CHAPTER-1

Chapter 1

Introduction & Review of Literature

1. Introduction

Lifestyle of people worldwide is influenced with increased modernization, changed dietary habits and concomitant rise in the stress level. They suffer from many life-threatening diseases like hypertension, cardiac diseases, cancer, diabetes mellitus, depression and many others (Shomali 2012).

1.1 Diabetes:

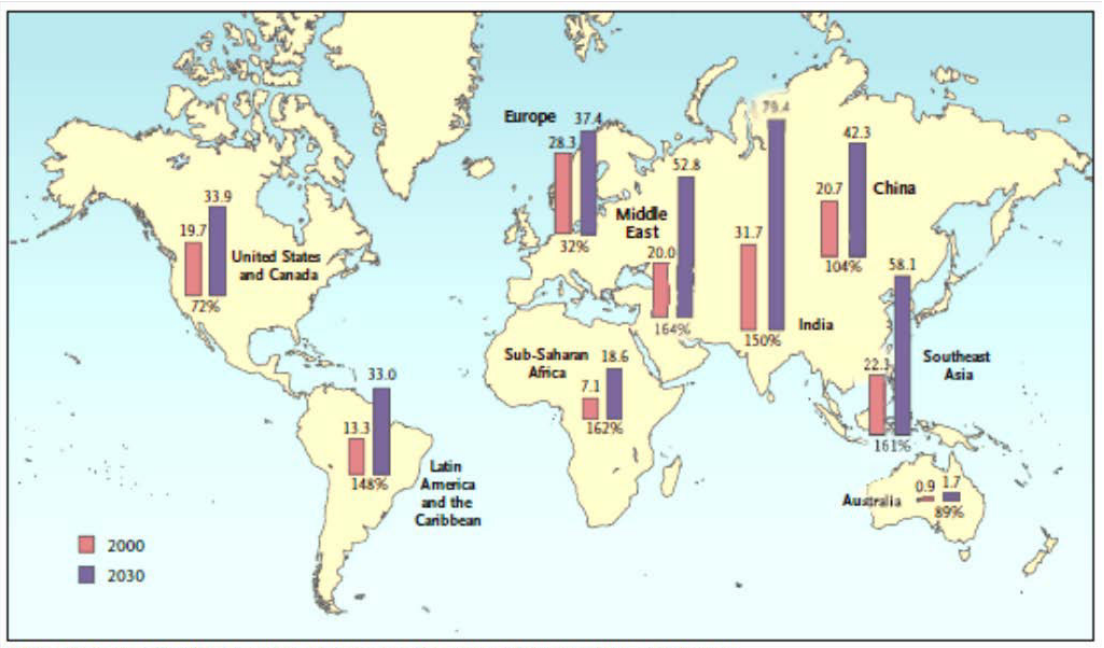
Diabetes mellitus is a multifactorial and complex metabolic disease. It is characterized by alterations in the metabolism of carbohydrate, fat and protein, which are caused by a relative or absolute deficiency of insulin secretion (IDDM), insulin action(NIDDM), or both (Joslin's 2005). It is a disorder resulting from both genetic predisposition and favoring environmental factors. It is called the disease of three "poly's" – Polyuria, Polydipsia, Polyphagia (Joslin's 2005). In the patients with long-standing diabetes, late complications develop which include microvascular alterations and leading to retinopathy with vision loss, nephropathy leading to renal failure, peripheral and autonomic neuropathy, severe cardiovascular, cerebrovascular and peripheral vascular atherosclerosis (Ananda Prabu K 2012).

1.2 Epidemiology:

Diabetes mellitus is the third most prevalent killer disease in the world. Epidemiology shows that it is one of the most common endocrine disorders and a major global health problem, affecting 8.3% of the world's population (Zimmet et al. 2001; Nam Han Cho et al. 2013). Approximately 382 million people suffer from diabetes worldwide (IDF 2013) and projections suggest that more than 592 million people will have diabetes by 2035. India and China are reported to have highest number of diabetics around the globe with India having 61.5 million diabetics (Nam Han Cho et al. 2013)The global cost of treating diabetes and its complications reaches US $1trillion annually (Shomali 2012; Nam Han Cho et al. 2013).

In many of the developing countries, a large section of population belongs to the rural areas and among them, many of their generations live in frequent starvation. These persistent low calorie dietary intakes for many generations have brought epigenetic modifications on energy homeostasis. Thus, an efficient metabolic engine for their survival has been created. India

being one of the developing countries in South-East Asia; there are lots of epigenetic modifications among people. Varied metabolic kinetics of Indian population makes them more vulnerable in managing high calorie load then their western counterparts. Constant migration of people from rural to urban areas contributed significantly in easier availability of high calorie food and lower physical activities that has lead to major metabolic dysregulation (Yajnik and Ganpule-Rao 2010). These facts of Asians especially Indians would make later the leading diabetic patients (Nakamura et al. 2006).



Millions of Cases of Diabetes in 2000 and Projections for 2030, with Projected Percent Changes.
Data are from Wild et al.[3]

Figure 1.1: Global projections for the diabetes epidemic: 2000–2030. Data show numbers of people with diabetes (millions) for 2000 and for 2030, as well as the percentage increase between the two time points (Hossain et al. 2007).

1.3 Classification:

Diabetes mellitus is broadly classified into three different types, however due to recent etiological studies, maternal diabetes is also considered as a subpart of diabetes mellitus and brain insulin resistance as Type III Diabetes.

1) **Type-I Diabetes mellitus** (TIDM); also called Insulin Dependent Diabetes Mellitus or Juvenile Onset Diabetes mellitus.
2) **Type-II Diabetes mellitus** (TIIDM) ; also called Non-Insulin Dependent Diabetes Mellitus or Maturity onset Diabetes mellitus (Joslin's 2005).

3) **Type III Diabetes mellitus** (TIIIDM): also known as brain insulin resistance.
4) **Gestational Diabetes mellitus** (Joslin's 2005).
    - Maternal insulin resistance

1.4 Etiology:

1.4.1 Type-I Diabetes mellitus (TIDM)

Type 1 diabetes is caused by an autoimmune reaction, where the body's defence system attacks the pancreatic insulin-producing β cells. As a result, the body can no longer produce required insulin. Other reasons include congenital absence of pancreas, genetic defects of β-cell; genetic defects in insulin action; other endocrine and exocrine dysfunctions and even viral infections of pancreas. The disease can affect people of any age, but usually occurs in children or young adults. It can be treated only by supplementing exogenous insulin. The two main forms of clinical type 1 diabetes are type 1a which is thought to be due to immunological destruction of pancreatic β cells resulting in insulin deficiency; and type 1b (idiopathic, about 10% of type 1 diabetes), in which there is no evidence of autoimmunity (IDF 2013). People with type 1 diabetes can lead a normal, healthy life through a combination of daily insulin therapy, close monitoring, healthy diet and regular physical exercise (Nam Han Cho et al. 2013).

1.4.2 Type-II Diabetes mellitus (TIIDM)

Type II diabetes mellitus also formerly known as NIDDM, is the most common form of diabetes mellitus where 80-90% of diabetic patients have this form of diabetes (ADA 2011). It is caused due to varying combinations of insulin resistance and insufficient compensatory insulin secretion. The disease has an insidious onset and remains asymptomatic and undiagnosed for long period. It starts with impaired glucose tolerance and mild hyperglycemia leading to hyperinsulinemia - the hallmark of Type II diabetes. The over-burdened β-cells die, leading to decreased insulin secretion in the later stages tending towards Type I diabetes (Balasubramanyam et al. 2008). The moderate hyperglycemia is able to induce severe diabetic complications at later stage. Thus the overall pathogenesis of TIIDM results from defects in insulin receptor (IR) function, IR-signal transduction, glucose transport and phosphorylation, glycogen synthesis, glucose oxidation and dysregulation of fatty acid metabolism which contributes to insulin resistance in target tissues and impairment of pancreatic insulin secretion (Saltiel and Kahn 2001).

Metabolic changes in TIIDM are less severe than the type 1 as the inadequately produced insulin restrains the development of ketoacidosis. Type II diabetes is strongly favored by genetic predisposition. Hyperglycemia is usually improved or corrected by diet, weight loss and oral hypoglycemic drugs (Lin and Sun 2010).

1.4.2.1 Etiological Factors in the Development of TIIDM:

1. Obesity:

There is an enormous amount of evidence implicating that obesity plays a major role in the development of diabetes. Studies within populations tend to show a gradation of diabetes prevalence and is least found in the lean people of any age. Longitudinal studies also show increasing likelihood of development of diabetes according to prevalence of obesity (Hossain et al. 2007). Data from the Nurse's Health Study demonstrate that, increasing body mass index (BMI), increases the risk of developing diabetes. Even a BMI of 24 kg/m^2, which is usually considered to be within the "normal" range carries a greater risk of developing diabetes than does a lower BMI (Colditz et al. 1990; Carey et al. 1997).

2. Lack of Physical Activity and Exercise:

Contracting skeletal muscle takes up more glucose from the circulation than it does in the rest state. This effect is partly mediated by adrenaline, and is responsible for the state of improved insulin sensitivity that is produced by exercise. The continuous increase in the glucose uptake that replenishes the glycogen stores and regulates carbohydrate metabolism is stopped due to lack of regular exercise. In addition, it has beneficial effects on lipid metabolism and its contribution to weight loss provides another mechanism whereby exercise may influence the development of type II diabetes (Manson et al. 1991).

3. Dietary Factors:

There seems to be no doubt that diet plays a significant role in the development of Type II diabetes. However, it has been remarkably difficult to point out the precise dietary constituents that are the key players. The increased risk of diabetes with increasing intake of total fat has been reported in several studies. A higher intake of saturated fat and trans fatty acids is associated with Type II diabetes, whereas higher intake of unsaturated fats especially polyunsaturated fatty acids appears to be protective (McCarty 2001; Salmeron et al. 2001). The relationship of carbohydrate intake to diabetes is less clear than for fat intake. Longitudinal studies showed that a low intake of dietary fiber increased the risks of diabetes (Rossouw et al. 2002).

4. Socio-cultural Factors:

Although much of the focus of research into the etiology of diseases such as diabetes is usually on the biomedical risk factors, and the unraveling of molecular mechanisms, socio-cultural factors can also play a major role. The change in many of the long-established social norms resulted in an explosion of diseases such as TIIDM and obesity. A study from the north of England found that the prevalence of TIIDM was nearly 30% higher in people living in areas with the worst quintile of deprivation scores, compared to those in the most affluent areas (Sadikot et al. 2004). In contrast, in a study from the southern India, those in the high income group were twice as likely to have diabetes when compared to the lower income group (Ramachandran et al. 2001).

1.4.3 Type III Diabetes (TIIIDM):

TIIIDM also known as brain insulin resistance corresponds to a chronic insulin resistance / insulin deficiency state observed in brain / neuronal cells. Insulin and insulin receptors are found throughout the central nervous system and can exert diverse effects though it was considered as an insulin independent tissue (Banks WA et al. 2012). The source of insulin in the brain might be either peripheral or local or both (Banks 2004). Regional and metabolic state-dependent differences occur in transport of insulin across the blood brain barrier through saturable transport mechanism and the distribution of insulin receptor within the CNS (Baskin DG et al. 1983). TIIIDM has been reported in relation to diabetes mellitus, obesity, aging and late onset of sporadic Alzheimer's disease and neurodegeneration. There are observations, where, there is reduced transport of insulin to brain because of down regulation of insulin receptor in blood brain barrier, down regulation of insulin receptor in neurons, insulin resistance because of reduced tyrosine phosphorylation of receptor as well as insulin receptor substrates. Increased activity of GSK3 beta leading to the tau hyperphosporylation has been observed in AD in response to hyperinsulinemia (Carro E and AI 2004; Hoyer 2004).

1.4.4 Gestational Diabetes (GDM):

Gestational diabetes mellitus (GDM) is defined by glucose intolerance of variable severity with onset of first recognition during pregnancy (Cheung KW 2011). The risk factors for GDM are advanced maternal age, obese/ overweight, diabetes in first degree relative, previous history of GDM, PCOS, etc. Hyperglycaemia during pregnancy is found to be associated with various maternal and perinatal adverse outcomes (Landon MB 2011). Patients with GDM are at higher risk for excessive weight gain, preeclampsia and caesarean

sections. Infants born to these mothers are at higher risk of macrosomia, birth trauma and shoulder dystocia (Wagaarachchi PT 2001). After delivery, these infants have a higher risk of developing hypoglycaemia, hypocalcemia, hyperbilirubinemia, respiratory distress syndrome, polycythmeia and subsequent obesity and Type II diabetes. In addition, having a history of Gestational diabetes mellitus makes mothers susceptible to a risk for development of TIIDM or recurrent GDM in the future. It is a common disorder affecting ~ 7% of pregnancies each year (ADA 2000).

Maternal Diabetes: A substantial body of evidence suggested that an abnormal intra-uterine milieu elicited by maternal metabolic disturbances as observed during diabetes programmes increases the susceptibility of the foetus to later develop chronic degenerative diseases such as obesity, hypertension, cardiovascular diseases and type II diabetes mellitus (TIIDM) (Reusens B et al. 2007). Hyperglycaemia and hyperinsulinemia are the major cause of maternal diabetes that alters foetal metabolic and circulatory homeostasis. This in turns leads to the ultimate insult targeting the loss of offspring beta-cell mass and propagates diabetes risk to the next generation again (Portha B et al. 2011) . Also the non-genomic mechanisms/epigenetics are involved in the installation of the programmed effect as well as in its intergenerational transmission. In maternal diabetes, the foetus increases its oxidative metabolism, becoming more hypoxemic. The increased placental weight as well as polycythaemia, which is common in infants of diabetic mothers, may represent positive adaptive mechanisms which protect the foetus from hypoxaemia (Maulik D 2002). Increase in the relative placental weight may protect foetus from maternal diabetes (Evers I M et al. 2003). This subset of foetuses among diabetic pregnancies is at highest risk for intrauterine death which may be a consequence of inadequate adaptation to pathophysiological disturbances.

1.5 Diagnostic Criteria for Diabetes mellitus:

In 1997, the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus published a new classification scheme and revised diagnostic criteria for diabetes mellitus (WHO 2013).

Diabetes symptoms (ie polyuria, polydipsia and unexplained weight loss) includes,

- a random venous plasma glucose concentration $\geq$ 11.1 mmol/l ($\geq$200mg/dl)
- a fasting plasma glucose concentration $\geq$ 7.0 mmol/l ($\geq$126mg/dl) (whole blood > 6.1mmol/l)

- two hour plasma glucose concentration ≥ 11.1 mmol/l (≥200mg/dl) two hours after 75g anhydrous glucose in an oral glucose tolerance test (OGTT).
- High diabetes risk having HbA1c 42-47 mmol/mol (6.0 – 6.4%)

In 1997 and 2003, The Expert Committee on Diagnosis and Classification of Diabetes Mellitus recognized an intermediate group of prediabetic individuals whose glucose levels do not meet criteria for diabetes, yet are higher than those considered normal.

- Impaired fasting glucose (IFG) :fasting plasma glucose (FPG) levels 5.6 mmol/l to 6.9 mmol/l (100 to 125mg/dl)
- Impaired glucose tolerance (IGT): 2-h values in the oral glucose tolerance test (OGTT) of 7.8 mmol/l to11.0 mmol/l (140-199 mg/dl)
- HbA1c:5.7 – 6.4%

1.6 Insulin signaling pathway

Insulin and the insulin-like growth factors (IGFs) control many aspects of metabolism, growth and survival in a wide range of mammalian tissues (Shepherd et al. 1995). Insulin exerts its various effects through its receptor which is a heterotetramer consisting of two α (135 kDa molecular mass) and two β (95 kDa molecular mass) subunits (White MF 1993). The insulin receptor (IR) exists as two isoforms differing by the presence (IR-B) or absence of 12 amino acids (IR-A) at the carboxyl terminus of the A-subunit, as a result of alternative splicing of the sequence encoded by exon 11. IR-B is the more abundant isoform in muscle, liver and fat tissue. Insulin binds with similar affinity to both isoforms (Belfiore A 2009).

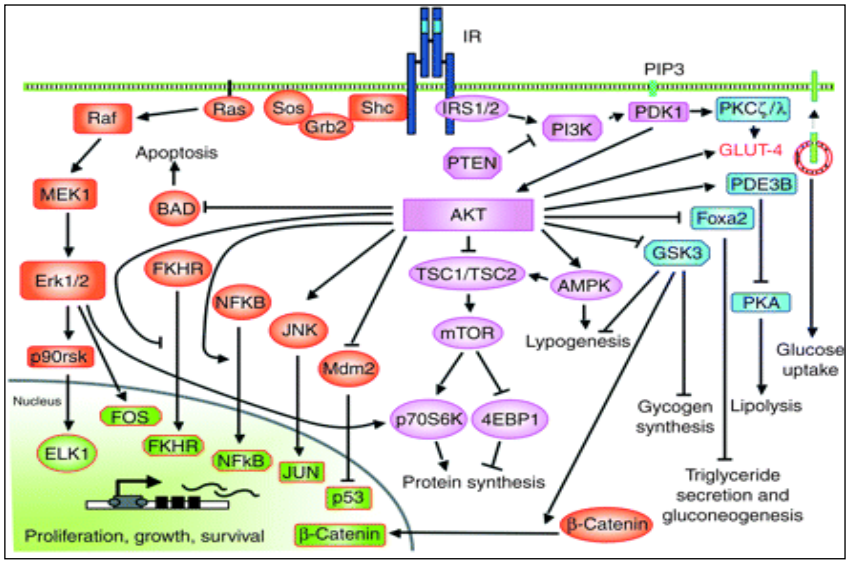


Figure 1.2: Insulin signaling pathway (Belfiore and Malaguarnera 2011)

Insulin binding to the α-subunit results in the dimerization of the receptor to form the α2β2 complex in the cell membrane and autophosphorylation of the β-subunit at Tyr1158, Try1162, and Tyr1163, the first step in the activation of IR. The activation of IR tyrosine kinase recruits and phosphorylates several substrates, including IRS1–4, SHC, Grb-2-associated protein (GAB1), DOCK1, CBL, and APS adaptor proteins, all of which provide specific docking sites for the recruitment of other downstream signaling proteins, leading to the activation of both the Ras/MAPKs and phosphatidylinositide-3-kinase (PI(3)K)/Akt signaling cascade (White 2003; Adams TE 2004; Taniguchi CM 2006; Laviola L 2007; Estall et al. 2009).

The Insulin Receptor Substrate (Erener et al., 2008) proteins are a family of cytoplasmic adaptor proteins that were first identified for their role in insulin signaling. IRSs are relatively specific substrates of IR/IGFR and are characterized by the presence of a NH2-terminal pleckstrin homology (PH) domain adjacent to a phosphotyrosine-binding (PTB)domain, followed by a COOH-terminal tail that contains numerous tyrosine and serine/threonine phosphorylation sites (Copps KD 2012) . The COOH terminal of each IRS protein has about 20 potential tyrosine phosphorylation sites that act as on/off switches to transduce insulin action, recruiting downstream signaling proteins, including PI(3)K subunit, phosphotyrosine phosphatase SHP2, and adaptor molecules such as GRB2, SOCS3, NCK, CRK, SH2B, and other molecules (White 2003; Sun X 2009).

The first family member to be identified was IRS-1. IRS-2 was discovered as an alternative insulin receptor substrate, initially named 4PS, in insulin-stimulated cells derived from IRS-$1^{-/-}$ mice. IRS-1 and IRS-2 are ubiquitously expressed and are the primary mediators of insulin-dependent mitogenesis and regulation of glucose metabolism in most cell types. Humans express one additional family member, IRS-4, which is more restricted in its expression pattern and is found primarily in brain, kidney, thymus and liver. A fourth IRS protein, Irs-3, is expressed in rodents, but not in humans. More distantly related IRS family members IRS-5 and IRS-6, also known as DOK4 and DOK5, share homology in their N-termini, but have truncated C-termini (Mardilovich K et al. 2009). In terms of downstream signaling, IRS1 appears to be linked to glucose homeostasis and IRS2 in regulation of lipid metabolism (Taniguchi CM 2005; Bouzakri K 2006; Thirone AC 2006).

1.6.1 PI(3)K/Akt pathway:

The activation of PI(3)K generates phosphatidylinositol (3,4,5)-triphosphate (PIP3), a second messenger activating 3-phosphoinositide-dependent protein kinase 1 (PDK1) and PDK2, which mediate the effect of insulin on metabolism and pro-survival. PDK1 and PDK2, in turn, activate the protein kinase Akt (PKB), by inducing phosphorylation at T308 and S473 respectively. PDK2 is also known as mTORC2 (Mammalian target of rapamycin complex 2) and activates other protein kinases such as protein kinase C (PKC) other than Akt thus controlling insulin stimulated Glut4 translocation, cell survival and energy homeostasis (Sarbassov DD and DM 2006; Hagiwara A and Heim MH 2012). mTORC2, through Akt, promotes the expression and activation of the sterol regulatory element binding protein 1 (SREBP1) transcription factor, a family member of the SREBPs that promote lipid and cholesterol synthesis (Yecies JL and Kwiatkowski DJ 2011) (Yabe D 2002). Moreover, mTORC2 and PDK1 suppress the fork head transcription factor(FoxO)-1 that promotes gluconeogenesis, mediating the effect of insulin on the suppression of hepatic glucose production (Hagiwara A 2012). Well-established Akt/PKB substrates include GSK-3 that promote glycogen synthesis, Rab GTPase activating protein AS160/TBC1D4 that regulate glucose transport, FOXO transcription factors and BAD that regulate apoptosis and PDE3B for cAMP degradation (Wang Y and M 2010) (Manning BD 2007; Vasudevan KM 2010).

1.6.2 Ras/ MAP kinase pathway:

IR and IGFR regulate cell growth-related gene expression via the Ras/MAP kinase pathway (Avruch 2007). The pathway is activated by insulin following the binding of Grb2 and the guanyl nucleotide-exchange factor SOS to cognate phosphotyrosines on IRS proteins Shc and Gab1. This binding triggers the activation of the small GTPase Ras and the subsequent activation of Raf, which triggers a kinase cascade that results in the phosphorylation and activation of the dual-specificity kinases MEK1 (MAPK and Erk 1) and MEK2, which in turn phosphorylate MAPK/Erk1 and Erk2 on threonine and tyrosine residues. The activated Erks phosphorylate various targets, including p90 ribosomal protein S6 kinase (p90RSK) and the transcription factor ELK1,there by promoting gene expression (Pouyssegur 2002). The small GTPases of the Rho family, Rac and CDC42, have also been implicated in insulin action. Both Rac and CDC42 are activated by the exchange of GDP to GTP, which triggers a conformational change that induces the interaction of the GTPases with downstream effectors, many of which are involved in the rearrangement of the actin cytoskeleton. This

property is thought to link Rac to GLUT4 translocation in skeletal muscle, whereas CDC42 might have this role in Adipocytes (Marcusohn 1995; Usui 2003).

Amongst the accessory pathway, c-Cbl proto-oncogene is tyrosine-phosphorylated by the IR and forms a complex with other molecules such as Cbl-associated protein (CAP) that results in the activation of the small GTPase TC10 and CAP–Cbl pathway that collaborates with the PI(3)K pathway in the stimulation of GLUT4 translocation (Baumann 2000).

1.6.3 Negative regulation of insulin signaling:

There are several mechanisms of feedback, one such class of regulatory proteins is tyrosine phosphatases among which PTP1B is most studied. This protein interacts directly with IR and dephosphorylates important tyrosine residues, thereby reducing its activity. Other proteins, such as suppressor of cytokine signaling-1 (SOCS1) and SOCS3 (Elchebly 1999; Ueki 2004) , growth-factor-receptor bound protein 10 (Grb10) and plasma-cell-membrane glycoprotein-1 (PC1) downregulate IR function by stearically blocking its interaction with the IRS proteins, or by modifying its kinase activity.

Thus, the overall action of insulin is to increase glucose utilization in muscle, liver and adipose tissue while depressing glucose production in the liver, which results in blood glucose lowering; to lower FFA level by refraining lipolysis, and to prevent ketone formation in the liver by opposing ketogenesis.

1.7 Insulin resistance

In an insulin resistance condition, such as TIIDM, insulin is secreted in adequate amount from the pancreas, however there are defects in the insulin action.Clinically, the term “insulin resistance” implies that higher-than-normal concentrations of insulin are required to maintain normoglycemia (Delghingaro-Augusto et al. 2004).

James Neel speculated that the tendency to develop insulin resistance is unlikely to be a genetic disorder. Instead, it must have evolved as an adaptive trait that later turned pathological due to changed life style and diet. He hypothesized that a "thrifty" genotype that helped survival in primitive life characterized by periods of "feast and famine" has now turned detrimental in the modern urban lifestyle and diet. Neel's hypothesis and its modified versions have dominated the etiology of recent epidemic of diabetes (Watve and Yajnik 2007).

Insulin resistance can occur because of defects in insulin action at pre-receptor, receptor or post-receptor level. Besides rare cases of abnormal insulin or presence of receptor antibodies (pre-receptor defects), reduction in the insulin receptor number is relatively a common factor contributing to insulin resistance. In addition, insulin binding may also be affected in rare conditions in which qualitative alterations occur in the receptor (e.g. decrease in the receptor affinity). Post-receptor mechanisms include the signals triggered by insulin binding to the receptor as well as the resulting changes in several key steps of intracellular metabolism (Basel 2000).

The mechanisms of post-receptor insulin effects can be distinguished into:

a. Short term regulation
b. Long term regulation

Short term regulation by either changes in concentrations of ions or regulatory compounds like change in phosphorylation level hence, change in activity of the enzyme of intermediary metabolism occurring within seconds or minutes.

Long term regulation encompasses induction/repression mechanisms leading to changes in synthesis of the enzyme proteins that takes longer hours.

1.7.1 Molecular Mechanism of Insulin resistance:

At the molecular level, insulin resistance can be acquired through multiple mechanisms as shown in **Figure** 1.3 (Biddinger S.S and Kahn 2006; Saini 2010).

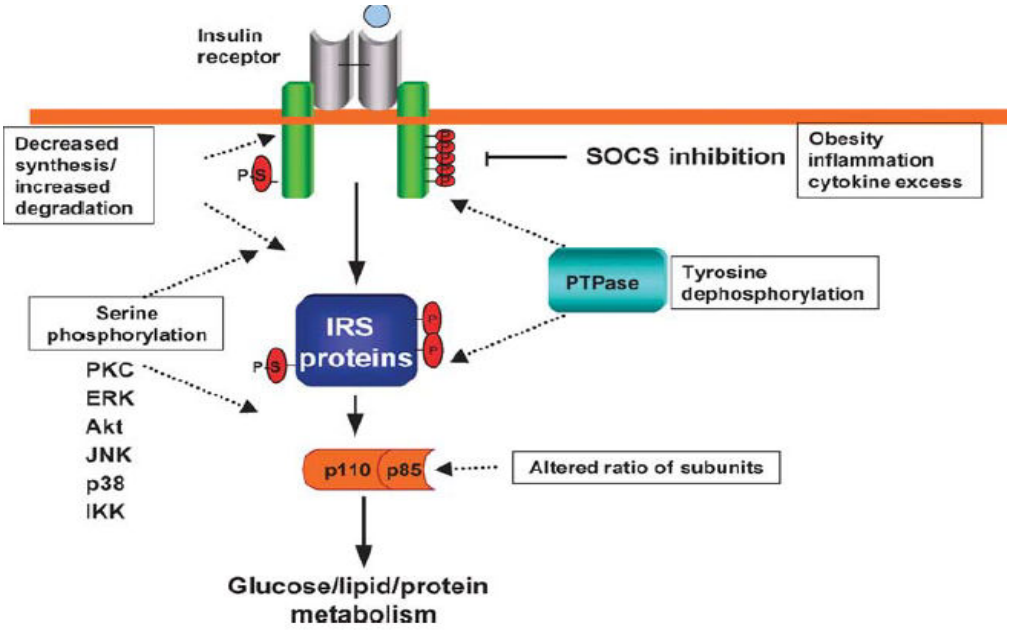


Figure 1.3: Molecular mechanism of Insulin resistance (S.S. Biddinger & C. Ronald Kahn, Annu Rev Physiol, 2006)

1. Important components of the insulin signaling cascade can be decreased by either increased degradation or by decreased transcription, as has been described for IRS-1 and IRS-2. Increased stimulation of SREBP-1c can lead to a decrease in IRS-2 levels.
2. Altered ratio of PI(3)K subunit. If the regulatory subunits of PI(3)K are increased, they can act as inhibitors of the normal dimeric form of the enzyme.
3. Any of the insulin signaling components can undergo posttranslational modifications that decrease or increase its activity. For example, the insulin receptor and the IRS proteins can undergo serine phosphorylation by PKC, Erk, and the stress kinases, JNK and IKKβ. Serine phosphorylation of the insulin receptor decreases its kinase activity. Serine phosphorylation of the IRS proteins impairs their ability to be tyrosine phosphorylated and increases their association with the 14-3-3 proteins, removing them from the insulin signaling cascade.
4. Insulin resistance can be produced by the interaction of inhibitory proteins with components of the insulin signaling cascade. For example, the suppressors of cytokine signaling (SOCS) proteins, which are induced by inflammatory cytokines, bind to the insulin receptor and block its signaling.
5. Insulin resistance can be due to increase in the activity or amount of the enzymes that normally reverse insulin action, including the phosphotyrosine phosphatases, e.g., PTP1b, and the PIP phosphatases, e.g., PTEN and SHIP.

1.8 Insulin sensitive peripheral tissues and their metabolism under insulin resistant state:

Type II diabetes associated insulin resistance leads to many metabolic changes in the peripheral tissues. Since the peripheral tissues (especially muscle and adipose tissue) are not able to take up glucose due to insulin resistance, there is increased gluconeogenesis and glycogenolysis in the liver as shown in Figure 1.4. The glucose produced by the liver is released into the blood stream for the use by other tissues like skeletal muscles and brain. Due to condition of depleted fuel availability, there is increased fat mobilization into adipocytes which leads to increased free fatty acids in the blood plasma. These free fatty acids (FFA) are taken up by the liver and esterified to form triglycerides which are released into the blood stream as VLDL. The proteins in the skeletal muscles are degraded to amino acids which are used as gluconeogenic precursors in the liver. Skeletal muscles also show increased glycogenolysis and use the glucose produced for their energy needs. Fatty acid oxidation is increased moderately in the liver which, in the long

run increases the ketogenesis. TIIDM is associated with many metabolic changes. Hepatic glucose output (HGO) and FFA further worsens the condition of hyperglycemia, insulin resistance and increases the risks of diabetic complications (Harvey 2011).

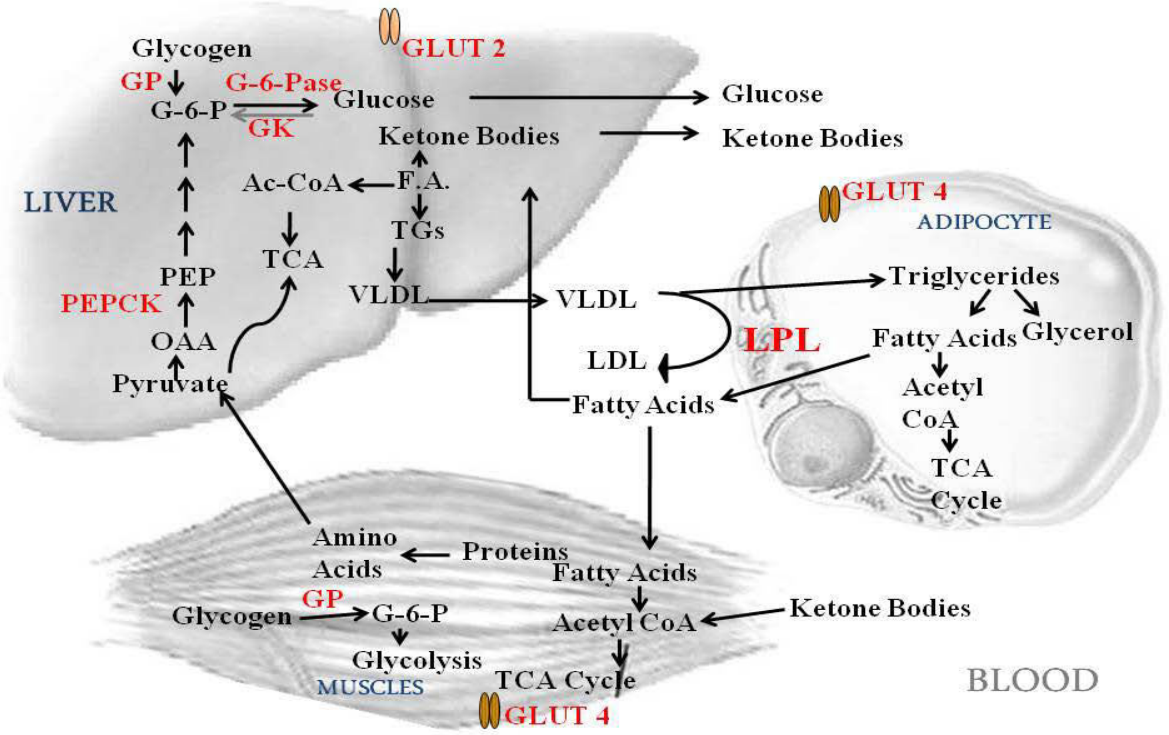


Figure 1.4: Intertissue relationship in TIIDM: Metabolism pathways and altered enzyme in insulin sensitive peripheral tissues during type II diabetics (Harvey 2011).

1.8.1 Hepatic carbohydrate metabolism:

Liver is the master organ where metabolism of all the biomolecules takes place. It is the main organ apart from skeletal muscles and adipocytes which maintains the concentration of glucose and even fatty acids in the blood. Under the fed condition, elevated insulin concentration increases glycogenesis, glycolysis and decreases gluconeogenesis in the liver (Harvey 2011) .

Under conditions of starvation, liver plays a major role in providing glucose for utilization, by other organs, especially the brain. There is increased level of glycogenolysis and gluconeogenesis during starvation under the effect of hypoglycemic hormones like glucagon and epinephrine. Alternatively, overexpression of key regulators of metabolism can lock hepatocytes in the fasted state (Collier and Scott 2004). PEPCK and G-6-pase are major regulatory enzyme of gluconeogenesis in liver and hence, has a major role in the hepatic glucose production (HGP). So, in TIIDM where liver may show insulin resistance, PEPCK expression increases by 2-3 folds despite of hyperinsulinemia and will lead to increase in

HGP further leading to increase in blood glucose (Scott et al. 1998; Sun et al. 2002). Particularly cAMP response element binding protein (CREBPs) is involved in the control of gluconeogenesis in the context of other known transcription factors using the PEPCK gene as a model promoter. CREB is essentially important to regulate the transcription of glucose-6-phosphatase gene in the liver (Thiel et al. 2005).

GLUT-2 is an insulin independent transporter present on Hepatocytes and β-cells of pancreas (Ferre 2004). GK expression is sufficient to promote fasted-to-fed transition. Once glucose influx is started, it can promote glycolysis and lipogenesis, and inhibits gluconeogenesis. Liver GK is a "Glucose sensor" of liver. It catalyses the reaction: Glucose to Glucose-6-Phosphate. The expression of glucokinase gene is regulated by insulin. During diabetes glucokinase activity and expression is altered. Lower glucokinase activity will decrease glucose utilization and energy expenditure which will reduce insulin sensitivity leading to hyperglycemia (Williams et al. 1999; Nawano et al. 2000; Otaegui et al. 2003; Collier and Scott 2004). Glycogen Phosphorylase catalyses the degradation of glycogen during starvation and provokes emergency energy requirement in liver and muscles. During diabetes increase in liver glycogen phosphorylase activity and expression further contributes to high hepatic glucose production (Poucher et al. 2007).

Peroxisome proliferator–activated receptor (PPAR)-γ coactivator-1 (PGC-1α) is one such transcriptional factor shown to play particularly important role in liver biology. PGC-1α can increase glucose production in hepatocytes and activates gluconeogenesis by increasing the transcription of key enzymes involved in glucose synthesis, such as PEPCK and G6Pase (Yoon et al. 2001). Hepatic PGC-1α binds to and activates multiple transcription factors, including FoxO1, glucocorticoid receptor, hepatic nuclear factor-4α, estrogen-related receptor α, and PPAR-α, resulting in increased expression of genes important for gluconeogenesis, fatty acid oxidation, lipid transport, and oxidative phosphorylation. Thus, the PGC-1(α & β) family of genes is at the centre of interactions between genetics, diet, and exercise – a crucial piece of the puzzle of TIIDM pathogenesis and new approaches to tissue-targeted interventions for both therapy and prevention.

1.8.2 Fat Metabolism and Obesity:

Pathophysiology of diabetes mellitus is not only about an insulin-glucose axis, but also 'fat derangements'. However, measurement of glucose tolerance has diagnostic importance, but mechanism/factor leading to pathophysiology of TIIDM is lacking (Raz et al. 2005). Obesity

is defined as a state of excessive adipose tissue mass and is best viewed as a syndrome or group of diseases rather than as a single disease entity. Specific syndromes of obesity, both in animal models and in humans, are associated with identified neural, endocrine, or genetic causes. Dyslipidemia and obesity in TIIDM are related to the complication like atherosclerosis, hypertension and CVDs (Abbasi et al. 2002)**.** Body weight is associated with age, blood pressure and levels of total and low-density lipoprotein cholesterol (LDL-C). Hyperglycemia is also associated with hypertension, hypertriglyceridemia and low levels of good cholesterol (HDL-C) and glucose stimulated hyperinsulinemia. An understanding of obesity and its consequences requires investigation of the many factors that control energy intake and energy expenditure, the two interrelated components of the energy-balance equation majorly in adipose tissue (Joslin's 2005). Adipose tissue comprises the deposition of fat cells called adipocytes, distributed throughout the body of mammalian and non-mammalian species. They are distributed basically into three different regions namely subcutaneous, dermal and visceral. White adipose tissue is most profoundly found in changing its mass throughout the lifespan of an organism and is involved in obesity associated complications. Subcutaneous fat depots are found to be less active when compared to that of visceral (Harvey 2011).

Adipose tissue is functionally very complex organ that plays a major role in peripheral and brain metabolism. Three major functions of adipose tissue are known. The well known amongst them is fat storage in form of triglyceride followed by controlled release of fatty acids and glycerol to meet the energy demand by various hormonal stimuli. Endocrine functions of this organ play a vital role in metabolism. The third most prominent function of this tissue is to regulate the signaling and metabolism by secretion of various bioactive molecules called as adipokines (Harvey 2011).

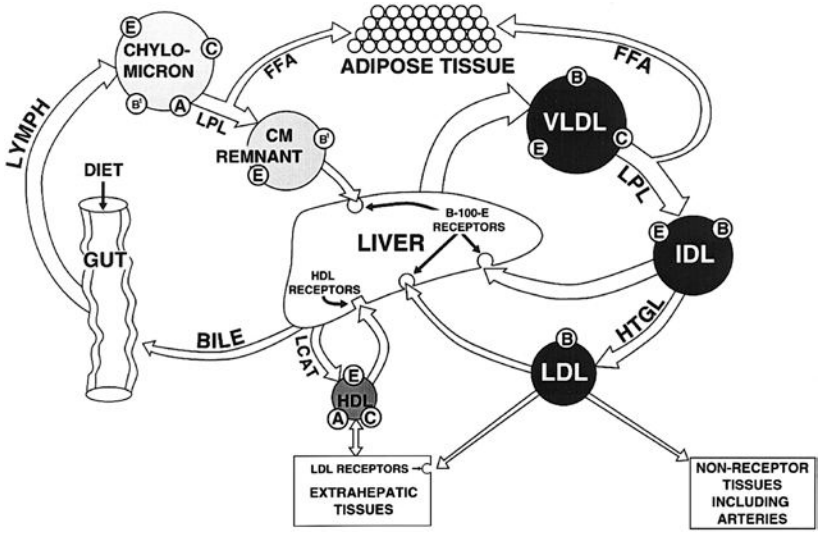


Figure 1.5: FFA and Lipoprotein metabolism in peripheral tissues

In post absorptive state, dietary fat are packaged in the intestinal mucosa and transported to circulation by lymphatic system. Triglycerides rich particles are cleared rapidly mainly by adipose tissue. CD36 and fatty acid transport proteins (FATPs), transports FFAs into adipocytes, which synthesize TG in well fed state or oxidize for energy during starvation. Lipoprotein lipase (LPL) is associated with the plasma membranes of the adipocytes. TAG synthesis begins with the uptake of fatty acids for storage by LPL. The expression of lipoprotein lipase is suspected to decrease in diabetes, thus increasing the levels of plasma lipoproteins that is regulated by one of the major regulatory enzyme ACC-1. Adipose TAG releases more than 50% of fatty acids that are re-esterified by glycerokinase to glycerol-3-phospate. This enzyme is absent in white adipose tissue and the phosphorylated glycerol is produced by incomplete version of gluconeogenesis known as glycerogenesis (Raz et al. 2005). Remnant particles cholesterol rich chylomicrons are cleared by the liver by binding to LDL on hepatocytes. VLDLs are predominantly composed of endogenous triacylglycerol (60%) and are produced in the liver, whose function is to carry lipid from the liver to the peripheral tissues (Figure 1.5). Imbalance between hepatic TAG synthesis and VLDL secretion leads to "Fatty Liver" (hepatic steatosis). This ailment is found in obesity, uncontrolled diabetes mellitus and chronic ethanol ingestion. VLDL is converted to LDL via intermediate-density lipoproteins (IDL). The produced LDL provides cholesterol to the peripheral tissues or refluxes back to the liver by binding to their cell surface membrane receptors those specifically recognized apo B-100 only. In diabetes, lower degradation of lipoprotein by LPL in adipose tissue increases the plasma chylomicron and VLDL levels that

ultimately results into hypertriacylglycerolemia (Yu and Ginsberg 2005). Hyperinsulinemia is known to enhance hepatic very-low density lipoprotein (VLDL) synthesis and thus leads to the increased plasma triglyceride and LDL cholesterol levels (Stalder et al. 1981; Sadur et al. 1984). Reduced level of HDL cholesterol in insulin resistance despite of its enhanced synthesis leads to decrease in plasma HDL cholesterol that is accounted entirely by an increase in the rate of apolipoprotein A1 (Golay et al. 1987).

Lipolysis of triglycerides release free fatty acids. The hydrolytic activity on TAG is initiated by phosphorylated hormone-sensitive lipase (HSL) which is responsible for mobilization of stored fat. HSL gets inactivated by its dephosphorylation in presence of high plasma insulin and glucose levels. When capacity of fat storage of white adipose tissue is reduced, it mainly pours off free fatty acids (FFA) to other peripheral organs like skeletal muscle, heart, kidney, pancreas and liver and leads to ectopic fat deposition (Randle et al. 1988) These depot are major reason for central obesity which increases gluconeogenesis, triglyceride synthesis, insulin resistance and β-cell dysfunction, further increasing the pathogenesis of TIIDM. (Shulman 2000; Arner 2001).

Adipose tissue secrete adipokines like adiponectin and leptin exclusively, wherein the former enhances while the later reduces insulin sensitivity and is involved in appetite regulation and energy homeostasis. Adiponectin plays a major role in insulin signaling that is regulated by glucose uptake in the skeletal muscle by activating AMPK signaling (Tomas et al. 2002). It increases muscle fatty acid oxidation, thus reducing the intracellular ratio of [fatty acids]/[fatty acyl-CoA] and their adverse effects on insulin-stimulated glucose utilization. Adiponectin expression is decreased in situations concomitant with insulin resistance (obesity, type II diabetes) in both animal models and humans (Ferre 2004). Proinflammatory cytokines like TNF-α, IL-6, resistin, adiponectin, etc. are the primary factors playing role in insulin resistance (Lyon et al. 2003).

Major metabolic pathways in adipose tissues are governed by important transcriptional factors like PPAR-γ, SREBP-1c, FoxO etc. PPAR-γ is a nuclear receptor that increases fat storage capacity of adipocyte with decreasing the chance of occurrence of obesity and hence induces insulin-dependent Glut4 translocation in adipocytes (Ferre 2004). Overall PPAR-γ helps in preventing insulin resistance arising due to high plasma free fatty acid levels in the skeletal muscles. In diabetics and obese people, its expression may decrease, thus leading to

insulin resistance (Savage 2005). In adipocytes FoxO improves insulin sensitivity by regulating PPARs (Nakae et al. 2008).

The other transcription factor Sterol Regulatory Element Binding Proteins (SREBP)-1c has a major role in the regulation of genes involved in carbohydrate and lipid metabolism in the liver, adipose tissue, muscles and pancreatic β-cells. SREBP isoforms, SREBP-1a and 2, are involved in the regulation of genes in the pathway of cholesterol metabolism. Overexpression of this factor in adipocyte cell lines stimulates the expression of FAS and ACC (Kim et al. 1998a; Kim et al. 1998b) and known to be associated with number of pathological conditions such as hypertriglyceridemia, insulin resistance, and TIIDM. Due to its anabolic effect on genes involved in glucose and lipid storage, SREBP-1c can be considered as a thrifty gene (Nakae et al. 2008).

1.8.3 Adipogenesis:

Adipose tissue, the storehouse of body fat, plays a key role in controlling glucose and fat homeostasis in the entire body. Hence being obese with an abnormal accumulation of fat in adipose tissue disturbs their normal functions, which leads to development of type II diabetes. High-fat-diet induced adipocyte hypertrophy (large adipocyte) causes decrease in expression and secretion of insulin sensitizing hormone and increase in insulin-resistant hormone, leading to insulin resistance in obesity which is termed as lipoatrophic diabetes (Figure 1.6) (Kadowaki et al. 2003).

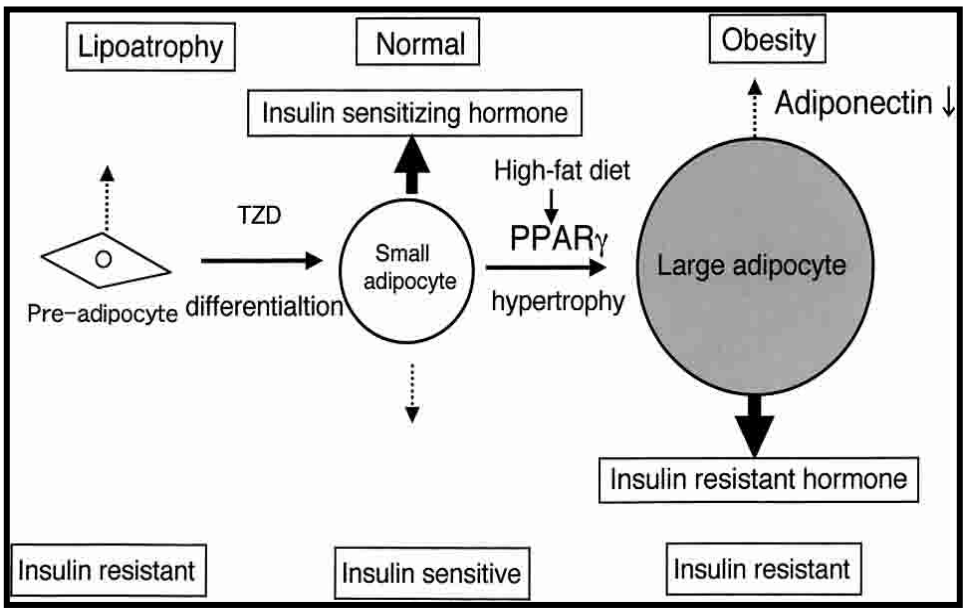


Figure 1.6: Remodeling in adipose tissue in different physiological conditions

White adipose tissue (WAT) mainly governs storage of energy and its mobilization. The production of adipokines by WAT also helps to regulate food intake, energy substrate metabolism, and metabolic rate, which are one of the three important aspects of energy balance (Ahima and Lazar 2008). Brown adipose tissue (BAT), in contrast, stores fewer lipids and contains a high density of mitochondria. The primary functions of brown adipocytes are basal and adaptive thermogenesis as well as energy expenditure. Adipocyte development *in vitro* is characterized by a multistep process from multipotent mesenchymal stem cells (MSCs) which are progressively determined, then committed to the adipocyte lineage, and ultimately differentiate into mature adipocytes (Otto and Lane 2005). Undifferentiated MSCs have the capacity to develop into several mesodermal cell types: osteoblasts, chondrocytes, myoblasts, and adipocytes (Young et al. 1995).

The 3T3-L1 cell line serves as one of the best-characterized and reliable in vitro model for

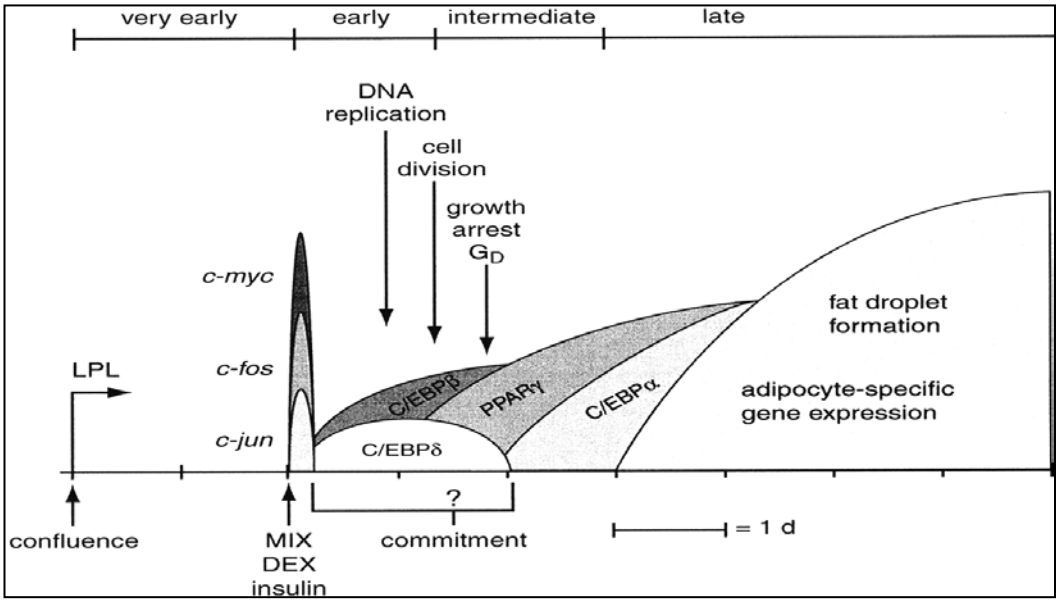


Figure 1.7: Stages of 3T3-L1 pre-adipocyte differentiation (Ntambi and Young-Cheul 2000).

studying the process of differentiation of pre-adipocytes into adipocytes. In culture, differentiated 3T3-L1, pre-adipocytes have an inherent ability to accumulate intracellular fat. Accumulation of intracellular fat droplets and the resultant changes in ultrastructural characteristics are similar to mammalian adipocyte hypertrophy (Green 1974; Novikoff 1980). A defined adipogenic cocktail can help differentiate confluent 3T3-L1, pre-adipocytes. Maximal differentiation occurs on treatment with a combination of insulin (a glucocorticoid that can elevate intracellular cAMP levels) and fetal bovine serum (Student 1980). Insulin acts through the insulin-like growth factor 1 (IGF-1) receptor and hence, IGF-

1 can be substituted for insulin in the adipogenic cocktail (Smith 1988). Dexamethasone (DEX), a synthetic glucocorticoid agonist, is traditionally used to stimulate the glucocorticoid receptor pathway whereas, methylisobutylxanthine (IBMX), a cAMP-phosphodiesterase inhibitor, is traditionally used to stimulate the cAMP-dependent protein kinase pathway.

The differentiation of preadipocytes to adipocytes is arbitrarily divisible into four steps. First, the preadipocytes withdraw itself from the cell cycle and, down regulate genes responsible for the "preadipocyte phenotype". The second step is called the "mitotic clonal expansion", where cells undergo at least one round of DNA replication and cell division. By 2^{nd} day of differentiation, the cells complete the post confluent mitosis and enter into an unusual growth arrest called G_D stage of cell cycle (Scott 1982). The growth arrest is required for subsequent differentiation. The mitosis is believed necessary, to unwind DNA, allowing access for transcription factors to the regulatory response elements present in genes involved in the modulation of mature adipocyte phenotype (Cornelius 1994). Next, 48 hours after the initiation of differentiation, the cells start to acquire the "early adipocyte phenotype", which represents the third step. C/EBP-β and -δ are able to associate with DNA in chromatin context within the first few hours after the induction of differentiation (Salma et al. 2004). C/EBP-β seems to mark a subset of early transcription factor hotspots before the initiation of differentiation and chromatin remodeling of these hotspots is required before the binding of other transcription factors (Siersbaek and Mandrup 2011). After the growth arrest, cells are committed to become adipocytes and start expressing late markers of differentiation by 3^{rd} day. Fourth, in the "differentiated adipocytes", genes already expressed at low levels in the early adipocyte phenotype, are now at their maximal expression levels, genes involved in energy storage and fat metabolism such as PPAR-γ are found to be expressed maximally (Ntambi and Young-Cheul 2000). Remodeling of the PPAR-γ2 promoter takes place before transcriptional activation of this gene (Xiao et al. 2011). C/EBP-β remodels the chromatin structure at putative enhancers to assist subsequent binding of PPAR-γ in late adipogenesis (Siersbaek et al. 2012). These late markers consist of lipogenic and lipolytic enzymes, as well as other modulatory proteins. The cells then round up accumulate fat droplets and become terminally differentiated adipocytes by 5^{th} to 7^{th} day (Figure 1.7).

In vivo monitoring of progression of preadipocyte differentiation in an experimental model is cumbersome. (Holm 2003). Hence, 3T3-L1 is most commonly used cell line for assessing *in vitro* anti-obesity potential of any therapeutic agent. Anti-obesity potential of herbal extracts

is also assessed *in vitro* using 3T3-L1 pre-adipocytes as these cells accumulate TG and release Leptin when they differentiate into adipocytes (Rayalam et al. 2009).

1.9 Poly (ADP-ribose) Polymerase

Poly(ADP-ribose)polymerase-1(PARP-1), is an abundant and ubiquitous chromatin associated nuclear protein amongst 17 PARP family members.

1.9.1 PARP Structure

PARP-1 is a 116-kDa protein comprising an N-terminal DNA-binding domain, which contains three zinc-binding domains (Zn1, Zn2, and Zn3) and a nuclear localization sequence (NLS); central automodification domain (AMD), which contains several glutamate, aspartate, and lysine residues as putative acceptors for auto(ADP-ribosyl)ation, a leucine zipper motif that mediates homodimerization or heterodimerization, and a BRCA1 C-terminal (BRCT) phosphopeptide-binding motif; a C-terminal catalytic domain, which contains a tryptophan, glycine, and arginine-rich (WGR) domain and the ‘‘PARP signature’’ sequence required for the catalysis of PAR synthesis (as shown in Figure 1.8) (D'Amours et al. 1999; Schreiber et al. 2006; Krishnakumar and Kraus 2010). The DNA binding domain (DBD) contains two Cys-Cys-His-Cys zinc fingers (FI/Zn1 and FII/Zn2) that mediate binding to DNA, a newly discovered third zinc binding domain (FIII/Zn3) that mediates inter-domain contacts important for DNA-dependent enzyme activation and a caspase-3 cleavage site (Schreiber et al. 2006). The AMD contains a BRCT (BRCA1 C-terminus) fold, which mediates protein-protein interactions (e.g., with DNA repair enzymes). The CD, which is the most conserved domain across the PARP family, contains a PARP signature motif, which binds NAD^+, as well as a "WGR" motif, which is named after the most conserved amino acid sequence in the motif (Trp, Gly, Arg) and which has an unknown function. Together, the structural and functional domains of PARP-1 confers the activities required for the broad range of functions of PARP-1 in the nucleus.(Krishnakumar and Kraus 2010)

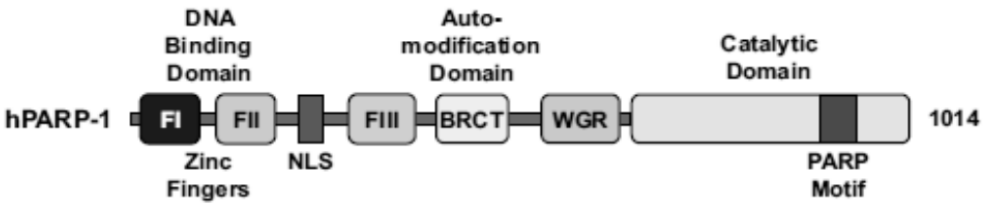


Figure 1.8: Structure of human PARP-1 protein.

1.9.2. Poly (ADP-ribose) Polymerase activity

PARP-1 catalyzes the nicotinamide adenine dinucleotide(NAD^+)-dependent addition of polymer of ADP-ribose(PAR) onto a variety of target protein that functions as posttranslational protein modification. Most PAR in the cell are produced by catalytic activity of PARP-1 which is the major ADP-ribosyltransferase in the nucleus.

1.9.3. Role of PARP in Physiological Functions and Adipogenesis

PARPs regulates many physiological functions like DNA repair and maintenance of genomic integrity, chromatin structure, DNA methylation, cellular signaling, inflammatory responses, energy homeostasis, metabolism, stem cell maintainance and cellular differentiation (Hakme et al. 2008). These multiple function of PARP-1 can be achieved by regulating chromatin structure and composition functioning as a classical coregulator with a wide variety of signal-regulated, sequence specific DNA binding transcriptional activators; functioning as a direct enhancer-binding factor; and regulating the actions of insulators and insulator-binding factors (Krishnakumar and Kraus 2010).

For differentiation to occur during developmental process, transcriptional machinery should be accessible. To break through the chromatin barrier, eukaryotic organism has evolved the strategy of using poly (ADP-ribose) polymerase 1 (PARP-1) to modulate chromatin structure and initiate key step triggering the gene expression (Rajamani Saranya et al. 2012). PPARγ is poly(ADP-ribosyl)ated by PARP-1 that prevents the transactivation of PPARγ and inhibits adiponectin and AdipoR1 expression in cardiac fibroblasts (Huang et al. 2009).

PARP antisense RNA showed no increase in ADP-ribosylation and is unable to complete the round of DNA replication required for differentiation into adipocyte. PARP may play a regulatory role within the replicative apparatus as a molecular nick sensor controlling the progression of the replication fork by modulating replicative enzymes and factors in the complex, by directly associating with them or by catalyzing their poly (ADP-ribosyl)ation (Simbulan-Rosenthal et al. 1996). PAR formation by PARP-1 could affect adipogenesis in multiple ways:

1) By excluding or retaining transcription factors from a special chromatin site (Rouleau et al. 2010);

2) By dissociating corepressors that occupy PPAR-γ2 and its target promoters during adipocyte differentiation, allowing the recruitment of transcription coactivators;

3) Regulate histone modifying enzymes by subsequently altering histone modifications (Krishnakumar and Kraus 2010).

PARP-1 activity is necessary for adipocyte differentiation, and increased PARylation can be observed in differentiating 3T3-L1 adipocytes (Erener et al. 2012). PARP-1 is recruited to PPARγ target genes in a PAR-dependent manner, allowing sustained expression of PPARγ and its target genes (Erener et al. 2012). Subsequently, PARP-$1^{-/-}$ mice display reduced fat mass deposition (Erener et al. 2012; Li et al. 2012). In addition, adipocyte derived stem cells from PARP-$1^{-/-}$ mice displayed decrease in adipocyte size, lower expression of PPARγ target genes upon differentiation, as well as a reduced ability to accumulate triglycerides (Erener et al. 2012b). Therefore, PARP-1 acts as a positive regulator of adipogenesis and adipocyte function. Transgenic mice harboring an ectopic integration of human PARP-1 (hPARP-1 mice) display enhanced adiposity (Mangerich et al. 2010; Li et al. 2012).

PARP-$2^{-/-}$ mice also showed a defect in adipose tissue function and a decrease of adipocyte differentiation. *In vivo*, the adipose tissue depots had smaller weight while histological analysis show reduction in adiopogenic phenotype. DNA-dependent interaction of PARP-2 and PPARγ/RXR heterodimer has been demonstrated by chromatin immunoprecipitation. PARP-2 modulates the activity of PPARγ/RXR nuclear receptor complex, a key transcription factor involved in the pathogenesis of several important diseases such as obesity, insulin resistance, type II diabetes, atherosclerosis, and lipodystrophy (Li et al. 2012).

1.10 TGF-β Super family and Adipogenesis

The search for biological processes that affect adiposity has revealed contributions from the neurohormonal axis, ligand-receptor signaling pathways, and regulators of energy homeostasis, cell differentiation, and classical metabolic pathways (Bluher et al. 2002), yet almost all current anti-obesity drugs function in the brain as appetite suppressants. Nevertheless, the efficacy of these drugs is limited, and they can cause a kind of undesirable side effects (Snow et al. 2005). A recent breakthrough in the TGF-β field that pertains to the control of adipocyte differentiation reduced adipose tissue mass in animal models, also our awareness of TGF-β superfamily signaling serves as an important contributor to the regulation of fat mass highlights its clinical relevance for the treatment of human obesity.

TGF-β Receptors are serine/threonine kinase transmembrane glycoproteins that are divided into two groups: type I receptors that contain a unique GS domain preceding the kinase

domain and type II receptors (Wrana et al. 1994), (Chang et al. 2002). TGF-β super family ligands include activins, inhibins, bone morphogenetic proteins (BMPs), Nodal and growth differentiation factors (GDFs) (Kingsley 1994). In general, the TGF-β/activin/Nodal branch initiates transcription by activation of Smads 2/3, whereas the BMP branch activates Smads 1/5/8 (Feng and Derynck 2005). Once a single R-Smad is phosphorylated by an activated type I receptor, it associates with the common Smad, Smad4 (Co-Smad) and the complex shifts its equilibrium from the cytoplasm to the nucleus to drive the transcription of downstream target genes (Lin et al. 2006; Hill 2009) (Kato et al. 1996). TGF-β superfamily ligands can also bind to various types of receptor on permutation and combinational basis; which gives its flexibility to carry out much larger physiological effects.

1.10.1 Positive and negative modulators of Adipogenesis

BMP (BMP2, -4, -6, -7, and -9) members shows dual roles in regulating adipogenesis and osteogenesis (Ahrens et al. 1993). Among all BMPs, BMP4 is mostly recognized for its proadipogenic influence. In mouse embryonic stem cells, BMP4 can induce adipocyte formation in a dose dependent manner in the absence of standard adipogenic "cocktails" (Taha et al. 2006). Its role in directing commitment to the adipocyte lineage was also demonstrated by treating multipotent *C3H10T1/2* cells with exogenous BMP4 (Tang et al. 2004). (Bowers et al. 2006). During proliferation, C3H10T1/2 cells normally have very low Bmp4 mRNA levels, which subsequently increase as the cells approach confluence. In contrast, in A33 cells committed preadipocyte, Bmp4 mRNA and protein levels, the phosphorylation of downstream Smads 1/5/8 peak during early proliferation and markedly decrease as the cells reach confluence and growth arrest (Bowers et al. 2006). Moreover, treatment of A33 cells with noggin, (Piccolo et al. 1996), decreases cytoplasmic triglyceride accumulation and *Pparg* expression, indicating that the timing of BMP4 expression is important for commitment to the adipocyte lineage in *C3H10T1/2* cells (Bowers et al. 2006) as shown in Figure 1.9.

Within the context of adipogenesis, TGF-β inhibits the early stages of 3T3-L1 differentiation into mature adipocytes, a property that is restricted to the first 35–40 h after onset of differentiation. TGF-β increases proliferation of *3T3-F442A* preadipocytes (Choy et al. 2000), promoting an increase in the progenitor population while simultaneously inhibiting their differentiation. TGF-β inhibits lipid accumulation (Richardson et al. 1989).

For the development of terminally differentiated adipocytes, the MSCs must first progress to the committed preadipocyte stage, a process that can be strongly influenced by BMP

signaling (Bowers et al. 2006). BMP (2/3/4/7) induces adipogenesis by increasing PPAR-γ expression (Hata et al. 2003). GDF38/myostatin promotes adipogenesis and inhibits

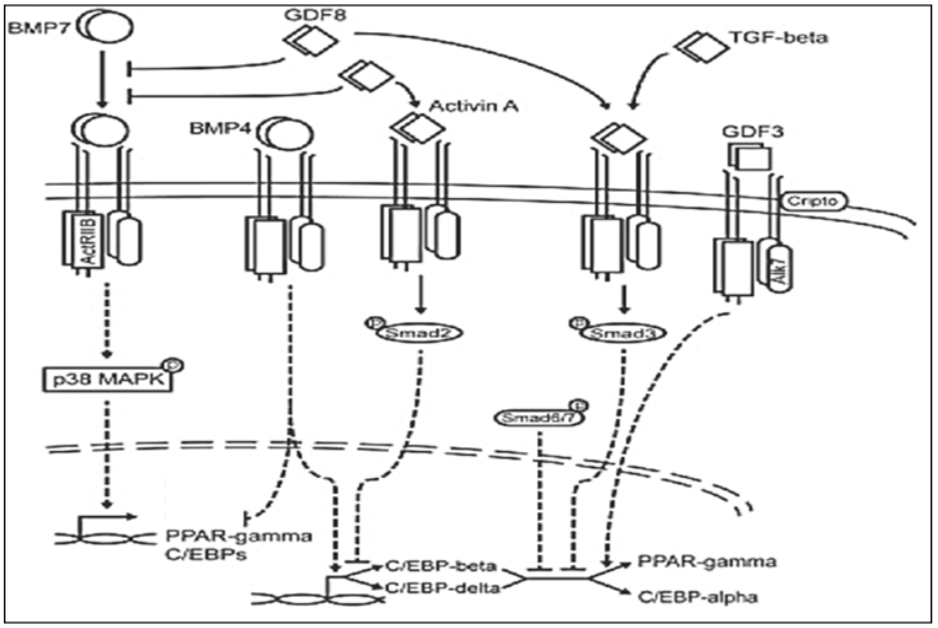


Figure 1. 9: TGF-β signaling which is enhanced by BMPs and antagonized by activin A and TGF-β ligands. Modified Figure : (Zamani and Brown 2010).

myogenesis in *C3H10T1/2* cells (Artaza et al. 2005). Similar to TGF-β, role of activin A in adipogenesis is mostly inhibitory, with positive effect on the proliferation of human adipocyte precursors, but negative effect on their subsequent differentiation (Hirai et al. 2005; Zaragosi et al. 2010). Activin A promotes 3T3-L1 cell proliferation, inhibits adipogenesis whereas, Activin B inhibits lipolysis and both of them inhibit smad2 mediated differentiation (Hirai et al. 2005; Zaragosi et al. 2010). In contrast to TGF-β, however, the inhibition of differentiation by activin A is mediated by Smad2 and C/EBP-β, rather than Smad3 (Itman et al. 2009). BMP3 increases proliferation, but neither the commitment nor the differentiation of MSCs or 3T3-L1 preadipocytes (Stewart et al. 2010). The adipocyte proliferation is mainly regulated by overexpression of Smad 3/6/7. Smad3 overexpression is mainly responsible for enhanced proliferation and inhibit differentiation without Smad6/7 (Choy et al. 2000). The mechanism of Smad3 in inhibition of adipogenesis was further elucidated by decreased expression of *Pparg* and *Cebpa* by functionally repressing their upstream factors, *Cebpb* and *Cebpd* (Choy and Derynck 2003). Smads 3/4 physically interact with the two upstream proteins and disrupt their function, thereby preventing the subsequent transcription of downstream targets essential for adipogenesis. The mechanism of inhibition

is thought to include effects on the nuclear localization of C/EBP-β, with consequential reduction in transcription of *Cebpa* (Satyavati GV 1989). In human MSCs, Smad3-mediated inhibition by TGF-β also involves the up-regulation of genes from the Wnt signaling pathway (Ross et al. 2000). Thus, several processes working together appear to mediate TGF-β's inhibitory role in adipogenesis.

1.11 Rodent models for TIIDM and insulin resistance.

Most of the studies pertaining to expression levels are carried out using mice/ rat models. Various NIDDM/TIIDM rat models are available.

1.11.1 Obese and non obese Type II diabetic models (Srinivasan and Ramarao 2007):

Obese diabetic models	Non obese diabetic model
ob/ob mouse	Cohen rat
db/db mouse	GK rat
KK mouse	Akita mutant mice
KK/Ay NZO mouse	Torri rat Non obese C57BL/6
TSOD mouse	ALS/Lt Rat
M16 mouse	
NONcNZO10 mouse	

1.11.2 Genetic rat models :

i. Zucker fatty rats (ZFR) have a mutation in the gene coding the leptin receptor (*fa/fa*) that results in obesity and hypertension with associated renal and cardiovascular disease.

ii. Goto Kakizaki rats (GK) is a nonobese, diabetic strain that presents primary β-cell defects and peripheral insulin resistance in both males and females.

iii. Zucker diabetic fatty (ZDF) rat also carries the same mutation and, additionally, a mutation that results in spontaneous hyperglycemia.

1.11.3. Transgenic/knock-out diabetic models:

HIP rat coexpress hIAPP (human islet amyloid polypeptide) with insulin and develop hyperglycemia and extrahepatic insulin resistance between 5 and 10 months.These animals also showed hepatic insulin resistance and impaired fasting glucose.

Insulin Receptor Substrate Knockout Mice Models with specific knockout in Irs-1, 2, 3, 4 alone and in combination has been generated and Irs 1 and 4 knockout mice demonstrated growth retardation and insulin resistance. Global insulin receptor knockout mice model had increased glucose as well as insulin levels (Joshi et al. 1996). Mice models were studied with specific knockout of insulin receptor and Glut4 were studied in different tissues (Bruning JC 1998).

1.11.4 Diet induced diabetic models:

i. Sand rat (*Psammomys obesus)* is a gerbilid rodent found in North Africa and the Middle East and is used as a model of nutrionally induced diabetes.
ii. Fructose/sucrose induced rats showed hyperglycemia and insulin resistance in various rats' strains like Wistar, Charles foster and SD rats.
iii. C5BL/6 mice showed insulin resistance after feeding high fat diet for more than 6 weeks. This model is authentic for showing systemic metabolic dysregulation.

1.11.5 In-utero metabolically programmed/neonatal diet induced models:

Permanent disturbances in metabolism and physiology in human adult life, such as obesity, TIIDM, and cardiovascular disease, may be "metabolically programmed" by malnutrition during critical stages of intrauterine development. Several methods have been developed for modeling this in rats. These include nutritional modulation in utero by reduced maternal dietary protein, ligation of the uterine artery and in the neonate by dietary protein restriction or by feeding a high-carbohydrate milk diet.

1.11.6 Surgically induced diabetic models:

i. VMH (ventromedial hypothalamus) lesioned diet induced obese diabetic rat has been developed and is characterized by marked obesity, hyperinsulinemia, hypertriglyceridaemia, insulin resistance, impaired glucose tolerance, moderate to severe fasting hyperglycemia and defective regulation of insulin secretory response despite extremely high insulin secretory capacity.
ii. Partial pancreatectomized diabetic rat: Partial pancreatectomy in animals performed as 70 or 90 per cent (usually 90%) dissection of pancreas has been reported in various animal species mostly in dogs, pigs, rabbit and also rats. It does not cause severe form of diabetes and is characterized by moderate hyperglycemia with neither reduction in body weight nor reduction in plasma insulin levels.

1.11.7 Chemically induced diabetic models:

i. Streptozotocin (STZ) induced neonatal diabetic rats (n0-rats): *Pascoe and Storlien (1990)* developed a model of mild TIIDM with slight hyperglycemia (8.6 mM) by dosing 2-day-old neonatal rats with 45 mg/kg STZ followed by feeding with a high-fat diet for 1 week when the animals had reached 8 weeks of age (Pascoe and Storlien 1990).

ii. Glucocorticoid induced insulin resistance: Dexamethasone is known to induce insulin resistance along with hyperglycemia through PI(3)Kinase and Glut4 translocation mechanism (Davis et al. 2010).

iii. Streptozotocin (STZ) + Niacinamide (Balasubramanyam et al.) induced adult diabetic rats: Niacinamide is added as it offers protection against STZ inhibiting β-cell death. It acts as a free radical scavenger at high doses, a potent inhibitor of PARP and protects against depletion of intracellular NAD (Masiello et al. 1998; Joslin's 2005). NA-STZ animal model showed reduced insulin content (40-60%) in pancrease and related metabolic alterations .This is non obese experimental NIDDM which mimics later stage of TIIDM.

1.12 Current treatment of TIIDM:

1.12.1 Life style change: Dietary treatment and exercise:

Diet is the corner stone for the treatment of TIIDM. Simple initial advice for calorie restriction and avoidance of sweet foods and drinks along with regular physical exercise can lead to symptomatic improvement and a fall in blood glucose levels before any reduction in body weight is detectable. A rate of weight loss of about 5 kg/week is seen.

1.12.2 Oral Hypoglycemic and hypolipidemic drugs:

Oral hypoglycaemic agents are also useful in the treatment of TIIDM. They include sulphonylureas, biguanides, alpha glucosidase inhibitors, meglitinide analogues, and thiazolidenediones. The main objective of these drugs is to correct the underlying metabolic disorder, such as insulin resistance and inadequate insulin secretion. They should be prescribed in combination with an appropriate diet and lifestyle changes.

i. Sulfonylureas:

Sulfonylureas are structurally related to sulfonamides that were discovered accidently in 1942, when it was noted that some sulfonamides caused hypoglycemia in experimental

animals. Sulfonylureas act by stimulating pancreatic β-cell insulin secretion in a glucose-independent manner.

Examples: The first generation of sulfonylureas includes Tulbutamide, Acetohexamide, Tolazamide, and Chlorpropamide. A second generation of sulfonylureas has emerged which includes Glibenclamide (Glyburide in USA), Glipizide, and Glimepiride.

ii. Meglitinides analogs:

Meglitinides may offer an alternative oral hypoglycemic agent of similar potency to metformin, and may be indicated where side effects of metformin are intolerable or where metformin is contraindicated. However, there is no evidence available to indicate what effect meglitinides will have on important long-term outcomes, particularly mortality.

Examples: Repaglinide, Nateglinide

iii. Alpha- Glucosidase inhibitor:

Alpha- Glucosidase inhibitors, mitigate postprandial glucose levels by partially blocking intestinal carbohydrate absorption. Alpha-Glucosidase inhibitors are used to establish greater glycemic control over hyperglycemia in diabetes mellitus Type II, particularly with regard to postprandial hyperglycemia. They may be used as monotherapy in conjunction with an appropriate diabetic diet and exercise, or they may be used in conjunction with other anti-diabetic drugs (Soe et al. 2011).

1.12.3 Insulin sensitizers

i. Biguanides:

The popular drug metformin belongs to this class of oral hypoglycemic drugs. The other examples include Phenformin and buformin; however both of these have been removed from usage in many countries due to its adverse effects.

Metformin: Mechanism of Action:

Antihyperglycaemic therapy is often initiated with Metformin, which acts on hepatic glucose production, hyperinsulinaemia tends to decline, improvement in lipids as a result of liver insulin sensitization, reduced plasma levels of the procoagulant factor plasminogen activator inhibitor-1 (PAI- 1), as well as improvement in vascular reactivity, endothelial function and microvascular function.

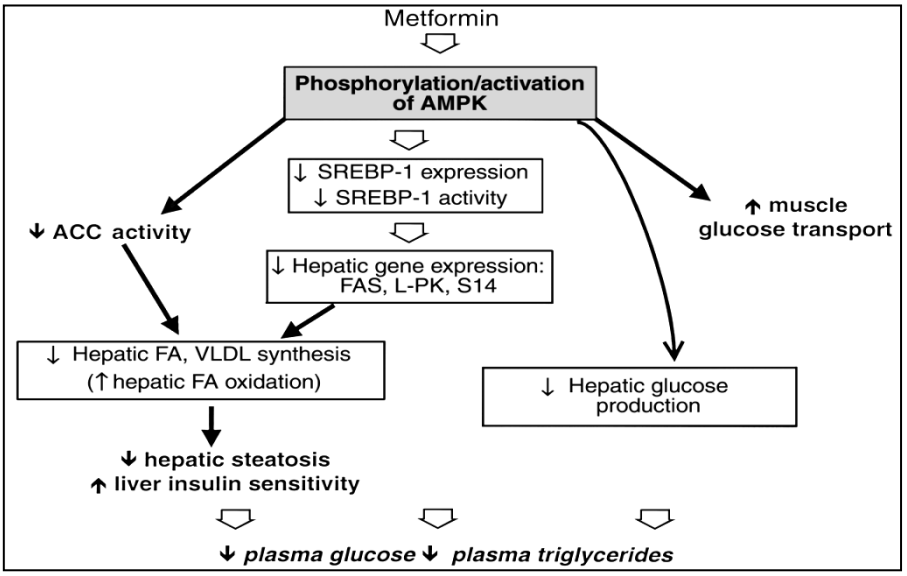


Figure 1.10: Model for the mechanism by which metformin mediates effects on lipid and glucose metabolism. FA, fatty acid.

Metformin has no significant effect in the secretion of glucagon, cortisol, growth hormone or somatostatin. It has been shown to increase peripheral uptake of glucose, and to reduce hepatic glucose output by approximately 20-30% when given orally but not intravenously. Metformin suppress hepatic gluconeogenesis through phosphorylation of CRE binding protein phosphorylation at Ser436 of insulin signaling pathway as critical for its therapeutic effect, and as a potential target for pharmaceutical intervention (He et al. 2009).

ii. Thiazolidinediones (TZDs):

Thiazolidinediones are selective agonists for nuclear peroxisome proliferators activated receptor – Gamma (PPARγ). The PPARγ is the family of transcription factor having subtypes - PPARγ1 and PPARγ2, which are found in key target tissue for insulin action in adipose tissue, skeletal muscle and liver. There is strong evidence to indicate that these receptors may be important regulators of adipocyte differentiation, lipid homeostasis, insulin action and vascular endothelial function. The TZDs bind to PPARγ which, in turn activate insulin responsive genes that regulate carbohydrate and lipid metabolism. They require presence of insulin for their action.

1.12.4 New class of insulin sensitizers: mTOR-modulating insulin sensitizers

Recently two pharmacofore which are close structure to PPAR-γ agonist , MSDC-0160 and MSDC-0602 and its major hydroxymetabolite have much reduced ability to bind to PPARγ (> 250- and > 50-fold respectively), versus rosiglitazone and yet maintains the mTOR-

modulating, insulin-sensitizing pharmacology . In a recently completed Phase IIb clinical trial in patients with Type II diabetes, once daily treatment with the compound produced similar lowering of glucose (fasting plasma glucose and hemoglobin A1c) as did 45 mg pioglitazone, but with approximately half the reduction of hematocrit and half of the increase in adiponectin levels. These data support the hypothesis that the ability to bind to and activate PPAR-γ can be removed from agents without compromising the ability of the compounds to be effective as antidiabetic agents (Colca et al. 2014).

1.12.5 Herbal plant and active principles in treatment of TIIDM:

Many allopathic medicines are available for moderating the conditions of diabetes. Plants used in traditional medicines for treating diabetes represent a valuable alternative for control of the disease. In recent years, there has been renewed interest in the treatment of diabetes using herbal drugs, as World Health Organization (WHO) has recommended evaluation of the effectiveness of plants due to side effects of modern drugs (Baby Josheph 2011). Around 1200 species of plants are known to have anti-diabetic potential; only 50% of which have been experimentally evaluated and very few have been evaluated for mechanism of action. Large numbers of herbal extracts and their active principal compounds have demonstrated hypoglycaemic activity for treating diabetes, thus representing a valuable alternative for control of the disease.

Momordica Charantia, *Trigonella Foenum Graecum* and *Gymnema Sylvestre*, real magic herbs in treatment of Diabetes are most explored for its mechanism of action in India worldwide as a shown in the Table.

Many herbs work at the level of glucose absorption in the intestine and its transport into the peripheral tissues, like liver, skeletal muscles and adipocytes. Many plant extracts like *Momordica charantia, Gymnema sylvestre, Azadirachta indica, Bougainvilea spectabilis, Trigonella + vanadate* have been shown to help, liver and muscle cells by increasing GLUT-4 translocation in the adipocytes and muscles and increasing expression of GLUT-2 in the liver in (Tiwari and Madhusudanarao 2002; Fernandes et al. 2007; Klein et al. 2007) (Tiwari and Madhusudanarao 2002; Siddiqui et al. 2006).

- Various herbs are shown to have insulin like activity. Some herbs like *Canna indica, Artemisia dracunculus, Momordica charantia, Dang Gui Bu Xue Tang* also show insulin sensitizing activity affecting the downstream signaling of insulin, especially the Phosphatidylinositol-3-Kinase and IRS-1 activity leading to reduced insulin resistance in

the peripheral tissues (Purintrapiban et al. 2006; Cao et al. 2007; Govorko et al. 2007; Sridhar et al. 2008; Liu et al. 2009).

- Hypoglycaemic activity of different herbs like *Coccinia indica, M. charantia* and *Mucuna pruriens* in diabetic rats leads to decreased activities of hepatic gluconeogenic enzymes glucose-6-phosphatase and fructose-1, 6-bisphosphatase and increased activity of Glucokinase and phosphofructokinase which are the major regulatory enzymes of glycolysis (Shibib et al. 1993; Rathi et al. 2002).

- Plant extracts like *Tarralin (Artemisia dracunculus), Cafeic acid, isoferulic acid, Trigonella foenum graecum, Syzygium aromaticum (L.)* and green tea decrease phosphoenolpyruvate carboxykinase (PEPCK) mRNA expression in NIDDM rat models. (Koyama et al. 2004; Prasad et al. 2005; Ribnicky et al. 2006; Liu and Cheng 2008)

- Lipid metabolism is another aspect which is altered to a large extent during NIDDM. These changes lead to increase in insulin resistance and hence hyperglycaemia is observed. Lipid metabolism is affected by many factors like PPAR-γ, adiponectin, leptin and lipoprotein lipase along with the enzymes of fat metabolism like fatty acyl CoA oxidase and fatty acid synthase. *Momordica charantia* extract down regulates lipogenic genes in adipocytes in the high-fat diet induced diabetes. *Korean red ginseng, banana leaf* water extract and respective combination effectively reduced blood glucose along with increased expression of liver PPAR-α and PPAR-γ (Chao and Huang 2003; Chan et al. 2005; Park et al. 2005; Fernandes et al. 2007; Luo et al. 2007). NIDDM is associated with increased oxidative stress in the tissues, especially the β-cells. This condition is tackled by many herbal products like Dandelion water extract and fenugreek–Vanadate mixtures which either scavenge ROS or increase the expression and activities of the anti-oxidant enzymes (Cho et al. 2002; Genet et al. 2002a).

Important plants and its active ingredient which are effective in the treatment of diabetes are as follows:

Plant	Active component extracted	Effect on metabolism	References
Artemisia dracunculus	Tarralin ™, 6-demethoxycapillarisin, 2,4-dihydroxy-4-methoxydihydrochalcone	Decreases PEPCK expression, mimics insulin action through PI(3)K and AMPK pathways	*(Ribnicky et al. 2006; Govorko et al. 2007)*

Swertia punicea	Methylswertianin and bellidifolin	Increased expression of key proteins involved in insulin signaling such as IR, IRS, PI(3)K; decreased activity of GK and increased activity of G6Pase	*(Tian et al. 2010)*
Capparis moonii	Gallotannins	Insulin mimetic activity	*(Kanaujia et al. 2010)*
Curcuma longa	curcumin, demethoxycurcumin	Activation of PPAR-γ	*(Kuroda et al. 2010)*
Morus alba	Quercetin-3-(6-malonylglucoside), rutin (quercetin 3-rutinoside), isoquercitrin (quercetin 3-glucoside)	α-glucosidase inhibitory effect	*(Yang et al. 2012)*
Azadirachta indica and Bougainvillea spectabilis	Chloroform extract Aqueous extract	Increase islet regeneration, and insulin release Increase G-6-PDH activity and glycogen content in liver and skeletal muscles.	*Meenakshi Bhat et al., 2009*
Canna indica(L)	Crude extract	Affects glucose transport into muscles by increasing expression of GLUT-1 protien and activation of PI(3)K pathway.	*(Purintrapiban et al. 2006)*
Cinnamon	Crude extract	Affects expression of Insulin receptor, GLUT-4 and tristetraprolin in mouse 3T3-L1 adipocytes.	*(Cao et al. 2007)*
Dang Gui Bu Xue Tang (DBT)	Crude extract	Improves insulin sensitivity through by activation of IRS-1 associated PI(3)K pathway and increased translocation of GLUT-4	*(Liu et al. 2009)*
Gymnema sylvestre	Crude extract	Ameliorates diabetic conditions by increasing β-cell regeneration and function and hence insulin release, improves serum lipid profile	*(Tiwari and Madhusudanarao 2002; Luo et al. 2007)*
Korean red ginseng and Banana leaf	Aqueous extract	Increase expression of liver PPAR-α and PPAR-γ.	*(Park et al. 2005)*
Momordica charantia	Aqueous extract	Enhances insulin release, increases glucose utilization by peripheral tissues, affects downstream insulin signaling, decreases activities of major gluconeogenic enzymes, down-regulates lipogenic enzymes.	*(Rathi et al. 2002; Chan et al. 2005; Fernandes et al. 2007; Huang et al. 2008; Sridhar et al. 2008)*
Syzygium aromaticum (L.) and green tea	Crude extract	Decrease PEPCK and G-6-Pase expression in diabetics	*(Koyama et al. 2004; Prasad et al. 2005)*
Trigonella foenum graecum	Seed powder with Sodium vanadate	Reverses altered gluconeogenic and lipogenic enzyme activity, regulates Na^+/K^+ ATPase activity, lipid peroxidation and GLUT-4 translocation, shows alteration in activities of oxidative enzymes	*(Raju et al. 2001; Genet et al. 2002b; Vats et al. 2004; Siddiqui et al. 2006)*

1.13 Enicostemma littorale

Enicostemma littorale (EL) a perennial herb, belonging to Gentianaceae family, is found widely distributed in diverse geographical locations of Asia, Africa and South America. Morphologically it is 5-30 cm tall, with sessile leaves, glabrous and cylindrical stem along with white and green flowers.

The herb is a famous folklore in Indian subcontinent and is known by various traditional names like "Chota Chirata" in Hindi, "Mamejevo" in Gujarati and "White head" in English. It is used for its anti-diabetic activity and anti-stomachic effect. In India, as an ayurvedic medicine EL is taken with other herbs for treatment of diabetes. This plant has potential nutrient value. The analysed nutrient value of this herb by ICMR is 140Kcal energy, 7gms of protein, 0.7g fat, 26.5 g of carbohydrate, 4.2 g of fibre, 8.4g of minerals and 49.9mg of Fe, 1.641mg of Ca and 81mg of P. The phyto-constituents of this herb are enormous. Studies by Natarajan and Prasad, 1972 reported that there are five alcohols, two sterols and volatile oil along with various mono and tri terpenes. Among the seven flavonoids, Swertisin is the important phyto-compound with various potential biological activities. Catechins, saponins, steroids etc. are also isolated from this plant. Swertiamarin is a bitter secoiridoid glycoside, an important major constituent of this plant.

1.13.1 Hypoglycaemic activity of EL:

First Few reports on hypoglycaemic action of EL were published from our lab. The whole plant of EL aqueous extract administered to alloxan-induced diabetic rats demonstrated hypoglycaemic effect (Vijayvargia et al. 2000)**.** Reports by Maroo et al depicted hypoglycaemic activity by administering EL methanolic extract (2.5 g/kg body weight/day) for 20 days on alloxan induced diabetic rat model by reduction in blood glucose levels from 466.50 to 237.20 mg/dL (Maroo et al. 2003) similarly other group showed significant decrease in various parameters of diabetes like blood glucose, TBARS, hydroperoxides in liver, kidney and pancreas after 45 days of EL extract administration (Prince PSM 2005). Administration of hot and cold aqueous extract of EL for three weeks to STZ induced diabetic rats showed similar hypoglycaemic activity along with decrease in food intake, water intake and rejuvenated the hampered lipid profile (Vishwakarma et al. 2010). EL aqueous extract was also used to treat chronic diabetes by restoring the elevated serum insulin, glucose, cholesterol, triglyceride, creatinine and increased HDL level (Murali et al. 2002).

1.13.2 Islet neogenic property of EL:

Our lab first time reported islet neogenic property of a small molecule SGL-1(Swertsin) derived from EL that very effectively differentiated extra-pancreatic cells like PANC-1 and NIH3T3 into islet like cell clusters (ILCC). Swertisin was proved to be a lucrative islet differentiating agent by transplantation of swertisin induced ILCC into STZ treated Balb/c mice that restored normoglycemia (Sarita Gupta 2010; Nidheesh Dadheech 2013).

1.13.3 Hypolipidemic activity of EL:

EL is proved to possess anti-hypercholesterolaemic activity. The aqueous extract was administered in NIDDM human patients wherein the effects showed significant decrease in fasting & postprandial blood glucose and in glycosylated haemoglobin levels and serum insulin levels. The lipid profile of the patients improved remarkably with decrease in serum total cholesterol and TG levels with concomitant increase in serum HDL cholesterol levels with inproved antioxidant status (Vasu et al. 2003). The author also proved hypolipidemic and antioxidant effect of EL extract for the first time by reducing serum cholesterol, triglyceride, LDL, VLDL, LDL/HDL ratio in cholesterol fed rats (Vasu et al. 2005). Reduction in the activities of erythrocyte catalase, superoxide dismutase and lipid peroxidation were observed along with increase in reduced glutathione levels in cholesterol fed rats. Liver and kidney cholesterol levels were also significantly reduced.

1.13.4 Antioxidant activity of EL:

Potent antioxidant activity was observed for all extracts of *E. littorale* from our lab. The crude extract form the whole dried plant, showed enzymatic and non-enzymatic antioxidant activity after treatment, with decrease in activities of erythrocyte catalase, superoxide dismutase and lipid peroxidation levels and increase in reduced glutathione levels as compared to diabetic and cholesterol fed untreated rats (Maroo et al. 2003; Vasu et al. 2005). EL also has anti-oxidant properties in liver and plasma in pDAB induced hepatocarcinoma in rats (Udayakumar 2008). EL extract reduces the activities of erythrocyte CAT and LPO levels with increased blood GSH level in various rat models (rajamani et al 2012). Also, our lab study showed antioxidant activity of EL extract on gentamycin induced nephrotoxicity (Bhatt NM et al. 2011). The *in vitro* antioxidant activity of aqueous, hydro-alcoholic, methanolic, chloroform and ethyl acetate extract of leaves of this plant has also been evaluated (Sharata L Derore 2008).

1.13.5 Anti-inflammatory and Anti-tumor activity:

The dose dependent study of alcohol extract and its ethyl acetate fraction showed a significant anti-inflammatory activity in carrageen induced rat hind paw edema as well as formalin induced rat hind paw edema chronic model in rats (Arivukkarasu et al. 2009).

The anti-tumor activity of the methanolic extract of EL was observed on peritoneal exudate cells of normal mice. Administration of the EL extract probably enhanced the adaptive immunity of mice and the cytokines and macrophages residing the peritoneal cavity pivotally inhibited tumor cell growth (Kavimani S 2000).

1.13.6 Antiparasitic activity of EL extract:

The herb is reported to have anti-parasitic activities like antimicrobial and antihelminth activities. Activity against *Aspergillus niger and Candida albicans* were observed in chloroform, methanol and ethanol extract of EL. Beneficiary effect of EL methanolic extract on in vitro schizont maturation was reported for the first time by our lab where swertiamarin was found to be potent phytochemical responsible for anti-malarial activity (Soni and Gupta 2009). Death was observed in Indian earthworm Perethema posthuma by administration of five different concentrations of ethanol extract (Mishra S 2011).

1.13.7 EL a master key for the diabetic complications:

Neuro-protective activity: Nociception was reduced in 45 days in diabetic rats when treated with EL extract that significantly increased MDA levels and reduced activity of antioxidant enzymes along with improved Na-K-ATPase activity (Bhatt NM et al. 2011).

Nephro-protective activity: Bhatt et al. proved the nephroprotective effect in diabetic rats upon treatment with EL extract. The nephrotoxicity induced by Gentamicin in form of mitochondrial oxidative stress in kidney was ameliorated by administration of EL extract for 45 days (Bhatt NM et al. 2009) .

Cardio-protective activity: Protective effects of *E.littorale* Blume (EL) extract on hypertension and insulin resistance along with its associated cardiovascular complications in high fructose (HF) fed rats was also observed. The study showed that HF-fed rats treated with EL showed improved insulin resistance, along with reduced hypertriglyceridaemia, hypertension, platelet aggregability, blood coagulation, serum enzymes (CK-MB, SGOT, LDH and SGPT), and vascular reactivity (Bhatt et al. 2012).

1.14 Swertiamarin: An Active lead from EL

1.14.1 Structure and pharmacokinetics:

A bitter glycoside, swertiamarin is a major constitutent of various plants belonging to Gentianaceae family like *Enicostemma allixare, Swertia chirata Clarke, S. japonica, S. angustifolia* and aids therapeutic value to them. Chemically this compound is secoiridoid glycoside in nature. UV, IR, Mass and NMR were used to detect the chemical structure of this compound. Mass spectra depicted the molecular weight of the compound to be 375 and its molecular formula to be $C_{16}H_{22}O_{10.}$ Hydroxyl, carboxyl and double bonds are present in the compound. Currently a rapid and very sensitive UFLC-MS/MS method has been reported for simultaneous detection of swertiamarin and gentiopicroside from rat plasma, with limits of detection 0.5ng/ml and 4ng/ml respectively (Feng et al. 2014). It is hydrophilic in nature.

Bioavailability of swertiamarin with an oral dosage of 20mg/kg in rats showed maximum plasma concentration to be 1920.1ng/ml, the elimination half life was found to be 1.10h and the time required to reach its maximum concentration in plasma was 0.954h (Li HL et al. 2011). After intravenous (4mg/kg) and oral (25mg/kg) administration of this compound into rats, pharmacokinetics showed that it is rapidly absorbed into the circulatory system and reached its peak concentration after 1h of administration. The absolute bioavailability was found to be 10.3%. High accumulation of swertiamarin in liver and kidney indicates that probably it is absorbed through liver and eliminated rapidly through kidney. Due to high hydrophilicity, the concentration was found to be very low in brain (Li HL et al. 2011).

1.14.2 Pharmacological activity:

Plants under *Gentianaceae* are known for their therapeutic values. The major constituent swertiamarin is a known potent compound for complementary and alternative medicine for many of human disease. Evidences suggest mutlifactorial effects of this compound.

1.14.2a Hypoglycaemic and anti-diabetic activity:

Swertiamarin was administered orally to the STZ induced type 1 diabetic rats wherein reduction in serum glucose, urea, creatinine, lipid profile and water intake were observed indicating amelioration of diabetic nephropathy in TIDM. The same was also supported by histological studies of the improvement in the glomerulus of the diabetic rats (Sonawane et al. 2010). Swertiamarin isolated from *E.axillare* was found to be a potent antinociceptive compound, which increased the tail withdrawal reflex (Jaishree et al. 2009). Structural docking of swertiamarin to glycogen phosporylase (GP) may affect its activity, suggesting

GP would be a one of the potent target for Swertiamarin in the treatment of diabetes (Vaidya et al. 2013). The active metabolite of this compound gentianine was found to be responsible for antidiabetic activity. The *in vitro* studies on 3T3-L1 showed marked reduction in the levels of expressions of PPAR-γ, GLUT-4 and adiponectin (Vaidya et al. 2012a).

1.14.2b Hypolipidemic activity:

Swertiamarin was administered (50 mg/kg b.w, i.p.) daily for six weeks to STZ induced diabetic rats. Significant reduction was observed in serum triglycerides, cholesterol and LDL in diabetic rats when compared to the control animals. Serum fasting glucose was reduced and insulin sensitivity index was found to be elevated (Vaidya et al. 2012b) (Udayakumar 2008).

Hyperlipidemia and hypercholesterolemia was induced in rats by intraperitoneal administration of poloxamer 407(400mg/kg). Intraperitoneal administration of swertiamarin reduced the levels of total cholesterol and serum LDL/HDL cholesterol ratio. Thus, the report suggested that swertiamarin is a potent lipid lowering compound (Vaidya et al. 2009a). The author also reported significant inhibition of 3-hydroxy 3-methyl glutaryl CoA (HMG-Co A) reductase activity on treatment of swertiamarin to the cholesterol fed hyperlipidemic rats (Vaidya et al. 2009b). Another recent property of swertiamarin as a potent lipid lowering agent comparable to atorvastatin which may contribute to its cardioprotective and antiatherosclerotic property was reported (Vaidya et al. 2009b).

1.14.2c Anti-inflammatory activity of swertiamarin:

Recent report from Saravasana et al, 2014 depicted dose dependent reduction in proliferation of cells along with concomitant lowered levels of nitric oxide in IL-1b induced fibroblast-like synoviocytes with swertiamarin administration,. The inflammatory responses were reduced by lowered gene and protein expressions of apoptotic markers, osteoclastogenic mediators, various proinflammatory mediators and p38 MAPK and its release. The same group also published that release of NF-kB, p65, p-IkBα, p-JAK2 and p-STAT3 signaling protein in both experimental and LPS induced cells (Saravanan et al. 2014).

1.14.2d Hepato-protective activity:

The hepatoprotective properties were very well proved by swertiamarin isolated from *Enicostemma axillare* administered at 200mg/kg body weight orally for 8 days to the D-GalN induced hepatotoxicity in rats (Vaijanathappa and Badami 2009). Study signified the role of EL ethanolic extract that owed hepatomodulatory activity against stress induced liver injury by CCl_4 in rats through antioxidant activities and reducing fat metabolism (Gupta RS 2007).

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