue, the IRS proteins mediate activation of MAP Kinase pathway which regulates gene expression that controls lipogenic-lipolytic balance and cellular differentiation. The expression of the lipogenic proteins –fatty acid synthase (FAS) and sterol regulatory element binding protein (SREBP) are induced by insulin via MAPK pathway. FAS play a central role in *de novo* fatty acid synthesis and SREBP causes an increase in uptake of fatty acids which leads to increased synthesis of triglycerides\(^ {13,14}\). Apart from the MAPK induced genetic modulation, insulin also exerts direct inhibitory effect on Hormone sensitive lipases (HSL) and perilipins to prevent lipolysis\(^ {15,16}\).

Insulin influences metabolic processes in liver mainly by its effect on post-translational modification of many hepatic enzymes. Insulin suppresses the gene expression of phosphoenolpyruvate carboxykinase\(^ {17}\) (PEPCK) and glucose-6-phosphatase (G-6-Pase) and thus regulates gluconeogenesis and hepatic glucose output. Also, glycogen synthase kinase is inactivated by insulin mediated PI3 kinase and Akt/PKB pathway which activates the enzyme glycogen synthase and converts glucose to glycogen\(^ {18}\). This pathway is active in adipose tissue and skeletal muscles also which helps in the storage of excess glucose.
The actions of insulin can be positively or adversely affected by many factors. Phosphotyrosine phosphatase enzymes, Serine–threonine phosphorylation or insulin receptor or IRS, Grb proteins, TNF – α, NF - κB, Suppresser of cytokine signaling(SOCS-s) etc. are a few factors which can inhibit...
insulin receptors and/or IRS proteins. Persistent hyperglycaemia can activate hexosamine pathway and can cause O-glycosylation and inactivation of insulin receptor, IRS, Akt/PKB, GLUT-4 and GSK. The glycosylation can also trigger NF-κB mediated transcription of proinflammatory genes by preventing the IκB inhibiton of NF-κB. Excess of glucose, amino acids and fatty acids can activate Mammalian target of rapamycin (mTOR) signaling pathway which phosphorylates and desensitizes IRS proteins and adversely affects PI3 kinase and Akt/PKB signaling.

Of the factors that can enhance the actions of insulin, the cellular fuel gauge AMPK and peroxisome proliferator activated receptors (PPARs) have an important role. AMPK activation occurs during nutrient deprivation which increases the AMP : ATP ratio. AMPK inhibits mTOR pathway and helps improve insulin sensitivity, increases glucose uptake, increases lipid oxidation and inhibits triglyceride and cholesterol biosynthesis by inhibiting the enzyme HMG CoA reductase in liver.

Regulation of metabolism through peroxisome proliferator activated receptors occurs by control of gene expression. Apart from metabolism, they also regulate inflammation, growth and development. The subtypes of PPAR, α and γ have discreet functions in glucose and lipid metabolism. PPARα promotes fatty acid uptake, mitochondrial β-oxidation and thermogenesis in skeletal muscles by inducing uncoupling proteins UCP-1, -2 & -3. PPARγ on the other hand, promotes development of adipose tissue and increases insulin sensitivity by promoting free fatty acid oxidation. The anti-hyperlipidaemic drugs fibrates, act by activating PPARα receptors. PPARγ activation also enhances the expression of a number of factors involved in energy homeostasis and insulin signaling like UCP-1, IRS-1, GLUT-4 translocation etc. The anti-diabetic drugs-thiazolidinediones are PPARγ agonists.

In the context of insulin signaling, though no drugs have been identified which directly activate insulin receptor and control the metabolic processes, many molecules are known to influence the biomolecules that mediate the insulin signaling pathway (like AMPK activators, PTP-1B inhibitors etc.) and are good candidates for anti-diabetic drugs.

b) **Effect of adipokines and adipo-cytokines in metabolic processes and derangements:**

Apart from its role as a lipid storage organ, adipose tissue is now recognized as an endocrine organ with many hormones and cytokines being secreted from. Hormones like leptin, adiponectin, resistin, retinol binding protein-4 etc are intricately associated with energy homeostasis. Leptin acts on the central nervous system and adipose tissue and controls food intake. Deficiency of the hormone or its abnormal signaling pathway causes hyperphagia and obesity. In the case of adiponectin, the high molecular weight isoforms acts to increase insulin sensitivity peripherally
through AMPK activation and increased expression of PPAR target genes whereas the low molecular isoforms act centrally to control food intake. Increased levels of resistin and retinol-binding protein-4 are associated with insulin resistance and type-2 diabetes. Chronic inflammation originating in adipose tissue is identified as one of the etiological factors of diabetes. Inflammation, due to reasons not yet known, in the adipose tissue in obese condition causes increased infiltration of macrophages. Chronic activation of these resident macrophages and their subsequent release of cytokines like TNF – α, IL-6, IL-1 etc. (called adipo-cytokines) and activates NF - κB. These inflammatory mediators causes insulin resistance by inducing protein tyrosine phosphatase (PTP)-1B and suppressor of cytokine signaling (SOCS) mediated insulin receptor degradation.

2. **Drug for diabetes and dyslipidaemia - current drugs and emerging targets**

Transition from normal to pre-diabetic to frank-diabetic state is a chronic event that involves recalibration of multiple homeostatic processes. Controlling the spread of damaging effects of these changes, if not resetting them to their original state, should be the primary function of effective anti-diabetic and anti-hyperlipidaemic drugs. The currently employed most effective drugs act by one of the following mechanisms – promotion of insulin secretion from pancreas (sulfonylureas), enhancing insulin sensitivity in skeletal muscles and adipose tissue (biguanides and thiazolidinediones), decreasing cholesterol & triglyceride synthesis in liver and adipose tissue (statins and niacin respectively) inhibiting lipolysis in adipose tissue and thus reducing the plasma free fatty acids levels (niacin) and enhancing fatty acid oxidation in liver adipose and skeletal muscles (fibrates). But modulation of CNS to regulate energy homeostasis by mediators like leptin and adiponectin has not been successful so far.

A few other classes of drugs like dipeptidyl peptidase inhibitors, glucagon like peptide analogues, alpha-glucosidase inhibitors, cholesterol absorption inhibitors etc. are employed as adjuvants to the main therapy. A recent review on the trends in diabetes research mentions 20 new emerging targets to combat diabetes and dyslipidaemia which include - Glucokinase (GK) Activators, Glycogen Phosphorylase (GP) Inhibitors, Sodium/Glucose Co-Transporter (SGLT) Inhibitors, Activators of AMP-Activated Protein Kinase (AMPK), Retinoic Acid X Receptor (RXR) Modulators, Farnesoid X Receptor (FXR) Modulators, Protein Tyrosine Phosphatase 1B (PTP1B) Inhibitors, Glycogen Synthase Kinase-3 (GSK3β) Inhibitors etc.

With all the therapeutically employed classes of drugs, the treatment of dyslipidaemia and diabetes is still far from satisfactory. Inadequate control and resistance to treatment, high cost of therapy and
higher incidence of adverse effects limit their use. The drug discovery research in metabolic disorders is still pressing forward to uncover newer ‘process mechanisms’, targets and drug molecules.

3. Assessment of anti-diabetic and anti-hyperlipidaemic activity: In-vivo and in-vitro models

A. In-vivo models

A1. Evaluation of incretin effect and/or insulin release –
Oral glucose tolerance test (OGTT) in rats or mice is an important test to evaluate the insulin release and metabolic actions of insulin. Together with intra-peritoneal glucose tolerance test (IPGTT) or intravenous glucose tolerance test (IVGTT), OGTT can be used to evaluate the incretin effect. The procedure involves oral administration of glucose (1.5-2 g/kg body weight) in 6-8 hour fasted animals and measurement of their plasma glucose levels at 0, 15, 30, 60, 90 and 120 minutes of glucose load. The area under the curve (AUC) of plasma glucose concentration-time is an index of the glucose tolerance in the animals, where animals with impaired glucose tolerance exhibiting larger incremental AUC and vice versa. Glucose disposal in the body after its oral administration occurs due to result of complex and simultaneous multiple events that include absorption, incretin effect, insulin release, peripheral utilization and hepatic glucose output. But glucose tolerance and insulin sensitivity are not the same. To estimate insulin sensitivity, the rate and extent of decline in glucose concentration after an intraperitoneal or intravenous bolus of insulin is measured. Another model of insulin sensitivity includes Homeostasis Model Assessment (HOMA) which is an index of both glucose and insulin dynamics and represents pancreatic beta cell function.

The different animal models of diabetes include genetically spontaneous models, diet induced models, chemically induced models, surgically induced and genetically modified animal models. In the current research works, diet induced (high fat diet and high sucrose diet) and chemically induced (streptozotocin) models have been employed.
Streptozotocin (STZ) is widely used in the induction of type-1 as well as type-2 diabetes. In mice doses ranging from 150 mg/kg to 200 mg/kg have been employed to induce different degrees of hyperglycaemia. STZ when injected intraperitoneally or intravenously is selectively taken up by the beta cells of pancreas and causes DNA damage in beta cells and their destruction. In STZ diabetic model, apart from the glucose levels, the lipid profiles and insulin levels are also deranged. High-fat diet fed (HFD) and high-sucrose diet (HSD) fed models also cause glucose intolerance and
hyperlipidaemia in rodents\textsuperscript{36, 37}. But the plasma insulin levels in these models depend upon the duration of the diets fed. Though there are different compositions of HFD and HSD, the diets used in the study are adopted from previous studies\textsuperscript{38} conducted in this college where their effect has been established.

B. In-vitro models

B1. Glucose uptake assays and mechanistic studies -
Assessment of glucose uptake can be done by using animal tissues or cells lines. The hemi-diaphragm of rat is commonly used to assess glucose uptake\textsuperscript{39}. The hemi-diaphragm is incubated for 30-45 min, in presence of drug, in Tyrode solution containing known amount of glucose (100 – 200 mg/dl). The decline in glucose concentration in the solution after the incubation period is an indicator of the ability of glucose uptake mediated by the drug. The thigh muscle of rat or frog can used also be used instead of hemi-diaphragm.

Cell lines like L6 myoblast or 3T3-L1 pre-adipocytes are used to estimate glucose uptake. Radioactive 2-deoxyglucose\textsuperscript{40} or non-radioactive fluorescent NBDG\textsuperscript{41} are used as substrates whose cellular uptake in the presence of drug is measured.

L6 myoblast or 3T3-L1 pre-adipocytes are also models to measure AMPK activation and GLUT-4 translocation\textsuperscript{42}. The lipogenesis can be measured by TG formation in 3T3 cells. Expression of PPARs α and γ can also be estimated using these cell lines.


The understanding that metabolic disorders are the result of inflammatory conditions has put the research focus on inflammatory mediators as well. Suppression of pro-inflammatory mediators like IL-6, TNF-α and NF-kB helps in the progression of atherogenesis and cardiovascular disorders. Cell culture and animal models are available for assessing the anti-inflammatory effect. LPS treated L6 myoblast and the macrophage cell line RAW 264.7 can be used to evaluate intracellular nitric oxide production\textsuperscript{43}, NF-kB and other inflammatory mediator expression using western blot.

The current study employs the above mentioned cell culture studies and animal models to determine the efficacy of the molecules and to determine their possible mechanisms of action.

4. References