1.1. THE ADIPOSE TISSUE

During embryonic development, adipose tissue is derived from the mesoderm and is present in every mammalian species, found throughout the body. It is a dynamic tissue metabolically that is the major site of storage for surplus energy but it also functions as an endocrine organ capable of synthesizing various biologically active compounds that controls metabolic homeostasis (Coelho et al., 2013). This dynamic tissue is comprised mainly of adipocytes and also of other cell types mainly the stromal vascular fraction, composed of blood cells, endothelial cells, pericytes and adipose precursor cells (Ahima and Flier, 2000).

Stromal vascular fraction

Stromal Vascular Fraction (SVF) is a component of the lipoaspirate obtained from liposuction of adipose tissue. Although lipoaspirate is the waste product of liposuction, it contains different subsets of cells which has regenerative potential. It contains a heterogeneous population of cells derived from enzymatically digested adipose tissue. SVF preparations are believed to be comprised of unknown numbers of stem cells mainly hematopoietic cells, adipose tissue stromal cells and endothelial progenitors cells. Fibroblasts, immune cells, endothelial cells, pericytes and additional uncharacterized cells are also found in SVF (Gimble et al., 2007)(Bourin et al., 2013).
Figure 1: Cellular subsets within the SVF (adapted from Dykstra et al., 2017)

SVF has rapidly gained attention due to its easy isolation, therapeutic potential and lack of ethical concern. It particularly enriches a population of stem cells, a type of mesenchymal stem cells (MSCs), which has been taken into consideration over the past years for their therapeutic properties (Dykstra et al., 2017). SVF has been shown to regenerate the tissues through various mechanisms. SVF promotes angiogenesis, moderately through the secretion of numerous growth factors such as Vascular Endothelial Growth Factor (VEGF) (Rehman
et al., 2004). It has also been shown that SVF has anti-inflammatory effects (Premaratne et al., 2011) and the preparations from SVF contain higher amount of IL-8, IL-15 and IL-1b and lower levels of the anti-inflammatory cytokines IL-13 and IL-10 when compared to the adipose derived mesenchymal stem cells, recommending SVF may have distinct immunomodulatory properties (Fu et al., 2013).

**Mesenchymal stem cells**

The most studied and well characterized cells of the SVF are the mesenchymal stem cells (MSCs) which appeared first in the annals of science in 1860s. Cohnheim and colleagues demonstrated the existence of non-hematopoietic, plastic adherent, fibroblast-like cells from the bone marrow, proposing that these cells were involved in the wound healing process (Owen and Friedenstein, 1988). There are lot of advances in this area of research since then. Finally, differentiation paradigm of MSCs derived from Bone Marrow (BM-MSC) was more expanded when Caplan and colleagues differentiated these cells into adipocytes, osteoblasts and chondrocytes (Caplan, 1991). Based on the limited differentiation potential, these cells were named as MSCs. It has not been fully understood that MSCs from adipose and from different sources possess same therapeutic potential or not. Comparison of MSCs from different sources for its potency is still argued (Strioga et al., 2012).

MSCs vary significantly in their paracrine secretions based on the tissue of origin (Burlacu et al., 2013). This difference is probably required for the maintenance of the tissue homeostasis in which they are residing. All the MSCs have immune regulatory properties (Krampera et al., 2007). This raises a theoretical possibility that MSCs from different organs may have differential differentiation capacity. It is quite likely that each organ in our body has organ specific MSCs, essential for maintenance of status quo, tissue repair, reconstruction and remodelling. Therefore these organ resident MSCs might have a separate pool of their own autocrine and paracrine secretions to support tissue architecture and eventually the
functionality of that organ. For example, MSCs from the liver would differ from those of pancreas although they are endodermal in origin whereas, MSCs from heart will differ from those of muscle, bone or adipose tissue although they are derived from the same germinal layer. This may also hold true for skin and brain derived from ectoderm. Hence, MSCs must be responsible for retaining organ specific structure and function without any alteration. At the same time these tissue/ organ resident MSCs may also have inborn capacity to transdifferentiate into another organ of same lineage in case of pathological or extreme physiological conditions demanding such as trans differentiation (Liver to Pancreas) (Dabeva et al., 1997). It is clear from the foregoing account that organ specific residential MSCs pool is responsible for maintaining tissue homeostasis.

Endothelial Progenitor Cells

Endothelial progenitor cells (EPCs) were first discovered in the peripheral blood of adults and demonstrated the capability of proliferating, migrating, and differentiating into endothelial lineage cells, as well as the de novo formation of new vessels (Asahara et al., 1997). During early embryo development, endothelial progenitor cells (EPCs) are required for vasculogenesis. On the other hand, adult vascular growth progresses from fully differentiated endothelial cells via angiogenesis (Risau, 1997). However, further findings have demonstrated the existence of postnatal, circulating EPCs that share phenotypic features with their embryonic counterpart, suggesting an angiogenic role for EPCs (Hristov and Weber, 2004)(Shi et al., 1998).

The transplantation of EPCs has been extensively applied in regenerative medicine for the management of ischemic diseases (Kawamoto et al., 2001). EPCs also have the potential for being used as the source of cell in the vascularization of tissue-engineered bladder. It has also been demonstrated that the formation of vasculature in a model of chorio allantoic membrane using EPCs (Sharma et al., 2009). A large number of EPCs can be isolated from
the SVF and it is possible that the SVF may constitute of a superior source compared to whole blood. However, before EPCs derived from SVF are considered for angiogenic therapies, some definitive markers for the identification of EPCs is needed. Hence, describing the phenotype and composition of the EPCs in the SVF is necessary to elucidate its full potential (Dykstra et al., 2017).

**Hematopoietic Stem Cells**

Hematopoietic Stem Cells (HSCs) are the cells isolated from the bone marrow or blood capable of renewing itself and differentiating to a variety of specialized cells within the hematopoietic lineage (Eaves, 2015). Adipose tissue is known to be an extramedullary reservoir for HSCs (Han et al., 2010). HSCs transplantation has been used for the treatment of a range of disorders related to blood, which includes inherited anemia, destruction of cancerous hematopoietic cells and most recent one being the autoimmune diseases (Hügle and Daikeler, 2010). Colony forming cell assays using the SVF from recipient mice showed that all SVF-HSCs originated from the bone marrow.

Additionally, HSC mobilization using Granulocyte Colony Stimulating Factor (G-CSF) improved the number of functional HSC in the SVF (Han et al., 2010). These outcomes support the use of SVF as an alternate source of HSCs. It is possible that the phenotype and longevity of HSCs in human SVF could be revealed using similar phenotypic profiling or animal models. Although, these are interesting findings, further studies are required to outline the differentiation capacity of HSCs under numerous physiological and pathological conditions (Dykstra et al., 2017).

**Monocyte/ Macrophage**

It is well-known that SVF contains monocytes and macrophages. Based on CD14 expression, it is estimated that the monocyte/macrophage compartment constitutes approximately 10% of the SVF (Astori et al., 2007). The macrophages found in the SVF are
known to express phenotypical marks of M2 macrophages viz. CD163 and integrin avb5 and they secrete interleukin-10 and interleukin-1 receptor antagonist (Zeyda et al., 2007). The phenotype characteristics of M2 macrophage is the opposite of their counterpart M1 macrophages. Historically, M1 macrophage have been known to mediate inflammatory responses (Mills, 2012). M2 macrophages are believed to exert anti-inflammatory properties and hence suggest a novel therapeutic potential. Studies on animal models demonstrate that the recruitment of inflammatory cells can be inhibited by modulating macrophages towards an M2 macrophage resulting in remarkable protection against atherosclerosis (Cardilo-Reis et al., 2012)(Mallat et al., 2001).

In obesity, monocytes/macrophages present in the adipose tissue are affected due to the accumulation of macrophages in the adipose SVF. Furthermore, these accumulated macrophages are found to be of M1 phenotype partially associated with chronic inflammation by secreting pro-inflammatory cytokines (Subramanian and Ferrante, 2009). One of the recent study showed that the ratio of monocyte/macrophage phenotype was skewed in adipose tissue derived macrophages isolated from obese patients with a greater number of macrophages expressing M1 markers as compared to non-obese patients (Silva et al., 2015). Interestingly, the levels of M1 macrophage accumulation was reduced in post bariatric surgery patients as compared to pre surgery levels, supporting the fact that the inflammatory environment is driven by the accumulation of adipose. Thus, the composition of macrophage from each and every patient’s SVF and its impact on modulating inflammation must be considered (Silva et al., 2015).

**Regulatory T cells**

Regulatory T cells (Tregs) are the immunosuppressive subpopulation of T cells which is known to inhibit the induction and proliferation of effector T cells thus mediating autoimmunity, inflammation, infection, allergic and tumor responses (Sakaguchi et al., 2008).
The main difference between the Treg residing in the visceral adipose tissue (Fat Tregs) and Tregs derived from the lymphoid is that fat Tregs contain higher fraction of CD positive T cells. Genes primarily associated with lymphocyte migration, extravasation and lipid metabolism express differentially in fat Tregs when compared to lymphoid derived Tregs (Cipolletta et al., 2011). Interestingly, fat Tregs express higher level of IL-10 in comparison with lymph node Tregs (Feuerer et al., 2009), resulting in a higher anti-inflammatory response.

The adverse effects of obesity are obvious in these cells. The number of Fat Tregs in visceral adipose are significantly reduced in insulin resistant animal models of obesity which is found in abundance in the lean mice (Feuerer et al., 2009)(Deiuliis et al., 2011). Altogether, the application of anti-inflammatory and immunomodulatory cells from the adipose tissue, although promising needs further considerations.

**Pericytes**

Two interacting cell types namely the endothelial cells and perivascular cells form blood vessels throughout the body. During the maturation of developing vasculature, recruitment of pericyte is crucial. After the formation of primary capillary plexus, a functional vessel network is formed by encompassing trimming and sprouting of vessels. As soon as newly formed sprouts ceases proliferation, growth factors like PDGF-B is secreted which attracts pericytes to enclose vessels in the brain, kidney, heart, lung and adipose tissue (Betsholtz, 1995). Pericytes like smooth muscle cells, work on vessels with large diameter and regulate blood flow by controlling vasoconstriction and vasodilation (Bergers and Song, 2005). Interestingly, pericytes are known to perform definite functions in different organs. Brain pericytes may constitute a microglia precursor which has a phagocytic activity (Thomas, 1999). Liver pericytes, called the hepatic stellate cells are known to regulate extracellular matrix remodelling, metabolism of vitamin A (Sato et al., 2003) and recruitment of
inflammatory cell resulting from liver diseases (Knittel et al., 1999). Pericytes from kidneys are crucial for the elevated capillary surface area (Gerhardt and Betsholtz, 2003).

Recently, it has been shown that the transplantation of pericytes improved heart function and contractility, decreased fibrosis and reduced inflammation in a disease model of ischemic heart disease model (Chen et al., 2013). Survival of pericyte is affected by stress conditions. Loss of pericyte is an initial hallmark of diabetic retinopathy resulting in microaneurysm due to reduced vessel integrity (Hammes et al., 2002). As discussed, the critical role for pericytes in vascular structure and function is known. Therefore, further studies could reveal a therapeutic role for pericytes in health and disease (Dykstra et al., 2017).

1.2. TYPES OF ADIPOSE TISSUE

Adipose tissue is scattered throughout the body and has a capacity to expand to accommodate excess energy in different forms (Gesta et al., 2007). In mammals, there are two main types of adipose tissues, White Adipose Tissue (WAT) and Brown Adipose Tissue (BAT). Structurally, WAT includes two major depots, subcutaneous WAT and visceral WAT surrounding internal organs. VAT is concentrated in the abdominal cavity and further divided into mesenteric, omental, perirenal, and peritoneal depots (Item and Konrad, 2012). BAT derives its color from extensive vascularization and the presence of many densely packed mitochondria. It contributes to non-shivering thermogenesis through lipid oxidation (Smith and Horwitz, 1969). BAT is traversed by numerous blood vessels compared to WAT which assists in delivering fuel for storage, oxidation and also in dispersing heat generated by the numerous mitochondria to other parts of the body (Kiess et al., 2008). Although BAT is readily available in both infant and rodent adults, it has been proposed that BAT in humans is limited to neonates and is progressively replaced by white adipose tissue with aging (Richard and Picard, 2011). WAT may represent the largest endocrine tissue of humans. The key functions of WAT are insulation and energy storage.
1.3. ENDOCRINE FUNCTION OF ADIPOSE TISSUE

Being an endocrine organ, adipose tissue is accountable for the synthesis and secretion of numerous hormones. Adipocytes are known to release hormones and other molecules that can act on nearby tissues and travel via the vasculature to distant sites mainly the brain, skeletal muscle, and liver (Stehno-Bittel, 2008). These are active in a variety of processes viz. regulator of nutritional intake are leptin and angiotensin, insulin sensitivity and inflammatory processes are mediated by Tumor necrosis factor α (TNF-α), Interleukin-6 (IL-6), Adiponectin, Resistin and Visfatin. Inflammatory pathways are regulated by Plasminogen activator inhibitor 1 (PAI-1) and Acylation Stimulating Protein (ASP) (Coelho et al., 2013).

Under normal weight conditions, these signals aid the body to suppress hunger, utilize the glucose, and decrease the risk of cardiovascular disease. However, in obesity, the hormones or the proteins that bind the hormones are not normal and can give rise to a state of chronic inflammation resulting in diabetes and heart disease. Additionally, excess amount of fat results in the accumulation of lipid droplets in nonfat cells, including skeletal and cardiac muscle. While some lipid droplets are useful as an immediate source of energy for the cells, huge amount of stored droplets can lead to cellular damage and cell death (Stehno-Bittel, 2008).
Figure 3: Adipokines secreted by adipose tissue

Leptin

Leptin, a 16 KDa small peptide is a pre inflammatory cytokine which belongs to IL6 cytokine family (Galic et al., 2010; Itoh et al., 2011). It is encoded by ob gene expressed in adipocytes. Leptin receptor is expressed in central nervous system as well as in peripheral tissues like hematopoietic and immune cells. It has a wide variety of physiological functions which includes regulation of appetite and energy expenditure by signalling the brain about body fat stores (Coelho et al., 2013). Concentrations of leptin in adipose tissue and plasma are dependent on the amount of stored energy and the status of energy balance. Thus, in obese individuals leptin levels are higher and further increases with overfeeding. Conversely, leptin levels are lower in lean individuals and fasting leads to reduction in circulating leptin. It is also believed that insulin also plays a role in mediating nutritional regulation of leptin, as in response to low insulin levels, as leptin decreases and rises in response to insulin or with feeding (Laclaustra et al., 2007). Synthesis of leptin is lesser in visceral adipose tissue than in subcutaneous and that is the reason females have higher levels of leptin due to greater proportion of subcutaneous fat in females. Leptin plays other roles too, which includes, glucose metabolism, modulation of the reward circuitry for feeding, lipid oxidation, substrate partitioning, and adipocyte apoptosis. (Galic et al., 2010; Itoh et al., 2011).
Adiponectin

Adiponectin is a 30kDa, full length protein known to circulate in trimeric, hexameric and higher molecular weight complexes (Weisberg et al., 2006). Adiponectin gene is located on chromosome 3q27. Adiponectin is exclusively secreted from adipose tissue (Palanivel et al., 2007). Adiponectin is associated with type 2 diabetes exclusively due to reduction in the levels of the circulating high molecular weight isoform, without any reduction in the levels of other two oligomeric forms (Schraw et al., 2008). The two identified receptors of adiponectin are AdipoR1 and AdipoR2. They constitute 7 transmembrane domains which differ structurally and functionally. Recently, it has been shown that abundant levels of AdipoR1 and AdipoR2 is present in the skeletal muscle but the liver predominantly expresses AdipoR2 (Galic et al., 2010). There is no significant fluctuation in the blood stream displayed by adiponectin, which means that adiponectin secretion is not acute but controlled by long-term metabolic changes (Kadowaki and Yamauchi, 2005). It has been shown that adiponectin regulates energy expenditure through the activation of AMPK in the hypothalamus, where the adiponectin receptor, AdipoR1 and AdipoR2 co-localize with the leptin receptor, ObR (Galic et al., 2010). The major actions of adiponectin and leptin have reciprocal functions for a homeostatic mechanism to retain energy stores and fat levels by the stimulation or suppression of appetite and energy expenditure (Itoh et al., 2011).

Tumor Necrosis factor α

Tumor Necrosis Factor α (TNFα), when synthesized is a 26 kDa transmembrane protein which is later cleaved by a metalloproteinase and released into the circulation as a soluble TNFα molecule (17 kDa) (Laclaustra et al., 2007). Isolated as well as differentiated adipocytes are capable of producing TNFα. For some years it was suggested that adipocytes are the principal source of elevated TNFα levels in obesity (Weisberg et al., 2006). The primary source of TNFα is the macrophage within the SVF, which is present in larger
quantities in visceral adipose as compared to subcutaneous adipose (Coelho et al., 2013). It is also postulated that the elevated TNFα levels in obesity is due to the increased infiltration of M1 macrophages into the adipose tissue (Weisberg et al., 2006). The quantity of macrophage corresponds to the fat mass as evidenced in both human and mice. It was observed that adipocytes contribute to the infiltration of monocytes from the circulation which supports diversification and maturation of monocytes by secreting Monocyte Chemotactic Protein (MCP1), Macrophage Inflammatory Proteins (MIP1α), Macrophage Migration Inhibition Factor (MIF1), Macrophage Colony Stimulating Factor (M-CSF) and chemokine CCL5 (RANTES) (Ouchi et al., 2011; Schäffler and Schölmerich, 2010). The first adipokine proposed to represent a link between inflammation, obesity and diabetes was TNFα. It has also been demonstrated that mRNA expression levels of TNFα in adipose tissue in obesity is strongly associated with pathogenesis of insulin resistance as TNFα is known to impair insulin signaling in adipose tissue and hepatocytes (Cai et al., 2005).

**Interleukin-6**

Interleukin-6 (IL-6) is a 21 kDa protein encoded by *IL6* gene. It is a proinflammatory cytokine and an anti inflammatory myokine (Ferguson-Smith et al., 1988). It has been shown that adipose tissue is the source for approximately 30% of circulating IL-6. Similar to TNF, the highest amount of IL-6 is derived from the SVF. Higher concentrations are observed in visceral fat in comparison to subcutaneous fat. IL-6 levels rise up in obesity and are stimulated by TNF and interleukin-1 (IL-1). Increased levels are associated with increased risk of atherosclerosis, coronary artery disease and angina. Generally, IL-6 inhibits lipase lipoprotein, induces lipolysis and enhances glucose uptake. In type 2 diabetes, elevated
levels of IL-6 positively correlates to the plasma free fatty acid concentration and the body mass (Coelho et al., 2013).

**Plasminogen Activating Factor-1**

Plasminogen Activating Factor-1 (PAI-1) is a single chain 45kDa glycoprotein containing 379 to 381 amino acids. PAI-1 gene is located on chromosome 7q21.3- q22. Main sources of PAI-1 are endothelial and vascular smooth muscle cells. However, it has been shown that other cells, such as hepatocytes, fibroblasts, monocytes, macrophages, platelets, mesangial cells, adipocytes, and stromal cells invading the adipose tissue also secrete PAI-1 (Correia and Haynes, 2006). The greater the adipose tissue and fat cell size, the greater is the contribution of adipose for the production of circulating PAI-1. Visceral adipose tissue has a greater capacity to produce PAI-1 than subcutaneous adipose. PAI-1 is involved in processes like fibrinolysis and is well known to be altered in obesity thereby increasing the risk of cardiovascular diseases (Mertens and Van Gaal, 2005; Skurk and Hauner, 2004).

**Angiotensin**

All the components of Renin-Angiotensin-Aldosterone System (RAAS) which includes, renin, angiotensinogen, angiotensin I-converting enzyme and angiotensin II type 1 receptor is expressed by adipose tissue (Ahima and Flier, 2000). Angiotensin II stimulates adipocyte differentiation and lipogenesis. Moreover, angiotensinogen mRNA and protein levels from adipose tissue are regulated by nutrition, resulting in decreased levels with fasting and elevates with refeeding (Carey et al., 2006). It is possible that RAAS peptides secreted by adipose tissue regulate blood pressure and cardiovascular responses in obese individuals by acting on the vasculature and distant targets. It also has recognized effect on cardiovascular function such as hypertension and hemostasis.
Acylation Stimulating Protein

Acylation Stimulating Protein (ASP) is produced through a two-step process which involves three proteins of the alternate complement system: C3, factor B and adipin, all of these are synthesized and secreted by adipocytes. ASP has a crucial effect on the enhancement of lipogenesis by glucose transporter type 4 (GLUT4) translocation and in the activity of Diglyceride Acyl Transferase (DGAT), an enzyme for triglycerides synthesis (Cianflone et al., 2003; Paglialunga et al., 2008). Plasma ASP rises with meals and enables the synthesis and storage of triglycerides. ASP levels are higher in obesity, Type 2 diabetes and cardiovascular disease whereas weight loss or exercise reduces ASP levels. Moreover, similar to insulin resistance, a deleterious ASP resistant state has also been proposed contributing to the dysregulated adipose tissue metabolism and dyslipidemia common to cardiovascular disease and diabetes (Coelho et al., 2013).

Resistin

Resistin is a 12.5 kDa small peptide synthesized with 108 amino acids, containing high amounts of cysteine (Coelho et al., 2013). Structure of resistin is strikingly similar to that of adiponectin (Patel et al., 2004). It is secreted by adipocytes as well as a large number of cells, especially, immunocompetent cells. Circulating levels of resistin rise in mouse models of obesity as well as in obese humans and are reduced by the anti-diabetic drug rosiglitazone (Steppan et al., 2001). Likewise, resistin has been implicated in the pathogenesis of diabetes and its complications. Release of resistin is stimulated by inflammation, interleukin-6, hyperglycemia, lipopolysaccharide, growth and gonadal hormones. Resistin released within the adipose tissue acts on adipocytes themselves giving rise to insulin resistance (Guzik et al., 2006). Moreover, it promotes insulin resistance by activating hepatic gluconeogenesis (McTernan et al., 2002). It also stimulates endothelial
cells to secrete antagonists of adiponectin like monocyte chemoattractant protein 1, vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 (Ouchi et al., 2011).

**Visfatin**

Visfatin is a 52 kDa highly conserved predominant in visceral adipose tissue. It is also called Pre-B cell colony-Enhancing Factor (PBEF), primarily produced by adipocytes, also by macrophages of the visceral adipose tissue, and in lesser quantities by subcutaneous adipose tissue. mRNA expression of visfatin considerably increases during the differentiation of preadipocytes to adipocytes (Olszanecka-Glinianowicz et al., 2012). It is regarded as a pro-inflammatory cytokine which induces activation of leukocytes and stimulates the production of TNFα and IL-6 (Moschen et al., 2007; Varma et al., 2007). Visfatin is preferentially expressed in visceral adipose tissue and its expression upregulates in animal models of obesity (Adeghate, 2008).

1.4. **THE METABOLIC SYNDROME**

Metabolic Syndrome (MS) is characterized by a collection of interconnected biochemical, physiological, clinical and metabolic factors that is directly proportional to increase in the risk of atherosclerotic cardiovascular disease, type 2 diabetes mellitus, insulin resistance, visceral adiposity, dyslipidemia, endothelial dysfunction and chronic stress (Kaur, 2014). The adipose tissue is crucial for the development of metabolic diseases and the underlying cause proposed is the adipose tissue dysfunction. Adipose tissue can expand by two ways: Hypertrophy (enlargement of the adipocyte) and Hyperplasia (increase in the number of adipocytes by recruitment of new adipocytes) (Rosen and Spiegelman, 2001). Out of which, hypertrophy is considered to be the one being more responsible for obesity, type 2 diabetes, dyslipidemia, insulin resistance and other metabolic diseases (Kusminski et al., 2016).
Inflammation

Systemic inflammation is directly augmented by adipose depots and stromal cells present within adipose tissue of an obese subject. The risk factor for the development of central obesity is that directly dictates insulin resistance and subclinical inflammation, which is followed by the metabolic syndrome and cardiovascular disease (Henninger et al., 2014). In the rat model of alcoholic liver disease; inflammatory cytokines, such as TNF-α and IFN-γ, induce liver injury (Kawaratani et al., 2013). In skeletal muscle, abnormal muscle repair can occur as myofiber degeneration and/or inflammatory infiltration persists (Kharraz et al., 2013).

Obesity

Obesity is a natural consequence of chronic overnutrition and sedentary lifestyle. Obesity that persists causes metabolic dysregulation which includes processes like insulin action on various metabolism and strictly affects processes regulating blood glucose, blood pressure, and lipid profile. Hence, gives rise to a group of conditions such as dysglycemia, hypertension, dyslipidemia and procoagulant state, collectively known as the metabolic syndrome (Grundy, 2003). As suggested by the data, obesity and the metabolic syndrome are direct precursors of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD).
Effect of obesity, the metabolic syndrome, T2DM, and CVD have increased in developing countries creating an urgent necessity to strategize health policies (Misra and Khurana, 2008).

**Type 2 Diabetes Mellitus**

Diabetes mellitus (DM) is perhaps one of the oldest diseases known. Over 3000 years ago, it was first reported in an Egyptian manuscript (Ahmed, 2002). In 1936, the difference between Type1 DM (T1DM) and Type2 DM (T2DM) was clearly described. In 1988, T2DM was first designated as a constituent of metabolic syndrome (Patlak, 2002). T2DM known as non-insulin dependent DM is the most common form of DM characterized by hyperglycemia, insulin resistance, and relative insulin deficiency. Type 2 DM is a result of interaction between genetic, environmental and behavioral risk factors for Type 2 DM can be prevented through lifestyle modification and control of diet, overweight and obesity (Olokoba et al., 2012).

**Dyslipidemia**

The three major characteristics of dyslipidemia associated with the metabolic syndrome are elevated fasting and postprandial triglyceride rich lipoproteins, reduced levels of high density lipoprotein (HDL) and increased small, dense low density lipoprotein (LDL). Persistant insulin resistance and compensatory hyperinsulinemia lead to the overproduction of very low density lipoprotein particles. A relative scarcity of an insulin-sensitive enzyme, called lipoprotein lipase is partially responsible for the reduced clearance of fasting and postprandial triglyceride rich lipoproteins, and the diminished production of HDL particles. All the lipoprotein defects add up largely to the elevated risk of cardiovascular disease in individuals with insulin resistance (Ruotolo and Howard, 2002).

**Insulin resistance**

The physiological condition where cells fail to respond to normal levels of insulin is called Insulin Resistance (IR). Although the supply of insulin from the β cells of pancreas is
adequate, cells are unable to use it effectively leading to a variety of disorders called the metabolic disorder. Primarily, organs like adipose, muscle and liver respond to insulin significantly but in IR condition they fail to do so. As a result, body needs higher levels of insulin for the uptake of glucose by the cells. At first pancreas makes enough insulin as required but later it is not able to produce insulin to keep the blood glucose to normal levels resulting in Type 2 Diabetes Mellitus (T2DM) (Wilcox, 2005). Normally, insulin suppresses glucose release from liver; however, the liver inappropriately releases glucose in the blood as a result of IR and T2DM. In the adipose cells, high accumulation of lipids, lipotoxicity, and increased breakdown of lipids result in remarkable increase in circulating free fatty acid levels. Reduced insulin stimulated glucose uptake, possibly by accumulation of lipid inside the cell and dysfunction of mitochondria, turn up in the skeletal muscle. Over the time, impairment of insulin secretion by pancreatic β cell can occur due to excess demand of insulin resulting in β-cells exhaustion. Inappropriate regulation of metabolism by the central nervous system is also one of the mechanisms associated with T2DM (Bhattacharya et al., 2007). Glucose is the major precursor of the glycerol breakdown. Glucose, through glycolysis or lactate, through glyceroneogenesis are the sources of glycerol-3-phosphate responsible for triacylglycerol synthesis in adipocytes. Enhanced glucose uptake is supposed to result in increased synthesis of triacylglycerol leading to obesity (Muñoz et al., 2010).

1.5. METFORMIN FOR METABOLIC SYNDROME

Metformin is a biguanide class of drug used to treat type 2 diabetes. The mechanism of action of metformin on body weight is still not clear. However, it has been shown that metformin is likely to inhibit weight gain primarily by anorectic effect directly or probably indirectly by stimulating glucagon-like peptide 1 (GLP-1) secretion.

After over 80 years of clinical use for the treatment of type 2 diabetes (T2D), metformin has proven to be safe as well as affordable, now making it the most commonly prescribed
oral anti-diabetic agent worldwide, taken by more than 150 million people each year. It has been demonstrated by Freemark and Bursey that metformin shows greater weight loss and improved insulin sensitivity in obese subjects with hyperinsulinemia on metformin 500mg twice daily compared to the placebo group without any dietary restrictions (Freemark and Bursey, 2001). Although metformin plays a very effective role in improving insulin sensitivity, there are some side effects associated with the treatment since it is a chemically synthesized compound, the most common ones include nausea, bloating, flatulence and diarrhea at beginning of the therapy. Vitamin B\textsubscript{12} deficiency has been also observed. Furthermore, one serious complication of metformin treatment is lactic acidosis, which occurs predominantly in patients with renal insufficiency.

1.6. MESENCHYMAL STEM CELLS FOR METABOLIC SYNDROME

There are various methodologies followed so far for the treatment of metabolic syndrome using stem cells and their conditioned media. However, the mechanism through which these cells tend to alleviate the disease still remains elusive. MSCs tested for its role in ameliorating metabolic syndrome are described below:

**Bone Marrow derived Mesenchymal Stem Cells (BMMSCs)**

The first report for the role of MSCs in improving insulin sensitivity by Si et al states that there are several mechanisms involved in the amelioration of hyperglycemia in T2D when treated with BMMSCs. There was an increased GLUT4 expression and the membrane translocation in the peripheral target tissues via an insulin dependent fashion (Si et al., 2012). MSCs have been associated in a lot of hepatic injury, the mechanism via which they contribute to diabetic liver disease is yet to be elucidated. It has been revealed that rat BMMSCs and their Conditioned Media (CM) administered to high-fat diet fed type 2 diabetic mice and streptozotocin induced insulin-deficient diabetic mice showed trophic effects of MSCs on liver damage. After 8 weeks of treatment, inspite of persistent hyperlipidemia and
hyperinsulinemia in HFD mice and persistent hyperglycemia in STZ mice beneficial effects of MSC and MSC-CM therapies were similar because both ameliorated the augmentation of aspartate aminotransferase and alanine aminotransferase. Moreover, hepatocyte regeneration in STZ-diabetic mice was induced and both therapies also prevented unnecessary lipid accumulation and apoptosis of hepatocytes and treated insulin resistance in high fat diet induced diabetic mice (Nagaishi et al., 2014).

It is very well known that bone marrow MSCs secrete factors that act in a paracrine manner to promote angiogenesis, modify cell migration and inhibit apoptosis. The protective outcome of CM was analysed in a study which revealed that multiple pro-survival factors in addition to MCP-1 are secreted by MSCs which act on numerous pathways. Future studies will describe the exact pathway used by MCP1 and the other known factors and whether they contribute to the favorable effects of stem cell therapy following tissue damage after myocardial infarction (Boomsma and Geenen, 2012). The capacity of multiple infusions of BMMSCs to promote prolonged decrease in hyperglycemia and apoptosis in pancreatic islets and increase in insulin sensitivity in high fat diet fed mice has been demonstrated (Bueno et al., 2015a). A novel diabetic foot ulceration model showed that the impaired healing process in diabetic rats was ameliorated by transplantation of BMMSCs. This improvement might be due to the alteration of keratinocyte functions (Kato et al., 2014). Furthermore, it has been shown that single-dose MSCs infusion ameliorates hyperglycemia but fails to restore normoglycemia in diabetic animals (Hao et al., 2013). Then, the question arises that; will multiple intravenous MSCs infusions reverse hyperglycemia in type 2 diabetes (T2D) rats? One of the study described the effect of administration of serial allogeneous BMMSCs infusions (1 × 10(6) cells/infusion) via the tail vein once in every 2 weeks to high fat diet induced and streptozocin (STZ) administered T2D rats and suggested that a multiple-MSC infusion strategy offers a viable clinical option for T2D patients (Hao et al., 2013). BMMSCs
CM could also improve the insulin sensitivity in HepG2 cells pretreated with palmitic acid through upregulation of insulin signalling component expression (Sun et al., 2015). Human BMMSCs CM has been shown to have remarkably higher levels of angiogenic factors viz. IL-6 and VEGF. It has also been demonstrated that MSC-CM which has been delivered in gelatin sponges promotes angiogenesis and facilitates the fracture healing in a rat model of diabetes. Hence, MSCs CM treatment may be used as an alternative approach for treating fracture non-union in diabetic patients (Wang et al., 2012a).

One of the major clinical problems in patients with diabetes is wound healing. Hyperglycemia and chronic inflammation at the site of the wound may be due to improper keratinocyte migration and proliferation during re-epithelialization. There was a decreased production of ROS when the rat keratinocytes were treated with rat BMMSC-CM. In addition, MSC-CM reversed the downregulation of phosphorylation of MEK1/2 and Erk 1/2, induced by high glucose and/or liposaccharides without affecting total levels (Li et al., 2015b). The safety of mesenchymal stem cell (MSC)-based therapy impacting on atherosclerosis has also been evaluated. Allogeneic MSCs obtained from rabbit bone marrow aspirates and expanded in vitro were infused into rabbits with hypercholesterolemia. The aortic sinus lesion size drastically increased in the MSCs treated rabbits when compared to the controls. Moreover, in MSC-treated aortas, vasa vasorum networks were more numerous and had improved capillary density. Allogeneic MSC transfusion may result in an increase in atherosclerotic lesion size. In cellular therapy either with MSCs or cell populations containing MSCs; strategy to attenuate the high potential of MSCs involved in atherogenesis of atherosclerosis should be taken in account (Liu et al., 2009).

**Umbilical Cord/ Wharton Jelly Mesenchymal Stem Cells (UCMSCs/ WJMSCs)**

It is very well known that the dysregulation of glucose metabolism is a common feature which is linked to insulin resistance. A rat model of liver cirrhosis was developed by
the treatment of CCl4 for weeks, human umbilical cord blood mesenchymal stem cells were infused and insulin resistance was shown to be improved associated with increased glucose levels and decreased insulin sensitivity in cirrhotic rats thus contributing to homeostasis (Jung et al., 2011). It is believed that MSCs may be a new treatment for obesity-related insulin resistance and T2D concerning macrophage polarized effects. Infusion of human umbilical cord derived MSCs produced anti diabetic effects and promoted insulin sensitivity in T2D. Additional analysis proved that UCMSCs can improve insulin resistance in part by production of IL-6 that prompts M2 polarization (Xie et al., 2016).

Wound healing is a major concern in diabetes and current treatments have not been so promising till now. Human WJMSCs when evaluated for its wound healing properties showed increased expression of several miRNAs associated with wound healing compared to human skin fibroblasts. It has been demonstrated that hWJSCs enhances healing of excisional and diabetic wounds through differentiation into keratinocytes and release of vital molecules (Fong et al., 2014). A few report states that the synergistic effect of mesenchymal stem cells with an anti diabetic compound works better for the reversal of the disease. One such report suggests that Liraglutide treatment, an anti-hyperglycemic drug in combination with umbilical cord mesenchymal stem cells improves glucose metabolism and the beta cells function in patients with type 2 diabetes (Chen et al., 2016). One of the major complications of diabetes is vasculopathy. The critical factor for diabetic endothelial dysfunction is the impairment in mitochondrial biogenesis and bioenergetics due to oxidative stress. An investigation focusing on the curative potential of human umbilical cord MSC-CM in diabetic endothelial dysfunction showed that MSC-CM perfusion in diabetic rats had protective effects on endothelial cells, with respect to glucotoxicity, by improving the mitochondrial function via PI3K/Akt/ Sirt1 expression. Sirt1 potentiated mitochondrial biogenesis, through Sirt1/AMPK/PGC-1α pathway (Yuan et al., 2016). UCMSCs cultured in vitro for six days in
high glucose (40mmol/l or 60 mmol/l) has shown significant DNA methylation. Activation of Notch Signalling pathway is responsible for the potency of UCMSCs differentiation into insulin secreting cells (Pan et al., 2017). Infusion of human UCMSCs show anti diabetic effect on T2D rats by promoting insulin sensitivity. These MSCs induced M2 macrophages and ameliorated IR caused by M1 macrophages. This report also showed that the increase in IL6 is due to stimulation of UCMSCs by M1 which upregulates IL4R expression, promotes phosphorylation of STAT6 in macrophages and sequentially polarized macrophages to M2 macrophage (Xie et al., 2016). Human Umbilical Cord Blood MSCs (hUCB-MSCs) directly injected into the quadriceps thigh muscle in diabetic patients with foot disease under insulin therapy showed significant reduction in blood glucose levels and insulin dosage. After 4 weeks of transplantation, serum levels of VEGF peaked and the levels of TNFα and CRP significantly decreased. The ratio of Treg/Th17 correlated positively to VEGF levels plasma and inversely to plasma IL-6 levels. Immune disorders are linked with the development of type 2 diabetes and its complications as the role of Treg cells in the onset and development of T2DM has been demonstrated (Li et al., 2013). It has been indicated that transplantation of allogeneic UCMSCs may be an approach to improve islet function in patients with T2DM. The levels of fasting C-peptide significantly increased following transplantation and continued to be high throughout the follow up period. Few patients became insulin free after the therapy, others required less insulin and continued to be on insulin. Fasting insulin was found to be comparatively stable in all the patients. There was no toxicity observed associated with MSCs injection within the follow up period (Guan et al., 2015). Attempts to transfuse UCMSCs has been shown to be safe and well tolerated, efficiently improves blood glucose, and the generation of C-peptide levels and Tregs are increased in a subcategory of T2DM patients (Kong et al., 2014).
Combined therapy of WJMSCs and sitagliptin in diabetic wistar rats can remarkably ameliorate hyperglycaemia, stimulate regeneration of islet $\beta$ cells and suppress generation of islet $\alpha$ cells. Although the exact mechanisms are not clear, no symptoms of rejection and toxic effect were witnessed and hence is being presented as one of the new therapies for type 2 diabetes (Hu et al., 2014).

**Dental Pulp Mesenchymal Stem Cells (DPSCs)**

The most frequent complication of diabetes is diabetic polyneuropathy in patients, a result of impaired blood flow and metabolic disorder (Vinik et al., 2000). When DPSCs isolated freshly from Sprague-Dawley rats and cryopreserved DPSCs were transplanted into the STZ induced diabetic mice, amelioration of sciatic nerve blood flow and sciatic nerve conduction velocity was observed. This suggested that DPSCs can be used as a tool for the treatment of diabetic neuropathy. Sciatic nerve conduction velocities and sciatic nerve blood flow was improved when rat DPSCs were transplanted into the unilateral hind limb skeletal muscles. DPSCs transplantation also upregulated the mRNA expression of M2 macrophages and significantly decreased the expression of M1 macrophages and tumor necrosis factor alpha which might be one of the therapeutic mechanisms for diabetic polyneuropathy (Omi et al., 2016). The effects of factors secreted by dental pulp stem cells from human exfoliated deciduous teeth (SHED) on $\beta$-cell function and survival has been reported. It is suggested that 1 ml SHED-CM when injected twice per day intravenously for 1st five days and later intraperitoneally for 9 days improved glucose intolerance, increased pancreatic insulin content and beta cell mass in STZ induced diabetic mice. The effect of SHED-CM was more prominent when compared to Exendin-4, an incretin based drug and BM-CM (Izumoto-Akita et al., 2015).
Adipose tissue Derived Mesenchymal Stem Cells (ADSCs)

Adipose tissue (AT) is emerging as a source of stem cells that can be obtained by a less invasive method and in larger quantities than from bone marrow. These cells can be isolated from human lipoaspirates and, like MSCs, can differentiate toward osteogenic, adipogenic, myogenic, chondrogenic, and neurogenic lineages. Due to their wide availability and ability to differentiate into other tissue types of the mesoderm—including bone, cartilage, muscle, and adipose—ADSCs may serve a wide variety of applications. It has been reported that ADSCs stimulate the phosphorylation of hepatic AMPK to recover from the glucose metabolism disorder in palmitate treated HepG2 cells in vitro (Xie et al., 2017). Our recent study revealed that ADSCs CM can act as an alternative insulin sensitizer providing stem cell solution to IR in vitro. In palmitate induced 3T3L1 and C2C12 cells, there was enhancement of glucose uptake. Increased phosphorylation of membrane GLUT4 and Akt at Ser 473 in C2C12 cells adds on to the insulin sensitizing effect of the CM derived from adipose tissue (Shree and Bhonde, 2017). In vivo studies in male Sprague Dawley mice fed on HFD and with low dose of STZ demonstrated that intravenous injection of adipose derived mesenchymal cells improve hyperglycemia by regulating hepatic glucose metabolism which is dependent on AMPK signalling pathway. Human adipose tissue derived mesenchymal stem cells have been shown to protect the podocytes from apoptosis which is a result of high glucose injury in the progression of diabetic neuropathy (Xie et al., 2017). A study by Seo et al demonstrated the effect of ADSCs treatment in experimental skin wounds in diabetic db/db mice. They showed that both topical Ex-4 treatment or local injection of ADSCs are beneficial. Nevertheless, a combination of Ex-4 and ADSCs have the best healing effect. They have also shown the angiogenic effect of Ex-4 on endothelial cells and angiogenic effects of ADSCs on both endothelial cells and keratinocytes which contributes to the acceleration of re-epithelization and wound healing (Seo et al., 2017). From a long time,
metformin is used as a leading insulin sensitizer. We have reported earlier that preconditioning of ADSCs with metformin could have a better therapeutic value for the reversal of type 2 diabetes. A significant decrease in hyperinsulinemia, triglyceridemia, serum IL6 and oxidised LDL were observed at the end of the study. This was the first report to demonstrate the synergistic effect of metformin preconditioning of ADSCs leading to reversal of hyperglycemia, hyperinsulinemia and triglyceridemia (Shree and Bhonde, 2016). Extracellular vesicles from ADSCs have therapeutic role in vascular and neurodegenerative disease (Gao et al., 2017). A study by Zhao et al showed that exosomes derived from ADSCs alternatively drove polarization of M2 macrophage, reduction in inflammation, and beiging of white adipose tissue (WAT) in diet induced obese mice (Zhao et al., 2018).
1.7. **SCOPE OF THE THESIS**

Since inflammation is the root cause of all the diseases including obesity, diabetes and insulin resistance. We aimed at disseminating the role of ADSCs and their secretome in reducing inflammatory milieu. We hypothesized that injection of ADSCs and/or its secretome will aid in reducing the inflammation bringing about decrease in insulin resistance leading to regulation of dysregulated metabolic profile.

*Figure 5: Diagrammatic representation of our hypothesis*
1.8. OBJECTIVES

We undertook the present study with the following objectives which is described in the following chapters:

Objective 1:
Examining the role of human adipose tissue derived mesenchymal stem cells (hADSCs) in the reversal of insulin resistance in vitro

Objective 2:
Evaluation of the role of hADSCs and its conditioned media on normalising acute inflammation induced by carrageenan in db/db mice

Objective 3:
Assessment of the effect of intramuscular injection of hADSCs in three different forms in diet induced obese model of mice

Objective 4:
Examination of the role of hADSCs transplantation preconditioned with metformin in the management of obesity in mice