Chapter 1
Introduction
1.1 Adipose Tissue

Adipose tissue is a specialized connective tissue found in all mammalian species. There are mainly two types of adipose tissue, brown adipose tissue (BAT) and white adipose tissue (WAT). Brown adipose tissue is mainly found in rodents and infants and is involved in thermo homeostasis of the body (Wozniak et al., 2008). Subcutaneous adipose tissue and visceral adipose tissue are the two main white adipose tissues in the body whereas interscapular and mediastinal are the main brown adipose tissues (Fig 1.1). White adipose tissue is the major form of adipose tissue present in adults and is the only tissue in the body that can change its mass even after adult size is reached (Bjørndal et al., 2011). Adipocytes are the major part of adipose tissue, though it also constitutes other non fat cells such as endothelial cells, monocytes, macrophages, fibroblasts, and stem cell precursors including preadipocytes. The fat mass varies in different people, 2% - 3% of body weight for an athlete to 60 % - 70% for an obese person. The normal fat mass for men is 9% - 18 % and for females it ranges from 14% - 28 % (Ahima, 2002; DiGirolamo, 2000). In the early 1950’s adipose tissue was just considered as a metabolically inert fat depot, which provides insulation and mechanical support for the organs. But later adipose tissue was credited with more fundamental functions. It was identified as a major site of energy regulation. Two major functions of adipose tissue are to store surplus energy as triglycerides and to release free fatty acids for energy production whenever required by the body (Etherton, 1982; Lafontan et al., 1985). When the food intake is more and energy expenditure is less, excess energy is stored in the adipose tissue as triglycerides. However, when food intake is less and energy expenditure is high, these stored triglycerides are converted to free fatty acids and glycerol which is transported to muscle and liver for fatty acid oxidation (Sypniewska, 1989).

The discovery of a satiety factor, leptin, in 1994 added a further dimension to the understanding of adipocyte tissue function. Adipocyte tissue secretes this molecule that is capable of regulating food intake and energy expenditure (Zang et al., 1994). Subsequent studies have identified that adipocytes secrete several other molecules which are involved in regulating various physiological functions (Trayhurn et al., 2008). These studies led to the recognition of adipocyte as a bonafide endocrine organ. The non fat cells of adipose tissue are also known to secrete various molecules. These secretory molecules play a crucial role in the control of whole body homeostasis and metabolism (Kershaw and Flier,
Any dysregulation of adipose tissue formation, lipoatrophy or obesity leads to abnormal secretion of these molecules, resulting in various metabolic complications including insulin resistance, type 2 diabetes and cardiovascular diseases (Roshini, 1985; Kahn et al., 2006; Grundy 2004).

1.1.1 Adipocyte Differentiation

Adipocytes are derived from the pluripotent stem cells which have the capacity to differentiate into adipocyte, myoblast, osteoblast and chondrocytes (Taylor and Jones, 1979; Koneiczny, 1984). The differentiation of these stem cells into adipocytes follows two distinct phases. In the first phase, pluripotent stem cell commits to adipocyte lineage and undergoes mitotic clonal expansion (Pairault and Green, 1979). In the second phase, the committed adipocyte precursors known as preadipocytes differentiate into mature adipocytes by acquiring all the necessary machinery for lipid synthesis and transport, insulin sensitivity and secretion of adipose specific proteins (Otto and Lane, 2005). Adipogenesis is accompanied by morphological differences. During differentiation, preadipocytes change their shape from a fibroblast structure to round fat cells with characteristic lipid droplets (Fig 1.2). It has been shown that during adipocyte differentiation, a transcriptional cascade is initiated by various signals emanating from...
both intracellular and extracellular environment culminating in the induction of various target genes (Rosen and MacDougald, 2006). The interplay of these gene products are involved in the formation of fully differentiated and functional adipocytes resulting in lipid accumulation and insulin sensitivity (MacDougald and Mandrup, 2002).

1.1.1.1 Transcriptional Regulation of Adipogenesis

Adipocyte differentiation involves a spatio-temporal regulation of gene expression. A network of transcriptional events regulate the expression of hundreds of genes involved in adipocyte differentiation. Peroxisome proliferator and activated receptor gamma (PPARγ) and CCAAT enhancer-binding proteins (C/EBPα, β and δ) family of transcription factors, play a key role in the regulation of the transcriptional events occurring during adipocyte differentiation (Rosen et al., 2002; Tang et al., 2004).

Fig 1.2 Schematic representation of adipocyte differentiation from its precursor cells.
1.1.1.1. A PPARγ

PPARγ is known as the master regulator of adipogenesis. It belongs to the nuclear-receptor super family of proteins. PPARα, PPARδ and PPARγ are the three isoform of PPARs. Of these isoforms, PPARγ is reported to be the most adipogenic one. PPARδ is ubiquitously expressed and has shown adipogenic effect, but its specific agonist did not promote adipogenesis in 3T3-L1 cells while PPARγ agonist significantly enhanced adipogenesis. This shows that PPARγ plays a major role in adipogenesis with a minor role for PPARδ (Amri et al., 1995; Berger et al., 1999; Brun et al., 1996). PPARγ is very crucial and sufficient for adipocyte differentiation. Ectopic expression of PPARγ in preadipocyte fibroblast cell induced adipocyte differentiation and so far not a single factor is identified that accelerates or induce adipogenesis in the absence of PPARγ (Tontonoz et al., 1994).

All the signaling pathways involved in adipocyte differentiation leads to PPARγ expression or activation. All the known pro-adipogenic factors so far identified at least work in part by activating PPARγ (Mac Dougald et al., 2002; Rosen et al., 2000; Tontonoz et al., 1994). PPARγ is highly expressed in adipocytes and it exists in two different isoforms PPARγ 1 and 2 which are formed by alternative splicing. Both these isoforms are involved in adipogenesis, however PPARγ 1 is also present in various cell type other than adipocytes. Ectopic expression of PPARγ 1 and 2 in PPARγ -/- fibroblast has shown that PPARγ 2 is more efficient than PPARγ 1 in promoting adipocyte differentiation (Chawla et al., 1994; Tontonoz et al., 1994; Zhu et al., 1995; Mueller et al., 2002).

The expression of PPARγ is very low in 3T3-L1 preadipocytes, and it rises dramatically during adipocyte conversion (Tontonoz et al., 1994). Cyclic adenosine monophosphate (cAMP) dependent ligand activity of PPARγ is observed during the initial period of adipogenesis (Tzameli et al., 2004). Upon the activation of PPARγ, it hetrodimerize with retinoid X receptor (RXR) and activate the expression of numerous genes regulating adipogenesis, lipid uptake and lipid metabolism and gradually convert preadipocytes to adipocytes (Tontonoz et al., 1994). Fatty acids and their metabolites are considered to be the endogenous ligands of PPARγ. Sterol response element-binding protein-1c (SREBP-1c) and C/EBPβ have been shown to increase PPARγ ligand
production. There are many synthetic ligands identified for PPARγ including thiazolidinediones (TZDs), a group of drugs which are used as insulin sensitizing agents. PPARγ ligands mediate their effects through binding to ligand binding domain (LBD). The LBD of PPARγ consists of 270 amino acid residues and this region lies in the C-terminus of the receptor. LBD also contain transactivation and dimerization regions. Biochemical and structural studies have elucidated the mechanism of PPARγ activation. During the activation, binding of ligand to LBD undergoes conformational rearrangement of the transcriptional activation function region (AF-2). This leads to the displacement of corepressor proteins and enhances the recruitment of coactivators essential for the transcription of various genes (Berger and Moller, 2002; Nagy and Schwabe, 2004; Li et al., 2003; Wilson, 2000; Nolte, 1998; Uppenberg, 1998).

PPARγ is not only required for the induction of adipogenesis but also necessary to maintain the adipocyte phenotype and function. Over expression of dominant negative PPARγ in adipocytes caused loss of triglyceride accumulation leading to dedifferentiation of adipocytes (Tamori 2002). Knockout of PPARγ in adipocyte resulted in adipocyte death and caused insulin resistance in liver and adipose tissue (Imai 2002). Though PPARγ alone is shown to be sufficient for initiating adipocyte transcriptional cascade and maintainence of adipocytes, a synergetic effect has been shown with C/EBPα. An ectopic expression of either of these has shown to enhance the expression of the other suggesting a mutual interaction for enhancing adipogenesis.

1.1.1.1.B C/EBPs

The members of C/EBP family of transcription factors C/EBPδ, C/EBPβ, C/EBPα, are involved in adipocyte differentiation in a temporal manner (Christy et al., 1991; Cao et al., 1991). Of these different isoforms, C/EBPα is crucial for adipogenesis and is known to enhance the expression of many adipogenic genes including PPARγ. C/EBPδ, C/EBPβ are known to induce the expression of C/EBPα by binding to its promoter in the initial phase of adipocyte differentiation. They are seen in the proliferating preadipocytes but diminish once it reaches confluency (Cao et al., 1991; Lin et al., 1993). In vitro and In vivo studies with double knock out for C/EBPδ and C/EBPβ provided varying results. It was seen that the expression levels of both C/EBPα and PPARγ in the double knock out mice was normal whereas a reduced expression of both C/EBPα and PPARγ was reported.
in cell line devoid of both C/EBPδ and C/EBPβ. These observations indicate that, apart from C/EBPβ and C/EBPδ, there might be some other factors that allow the expression of PPARγ and C/EBPα in vivo (Tanaka, 1997). Other studies have shown that both C/EBPβ and C/EBPα have the capacity to induce PPARγ expression in preadipocytes (Schwarz et al., 1997). C/EBPα has shown an auto activation of its transcription (Christy et al., 1991). Ectopic expression of C/EBPα was able to independently induce adipogenesis without any external adipogenic inducer (Freytag et al., 1994). Knock out of C/EBPα has shown reduced adiposity in mice (Wu et al., 1999). Even though C/EBPα is important for adipogenesis, this transcription factor cannot function independently without PPARγ. Ectopic expression of C/EBPα in PPARγ -/- fibroblast cannot induce adipogenesis, while ectopic expression of PPARγ in C/EBPα -/- cell was able to induce adipocyte differentiation, but insulin sensitivity was less in these cells. These studies indicate that a mutual co-operation between C/EBPα and PPARγ is necessary for adipocyte differentiation and functioning (Rosen, 2002; Wu et al., 1999).

1.1.1.1.C Other Adipogenic Transcription Factors

Apart from the above mentioned transcription factors, there are a number of other transcription factors involved in the complex process of adipogenesis. Another transcription factor that is involved in PPARγ activation and adipocyte differentiation is SREBP-1c. It belongs to basic helix loop helix leucine zipper family of transcription factors. SREBP-1c is abundantly present in adipose tissue and is known for its adipogenic activity (Tontonoz et al., 1993). Overexpression of SREBP-1c in 3T3-L1 cells accelerates adipogenesis by activating PPARγ. Preadipocyte expressing dominant negative SREBP-1c downregulates the adipogenic conversion. However, SREBP-1c alone cannot induce adipogenesis and it is shown that SREBP-1c facilitates adipogenesis by producing endogenous ligands for PPARγ (Kim et al., 1997). Kruppel like factors contributes to the initial phase of adipogenesis by stimulating the expression of C/EBPδ and C/EBPβ (Oishi et al., 2005). Kruppel box 20 (Krox 20) is known to induce adipogenesis by inducing C/EBPβ expression (Chen et al., 2005). Early B-cell factor family of transcription factors are expressed during adipogenesis. Though Liver X receptors are known to be involved in adipocyte differentiation, the exact mechanism has not been delineated so far. The signal transducer and activator of transcription-5a (STAT5a), Cyclic AMP response element-binding protein (CREB), Endothelial PAS
domain-containing protein 1 (EPAS1), Brain and muscle Arnt-like protein-1 (BMAL1) are other transcription factors involved in adipogenesis (Shimba et al., 2004; Nanbu-Wako et al., 2002; Floyd et al., 2003; Zhang et al., 2004; Shimba et al., 2005; Akerblad et al., 2002).

![Fig 1.3 Regulation of adipocyte specific gene expression by the sequential expression and coordinated activation of transcription factors.](image)

### 1.1.1.1.D Anti-adipogenic Transcription Factors

Many transcription factors are shown to be down regulated during adipocyte differentiation. These include members of GATA-binding and forkhead families, Krüppel-like factors, KLF2, KLF7 and C/EBP homologous proteins (CHOP). These factors do not bind directly to the DNA but inhibit mainly through protein-protein interactions. One such example is GATA 2 which binds to C/EBPα and inhibits transactivation of PPARγ by C/EBPα (Tong et al., 2005).

### 1.1.1.1.E Transcriptional Coregulators in Adipogenesis

A number of nuclear cofactors are reported to be involved in adipogenesis. These cofactors are either coactivators or corepressors that are part of transcriptionally active or inactive complexes. The co regulators have the capacity to directly modify the chromatin or recruit the chromatin modifiers. Several histone acetyl tranferases and histone
Deacetylases are involved in adipogenesis by the regulation of transcription. PPARγ interacts with several coregulators and control the expression of numerous adipogenic genes. Steroid receptor coactivator 1 (SRC-1), thyroid hormone receptor-associated proteins (TRAP) are some of the coactivators while, nuclear receptor corepressor (NCoR) and silencing mediator of retinoid and thyroid hormone receptors (SMRT) are corepressors involved in adipocyte differentiation (Spiegelman BM. and Heinrich, R 2005).

1.1.2 Signaling Pathways Involved in Adipogenesis

Adipogenesis is regulated by the activation of transcription factors. Various signaling pathways stimulated by both intra and extracellular signals are known to activate these transcription factors. Some of the key signaling pathways involved in adipocyte differentiation are described below (Fig 1.5).

1.1.2.1 MAPK Signaling Pathway

Mitogen-activated protein kinase (MAPK) family has shown to play a critical role in adipocytes differentiation. A biphasic effect of MAPKs on adipogenesis is reported. Extra cellular signal regulated kinase, ERK, is required for proliferation of preadipocytes and the inhibition of ERK in the early phase is known to inhibit adipocyte differentiation in 3T3-L1 cells. ERK42/44 has shown an increased phosphorylation in the initial hour of adipocyte differentiation upon the addition of adipogenic medium containing insulin, dexamethasone (Dex) and isobutyl methane xanthine (IBMX). This enhanced ERK42/44 phosphorylation is important in activating factors that regulate both C/EBPα and PPARγ expression. ERK 42/44 activation was necessary for CREB dependent expression of C/EBPα and PPARγ expression (Belmonte et al., 2001; Prusty et al., 2002). It is interesting to note here that ERK inhibits adipocytes differentiation in the late differentiation phase. ERK phosphorylates PPARγ and inhibits adipocyte differentiation. This shows that an initial rise in ERK activity is essential for adipogenesis while a reduction in activity is required for further progression of adipogenesis. The MAPK phosphatase regulates this ERK activity by dephosphorylating it. A unique proadipogenic role of p38 MAPK is reported in 3T3-L1 cell models (Aquadi et al. 2006).
1.1.2.2 Insulin Receptor IR/Akt Signaling.

Insulin receptor (IR)/Akt signaling pathway is important in transducing the proadipogenic effects of insulin (Manning and Cantley, 2007; Sakaue et al., 1998). Insulin signals are transmitted to the adipogenic cascade in different ways. IR signaling increases CREB phosphorylation, and enhance the transcription of genes required for adipogenesis (Klemm et al., 2001). The other way is by increasing Akt/PKB phosphorylation. Akt increases PPARγ expression and promotes adipogenesis by two different signaling mechanisms. Akt phosphorylates and inhibits a strong inhibitor of adipogenesis FOXO 1, a member of forkhead box O (FOXO) family of transcription factors (Armoni et al., 2006; Nakae et al., 2003) and activates mammalian target of rapamycin (mTOR) (Wullschleger et al., 2006). Many studies have shown that Akt mediated mTOR activation is essential for proper differentiation of preadipocytes (Bell et al., 2000; Cho et al., 2004; Kim and Chen, 2004).

1.1.2.3 Wnt/β-catenin Signaling

Wnt signaling attenuates adipocyte differentiation by blocking the expression of PPARγ and C/EBPα. A major component of the Wnt signaling pathway is the cytoskeletal associated β-catenin residing in the cytoplasm. The expression of β-catenin is maintained at a normal level through the ubiquitin proteasome degradation. Glycogen synthase kinase 3 beta (GSK3-β) phosphorylates βcatenin and target it for proteosomal degradation. The effector molecules of Wnt inhibit GSK3-β activity resulting in the accumulation of β-catenin in the cytoplasm, followed by its translocation to the nucleus. The nuclear β-catenin now binds to T-cell factor/lymphoid enhancer factor (Tcf/LEF) family of transcription factors and inhibits the transcription of genes involved in adipocyte differentiation. As the cell progress to differentiation, β-catenin is downregulated. This downregulation is seen at the onset of PPARγ expression. PPARγ is shown to promote GSK3-β mediated degradation of β-catenin. Troglitazone, a PPARγ ligand, has shown to downregulate through GSK3-β. This shows that an appropriate maintainance of β-catenin level is required for adipogenesis (Ross et al., 2000; Bennett et al., 2002; Moldes et al., 2003).
1.1.2.4 Adenosine Monophosphate Activating Protein Kinase, AMPK Signaling

AMPK is the sensor of cellular energy and plays a major role in regulating energy balance in response to metabolic requirements. AMPK is a heterotrimeric protein with a α catalytic subunit, a β subunit important for enzyme activity and targeting, and a γ regulatory subunit, that binds the allosteric activator, AMP. Different studies have reported an antiadipogenic effect for AMPK. For example, AMPK knockout mice exhibited a very high rate of adipose tissue and adipocyte hypertrophy compared to wild type mice (Villena et al., 2004). Aminoimidazole carboxamide ribonucleotide (AICAR), an activator of AMPK, inhibits adipogenesis in 3T3-L1 cells by blocking the expression of adipogenic marker genes such as fatty acid synthase and the transcription factors PPARγ and C/EBPα (Habinowski and Witters, 2001; Dagon et al., 2006). AICAR phosphorylates the α-subunit of the eukaryotic initiation factor-2 (eIF2α). This phosphorylation of eIF2α leads to the downregulation of proteins involved in adipogenesis (Yossi et al., 2005). AMPK phosphorylates and inactivates acetyl-CoA carboxylase (ACC), the rate limiting enzyme of fatty acid synthesis (Sim AT and Hardie DG, 1988). Moreover AMPK is known to phosphorylate hormone sensitive lipase and downregulate lipolysis in adipocytes. In general, AMPK is known to downregulate pathways that promote adipogenesis and thus prevent obesity. Therefore, recently, AMPK has been considered as a novel target for treating insulin resistance, obesity and type 2 diabetes (Rojas et al., 2011).

Fig. 1.4 Extracellular signals that directly or indirectly activate C/EBPα or PPARγ to activate the expression of adipocyte specific genes.
1.1.3 Factors Affecting Adipocyte Differentiation

Adipocyte differentiation is modulated by a number of biological factors. Several hormones, growth factors and other molecules are known to play a major role in adipocyte differentiation. Insulin, insulin like growth factor 1 (IGF-I), cAMP, glucocorticoids, and prostaglandins are some of the factors that are known to accelerate adipogenesis while some other growth factors (Transforming growth factor-α, platelet derived growth factor, epidermal growth factor), extracellular matrix protein fibronectin and transmembrane protein, preadipocyte factor-1 (Pref-1) downregulates adipogenesis. IGF-1 induces clonal expansion in 3T3-L1 preadipocytes (Boney et al., 1994). Nuclear hormones like glucocorticoids, retinoic acid and 3, 3′, 5-triiodothyronine are known to enhance adipogenesis by binding to its own intracellular receptors (Gaillard et al., 1991). A synthetic glucocorticoid dexamethasone induces adipogenesis in 3T3-L1 cells and is generally used in the cocktail for induction of adipogenesis (Calvo et al., 1991). Glucocorticoids induce expression of C/EBPδ leading to PPAR-γ expression. They are also known to enhance adipogenesis by increasing intracellular cyclic AMP levels. Arachidonic acid derivatives prostaglandins PGF$_{2α}$, PGE$_2$, PGD$_2$ are known to act as endogenous ligands for PPARγ (Hertz et al., 1996). Elevated cAMP increases adipogenesis via CREB activation (Vassaux et al., 1992). IBMX used in adipogenic cocktail is an inhibitor of phosphodiesterases and it mediates its effect via elevating cAMP levels (Calvo et al., 1991).

1.1.4 Adipocyte as Endocrine Cell

In the past years, several lines of evidence have demonstrated adipose tissue as an endocrine organ. Adipocyte secretes an array of biofactors known as adipokines to the blood stream that are involved in the metabolic regulation and over all physiological activities of the body. The diverse adipokines includes chemokines (Monocyte chemo attractant protein 1 MCP-1), cytokine and cytokine-like proteins (leptin, Tumor necrosis factor alpha TNFα, interleukin 6 IL-6), growth factors (Transforming growth factor beta TGF-β and Vascular endothelial growth factor VEGF), angiotensinogen, complement and complement related proteins (adiponectin) and factors such as resistin and visfatin (Fig 1.5) (Gnacińska et al., 2009). The cytokines are also secreted from other non fat cells in the adipose tissue. Major adipokines implicated in type 2 diabetes and insulin resistance
are briefly described below.

Fig 1.5 Adipokines secreted from adipocytes which are involved in various physiological functions of the body.

1.1.4.1 Leptin

Leptin is a polypeptide hormone secreted from adipocytes which are involved in regulating food intake and energy expenditure. It was the first protein recognised as an adipokine (Zhang et al., 1994). Leptin decreases orexigenic and enhances anorexigenic peptide expression in the hypothalamus resulting in suppression of food intake (Friedmann et al., 1998; Waters et al., 2000). Leptin enhances lipolysis in adipocytes. Leptin administrated rats have shown reduced triglycerides in adipocytes, muscles and liver and increased free fatty acid oxidation (Collins et al., 1996; Minokoshi, 2002). Insulin induces leptin production in adipocytes and leptin in turn by a feedback mechanism inhibits insulin production. Expansion of adipose tissue leads to increased secretion of leptin. It has been shown that long term hyperleptinemia down regulates insulin signaling (Moran and Phillip, 2003; ), on the other hand, some studies have shown that insulin resistance in obesity leads to hyperleptinemia ( Hintz et al., 1993).

1.1.4.2 Adiponectin

Adipocyte complement related protein or adiponectin is secreted from adipocytes and is found abundantly in plasma. Secretion of adiponectin from adipocytes is stimulated
by insulin (Hu et al., 1996; Scherer et al., 1995). Unlike other adipokines, adiponectin level is very low in obese and insulin resistant conditions (Arita et al., 1999). It is shown that adiponectin enhances insulin sensitivity by various modes (Tomita et al., 2001). The administration of adiponectin to obese mice alleviated insulin resistance by lowering triglyceride content in liver, muscle and adipocytes. This was established by increasing the expression of various proteins involved in fatty acid catabolism and energy dissipation. Adiponectin also lowers hepatic gluconeogenesis by down regulating the expression of enzymes involved in the process and also enhances glucose uptake in insulin sensitive cells (Combs et al., 2001). Adiponectin on the other hand lowers insulin resistance by activating insulin signaling molecules such as insulin receptor substrate 1, PKB and AMPK. Adiponectin also inhibits TNFα, secretion from macrophage cells and reduce inflammation (Yamauchi et al., 2001).

1.1.4.3 Resistin

Resistin is a polypeptide hormone identified in 2001. As its name implies resistin resists insulin action. Resistin is mainly expressed in adipocytes, and their level rises during adipogenesis. The synonyms of this protein are ADSF (adipocyte-specific secretory factor) and FIZZ3 (found in inflammatory zone). Elevated levels of resistin are observed in the blood plasma of obese mice (Steppan et al., 2001). Many studies suggested a negative correlation of insulin action and resistin level however, conflicting results are observed. In obese person, the resistin mRNA level in adipose tissue is significantly higher compared to normal weight persons (Patel et al., 2003). Resistin was shown to interfere insulin signaling by attenuating insulin-stimulated glycogen synthesis, phosphorylation of insulin receptor substrates (IRS), Akt/PKB, and by increasing the expression of SOCS-3, a known inhibitor of insulin signaling (Steppan et al., 2005; Niederwanger et al., 2007). However several other studies in human subjects failed to show any impact of resistin on obesity and insulin resistance.

1.1.4.4 TNFα

Tumor necrosis factor α is a transmembrane protein of 26 kDa. An alternative form which is a proteolytic cleavage product of TNFα with a molecular weight of 17 kDa is also known to exist and both these forms mediate biological response and are found in
non fat cells and adipocytes. TNFα expression is elevated in high fat diet induced obesity and is known to play a central role in establishing insulin resistance (Hotamisligil et al., 1993; Fain et al., 2004). TNFα induces insulin resistance mainly by enhancing the level of free fatty acids (Kern et al., 1995) which in turn interferes with the insulin signaling pathway (Kanety et al., 1995). A negative correlation with TNFα expression and adiponectin level is observed in obese mice. The elevated level of TNFα induces insulin resistance in hepatocytes, adipocytes and muscle. TNFα down regulation has shown to enhance insulin sensitivity in obese mice (Uysal et al., 1997).

1.1.4.5 MCP-1

Monocyte chemo attractant protein 1 (MCP-1) is produced in adipocytes and several other non fat cells such as macrophages and endothelial cells. In obese condition, white adipose tissue secretes enormous levels of MCP-1 which leads to infiltration of macrophages to fat depots and subsequent release of TNFα leading to induce insulin resistant state in these tissue (Harman et al., 2007). MCP-1 gene is insulin responsive in nature and it is sensitive to insulin even in insulin resistant state. Therefore in obese conditions and hyperinsulinemic state, the level of MCP-1 is very high (Sartipy et al., 2003). The target specific deletions of MCP-1 in white adipose tissue lead to improved insulin sensitivity and over expression leads to insulin resistant state (Kim et al., 2006).

1.1.4.6 Interleukin 6

Interleukin 6, is considered as a major pro-inflammatory cytokine, produced and secreted by leukocytes, adipocytes, and endothelial cells. The 30 % of IL-6 in circulation comes from adipocytes (Mohammed-Ali et al., 1997). Expansion of adipose tissue leads to secretion of IL-6. Elevated levels of IL-6 positively correlate with obesity and type 2 diabetes (Fernandez-Real et al., 2000; Pradhan et al., 2001). IL-6 down regulates the expression of insulin receptor in peripheral tissues. It inhibits glycogen synthesis and increases lipolysis in liver leading to secretion of free fatty acids to circulation which ultimately contributes to insulin resistance (Senn et al., 2000). In vivo introduction of recombinant IL-6 increased gluconeogenesis resulting in hyperglycemic state followed by hyperinsulinemic state (Stith et al., 1994). IL-6 also reduces adiponectin secretion. Elevated levels of IL-6 can thus leads to the development of T2D.
1.2 Adipose Tissue Dysfunction, Leading to Insulin Resistance and Type 2 Diabetes

The adipose tissue consists of 50% of adipocytes and 50% nonfat cells including fibroblasts, endothelial cells and macrophages (Trayhurn 2007). During increased food intake or positive calorie balance, excess energy is stored in already present adipocytes or form new functional adipocytes from precursor cells known as adipogenesis. An optimal balance of this process is required for normal physiological functions. Any alterations in the maintenance of adipose tissue in the body lead to serious metabolic complications. Too little or too much adipose tissue leads to lipoatrophy or obesity. The consequences of these states are insulin resistance and type 2 diabetes. Insulin resistance is a condition in which the insulin sensitive tissue cannot respond to normal physiological levels of insulin and leads to decreased glucose disposal resulting in hyperglycemic state. β cells fail to supply insulin to compensate this hyperglycemic state. β cell failure and peripheral insulin resistance together leads to type 2 diabetes.

1.2.1 Lipoatrophy Linking to Insulin Resistance and Type 2 Diabetes

The absence of adipose tissue or reduced adipose tissue is found in many persons. This syndrome is either congenital or acquired (Rossini, 1985; Seip and Trygstad, 1996). Insulin resistance and type 2 diabetes were present in all these patients. Recent studies have shown that HIV patients suffer from acquired lipodytrophic, and this is mainly seen in patients who were given medications with protease inhibitors (Carr et al., 1999). Protease inhibitors are known to inhibit adipocyte differentiation and promote apoptosis of adipocytes. Patients with lipodystrophies have impaired adipose tissue function with decreased adiponectin levels and increased free fatty acids levels. With impaired adipose tissue the storage of fatty acid is inadequate and leads to high levels of circulating free fatty acids resulting in lipotoxicity. This lipotoxic condition leads to storage of lipids in other sites like muscle, liver and pancreas leading to insulin resistance and type 2 diabetes (Haquae et al., 2002; Gavriloa et al., 2000).
1.2.2 Obesity, Insulin Resistance and Type 2 Diabetes

Obesity is an energy balance disorder in which nutrient intake exceeds energy expenditure (Dvorak et al., 1997). This leads to the expansion of adipose tissue (hypertrophy) or generation of new adipocytes (hyperplasia) (Khan, 1992). Since adipose tissue is known to play a crucial role in regulating glucose metabolism and insulin sensitivity, dysfunction of this tissue leads to adverse metabolic complications. Studies have shown that obesity or excess body fat content is the major risk factor for insulin resistance and type 2 diabetes (Colditz et al., 1990). The expansion of adipose tissue can occur at any time in the life span when nutrient intake is high (Ailhaud et al., 1992).

Two main theories have been proposed by two different groups about the development of insulin resistance and type 2 diabetes in obese conditions. The first theory states that excess food intake leads to expansion of adipocyte tissue by triglyceride storage and when its storage capacity is reached, the excess fat is deposited in other sites such as liver, muscle and pancreas contributing to insulin resistance, followed by hyperglycemic state and type 2 diabetes. The expansion of adipocytes also inhibits angiogenesis to that area there by limiting the generation of new functional adipocytes which can store this excess fat, and this further complicates the process. (Virtue and Vidal-Puig, 2008; Schrauwen, 2007). The second theory states that excess accumulation of fat in adipose tissue alters adipokines secretion. The stressed adipocytes in obesity secretes multiple chemokines and cytokines such as MCP-1, IL-6, TNFα, leptin, resistin and free fatty acids. In the second phase massive infiltration of macrophage to adipose
tissue occurs and these cells become the important source of inflammation in adipose tissue. These factors with its endocrine and paracrine effects eventually results in insulin resistance (Hotamisligil et al, 1993, Friedman et al., 1998, Weyer et al., 2000; Trayhurn and Wood, 2004; Skurk et al., 2007). The second theory seems to be more relevant because studies show that the biomolecules from fat cells and non fat cells together contributes to insulin resistance and type 2 diabetes (Weisberg et al., 2003).

![Diagram](fig1.7.jpg)

**Fig 1.7 β cell failure in obesity induced by adipokines, inflammation and free fatty acids. Figure adapted from Kasuga 2006.**

### 1.3 Obesity Epidemic

The prevalence of obesity is very high in both developed and developing countries. The rate of obesity is exponentially rising since 1980s. Obesity is responsible for 3-8% of health problems and 10-14 % of deaths in different countries. By the year 2015 approximately 2.3 billion adults will be overweight and more than 700 million will be obese (WHO, URL: [http://www.who.int/mediacentre/factsheets/en/](http://www.who.int/mediacentre/factsheets/en/)). This increase in obesity is mainly due the differences in life style adopted by people such as increased intake of high calorie foods and decreased physical activities, increased urbanization, changing modes of transportation, increased environmental pollution, habit of having tin foods containing several obesogenic compounds.
1.4 Compounds that Modulates Adipogenesis and Adipokine Expression

Several synthetic and natural compounds have shown to modulate adipogenesis and adipokine expression by different mechanisms. Bisphenol A, butylated hydroxyanisole, rosiglitazone are some of the synthetic compounds known to accelerate adipocyte differentiation (Masuno et al., 2005; Wang et al., 2004; Sheu et al., 2004; Galinier et al., 2006). Compounds like reversine, magnolol, harmine, H89, phloretin, emodin are the phytochemicals known to enhance adipogenesis in 3T3-L1 preadipocytes (Kim et al., 2007; Waki et al., 2007; Choi et al., 2009; Hassan et al., 2007 and Yang et al., 2007). Triterpenoids like ursolic acid, oleanolic acid inhibits adipogenesis (Sung et al., 2010; Huges et al., 2008). Flavanoids like quercetin, myrestin, geinstein also reduce adipocyte differentiation. Fucoidan, a polysaccharide reduces adipocyte differentiation (Kim et al., 2010). Some phytochemicals possessing antioxidant and anti-inflammatory effect has shown to exhibit anti-adipogenic activity. Guggulsterone, isorhamentin, esculetin, and genistein are such compounds that have shown to inhibit adipocyte differentiation (Lee et al., 2008; Yang et al., 2008; Yang et al., 2006; Hwang et al., 2005). Luteolin, naringenin, isoohumulone, resveratrol modulates inflammatory adipokine expression in 3T3-L1 adipocytes (Hirai et al., 2007; Ando et al., 2009).

1.5 Relevance of the Study

Adipocyte malfunction due to lipoatrophy or obesity leads to insulin resistance and type 2 diabetes. Alteration in adipokine homeostasis and the elevated levels of free fatty acids in circulation are the major causes for these complications. Any compounds/molecule that can modulate adipogenesis or the expression of adipokines involved in insulin resistance, could potentially be a therapeutic agent in managing obesity induced insulin resistance or type 2 diabetes. Functional characterization of such compound/s may lead to the identification of novel factor/s playing a role in adipogenesis. Though there are studies describing the identification of modulators of adipogenesis, very little efforts have been made to understand the basic molecular mechanism of the process using these molecules. The objectives of this study were not only to identify the molecules that modulate adipogenesis, but also to elucidate the molecular mechanism by which these molecules exert its effects. Such an approach may aid us in identifying novel molecular pathways that regulate adipogenesis. Therefore this work has the potential of both basic
and translational research. The aims of this study were to identify and functionally characterize small molecule modulators of adipogenesis.