INTRODUCTION & REVIEW OF LITERATURE

CHAPTER 1

1 INTRODUCTION & REVIEW OF LITERATURE

The modern world is suffering from high incidence of metabolic syndrome with clinical manifestation of diabetes, cardio vascular diseases (CVD) and obesity (Blüher, 2010).

1.1 Obesity

Obesity is extensively increasing with increment in economic disease burden globally. It is defined as body mass index (BMI) (Kg/m^2) of >30, as per World Health Organization (Figure 1.1.) (WHO) (Blüher, 2019). Prevalence of obesity was explored in 195 countries worldwide during 1980 to 2015. Surprisingly, 603.7 million adults and 107.7 million children were observed to be victim of obesity. United States of America (USA) and China had highest obese adults whereas India and China had highest obese children (Collaborators, 2017). Persistent hike in obesity has led to the prediction that till 2030, 57% of global population would be obese (Ferretti et al., 2019).

Distinct factors attribute to development of obesity and other metabolic disorders. Globalization and modernization are the major factors responsible for reduced physical exercise and transition in consumption of food from traditional fibrous and less fatty food to highly processed, less fibrous, high trans-fat, carbohydrate rich and animal meat. The unhealthy dietary habits as a part of western culture have immensely contributed in obesity and diabetes (Blüher, 2019; Fox et al., 2019). All these factors have instigated dreadful effects on health issues in developing countries of South East Asia and Africa (Collaborators, 2017).

1.2 Obesity a major risk factor in India

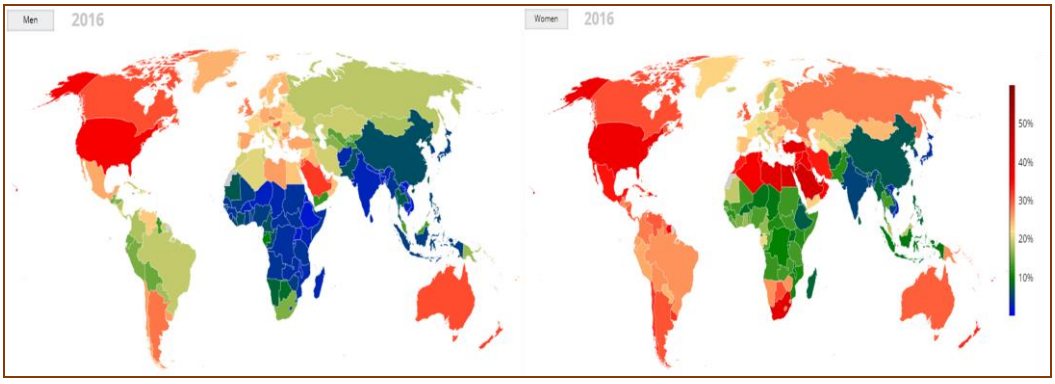

Figure 1. 1 Epidemiology of Obesity (WHO report 2016)

India is facing a dramatic rise in socioeconomic reforms in modern world and thus, is facing challenge against metabolic disorders prevailed due to obesity. India ranks second in the world for having highest numbers of obese children. Among 650 million obese worldwide, more than 150 million obese are Indians which itself is a major matter of concern in increasing socio-economic burden of the country (Ahirwar et al., 2018). More than 33% rural population and around 50% of urban Indians are obese (Behl et al., 2017).

Indians have three predisposed condition that contribute to development of obesity and diabetes amongst which, weight of the new born baby is very less compared to those of healthy Caucasian babies (Vasan et al., 2012). Second issue is the maternal malnutrition condition that induces metabolic disorder into the child. Third critical issue is the "thin-fat" phenotype which defines fat accumulation on the abdominal region whereas, torso and lower limbs are thinner compared to huge abdomen (Speakman, 2008).

Traditionally, India is known for unique culture and magnificent practice of vegetarian diet along with various spices that regulate energy homeostasis in the body. More of fasting, and consumption of high fibres and protein rich diet was practiced since ages (Shridhar et al., 2014). Additionally, hardships and physical work were enforced to earn regular livings assisted with malnourished population in most part of the country (Mishra et al., 2017).

Socioeconomic reforms in India have flourished availability of attractive western foods which are unhealthy, contain highly processed grain flours, excess of fat and are deep fried (Corsi et al., 2019). The changes in dietary composition have dramatically introduced use of saturated fats and oils, high carbohydrate content, trans-fat and reduced fibre, omega 3- FAs, poly unsaturated FAs etc (Gulati et al., 2017). With special reference to population of Gujarat, the habit of consuming more sweet food in regular diet has profoundly influenced the incidences of diabetes and obesity (Gulati et al., 2014). Moreover, the frequency and pattern of diet with disturbed circadian rhythm have attributed diabetes, CVD and several cancers in Indians (Sridhar et al., 2016).
Speakman in 2008, had explicitly explained that excess calories and fat accumulation leads to genetic drifts which give rise to "drifty genes" and not the "thrifty genes" as per the old notion of positive selection for activation of thrifty genes that would profoundly impact elevation in obesity and associated diseases (Speakman, 2008).

Since few years South Asians, specifically Indians have been delineated as distinct class of obese (Indulekha et al., 2015).

A comprehensive analysis had been performed among Indian population that segregated Indians into 4 classes based on BMI and metabolic alterations. Subjects those with BMI<25 with no metabolic syndrome (MS) which comprised hypertension, high waist circumference, diabetes and hypertriglyceridemia were considered as metabolically healthy non-obese (MHNO). Those individuals who had BMI>25 but no MS were metabolically healthy obese (MHO). Individuals having BMI $\leq$ 25 with MS were classified as metabolically obese non obese (MONO). And those who had BMI $\geq$ 25 with MS were known as metabolically obese obese (MOO) (Indulekha et al., 2015). Therefore, Prevention and Management of Obesity and Metabolic Syndrome group has re-categorized Indians as overweight with BMI between 23 and 24.9 and obese with BMI >25 (Aziz et al., 2014; Anoop Misra et al., 2009) Metabolically obese phenotypes had high propensity towards CVDs and diabetes. Pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-α), resistin, inter leukin (IL)-6, C-reactive proteins (CRP) were elevated in metabolically obese subjects among which resistin and CRP were found to increase persistently with severity of metabolically obese phenotypes. Thus, Indians being metabolically obese are more susceptible to metabolic disorders like diabetes and CVD (Indulekha et al., 2015).

1.3 Diabetes

There are two main types of diabetes. Type I diabetes mellitus (TIDM) is due to dysregulation of one of the major hormone insulin wherein, either insulin production and secretion by pancreatic islets is hampered and thus, is known as insulin dependent diabetes or juvenile diabetes as it occurs in young ones (Bajaj, 2018). Failure of insulin action due to inability to bind insulin receptor (IR) and downstream insulin signaling leads to Type II diabetes mellitus (TIIDM) or non-insulin dependent diabetes which develops after the age of 50s, but now it has emerged in young population of late 30's and sooner might be present in younger people (Petersen et al., 2018).

1.3.1 TIIDM

Obesity imparts TIIDM and CVD due to hyperglycemia, hyperlipidemia, artherosclerosis etc. A person diagnosed with Fasting plasma glucose (FPG) $\geq$ 126 mg/dL or oral glucose tolerance test (OGTT) using 75 g of anhydrous glucose with FPG $\geq$ 126 mg/dL and/or 2-hour (H) plasma glucose $\geq$ 200 mg/dL is considered as a

diabetic person with Glycated haemoglobin (HbA1c) $\geq 6.5\%$ or Random plasma glucose ≥ 200 mg/dL (Bajaj, 2018). Obese and diabetic people suffer from dyslipidemia with altered lipid profile. There is a positive association of BMI and waist circumference with triglycerides (TG) and low density lipoprotein (LDL)-Cholesterol and concomitant negative association with High density lipoproteins (HDL). If serum levels of total cholesterol >200mg/dl, LDL-C >130mg/dl and HDL-C less than 40mg then the subjects have dyslipidemia and are at the major risk of CVD (K. Bora et al., 2015).

1.3.2 Insulin and Insulin signaling in peripheral tissues

Insulin is the predominant hormone that regulates several metabolic processes in the body. The signaling pathway involves binding of insulin to the IR hetero-tetramer comprised of 2 alpha (α) and 2 beta (β) subunits. Insulin binds to the extracellular α subunits whereas, β subunits span the membrane and cytosolic domain consists of tyrosine kinase domain. Binding of insulin to IRβ induces conformational change which activate tyrosine kinase activity that leads to phosphorylation of tyrosine residues and recruits binding of IR substrates (IRS) (Belfiore et al., 2017). Upon activation insulin directs either mitogenic or metabolic signals. Activated IR downstream recruit's phospho-tyrosine binding scaffold proteins like SH2 domain-containing adapter protein B (SHB), SH1B, SH2B, GRB10, GRB14 which activate downstream effectors which propagates mitogenic and metabolic responses in the cell (U. Jung et al., 2014). Insulin persuades metabolic effects at a relative lower concentration than that required for mitogenic actions (Bedinger et al., 2015).

Mitogenic activity of insulin signaling are governed by GRB2 and SHC whereas, SH2B2 facilitates insulin mediated metabolic responses. These substrates have regulatory roles in insulin signaling (Desbuquois et al., 2013). CEACAM1, one of the IRS dephosphorylate IR by protein tyrosine phosphatase (PTPases) which are just inactivated once IR are internalized (Najjar, 2002). PTP1B is activated with a time delay and hence is inhibited after post IR activation through hydrogen peroxide (H_2O_2) produced by NAD(P)H oxidase 4 thus, amplifying downstream insulin signaling (Mahadev et al., 2004). Among all, IRS family have been extensively studied. There are six isoforms for IRS amongst all IRS-1 and IRS-2 are studied extensively and exert metabolic actions of insulin. IRS proteins have plextrin homology and PTB domains that target them to activated IR where long carboxylic acid (COOH) terminal tails are

replaced by tyrosine and serine threonine residues. Once IRS-PTB complex binds to IR, it phosphorylates the IRS at multiple tyrosine sites and thus drives the signaling ahead. IRS1 and IRS2 knock out (KO) mice downregulated IR and thus mitigated metabolism Figure 1.2. (Petersen & Shulman, 2018).

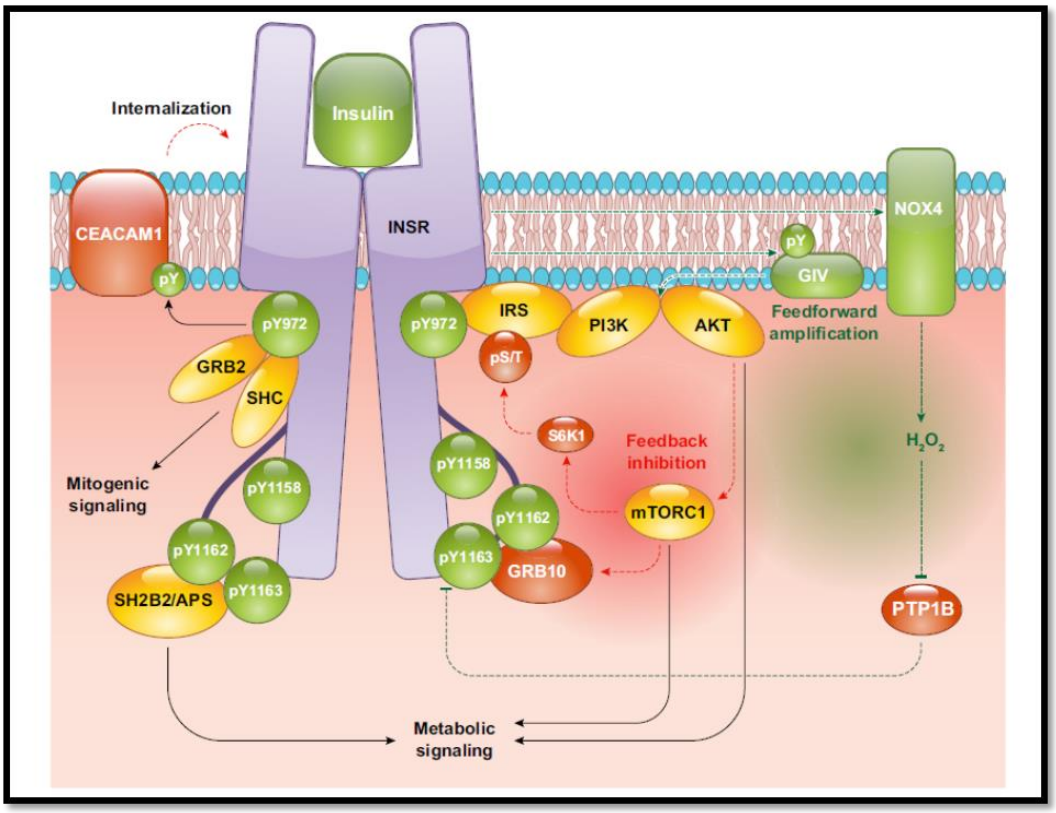

Figure 1. 2 Insulin signaling (Petersen & Shulman, 2018).

Insulin signaling is conserved in all the peripheral tissues, however metabolic results vary. Pancreas drain maximal insulin through portal vein compared to other organs. Insulin drives anabolic functions like synthesis of major macromolecules glycogen, lipids and proteins. It facilitates glycogen synthesis and its storage in liver. It suppresses hepatic glucose production (HGP) and glycogenolysis (Saha et al., 2004).

Muscle is a large and dynamic organ which requires lot of energy. Abundance of glucose is channelized to muscle through Glucose transporter type 4 (Glut4) which promotes glycogen synthesis. Insulin signaling is classically and efficiently activated by IRS-1 in myocytes (Peterson and Shulman, 2018). Adipose tissue is a central player in energy metabolism. Insulin inevitably promotes lipogenesis by re-esterification of circulating free FAs (FFA) or FAs within adipocytes and inhibits lipolysis and thus, regulates levels of non-esterified FAs (NEFA) in circulation. Anti-lipolytic action of

insulin promotes energy homeostasis and balances metabolic processes of the body. However, dysregulation in insulin signaling leads to insulin resistance which causes metabolic alterations in peripheral tissues that culminates into TIIDM and associated complications like obesity (Hotamisligil, 2000).

1.3.3 Insulin resistance- A hallmark of TIIDM and Obesity

Insulin resistance is a condition where IR gets phosphorylated at serine residue with downregulation of its tyrosine kinase activity. Further, IR undergoes ubiquitin mediated degradation which stammers insulin signaling with onset of insulin resistance. Moreover, phosphoinositide 3-kinase (PI3K) and Protein Kinase B(PKB)/AKT are also downregulated due to high inflammation. PTP1B phosphatases are activated and cleave IRs which induces insulin resistance. Suppression of GLUT4 translocation inhibits insulin mediated glucose uptake and hence, a state of hyperglycemia and hyperinsulinemia is triggered. Prevalence of elevated circulating FFAs with hyper triglyceridemia is characteristic of TIIDM and obesity (L. Chen et al., 2015).

Muscle is the first organ to be affected by insulin resistance. Inability of muscles to uptake glucose concomitantly inhibits glycogen synthase enzymes and hexokinases which render depletion of energy source for exercising muscles (DeFronzo et al., 2009). Insulin resistance in liver, upregulates gluconeogenesis and glycogenolysis with reduced glycogenesis. HGP amplifies insulin resistance in adipose tissue. Several genetic models have depicted HGP and adipose tissue insulin resistance, precisely liver specific IR (LIRKO) mice which lack IR are highly glucose intolerant and are incompetent to suppress HGP in hyperinsulinemic - euglycemic clamp studies. Hepatic insulin resistance is provoked by elevated expressions of Forkhead box protein O1 (FOXO1) and its nuclear translocation (Fischer et al., 2018). Ectopic deposition of FFAs into liver enforces the hepatocytes to undergo a transition from fibroblast to lipid laden adipocyte like cells (L. Chen et al., 2015). The parenchymal cells are distorted and fibrosis is initiated. Elevated lipid deposition, HGP and fibrosis drives hepatomegalies from non-alcoholic fatty liver disease also known as hepatic steatosis which culminates into fatal liver cirrhosis or liver cancer (Figure.1.3.) (Patel et al., 2016).

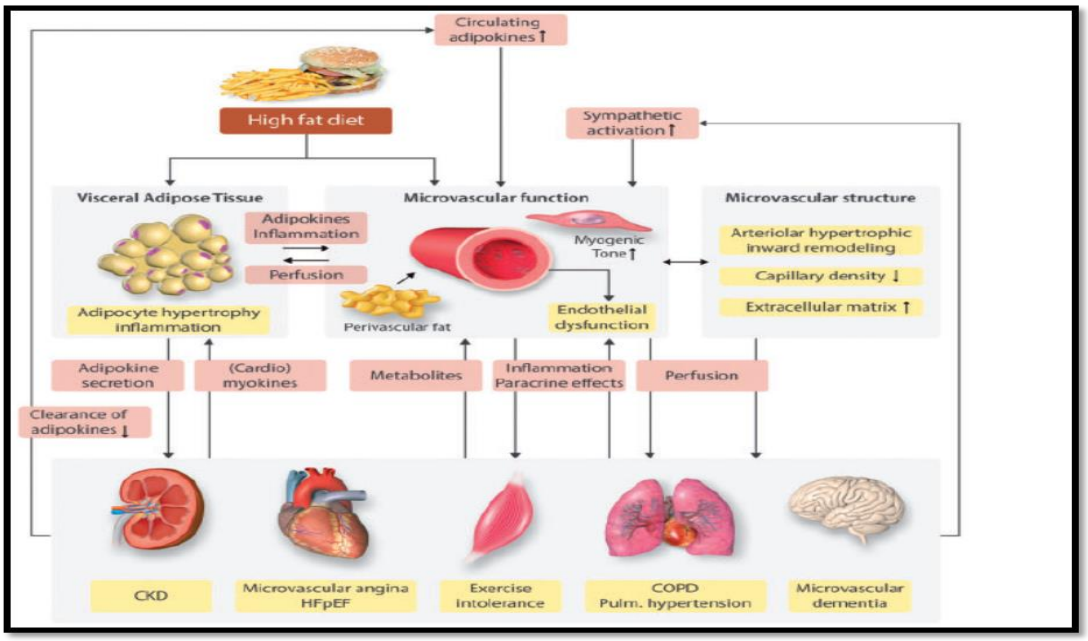

Figure 1. 3 Obesity embarks pathological disturbances in peripheral tissues.(Sorop et al., 2017)

In adipose tissue insulin resistance induces lipolysis, dysregulated lipid droplet formation and packaging. Perilipin coating on adipocytes for uptake of NEFA becomes ineffective. Dyslipidemia in adipose tissue is the major culprit for spread of metabolic alterations in all the peripheral organs (K. Sun et al., 2011). Obesity instigates heart failure by myo-cardio-lipotoxicity which leads to cell death that causes cardiac dysfunction. Diabetes independently assaults heart functions propelling CVDs that cause heart failure (Jahng et al., 2016). Persistent insulin resistance with hyperglycemia bestows burden on pancreas to produce more and

more insulin. As a result of prolonged stress, efficiency of insulin producing β cells decreases which causes hyperglycemia that induces glucotoxicity and oxidative stress leading to apoptosis of β cells (Kahn, 2003). This clinical stage of TIIDM resembles to that of TIDM patients, who are deficient in pancreatic beta cells and insulin levels (Petersen & Shulman, 2018).

Kidney plays an important role in maintaining body homeostasis. Obesity launches detrimental effects on kidney which leads to obesity related glomerulopathy (ORG) (Kovesdy et al., 2017). Incidences of ORG have increased by 7% compared to previous prevalence. Glomerular hyperfiltration are the predominant pathological intervention responsible for ORG which causes glomerular hypertension, microvascular stretching and glomerulomegaly. These pathophysiology leads to loss

of podocyte which undergoes focal and segmental glomerulosis (FSGS) (Praga et al., 2017).

1.4 Adipose Tissue Biology

Adipose tissue is the largest organ of the body which comprise 5 to 60% of whole-body weight and is extremely dynamic and multi depot, that plays an imperative role in energy homeostasis of human body (Wajchenberg, 2000). It stores energy in the fed state and provides fuel in the form of FAs and glycerol to other organs during the fasting state or starvation. There are two different types of adipose tissue named on the basis of their colour appearance i.e. brown (B) adipose tissue and white (W) adipose tissue which are allocated distinct origin, locations and functions (Cedikova et al., 2016).

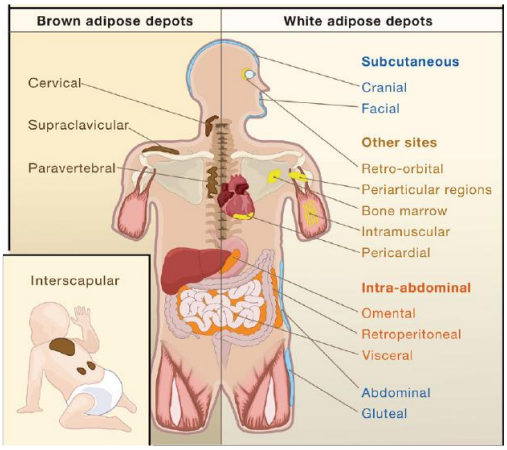

Figure 1. 4: Distribution of B-adipose tissue and W-adipose tissue. (Gesta et al., 2007)

1.4.1 W-adipose tissue

W- adipose tissue is broadly classified into two different types on the basis of their depots, namely subcutaneous (S) adipose tissue which spans the layer below the skin throughout the body along with breast, gluteal, femoral, abdominal, cranial and facial depots whereas, adipose tissue surrounding the intra-abdominal region around omental, mesenteric, retroperitoneal and perigonadal depots is known as visceral (V) adipose tissue (Raajendiran et al., 2016). W- adipose tissue is also found in periarticular, intermuscular, bone marrow and retro-orbital sites with vital functions (Figure 1.4.) (M.-J. Lee et al., 2013; A. Park et al., 2014).

S-adipose tissue contributes to approximately 80% of whole body whereas, V-adipose tissue is around 10-20% in men and 5-10% in women (M.-J. Lee et al., 2013). S-adipose tissue is the primary source of energy reserve and studies have described that

S-adipose tissue and V- adipose tissue distribution varies among races and ethnicity (Wajchenberg, 2000). Thicker s adipose tissue is protective in nature which behaves as shield against the adverse effects of obesity and other metabolic alterations that supports the healthy obese phenotype found in Caucasians. In contrast small s adipose tissue and large V- adipose tissue are highly vulnerable to metabolic dysregulations causing obesity and diabetes, which supports the metabolically obese phenotype as found in South Asians with special reference to Indians (Indulekha et al., 2015)

1.4.2 W-adipose tissue metabolism

W-adipose tissue metabolism is composed of round or oval shaped mature adipocytes which store neutral triglycerides in the form of large lipid droplets occupying 90% of the cell cytoplasm and have few mitochondria and small smooth and rough endoplasmic reticulum. These adipocytes are interconnected by a network of innervated connective tissue with lose vascularization (Gomez-Hernandez et al., 2016). Adipocytes uptake FAs liberated from liver and plasma through chylomicron and store them as triacyl glycerol (TAG). Hormone sensitive lipase cleaves TAGs to FA and glycerol contained in perilipin coated lipid droplets that gets transported to active muscles for energy production (Petersen & Shulman, 2018). *De novo* lipogenesis is also carried out by adipose tissue in lower animals which consume high carbohydrate diet. However, in humans' lipogenesis in adipose tissue is extremely low in comparison to that carried out by liver. But to dispose off high carbohydrate, adipose tissue carries out lipogenesis significantly (Lafontan, 2009).*De novo* lipogenesis is through FA synthesis from Acetyl Co-A by activating the rate limiting enzyme acetyl- CoA carboxylase (ACC) which stimulate the release of insulin from pancreas (Petersen & Shulman, 2018).

Insulin is an important hormone that plays an imperative role in regulation of energy homeostasis and metabolism. It facilitates translocation of GLUT4 to the plasma membrane to mediate glucose uptake in adipocytes. This insulin mediated glucose uptake provokes glycolytic and lipogenic enzymes with activation of sterol regulatory element binding protein (SREBP) -1c and carbohydrate response element-binding protein (CREBP), main transcriptional factors that regulate lipogenesis in adipocytes (Luo and Liu, 2016). SREBP-1c positively stimulates Peroxisome Proliferator-Activated Receptor (PPARγ), the master transcriptional factor which drive lipogenesis

by activating its dependent lipogenic genes like fatty acid synthase (FAS), lipoprotein lipase (LPL) etc. (Moseti et al., 2016).

The life span of human adipocyte has been reported to be around 9.5 to 10 years wherein, TG are replaced roughly 6 times during the life of an adipocyte and ~8.4 % of adipocytes are replaced every year irrespective of obesity category and age (White et al., 2019). Apart from mature adipocytes, W- adipose tissue comprises other cells like pre-adipocytes, endothelial cells, macrophages and Adipocyte derived mesenchymal stem cell (ADSC) which collectively attribute a dynamic endocrine function of W- adipose tissue (Figure 1.5.) (K. Sun et al., 2011).

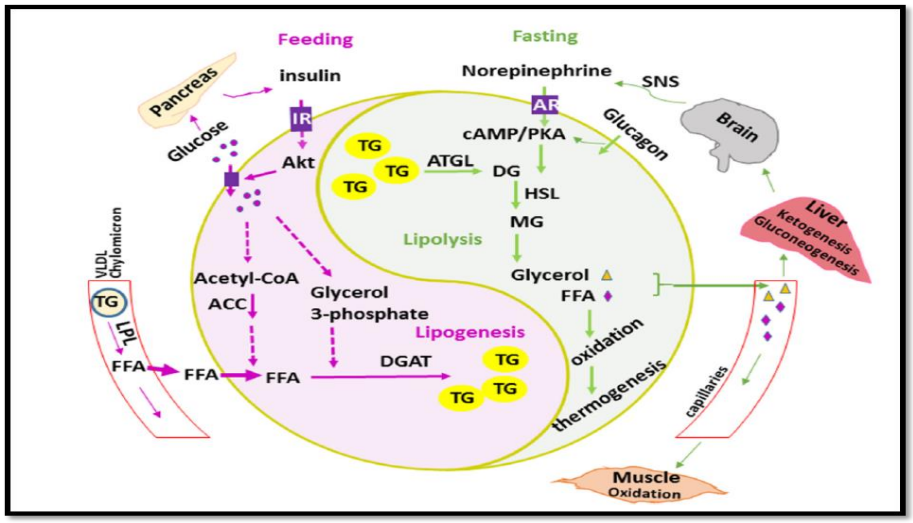

Figure 1. 5: Lipid metabolism in peripheral tissues: Lipogenesis and lipolysis pathway regulated by AT in response of nutrients (L. Luo et al., 2016).

1.4.3 W- adipose tissue as an endocrine organ

Adipose tissue secrete biologically active adipocyte secretory factors known as adipokines, which are versatile in function and action as they may be autocrine, paracrine and endocrine. These adipokines are hormones, growth factors, peptides, cytokines, angiogenic factors, micro RNA (miRNA) etc. secreted in response to the cellular stimuli which dynamically remodel adipose tissue and maintain energy homeostasis (L. Luo & Liu, 2016; K. Sun et al., 2011). Apart from adipocytes, endothelial cells, immune cells and specialized stem cells ADSC, present in adipose tissue secrete plethora of growth factors, angiogenic factors, cytokines, wound healing factors etc. (Kapur et al., 2013).

Adipocytes secrete two important hormones adiponectin and leptin which regulate energy homeostasis and insulin sensitivity. Resistin is also a major adipokine secreted

by adipose tissue resident macrophages. Whereas, other immune cells of adipose tissue and endothelial cells secrete adipokines like apelin, visfatin, TNF-α, IL-1α and β, IL-6, transforming growth factor (TGF)-β, plasminogen activator inhibitor (PAI-1) and monocyte chemoattractant protein (MCP)-1 etc. (Figure 1.6) (McArdle et al., 2013).

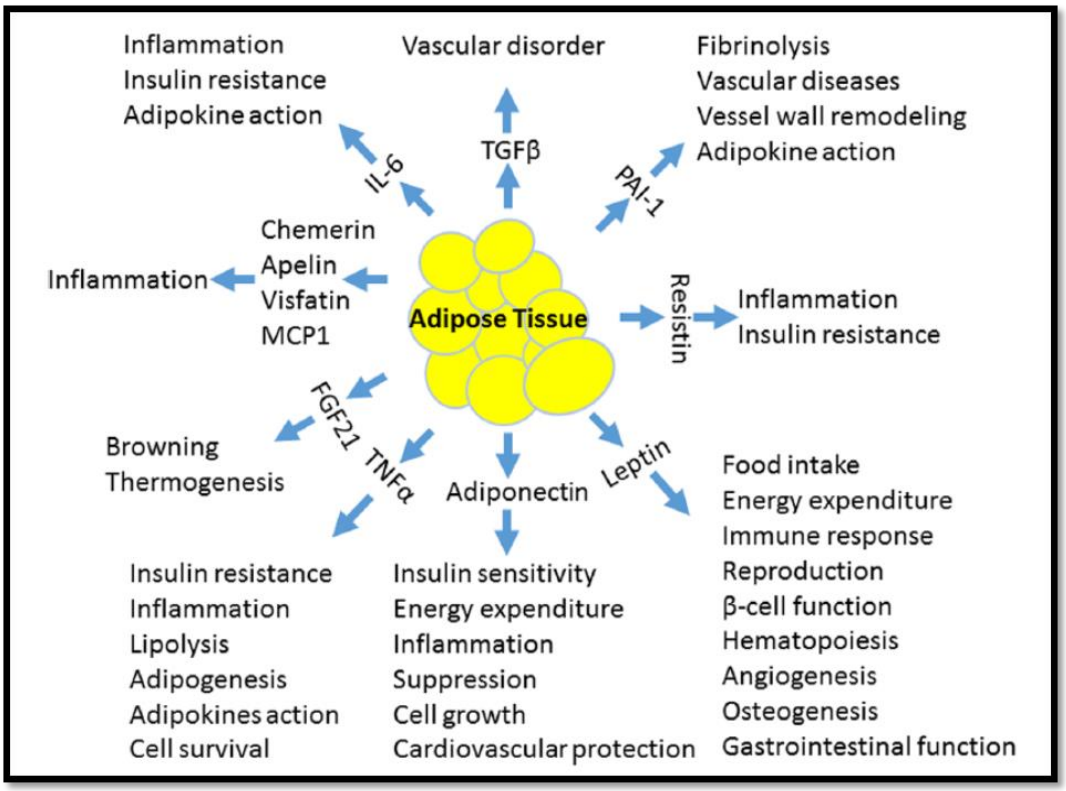

Figure 1. 6: Physiological functions of adipokines (L. Luo & Liu, 2016)

1.4.3.1 Leptin

Leptin produced by mature adipocyte, also known as satiety hormone was the first adipokine, discovered in 1994. It is a 16kDa peptide encoded by ob gene which regulates hunger and hyperphagia. It's concentration in circulation ranges from 6–15.25 ng/mL (Mahadik et al., 2008). Adipocytes primarily secrete leptin that crosses blood brain barrier and binds to leptin receptor present in the hypothalamus of brain which, is the regulatory centre of food intake (Ahima, 2008). It acts through Janus kinase- signal transducer and activator of transcription (JAK2- STAT3) signaling pathway and inhibits the orexigenic neurons like Neuropeptide Y(NPY), Agouti-related protein (AgRP), galanin, orexin, melanin concentrating hormone (MCH) and galanin like peptide. The satiety effect is also exerted by regulation of anorexigenic hormones like Proopiomelanocortin (POMC), Brain-derived neurotrophic factor (BDNF) etc. Leptin stimulates insulin secretion from pancreatic β cells and thus regulates glucose homeostasis. It also plays an important role in energy expenditure in

B-adipose tissue (L. Luo & Liu, 2016). However, higher levels of leptin are associated with obesity and metabolic syndrome (Mazor et al., 2018).

1.4.3.2 Adiponectin

Adiponectin, a 30kDa peptide is one of the major adipokine produced by mature adipocyte belongs to complement 1q family. It attributes multiple functions like maintaining insulin sensitivity, anti-inflammatory activity and cardio protection. It is abundantly found in circulation around 10-30ug/ml which is high enough compared to other hormones (Mahadik et al., 2008; Pajvani et al., 2003) Three forms of adiponectin exist: low molecular weight adiponectin (67kDa), intermediate adiponectin of 120kDa and higher molecular weight adiponectin >300kDa, among which the latter one depicts highest insulin sensitivity. It binds to adiponectin receptor I and adiponectin receptor II and activate downstream signalling through adenosine monophosphate activated protein kinase (AMPK) which suppresses gluconeogenic enzymes phosphoenolpyruvate carboxylase and glucose 6-phosphatase which reduces HGP and stimulates insulin sensitivity in liver (Z. V. Wang et al., 2016). It activates ceramidase which inhibits inflammatory response generated through ceramides. Adiponectin provokes insulin sensitivity in muscle and other tissues by modulating fibroblast growth factors and hepatic growth factors (HGF). Apart from insulin sensitivity adiponectin has been found to regulate energy homeostasis (Ghoshal et al., 2015).

1.4.3.3 Resistin

Resistin is a 12kDa cysteine rich peptide predominantly secreted by adipocytes and adipose tissue macrophage (ATM) in mice and humans respectively. It is pro-inflammatory adipokine which is secreted due to excess calorie intake that leads to metabolic alterations like obesity, insulin resistance and diabetes. It is found in the circulation in the range of 10.5 (7.25–12.9) ng/ml (Mahadik et al., 2008). It activates Mitogen Activated Protein Kinase (MAPK) and serine threonine kinases which phosphorylates IRS-1 and down regulates insulin signaling causing insulin resistance (Sorop et al., 2017). It assaults adipocyte maturation and leads to dyslipidemia. Detailed information on resistin is mentioned in chapter 5.

1.4.3.4 TNFα

TNFα is a multifunctional 17kDa cytokine, which governs several physiological processes like cell proliferation, wound healing, inflammation etc. It is one of the predominantly secreted cytokine. It is majorly secreted by all monocytes and

macrophages of the body. It is also secreted by adipocytes, ATMs and ADSC in response to inflammatory stimuli. It binds to TNF- receptor (R) and mediates inflammation by activating inflammatory pathways like nuclear factor κB kinase subunit β (NFκB), c-Jun N-terminal kinase (JNK) and MAPK and dysregulates adipocyte metabolism (Castoldi et al., 2016). These serine threonine kinases reduce auto-phosphorylation of IR with concomitant serine -307 phosphorylation and thus, downregulate tyrosine kinase activity which culminates into inhibition of insulin signaling cascade and glucose uptake (Hotamisligil, 2000). It inhibits adipogenesis and induces lipolysis thus increasing, the circulating FFAs and renders insulin resistance in adipocytes and peripheral tissues. It is secreted by adipose tissue during excess stored energy and in response of circulating FFAs (K. Sun et al., 2011).

It also provokes insulin resistance in peripheral tissues like liver, adipose tissue and muscle through ceramide production (Hotamisligil, 2000). Extensive information for TNFα is elaborated in chapter 5. Energy homeostasis is not only maintained by W-adipose tissue but B- adipose tissue also plays an imperative role in energy balance of an organism.

1.4.4 Origin and Dynamics of B-adipose tissue

Several phenomena have been documented for the presence of B- adipose tissue in adults which constitutes 1. Differentiation of ADSC into *de novo* brown adipocytes. 2. Trans-differentiation of existing white adipocytes into brown adipocytes which are known as beige or brite adipocytes. ADSC comprise a pool of population which show presence of myogenic factor 5 (MYF5) which is responsible for differentiation of ADSC into brown adipocytes or myocytes. Thus, it has been established that brown adipocytes and myocytes share a common pool of cells. Specifically, $MYF5^{+}$ cells which possess PR-domain containing 16 (PDRM16) transcriptional factor, are responsible for production of brown adipocytes (Barberá et al., 2001; Seale et al., 2008; Timmons et al., 2007). PPARγ coactivator alpha (PGC-1α) regulates mitochondrial biogenesis and stimulates adipogenesis of brown adipocytes, which further activate uncoupling protein (UCP)-1 in them. PDRM16 KO mice were deficient in mitochondrial functions, their number and they failed to regulate temperature on cold stimuli (Moonen et al., 2019). White adipocytes that are found to express UCP-1 and are produced from MYF5 negative population are known as beige or brite (brown in white adipocyte), which are produced by trans-differentiation of

white adipocytes to brown adipocytes. They do not express PDRM16 rather express Homeobox (HOX) genes (Cedikova et al., 2016).

B-adipose tissue plays an essential role in thermoregulation of an organism. In lower animals it regulates the body temperature during extreme cold in non-shivering manner. Classically in humans and higher primates it was observed that B-adipose tissue was present in infants. It is majorly located between the scapulae, in retroperitoneum and great vessels (Betz et al., 2015). It is composed of adipocytes having high numbers of mitochondria with under developed endoplasmic reticulum and multi lipid droplets. The heme cofactor of cyto-chrome oxidase enzyme of mitochondria imparts brown colour to this class of adipose tissue, hence named B-adipose tissue (Cedikova et al., 2016).

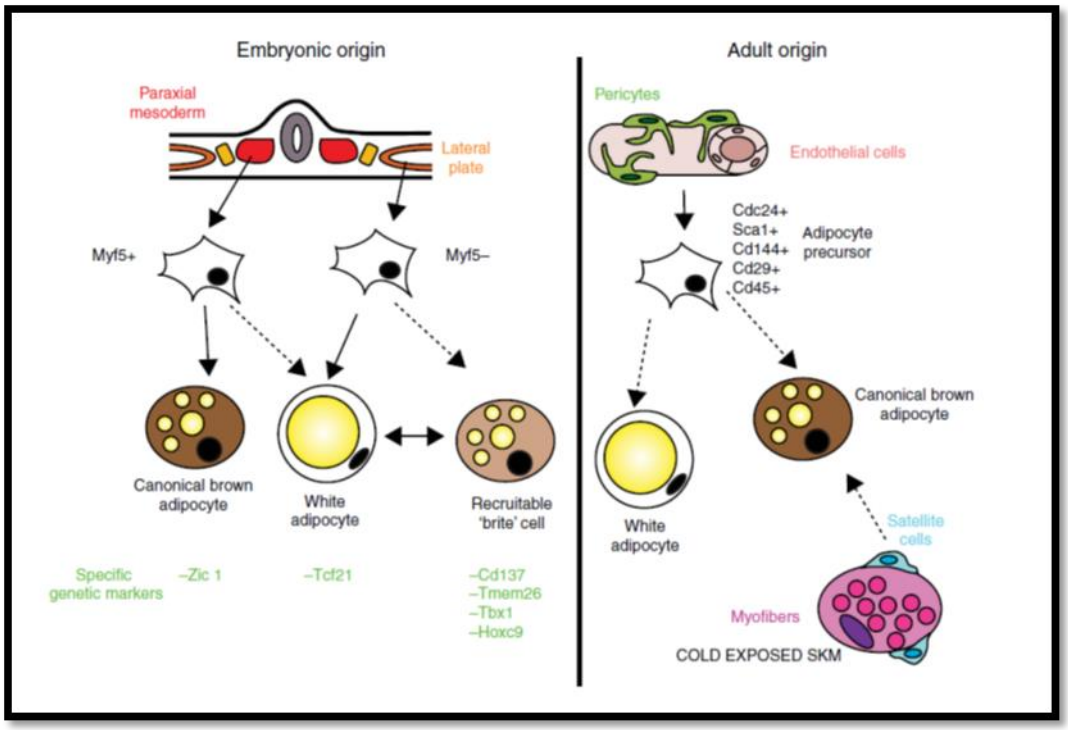

Figure 1. 7: Origins and kinetics of B-adipose tissue (Carobbio et al., 2013).

B-adipose tissue regulates body temperature through activation of two important transcriptional factor UCP-1 and PGC-1α. Cold stimuli stimulate norepinephrine to bind the β3 adrenergic receptor located on the brown adipocytes and activate UCP-1, which translocate protons and uncouples mitochondrial electron transport chain from adenosine triphosphate (ATP) synthase that liberates energy in the form of heat by β oxidation of FAs (Moonen et al., 2019). This phenomenon of heat production by mitochondria is known as "proton leak". FA β oxidation is high in B-adipose tissue

which liberates energy in the form of heat (Figure 1.7.) (Cedikova et al., 2016; Moonen et al., 2019).

1.5 Pathophysiology of obesity

Clinically, expansion of adipose tissue with elevated levels of FFAs with profound body weight is known as obesity. Excess of FA accumulation leads to expansion of adipose tissue where it is dramatically remodelled. Obesity occurs via two phenomena called as "hypertrophy and hyperplasia". Surplus FAs provoke adipose tissue expansion which accommodates maximum plausible lipids. Adipocytes become enlarged circular shaped with huge lipid droplets and progressive expansion of adipose tissue leads to hypertrophy (Reilly et al., 2017). Several cellular and molecular events that take place during hypertrophy includes secretion of TNFα, insulin like growth factor (IGF)-1 and other pro-inflammatory cytokines that provoke adipogenesis. The hypertrophic adipose tissue instigates adipogenesis which give rise to new adipocytes through adipocyte differentiation paradigm. Thus, with commencement of adipogenesis, adipocyte hypertrophy and hyperplasia occur simultaneously during obesity. Elevated levels of inflammatory cytokines activate adipose tissue resident immune cells and macrophages (de Ferranti et al., 2008). These macrophages are aggravated and get class-switch from M2 to M1 phenotype. These dendritic macrophages stimulate inflammatory pathways like NFKβ, JNK, MAPK which induce inflammation via activation of inflammasome complex through nucleotide binding domain and leucine rich repeat containing protein (NLRP)3 (U. Jung & Choi, 2014).

During obesity, adipose tissue undergoes drastic transition in cellular composition and endocrine function. With increased adiposity levels of pro-inflammatory cytokines and adipokines leptin, resistin, visfatin, CRP, IL-1β upsurge with reduction in adiponectin, omentin, IL-10, IL-4 thus, causing a state of inflammation (D.-m. Zhang et al., 2018). High fat diet in mice and humans both extravagant leptin secretion by adipocytes. Recent studies have deliberated that during obesity circulating levels of Matrix metalloproteinases (MMPs) 2 increases which cross blood brain barrier and cleaves the extracellular domain of leptin receptor (Morris, 2018). Thus, dysfunctional leptin receptors diminish downstream extracellular signal regulated kinase (ERK) and STAT3 signaling pathways. Thus, accumulated leptin cannot bind leptin receptors in hypothalamus and thus a state of leptin resistance occurs with hyperleptinemia. As a

result, there is dysregulation in feeding behaviour and satiety which culminate into hyperphagia and weight gain inducing obesity (Mazor et al., 2018).

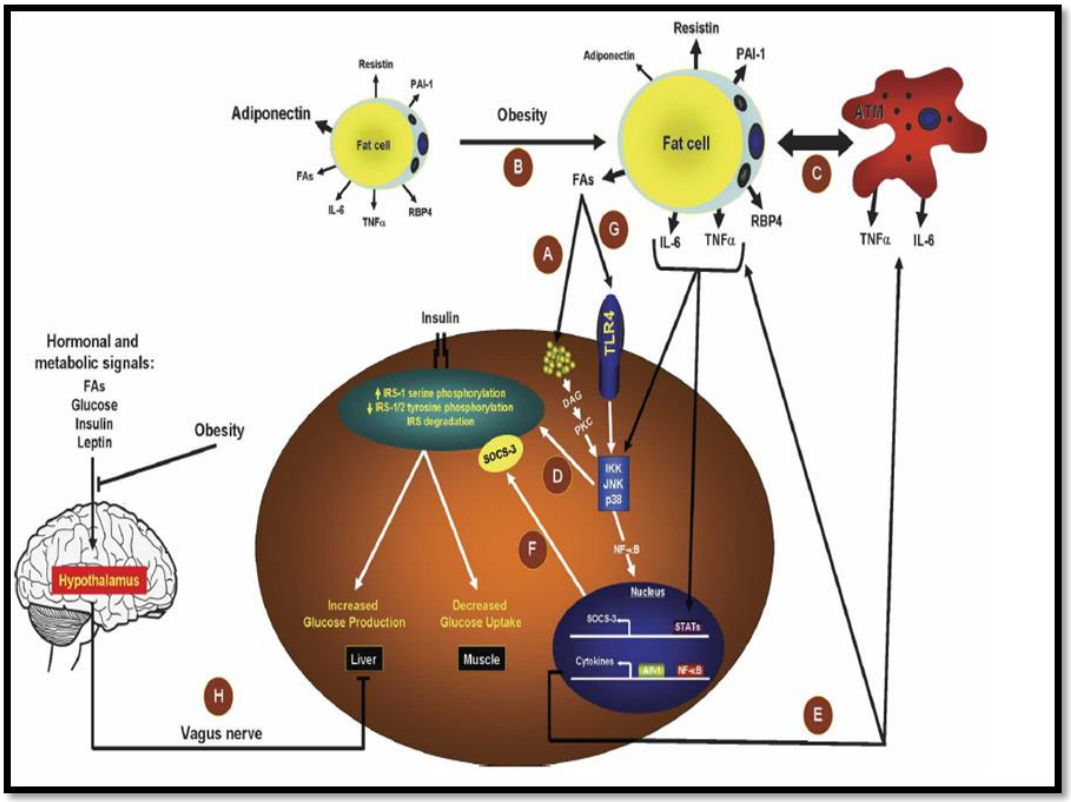

Figure 1. 8: Obesity mediated metabolic dysregulations. (Qatanani et al., 2007)

Adiponectin one of the major adipokine secreted by adipocytes for maintaining insulin sensitivity is drastically reduced in obesity. Excess of fat and circulating FFAs downregulates adiponectin transcript which reduces its production and secretion. Elevated oxidative stress and inflammation mitigates adiponectin action (Nigro et al., 2014). Moreover, it has been examined that AdipoR1 and AdipoR2 are downregulated as the adaptor protein-containing pleckstrin homology domain, phosphotyrosine-binding domain and leucine zipper motif 1 (APPL1) are altered and reduced during obesity. This event diminishes binding of adiponectin to the receptors and thus, causes adiponectin resistance. Eventually obesity stammers both adiponectin hormone as well as its receptors which in turn reduces AMPK activity. Adiponectin plays a major role in glucose homeostasis thus, its diminished levels elevate de novo HGP and lipogenesis which causes non-alcoholic fatty liver disease. Moreover, adiponectin inefficiently regulates insulin signaling in muscle which causes muscle insulin resistance (Engin, 2017). Obese subjects were deficient in adiponectin messenger

ribonucleic acids (mRNA) transcripts and reduced levels of adiponectin secretion. (Mahadik et al., 2008).

FAs act as ligand to TLR4 and activate production of pro-inflammatory cytokines TNFα, resistin, IL-1β etc. and stimulate inflammatory pathways like NFκβ, JNK etc. Moreover, activation of TLR4 and excess of FAs during obesity attract M1 macrophages infiltration (Jang et al., 2017). Hypertrophic adipocytes secrete several chemokines like MCP-1, MCP-2, MCP-3, Regulated upon Activation, Normal T cell Expressed, and Secreted (RANTES), chemokine (C-X-C motif) ligand. (CXCL)14 etc., that direct macrophages from bone marrow to adipose tissue. Transgenic mice subjected to lipoatrophy demonstrated infiltration of macrophages in the adipose tissue. Thus, macrophage infiltration rapidly increases in adipose tissue which worsens inflammation in the tissue (K. Sun et al., 2011). Interestingly, adipocytes transdifferentiate into macrophage and these manifests accountability of around 80% macrophages in adipose tissue during obesity. Macrophages rapidly undergo class switching from alternatively activated M2 to classically activated M1 macrophages which persists and result into inflamed adipocytes (Hill et al., 2018). Thus, a state of chronic inflammation is established in adipose tissue which finally culminates into systemic inflammation (Figure 1.8.) (L. Chen et al., 2015).

In advanced stage of obesity, as evident hypertrophic adipocytes are surrendered to hypoxia, inflammation, diminished angiogenesis and fibrosis (Ferretti & Mariani, 2019). Elevated inflammation in adipocytes activate serine threonine kinase that downregulates tyrosine activity of IR and thus, downregulate insulin signaling and embark insulin resistance in adipocytes which mitigates systemic energy homeostasis and causes peripheral insulin resistance (L. Chen et al., 2015). The severe cycle of hypertrophy and hyperplasia enhance lipolytic activity and dysregulated FA uptake in adipocytes, which leads to leaky adipocyte phenotype. Moreover, M1 macrophages surround the hypertrophic adipocytes which undergo apoptosis to form crown like structures (CLS) around the apoptotic adipocytes which are then phagocytosed by macrophages for their immune clearance (Hill et al., 2018; K. Sun et al., 2011). As a result, excess of FFAs is secreted in circulation which enter portal veins and ectopically deposit into other organs like liver, muscle, heart and kidneys which causes fatty liver diseases, muscular dystrophy, atherosclerosis etc. These alterations have been very well demonstrated in high fat and cafeteria diet induced obesity models and

in genetic models like ob-ob and db-db mice. (Mazor et al., 2018; Morris, 2018; Nigro et al., 2014).

Obesity not only mitigates functions of adipocytes, but other important cells of adipose tissue also. It is a highly orchestered connective tissue. Apart from adipocytes, macrophages, endothelial cells, ADSC also play important functions. They have been designated chief position in the field of regenerative medicine (P. Bora et al., 2017). It is therefore, incumbent to understand the stem cell biology and insights of ADSC.

1.6 Stem cells Biology

Stem cells are specialized type of cells which play crucial role in development, repair, regeneration and maintenance of whole body. They are present in all multicellular organisms (Kumar et al., 2010). There are several types of stem cells which are peculiarly present during different time periods of life span of the body. They are classified on the basis of their origin namely embryonic stem cells (ESC), adult stem cells which further comprises of hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC), induced pluripotent stem cells (iPSC). Stem cells are totipotent, pluripotent, multipotent and unipotent in nature. Zygote is the only totipotent cell of the body that gives rise to all types of body cells (Mahla, 2016).

Stem cells have indefinite capabilities to self-replicate, express low major histocompatibility complex (MHC) and have immense potentials to differentiate into multiple cell types (Shapiro et al., 2019). Stem cells have profound secretory function, they are attracted towards injured tissue site and repair the wound by secreting several biochemical factors like growth factors, vasculative factors, chemokines, anti-inflammatory cytokines, angiogenic factors collectively known as stem cell secretome (Kapur & Katz, 2013). They are highly dynamic and are immunomodulatory in nature which suppress inflammation and immune rejections. All these properties of stem cells have prioritized them in field of medicine, regenerative therapy, bio-pharma and stem cell industries. A special branch of stem cell biology has developed past 20 years and is tremendously growing with intricate developments and discoveries (Figure. 1.9.) (Shapiro et al., 2019).

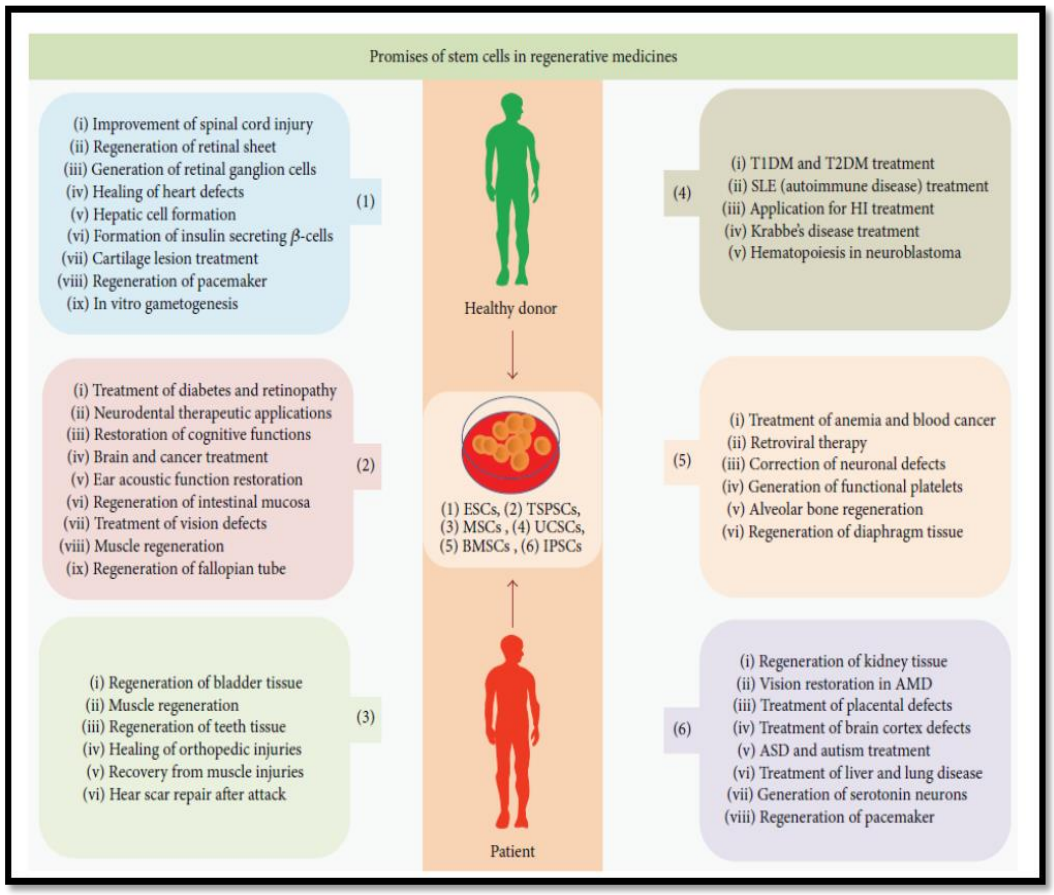

Figure 1. 9: Applications of stem cells in regenerative medicine and therapeutics (Mahla, 2016)

1.6.1 ESC

ESC are derived from inner cell mass of embryo of gastrula stage. These stem cells are pluripotent in nature which give rise to more than 200 different types of somatic cells including all the three germ layers namely mesoderm, ectoderm and endoderm (Cyranoski, 2018). During development ESC differentiate first into cells of ectoderm lineage like neurons, skin cells and pigment cells or into mesodermal origin cells like adipocytes, osteocytes, chondrocytes, myocytes and heart. ESC differentiates into endoderm lineage cells like pancreas, respiratory, digestive, lung, liver and intestine (Shiraki et al., 2014).

ESC express specific set of genes which govern pluripotency to this class of stem cells. They are octamer-binding transcription factor (OCT4), SOX2, NANOG, Kruppel-Like Factor (KLF) 4, c-MYC. OCT4, NANOG and SOX2 are core regulatory genes that regulate pluripotency and maintain self-renewal of ESC. Which has been deduced by lineage tracing studies. Amongst them OCT4 is required for self-renewal of the ESC by inhibiting its differentiation into trophectoderm (Z. Wang et al., 2012).

Epigenetic modifications like DNA methylation, DNA hydroxymethation and histone modifications attribute tight regulation of ESC. A group of Polycomb (PcG) protein complexes exist, which are evolutionary transcriptional repressors in ESC, regulate pluripotency of stem cells (Boland et al., 2014). They regulate HOX gene and cell cycle regulating genes of the stem cells. Almost all PcG genes target more than 2000 genes which mostly drive lineage specific differentiation (Wutz, 2013).

Despite of vast differentiation potentials of ESC, there are several regulatory affairs that restrict application of ESC in regenerative therapy. One of them include religious restriction in use of embryo as National Institute of Health (NIH) had levied restrictions on the use of embryos and generation of new human embryonic cell lines (Greely et al., 2007).Since January, 2009, twenty-two human ESC lines are available but are dangerous to use as they tend to accumulate mutations and diverge them towards cancerous cell formation. Ethical issues are also concerned with the health risk developed in women during retrieval of embryos and several other ethical concerns in use of embryos for research (Lo et al., 2009).

1.6.2 Adult Stem Cells

Stem cells are also present in the tissues of an adult. Almost all adult tissues have different kinds of stem cells. Bone marrow is a richest source of stem cells delineated into HSC and MSC. Adipose, muscle, dental pulp, placenta, etc. are repertoire of MSC. (Shapiro et al., 2019).

1.6.3 HSC

Cord blood is a rich source of HSC and are immune characterized by cluster of differentiation (CD)$34^{+}CD133^{+}CD31^{+}CD38^{-}$ etc. During the developmental phase, HSC are destined to aorto-gonado - mesonephros region which are shifted to fetal liver and finally to the bone marrow which is a permanent abode for HSC throughout the lifespan of higher animals. A single HSC can give rise to whole hematopoietic system which includes lymphoid lineage which comprise T cells, B cells, natural killer cells and myeloid lineages that comprise megakaryocytes, erythrocytes, granulocytes and macrophages. Dendritic cells are developed from common myeloid precursors which are found in adult tissues also (Ng et al., 2017). These cells are pluripotent in nature and give rise to all blood cells. They have been used to treat CVDs and ischemia. They are best promising tool for autologous or allogenic transplantation (Lo & Parham, 2009). Autologous or allogenic bone marrow has been a promising therapy to treat

several haematological problems. *In vitro* differentiation of HSC into majority of cell types are explored in regenerative therapy as they are capable of differentiating into neural cells, hepatic cells and cardio-myocytes. HSC are applied for amelioration of collagen disorder, muscular disorder to treat CVD and ischemia. Granulocyte colony stimulating factor is used to treat brain stroke and deformities generated in unions due to fractures (Ogawa et al., 2013). They are profound in ameliorating hematopoietic malignancies and reduce side effects of chemotherapy (Lo & Parham, 2009).

1.6.4 MSC

MSC are multipotent stem cells present in majority of the tissues of the body. They are fibroblastic stromal cells which maintain the cell pool and architecture of the tissues and organs. Their ability to adhere on plastic surface and to differentiate into multiple lineage have recognized them as MSC. They express CD44, CD105, CD71, CD90 and CD73 but do not express CD31, CD34, CD133, CD45, CD11b etc. (Chosa et al., 2018). They are multipotent tissue resident stem cells. Adult tissues like bone marrow, adipose tissue, peripheral blood, heart, muscle, dental pulp have profound niche of MSC. They are also found in birth associated adult tissues like placenta, cord blood, umbilical cord, whortman jelly, amniotic fluid, chorion villi etc (Berebichez-Fridman et al., 2018; Rizvanov et al., 2016).

MSC have self-renewal, multi lineage differentiation, immune suppressive, immune-modulatory, angiogenic and wound healing capacity. They are widely explored in regenerative therapy and have proved their abilities to cure several wounds, metabolic or immune deformities like burnt tissues, diabetes, diabetic foot ulcers, cardiac ischemia, muscle dystrophy, degenerative diseases like osteo and rheumatoid arthritis (Berebichez-Fridman & Montero-Olvera, 2018; Rizvanov et al., 2016). Their wide application and easy access and lesser ethical constraints compared to ESC have perpetuated them as better and alternative candidates in regenerative therapy (M. Wang et al., 2018).

MSC have more differentiation and healing capacity then HSC as observed in a comparative study of treatment of cardiac ischemia with HSC and MSC (Armiñán et al., 2010). HSC are used to treat blood disorders and post chemotherapy and radiations but they are attributed to generate cancer stem cells, which make them dreadful to be applied in regenerative medicine. (Rizvanov et al., 2016). MSC are preferred more over HSC which migrate and home the wounded tissue by secretion of biologically

active chemokines, activation of regulatory T Cells, modulation of tolerogenic dendritic cells, generation of immune reaction and apoptosis (Hass et al., 2011). They express very low MHC and have reduced immune response generation against antigens which makes them ideal cells for autologous and allogenic transplantation (Kadle et al., 2018).

MSC have profound secretory functions, human MSC secrete Indoleamine 2,3 Oxygenase (IDO), supresses immune response by suppressing proliferation and efficacy of immune cells like T cells and natural killer cells. Kidney grafts along with MSC are well tolerated as these IDO acts as immune suppressor (Gebler et al., 2012). During inflammation MSC induce nitric oxide synthase and exert immune modulation by inhibition of STAT5 thus, causing apoptosis of T cells. Pro-inflammatory cytokines like TNFα, IL-1 probably activate TNF stimulated gene 6 (TSG6) that renders anti-inflammatory actions and reduces inflammation and size of scar or infarction (Bai et al, 2012). Moreover, MSC secrete several anti-inflammatory cytokines and chemokines like IL-10, - C-C motif chemokine (CCL)2, prostaglandins which in coordination with IDO suppresses inflammation (M. Wang et al., 2018).

MSC from different tissues have different potentials. Bone marrow derived MSC (BONE MARROW STEM CELLS) are recognized as "Gold Standard stem cells" after they were discovered by Friedenstein and colleagues (Friedenstein et al., 1966). They have been differentiated into effective islet like clusters and are being transplanted into diabetic patients. They have been grafted for amelioration of renal failures. These MSC well differentiate into cardio-myocytes for treatment of cardiac ischemia. Moreover, BONE MARROW STEM CELLS have been implicated in the field of age related degenerative diseases like osteo and rheumatoid arthritis as they differentiate into the same lineage cells. Clinical trials on BONE MARROW STEM CELLS have been conducted for amelioration of TIDM, Cardiac ischemia, multiple sclerosis, osteoarthritis (OA), renal failures etc (Awad et al., 2019; Bhansali et al., 2017; Chahal et al., 2019; Fotino et al., 2010; Reinders et al., 2014; Uccelli et al., 2019).

However, currently comparative analysis of MSC from different tissues have been carried out among which, ADSC lead their stem cell potentials and ability of wound healing compared to that of BONE MARROW STEM CELLS. ADSC, placental MSC, BONE MARROW STEM CELLS, dental MSC etc. have been compared for

their stemness properties and many properties of ADSC have placed in the centre of stem cell biology (W. Zhou et al., 2019).

1.6.5 ADSC

Human W-adipose tissue is rich source of MSC known as ADSC. They comprise upto 1-10% of total stem cell population when compared to 0.1% of MSC population derived from bone marrow. Abundance and ease of availability of adipose tissue have proved ADSC to be priority stem cell type. They can be obtained from S-adipose tissue and V- adipose tissue excised surgically or through liposuctions. Adipose tissue and lipoaspirates are easily available with consent of the patients as they are discarded after surgery (Peptan et al., 2006). ADSC can be isolated from adipose tissue by collagenase I digestion and are obtained from stromal vascular fraction (SVF) (Cawthorn et al., 2012). They can be separated by sorting through flow cytometry or can be purified by sequential passages (#). Pure ADSC demonstrate increased expression of $CD105^+$, $CD44^+$, $CD90^+$. ADSC have magnificent potentials of rapid proliferation, wound healing, angiogenic, self-renewal, immune-modulation and multi-lineage differentiation capabilities (Roldan et al., 2011).

ADSC have marvellous differentiation capabilities. These cells express pluripotent genes like OCT4, SOX2, NANOG and KLF which paves them the way for differentiating into several cell types of all the three germ lines like mesoderm which constitute adipocytes, chondrocytes, osteocytes, myocytes, followed by endodermal lineage cells like islets, hepatocytes, cardiomyocytes and ectoderm lineage cells like neurons, skin cells etc. (Orgun et al., 2017; Pérez et al., 2015; U. Thakkar et al., 2014).

The environmental cues and the physiological conditions attribute towards functionality of ADSC. S-adipose tissue and V-adipose tissue differ drastically in their functions and properties (Peptan et al., 2006). ADSC isolated from S-adipose tissue of thighs and lower limbs are the most promising and healthy compared to those of abdominal region. Yield of ADSC ranges from $0.1x10^5$ cells/ml of lipoaspirate derived from abdominal tissue to $1.5x10^5$ cells/ml of lipoaspirate from thighs which denotes variability of yield of ADSC from different site of tissues (Tsekouras et al., 2017). They also differ in their proliferative abilities, secretory nature, propensity towards senescence and wound healing.

ADSC secrete plethora of growth factors, angiogenic factors, cytokines, chemokines etc. The bundle of secretory factors released by ADSC are termed as secretome.

Proteomic analysis has revealed almost thousand proteins to be present in ADSC secretome. Angiogenic factors like IGF-1, HGF, vascular endothelial growth factor (VEGF) and transforming growth factor β (TGFβ) are abundantly present in the secretome (Vizoso et al., 2017).

ADSC also secrete anti-inflammatory cytokines like IL-10, IL-4. ADSC and macrophage co-culture provoked M2 macrophage to secrete stromal cell derived factor-1 which reduces inflammation (Skalnikova, 2013). Thus, ADSC have superb immune-modulatory effects through which they suppress inflammation and graft rejection. ADSC secretome is rich in neurotrophic factors like mesencephalic astrocyte derived neurotrophic factors, meteorin, neuron derived neurotrophic factors which cumulatively render protection to neurons and other glial cells during brain injury (Kalinina et al., 2015).

ADSC secretome comprise nano-sized vesicles known as exosomes which are derived from cytoplasmic and plasma membrane content that are secreted by cells as a part of intercellular communication. They have a peculiar size of 100-250nm. They are formed by fusion of intermediate endocytic compartment, multi-vesicular bodies and plasma membrane and are released as intra-luminal vesicles (ILV) into the extracellular space (Niada et al., 2018). Exosome cargo encompass macromolecules like protein, deoxy ribonucleic acids (DNA), miRNA, mRNA, lipids, proteins etc. through which they ameliorate several metabolic disorders and wounds (Domenis et al., 2018).

ADSC secretomes have gained a cutting edge position in regenerative medicine and are best known as cell-free therapy for amelioration of several diseases and have been commercialized (Figure 1.10)(Zvonic et al., 2007).

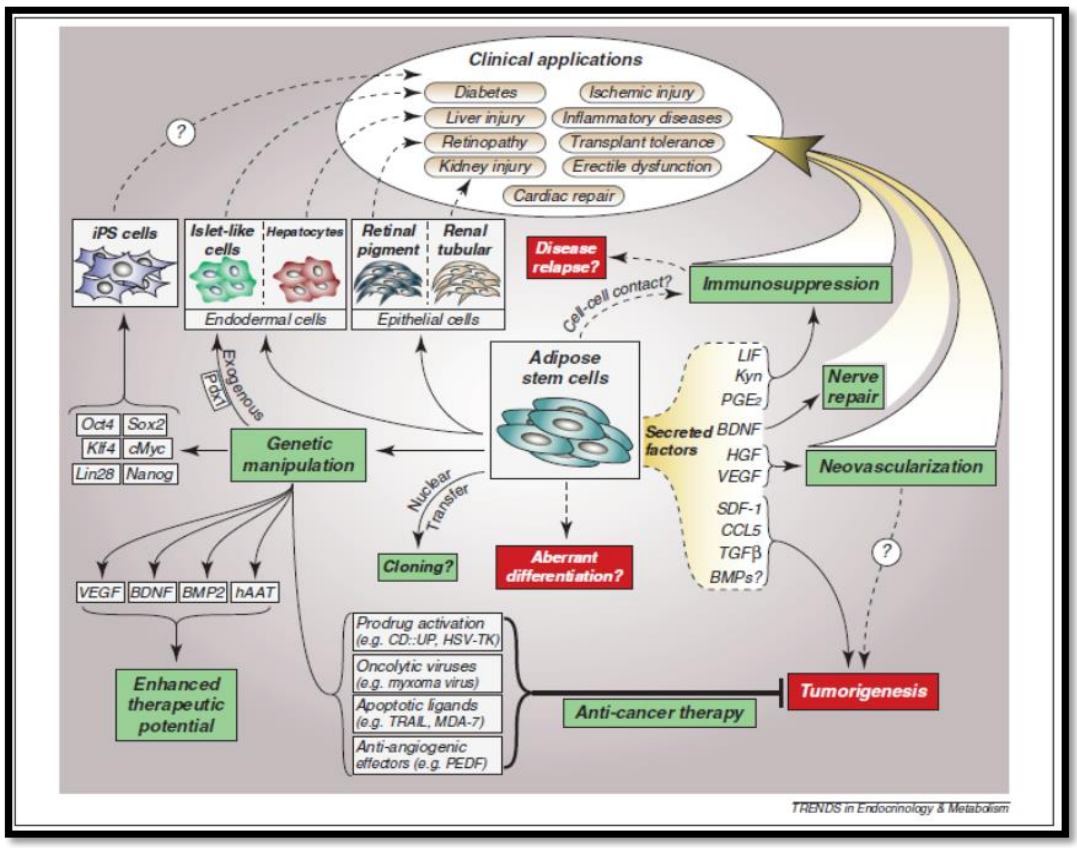

Figure 1. 10: Clinical applications of ADSC

1.7 Regenerative potentials of ADSC

Extensive comparative analysis between ADSC and BONE MARROW STEM CELLS have been performed which depicted that genome of BONE MARROW STEM CELLS is more heterogeneous than that of ADSC which is essential for ADSC to be transplanted for clinical relevance. Interestingly, ADSC do not depend on the mitochondrial respiration as they are more tolerant towards hypoxia which assists them maintain stemness when compared to BONE MARROW STEM CELLS (W. Zhou et al., 2019). Moreover, response of ADSC and BONE MARROW STEM CELLS towards pro-inflammatory cytokines revealed that ADSC had reduced immunogenicity with concomitant high immune-suppressive potentials which bestow them as ideal candidates for allogenic transplantation with respect to the patients who require multiple injections of allogenic ADSC. They secrete IL-33 which act on endogenous immune cells of the tissue and thus renders immunosuppression and protecting the transplanted graft from immune-rejection (Asada et al., 2017). At P#0, BONE MARROW STEM CELLS depicted higher number of immune cells when compared to ADSC, thus making it more susceptible towards immune-rejection (W. Zhou et al., 2019).

It is well known for its wound healing capabilities. Wounds created due to diabetes, chemo and radio therapy produce scar and ischemia. ADSC transplantation after chemotherapy and radiotherapy have rendered protection to the damaged tissues and cells. Fibroblast growth factors (FGF) secreted by ADSC play role in wound healing. It also comprises essential growth factors which protect against muscular dystrophy (Mitchell et al., 2019) and played a significant role in rendering protection against arsenic toxicity (Curtis et al., 2018).

The secretory proteins act through paracrine effect and produce collateral vessels around infarcts or ischemia with enhanced angiogenic potentials. ADSC secretome is cardio-protective attributed by the synergistic effect of IGF-1, IL-6 and VEGF. Angiogenesis is augmented by activation of VEGF/ mammalian target of rapamycin (mTOR)/AKT pathway thus protecting the animals and humans from ischemia (Orgun & Mizuno, 2017). Thus, the comprehensive analysis depicted above destines ADSC to be ideal candidates for application in regenerative medicine and cell therapy (W. Zhou et al., 2019).

1.7.1 Clinical Trials of ADSC

ADSC have been extensively applied as cell therapy for various metabolic diseases, immune diseases and several tissue and organ transplantations.

In TIDM, due to side effects and limitations of insulin and other medicines, stem cell therapy has been a promising therapeutic approach. Islets derived from ADSC were transplanted in patients suffering from TIDM, who recovered from hyperglycemia and β cell death for a long period of time (Dave et al., 2014). Moreover, ADSC have been used in treatment of diabetic foot ulcers (Gadelkarim et al., 2018). ADSC were differentiated into effective cardiomyocytes which were transplanted to the patients for treatment of CVD which depicted effective potentials of ADSC (Comella et al., 2016). OA is a commonly prevalent disease in middle aged and aged females and males. It does not have promising treatment. ADSC transplantations into OA patients have reduced inflammation, increased survival of osteogenesis, reduced osteo-clastogenesis and pain, which showed ADSC clinical trials for OA are very effective (Freitag et al., 2019).

Clinical trials of ADSC on lipopolysaccharide induced sepsis into humans, reduced fibrosis, pain, inflammation, increased angiogenesis and immunosuppression (Perlee

et al., 2018). ADSC have been implicated into autoimmune disease like multiple sclerosis (Fernández et al., 2018)

1.7.2 Clinical applications of ADSC in India

India has also made a lucrative progress in the field of stem cells biology. Stem cells are being explored for their regenerative therapy and are being modulated (Mahla, 2016). Amongst all bone marrow stem cells and ADSC have been extensively studied at pre-clinical levels. Several private clinics have started stem cell therapy for amelioration of diseases like diabetes, osteoarthritis, renal problems (Dave et al., 2014; Vanikar, Trivedi, Kumar, et al., 2014).

ADSC derived islet were co-infused with HSC into portal veins and thymus into TIDM patients for better retention of the graft and angiogenesis (Dave et al., 2014; Dave et al., 2013; U. G. Thakkar et al., 2015). Co-infused stem cells had promising immunosuppressive activity even after 5 years of post-operative follow up. Serum creatinine and other renal functions were restored in patients who received these therapy (Dave et al., 2013; Vanikar et al., 2012; Vanikar, Trivedi, Kumar, et al., 2014). Moreover, patients with renal failure under non-myeloid ablative conditioning received the co-infusion of ADSC and HSC when monitored till 6 years of transplantation were found to have 90% live renal grafts and 80% survival rate (Vanikar et al., 2018). Interestingly, ADSC were trans-differentiated into neural stem cells when co-infused with HSC into the patient with systemic neuronal and systemic brachial plexus disturbance showed recovery after 16 years of injury. Re-myelination of de-myelinated neurons in the patient which explicitly provided example of pluripotent ADSC for treatment of neuronal injuries (U. Thakkar et al., 2014).

1.8 Regulation of MSC

The abilities of MSC to self-renew, proliferate and differentiate into multiple cell types are regulated by several molecular and cellular mechanism. Majorly, miRNA regulate the stemness of ADSC. miR-16 targets Cyclin E to inhibit proliferation of MSC. miR-143 restricts entry of cells into synthesis (S) phase and thus, maintain stemness of MSC by negatively regulating cell cycle of MSC (T. Zhang et al., 2018). Contrary, there are several miRNAs which regulate MSC differentiation into multiple lineage cells. miR27 targets G2/M phase and thus promote differentiation of MSC into osteocytes (Mens et al., 2018).

Signaling pathways like bone morphogenetic proteins (BMP), Wingless type (WNT), NOTCH and Hedge Hog tightly regulate MSC differentiation into multiple cell types. BMP positively regulates both adipocyte and osteocyte, whereas WNT negatively regulate adipogenesis but positively stimulates osteogenesis (Cook et al., 2013; James, 2013).

Recently, Stimulator of chondrogenesis (SCRG)1/ Bone marrow stromal cell antigen (BST)1 ligand-receptor complex has been explored which regulate self-renewal and proliferation of MSC by upregulating expression of CD271. This ligand is secreted in extracellular space which binds to the receptor BST-1 which stimulates migration of MSC and inhibits differentiation into osteoyctes. This phenomenon is governed by activation of Focal adhesion kinase (FAK)/PI3K/AKT signaling pathway (Chosa & Ishisaki, 2018).

1.9 Signaling pathways that regulate Fate of ADSC

ADSC efficiently differentiate among various cells of all the germ layers like adipocytes, osteocytes, myocytes, chondrocytes, islets, neurons etc. Amongst all ADSC fate, adipocytes and osteocytes are the two main cell types obtained after differentiation. Fate of ADSC is governed by the signaling pathways (Figure 1.11) (Q. Chen et al., 2016).

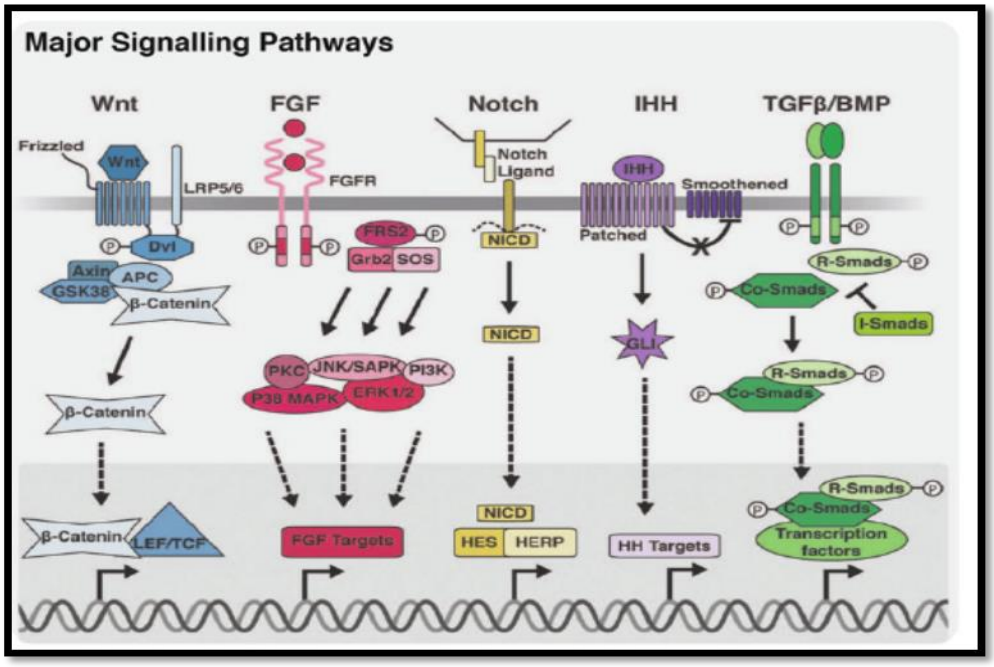

Figure 1. 11: Signaling pathways regulating ADSC fate. (Gaur et al., 2018).

1.9.1 TGFβ Super family signaling pathway

TGFβ is a super family of proteins dissected in two distinct signaling pathways based on receptors, ligands, signal transducers and effector responses. They are BMP signaling and TGFβ both of which antagonistically participate during adipogenesis (K. Luo, 2017).

1.9.1.a BMP signaling pathway

BMPs belong to the TGFβ super family of signaling molecules which were detected during ectopic bone formation. Commitment of ADSC towards specific cell type depends upon the type and concentration of BMP ligands secreted by the cells. More than 50 types of BMP ligands are secreted by cells which drive the differentiation of ADSC towards adipogenesis or osteogenesis. Precisely BMP-4 and BMP-7 at lower concentration induces adipogenesis however, increase in their concentration diverts ADSC towards chondrogenesis or osteogenesis (James, 2013).

BMP ligands bind to the receptors known as BMPR1A or BMPR1B which decides the fate of ADSC differentiation. Moreover, these ligands also bind to other class of receptors like Activin receptors which belong to the same family of proteins. BMPR1A have enhanced affinity towards BMP-2 and BMP-4 which drive ADSC towards adipogenesis. If ligands bind to BMPR1B receptors, ADSC are differentiated into osteocytes (Rahman et al., 2015).

As the ligand-receptor heterodimers are formed, receptor kinase phosphorylate thR-SMAD1, SMAD5, SMAD8 to activate the pSMAD1/5/8 complex. These R-SMADs have additional MAD homology (MH)1 and MH2 domains on N and C terminal where, MH1 binds to the DNA sequences while MH2 binds to BMPR1, thus driving the signaling (Beederman et al., 2013). Further, dissociation of SMADs from receptor enables them to interacts with comediator SMAD(CO-SMAD)4 and then gets translocated to the nucleus where, it binds specific DNA binding cofactors, repressors and activators which either activate or suppress the targeted transcription factors which govern adipogenesis, osteogenesis or myogenesis (K. Luo, 2017). Activation of BMP signaling stimulates transcription of adipogenic transcription factors (ATF) like C/EBPβ, C/EBPδ, PPARγ, C/EBPα, SREBP-1c to initiate adipogenesis. Further, once PPARγ is expressed, it's dependent target genes like FAS, LPL, Carnitine palmitoyl transferase (CPT-1), fatty acid binding protein 4 (FABP4), adiponectin, leptin etc. are

activated which drives the formation of proper lipid laden intact mature adipocytes (Schulz et al., 2009).

Similarly, BMP signaling also stimulates osteogenesis as BMP2, BMP6, BMP7 and BMP9 activate the signal through canonical BMP pathway. Osteogenic transcription factors (OTF) like Runt related transcription factor (RUNX) 2, Osterix, (Sirtuins) SIRT-1, osteoprotegerin etc. are activated which further target the target genes like Alkaline phosphatase, collagen Type I etc for formation of functional osteocytes (Beederman et al., 2013).

1.9.1.b TGFβ signaling pathway

TGFβ belongs to the super family of TGFβ super family of proteins. Here also BMP ligands bind to the TGF receptors or activing receptors. Upon binding this interaction activates SMAD2, SMAD3 by phosphorylating them. This activated pSMAD2/3 binds to the CO-SMAD4 which translocate together in the nucleus to act on their target genes. TGFβ signaling pathway inhibits adipogenesis by repressing the expression of the major ATFs C/EBPβ and C/EBPδ (Gaur et al., 2018).

1.9.2 WNT signaling pathway

WNT signaling was detected during developmental stage of drosophila. This pathway plays a central role in development of an organism, regulates stemness of stem cells. It activates stem cells to repair wounds or injury of assaulted tissues. WNT ligands bind to frizzled receptors and low density lipoprotein receptor related proteins (LRP) 5/6 co-receptors. This interaction activates intracellular dishevelled proteins which inhibits the proteosomal degrading complex comprised of β catenin (Q. Chen et al., 2016). Constitutive activation of WNT signaling accumulates dephosphorylated active β catenin which are translocated to the nucleus which in turn activates or inhibits the cascade of target genes. WNT signaling stimulates osteogenesis and inhibits adipogenesis, however but when over activated inhibit adipogenesis and osteogenesis (James, 2013).

1.9.3 Hedge Hog Signaling

Hedge hog signaling was identified in drosophila during developmental stage. These proteins bind to the receptors patched (Ptc) represses smoothened repressors and activate (glioma-associated oncogene)Gli family of transcriptional factors namely Gli1, Gli2 and Gli3. Smo activation represses transcriptional activation and thus

hinders expression of target genes. Indian Hedge-hog proteins are indispensable for activation of osteogenesis. This signaling negatively regulates adipogenesis, therefore, it is pro-osteogenic and anti-adipogenic (El-Safadi et al., 2019; James, 2013).

1.10 Fate Decision of ADSC: Adipocyte/ Osteocyte differentiation

Recent experimental evidences have depicted that differentiation of MSC into adipocyte or osteocyte is a very distinct phenomenon. Transcriptome analysis revealed that osteocytes are closer to MSC than that of adipocytes. Adipogenesis requires enhanced chromatin remodelling whereas osteogenesis commences with the niche of the OTF previously present. Anti-adipogenic or preosteogenic factors drive osteogenesis without much of DNA remodelling. Thus, evidently adipogenesis is complicated than osteogenesis and this could be the reason for rapid bone remodelling in adults and repair of bone fractures (Rauch et al., 2019).

1.10.1 Adipogenesis

ADSC differentiate into adipocytes through well orchestered process known as adipogenesis. The *de novo* adipogenesis in AT starts through differentiation of ADSC wherein, several ATFs participate in the process (James, 2013). Adipogenesis in culture is triggered when the cells become confluent by insulin, dexamethasone, glucocorticoid acts as mitogen drives the cells undergo atleast two cell divisions of cell cycle. Mitosis in cells re-organizes chromatin which triggers adipogenesis in cells (Tong et al., 2001). Krox20, an early growth element is upregulated in confluent cells when the cells re-enter the cell cycle and thus activate the early ATFs C/EBPβ and C/EBPδ. Proteins of E2F family are the regulatory proteins of adipogenesis (Moseti et al., 2016).

1.10.2 Transcriptional factors for adipogenesis

Adipogenesis is governed by transcription factors of two different families namely PPARs and C/EBPs. They drive adipogenesis from clonal expansion to formation of mature lipid laden adipocytes. Amongst all, PPARγ is the master regulator which turns on the molecular switch of adipocyte formation.

1.10.3 PPAR

PPARs are ligand activated transcriptional factors that belong to nuclear hormone receptor family (Kersten, 2002). They are named so, as PPARα on binding leads to proliferation of peroxisome. They have a non-conserved N-terminal domain, hinge

region and a C-terminal ligand binding domain. This family of transcription factors comprise of 3 members α, β and γ. PPARα is abundantly found in liver and regulates β oxidation. It is also expressed in kidney, skeletal muscle and heart where proliferation of peroxisomes is on pace. PPARα regulate nutrient metabolism including gluconeogenesis and amino acid metabolism (Dreyer et al., 1992; Nemali et al., 1988).

PPARβ is also present in many tissues but its function is still elusive. PPARγ is found abundantly in both W-adipose tissue and B-adipose tissue, muscle, liver, brain etc. PPARγ2 is abundantly found in W-adipose tissue and its main role is to propagate pre-adipocytes to adipocytes accumulated with lipid droplets. It also regulates insulin sensitivity and maintains glucose homeostasis. It forms heterodimer with retinoid X receptor (RXR) which enables it to target direct repeat (DR)-1 site of the target sequences of the genes (Chandra et al., 2008). PPARγ null mice were deficient in adipocyte formation, thus it is inevitable transcription factors for adipogenesis and lipogenesis (Moseti et al., 2016)

1.10.4 C/EBP

C/EBPs are conserved basic leucine zipper transcription factors having six members in the family among which C/EBPα, C/EBPβ and C/EBPδ are well established. All the three transcription factors play magnificent role during adipogenesis. Gain of function and loss of function experiments have deduced that mice deficient in either of the transcription factors cannot induce adipogenesis (Moseti et al., 2016)

C/EBPα deficient mice had immature adipocytes and their insulin sensitivity was lost and in vivo W-adipose tissue was not developed. Thus, C/EBPα augments and regulates insulin sensitivity in adipocytes (Farmer, 2006).

1.11 Transcriptional factors interplay during adipogenesis

Adipogenesis is a highly orchestered phenomena where ADSC are differentiated into adipocytes with temporally expressed ATF. The process is divided into early, middle and late differentiation program which include change in cell shape and size, cytoskeleton re-organization, differential expressions of target genes, lipid accumulation and expression of lipid laden phenotype of adipocytes. Lineage commitment of adipogenesis depends on chemical, physical and biological factors. The process is highly dynamic, chromatic remodelling with enhanced expression of enhancers drive adipogenesis stringently (Rauch et al, 2019). ADSC are induced to

adipogenesis in presence of adipogenic cocktail comprised of isobutylmethylxanthine (IBMX), dexamethasone, indomethacin and insulin. hADSC form mature and functional adipocytes after 3 to 4 weeks through adipogenesis (Pérez et al., 2015).

IBMX and dexamethasone are prime requirement to initiate the paradigm of adipogenesis. Human ADSC require specific factors to drive adipogenesis. Recently, transcriptome analysis of TERT4 MSC cell line depicted upregulation of glucocorticoid signaling gene expression during adipogenesis and osteogenesis. IBMX inhibits phosphodiesterase and elevates the levels of intra-cellular cyclic AMP which in turn activate transcription factors for adipogenesis (Cao et al., 1991). Dexamethasone induces expression of C/EBPδ after 4H of induction, however, C/EBPβ are activated when they are phosphorylated at Thr 188 and Thr179 or (Serine)Ser184 by MAPK or Glycogen synthase kinase (GSK) respectively. Thus, both C/EBPβ and C/EBPδ are initial transcription factors that induce adipogenesis. In human ADSC, these transcription factors drive remodelling of the cell to circular or oval shape from slender fibroblast cells after 3rd or 4th day(D) of the induction of adipogenic differentiation (Figure 1.12) (Moseti et al., 2016).

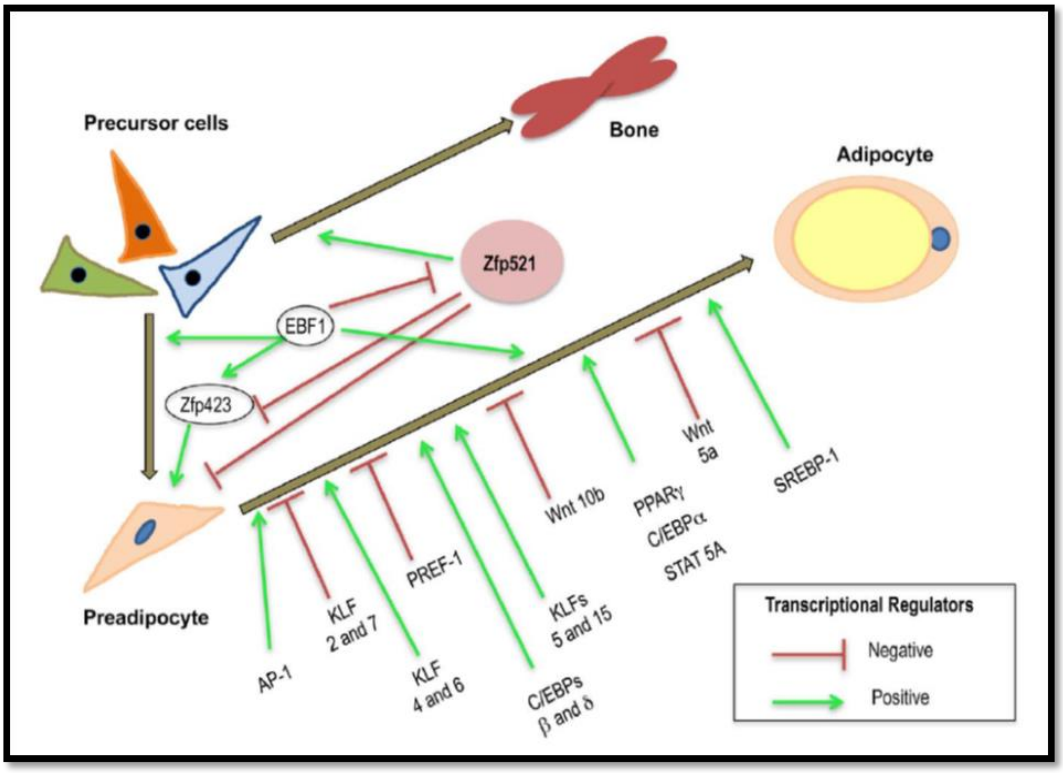

Figure 1. 12: Stages of adipogenesis (Stephens, 2012).

C/EBPβ binds to the regulatory elements in proximation of promoters of PPARγ and C/EBPα and activate them and positively stimulate the expression of final set of ATF.

Indomethacin stimulates activation of PPARγ and promote adipogenesis (Styner et al., 2010) driving the cells towards mitotic clonal expansion, one of the prime sequel for commencement of terminal differentiation of adipocytes. Halting the cells at these stage inhibits terminal differentiation of adipocytes. Further, PPARγ stimulates C/EBPα which retrospectively binds to the promoter region of PPARγ forming a self-regulated loop. Both of these transcription factors synergistically activate adipocyte specific target genes.

PPARγ bind to the regulatory binding element of the target genes like Glut4, LPL, activating protein 2 (aP2), Phosphoenolpyruvate carboxykinase (PEPCK), FA transport protein etc. whereas, C/EBPα transcriptionally activate its target genes like aP2, PEPCK, Glut4, stearoyl Co-A desaturase-1 (SCD-1) etc. by binding to their promoters thus, culminate the preadipocytes into mature adipocytes.

Insulin translocate Glut4 to the plasma membrane for insulin dependent glucose uptake. Leptin is activated by C/EBPα and SREBP-1c, whereas adiponectin is activated by several transcription factors among which PPARγ, C/EBPα and SREBP-1c are master regulator of adiponectin (Raajendiran et al., 2016). The above cascade of events during adipogenesis culminate in formation of mature adipocytes which eventually participates in energy homeostasis (Tong & Hotamisligil, 2001).

Adipogenesis is negatively regulated by PREF-1 and SIRT-1. PREF-1 binds to fibronectin which interacts with several integrin receptors and inhibits expression of ATFs. PREF-1 knock down *in vitro* or PREF-1 KO mice do not produce any adipocytes or W-adipose tissue. SIRT-1 inhibits expression of PPARγ and thus inhibits adipogenesis (Q. Chen et al., 2016; Moseti et al., 2016).

ADSC can be differentiated into osteocytes. There are several chemical, physical and environmental cues that may affect adipogenesis and divert ADSC towards osteogenesis.

1.11.1 Osteogenesis

ADSC differentiate into osteocytes efficiently. Osteogenesis is a cellular and molecular process that drives formation of osteocytes comprised of calcium deposits. Osteocytes are the fundamental cells for bone formation. Transcriptional factors that are involved in osteocyte differentiation are RUNX2, Osterix, SIRT-1 etc. (Kirkham et al., 2007).

1.11.1.a RUNX2

RUNX2 belongs to the family of RUNX comprised of three genes *Runx1/Cbfa2/Pebp2αB, Runx2/Cbfa1/Pebp2αA, and Runx3/Cbfa3/Pebp2αC* which contain DNA binding runt domain homologous to the Runt gene of Drosophila. RUNX proteins bind to the transcription co-activator core binding protein β (Cbfβ)/polyoma enhancer binding protein 2β (Pebp2β) (Shui et al., 2003).

All the three RUNX are attributed distinct functions. RUNX1 and Cbfβ are required for HSC differentiation. RUNX2 govern osteoblast differentiation whereas, RUNX3 are required in neurogenesis and regulation of epithelial cells growth. Further, RUNX2 and RUNX3 both are required for chondrocyte maturation (Shui et al., 2003). Cbfβ is involved in RUNX2 dependent osteoblast differentiation. $RUNX2^{-/-}$ mice are devoid of bones due to complete absence of intramembrane and endochondral ossification as they are devoid of osteoblast differentiation (Sinha et al., 2013).

1.11.1.b Osterix

Osterix belongs to the SP family which consist of three zinc finger motifs. Osterix arrives later in the process of osteogenesis. It drives maturation of osteocytes. It activates several osteogenic genes responsible for bone and skeletal formation. At the time of birth $osterix^{-/-}$ mice die due to lack of ribs formation which are inevitable for breathing. Moreover, perichondrial MSC deficient in osterix differentiate into chondrocytes. However, much information is not there regarding this gene as compared to well established RUNX2 transcription factor. Experimental evidence suggests that RUNX2 deficient mice do not express osterix but OSTERIX mice do express RUNX2 which defines that osterix is a later OTF (Komori, 2006).

1.11.1.c SIRT-1

SIRT play versatile role in different physiological functions and their regulations. Amongst all SIRT-1 is a unique Class III histone deacetylase that does not repress but enhances expression of RUNX2 through post-translation modifications. SIRT-1 interacts with RUNX2 and stimulates the downstream bone sialoprotein (BSP) and osterix, thus elevating osteogenesis. SIRT-1 KO mice are deficient in osteoblast and suffer from osteoporosis (Zainabadi et al., 2017).

1.11.2 Transcription cascade for osteogenesis

Osteogenesis can be rapidly induced by presence of pro-osteogenic cellular machinery. Bone formation is not ubiquitous as different bones and cartilages are

required for different structure and function of body parts like skull, ribs etc. Execution of osteogenesis is essentially carried out in presence of dexamethasone, ascorbic acid and β-glycerophosphate. Dexamethasone upregulates expression of RUNX2 and drives osteoblast differentiation. Ascorbic acid induces expression of Collagen Type-I for calcium formation. β glycerophosphate plays a crucial role in osteogenesis as it serves as the source of phosphate for the induction of osteogenesis (Moseti et al., 2016). It helps in production of hydroxylapatite mineral required for mineralization of bones. It is evident that intracellular signaling during osteogenesis is governed through ERK1/2. The paradigm of MSC to osteoblast to osteocyte formation takes place in three to four weeks (Langenbach et al., 2013). RUNX2 binds to major DNA binding sites of early osteogenic genes like osteopontin, osteocalcin, col1a, matrix metalloproteinase (MMP-1), bone sialo protein thus, induces osteoblast formation (Sinha & Zhou, 2013).

RUNX2 binds to the RUNX2 binding element on the promoter of osterix which induce expression of Osterix, later osteogenic transcription factor which drives transition of osteoblast to mature osteocyte and activate functional genes osteocalcin, osteonectin, osteopontin, bone sialoprotein and collagen type I responsible for mineralization and calcium deposition in osteocytes (Figure 1.13) (Langenbach & Handschel, 2013; Sinha & Zhou, 2013).

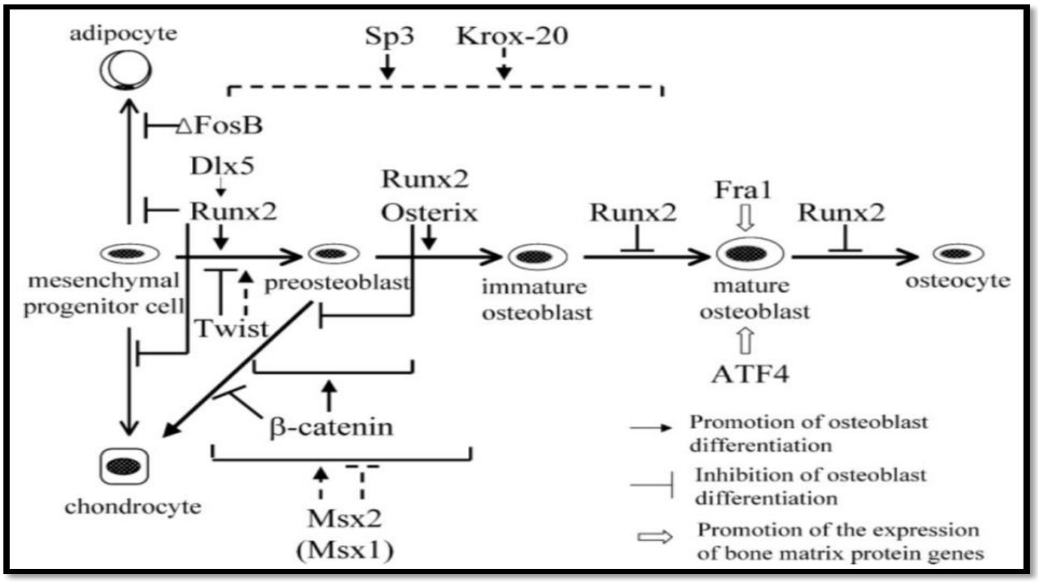

Figure 1. 13: Transcriptional cascade for osteogenesis, (Komori, 2006)

Osteogenesis influences skeleton, cranial and maxillofacial bones. Apart from three major OTF other transcription factors also govern osteoblast differentiation and bone

formation of specific parts of the body. They are Msh homeobox (Msx)1, Msx2, Distal-Less Homeobox (Dlx)5, Dlx6 etc. which direct bone formation of cranial, facial and several other parts of skeleton. Deficiency of these transcription factor provokes defects in the cranial, maxilofacial part of the body and also suffer from degenerative diseases (Li et al., 2018).

However, since a decade it has been evoked that hADSC from obese Caucasians are compromised in their stemness.

1.12 Detrimental effects of obesity on ADSC

ADSC are self-resilient stem cells which have bestowed them a major attraction in regenerative therapy. However, physiology, life style, dietary consumption and pattern of consumption, energy expenditure, ethnicity etc. influence the efficacy and functionality of the stem cells. In the present decade it has been profoundly observed that obesity short-circuits stemness network of ADSC. Transcriptome analysis of ADSC derived from obese Caucasian population have revealed increment in gene expressions of inflammatory pathways, apoptotic pathway and oxidative stress compared to those derived from control Caucasian subjects (Roldan et al., 2011). These ADSC had upregulation of PPARγ and other adipogenic lineage genes in undifferentiated cells, which demonstrated that ADSC derived from obese Caucasians were pre-committed towards adipogenesis. Moreover, ADSC derived from obese subjects had reduced proliferation, differentiation potentials and colony forming abilities (Oñate et al., 2013; Pérez et al., 2015). These cells were explored for their angiogenic and wound healing potentials and it was observed that ADSC of obese subjects failed to halt progression of autoimmune encephalitis in mice compared to ADSC derived from control subjects (Strong et al., 2016). Moreover, ADSC derived from diabetic subjects were more vulnerable than those of obese subjects. More detailed information on repercussions of obesity on ADSC is available in chapter 4. of the thesis.

In the modern scientific world, there is a dynamic progress in the field of medicine for treatment of metabolic disorders like obesity, diabetes, CVD etc.

1.13 Treatments for obesity and diabetes

Obesity mediated metabolic disorders have manifested economic burden and several line of actions have been implicated for the treatment of these dreadful diseases.

Pharmaceutical industries have been flourished with novel arenas for discovery and design of effective anectdotes for metabolic diseases since years. Several clinical trials have been performed for identification of candidate drugs for treatment of obesity and diabetes (Wilding, 2018).

Discovery of insulin and its action in treating diabetes was a major breakthrough in the field of medicine. There are different classes of drugs, which act differentially to control hyperglycemia either as insulin secretagogue or as insulin sensitizers. Sulfonylureas and thiazolidinediones (TZD) are effective anti-diabetic agents among which former enhances secretion of insulin from pancreatic β cells and the latter are classified as insulin sensitizers which reduces insulin resistance. Insulin secretagogues like sulfonylureas increase insulin secretion by affecting potassium channel of β cells (Proks et al., 2002).

Other class of drugs like dipeptidyl peptidase (DPP)4 inhibitors which inhibit the dipeptidyl peptidase 4 enzyme to increase the levels of glucagon-like peptide (GLP-1) and other incretins. These drugs are weight neutral however; GLP-1 receptor agonist reduce weight upto 5.3 Kg (Klonoff et al., 2008; Pratley et al., 2009).

Medication (Trade Names)	Mechanism of Action	Five Most Common Side Effects	Possible Safety Concerns*	Mean 1-Year Weight Loss Compared to Placebo(dose)
Decreases absorption				
Orlistat, (Alli, Xenical)	Lipase inhibitor	Abdominal pain, flatulence, fecal urgency, back pain, and headache	Fat-soluble vitamin deficiencies, altered absorption of medications, cholelithiasis, nephrolithiasis	3.4 kg, 4.0% (120 mg TID)
Suppresses appetite				
Lorcaserin (Belviq)	Serotonin receptor agonist	Headache, nausea, dizziness, fatigue, and nasopharyngitis	Serotonin syndrome, hypertension, edema, avoid in liver and renal failure	3.3 kg, 3.6% (10 mg BID)
Phentermine/ Topiramate (Qsymia)	Norepinephrine release, GABA receptor modulation	Constipation, paresthesia, insomnia, nasopharyngitis, and xerostomia	Birth defects, cognitive impairment, acute angle-closure glaucoma, lactic acidosis with metformin, avoid in renal	6.7 kg, 6.6% (7.5/46 mg daily) 8.9 kg, 9.0% (15/92 mg daily)
Naltrexone/ Bupropion (Contrave)	Opiate antagonist, decreased re-uptake of norepinephrine	Constipation, nausea, headache, xerostomia, and insomnia	Depression, anxiety, acute angle-closure glaucoma, avoid in patients with uncontrolled hypertension and renal failure	4.1 kg, 5.2% (16/80 mg BID)
Liraglutide (Saxenda)	GLP-1 receptor agonist	Hypoglycemia, constipation, nausea, headache, and indigestion	Gastroparesis, suicidal ideation, increased heart rate, caution in pancreatitis and cholelithiasis	4.5 kg, 5.6% (3 mg daily)

Figure 1. 14: Details of drugs applied for treatment of obesity and diabetes (Bramamte et al, 2017)

Metformin (Met) a gold standard which activates AMPK which provokes insulin signaling and thus, renders insulin sensitivity. Met is a weight reducing anti-diabetic drug which accounts for 3 Kg weight loss. Further, it acts on glucagon by inhibiting mitochondrial function which alters cyclic AMP signaling and confers anti-hyperglycemic activity (Pernicova et al., 2014). Another class of drug is sodium glucose transporter (SGLT) which is most applied in recent therapy. They are broadly classified as SGLT1 and SGLT2 inhibitors which function independent of insulin and inhibits glucose absorption. SGLT2 reabsorbs 90% glucose in kidneys whereas, SGLT1 control 10% of glucose absorption by intestine (Hsia et al., 2017). Both SGLT inhibitors function independent of insulin. SGLT2 are upregulated in TIIDM and glucose is lowered by SGLT2 specific inhibitors (van Baar et al., 2018). Glucosuria, dehydration and 2.4 Kg weight loss are the major drawbacks of these drugs. However, SGLT2 inhibitors are not administered independently but in combination of Met or insulin and rehydrating electrolytes and drugs (Bramante et al., 2017). Exogenous administration of insulin for treatment of chronic diabetes induces weight gain. Treatment of obesity with several kinds of drugs has always been crucial and controversial. Statins are the group of drugs used for reducing obesity. Orlistat is one of the effective drug which is used for reducing weight and treat obesity. Others are Lorcaserin, Liraglutide, phentermine etc. which reduce weight by several mechanisms as in (Figure 1.14.). In principle weight lowering drugs reduce diet consumption and increase energy wastage but none of the drugs activate browning of adipose tissue or increase energy expenditure. However, mostly all synthetic drugs have side effects (Bramante et al., 2017).

With advancement of science and knowledge, medicine field has developed enormously and Ayurveda has been now recognized internationally for its proficiency and health benefits. Abundant medicinal plants are omnipresent and are being explored for their therapeutic efficiency (Teoh et al., 2018). More than 1200 plant species like *Enicostemma littorale (EL), Aloe vera, Momordica Charantia* etc. were screened for anti-diabetic properties and approximately 200 phytocompounds having anti-diabetic properties have been reported (Teoh et al., 2010). Phyto-compounds like Catechins, Alkaloids, Flavonoids, Biguanides, Xanthones, Saponins, Sterols, Phenolic Acids, Carotenoids, Triterpenoids etc. attribute safe and effective health benefits without any harm to human body (Figure 1.15.) (Teoh & Das, 2018).

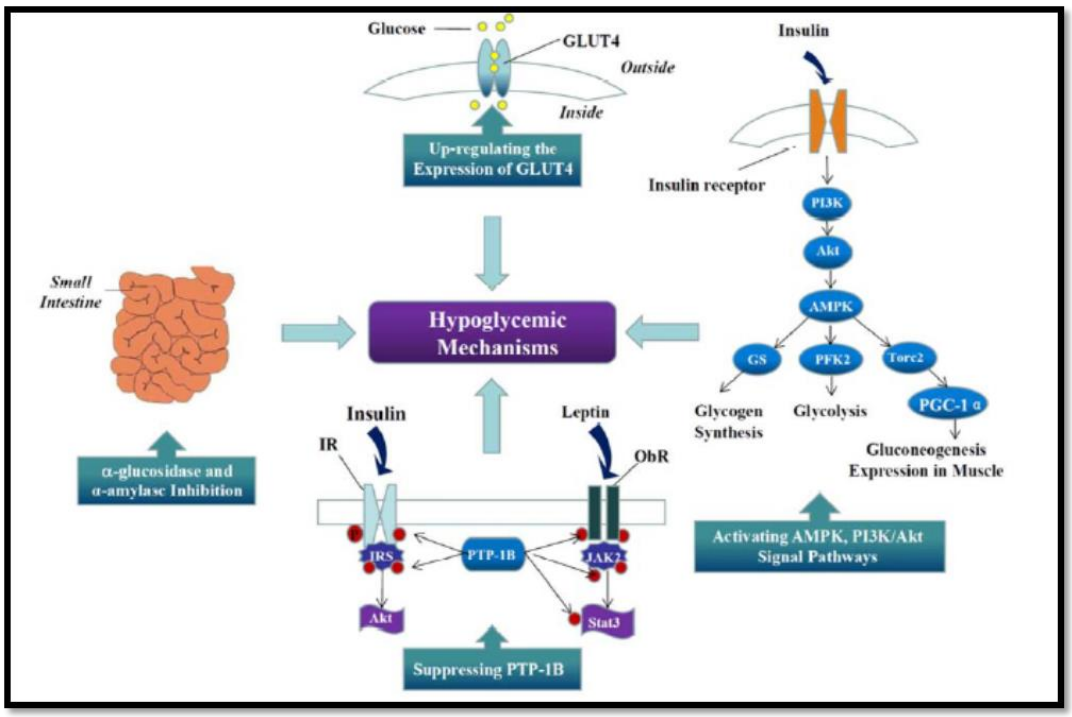

Figure 1. 15: Mechanism of phytochemicals hypoglycaemic effects (M.-j. Chen et al., 2017)

These phytocompounds like alkaloids, flavonoids have specific mechanism of action. Flavonoids isolated from several plants alleviate insulin resistance by targeting insulin signaling proteins. They reduce HGP, hyperlipidemia. They are rich in anti-oxidants thus scavenge extraneous oxidative stress generated during obesity and diabetes. Alkaloids regulate fasting blood glucose, AMPK, hepatic glucose and glycogen metabolism etc. (M.-j. Chen et al., 2017)

1.13 EL amongst many antidiabetic medicinal plant

EL belongs to the family *Gentianaceae*, is a perennial folk-lore Indian traditional plant also known as Chotta chirata, Mamejava and many other vernacular names. It is commonly found in Saurashtra region of Gujarat state. It has anti-obesity and anti-diabetic activity. It is used in several ayurvedic formulations implicated for treatment of several metabolic diseases (Mishra et al., 2017).

EL has extensively been explored for several therapeutic properties and blume of the plant is used for therapeutic purpose. This plant has wide range of therapeutic activities like anti-diabetic, anti-noceceptive, anti-oxidative, hypolipidemic, anti-malarial, anti-helminthic, anti-ulcer, anti-inflammatory and anti-tumorous activities (Patel et al., 2013).

Since 22 years, our lab has executed extensive research for exploring the therapeutic potentials of *EL*. We have explored the molecular mechanism of EL as anti-diabetic therapy for both Type I and Type IIDM. Alloxan induced diabetic animals were administered EL aqueous extract which lowered the blood sugar levels of diabetic animals (Vijayvargia et al., 2000). Then after, methanolic extract of EL demonstrated remarkable anti-hyperglycemic potentials as it reduced the blood glucose levels from 466.50 to 237.20 mg/dl. Other labs have also demonstrated glucose lowering potentials of EL extracts as it scavenged hydroperoxides from liver, pancreas and kidney (Prince PSM, 2005). Hot EL extract efficiently ameliorated hyperglycemia in diabetic animals. It was found that swertiamarin (SM) was abundantly present in the extract (Mishra et al., 2017).

EL has potential anti-oxidative and anti-inflammatory activities. Cholesterol fed rat were administered EL extract which reduced reactive oxygen species (ROS) by increasing anti-oxidant enzymes. (Maroo et al., 2003; Vasu et al., 2005). It also demonstrated anti-oxidant potentials in nephrotoxic diabetic rats (Niraj Mukundray Bhatt et al., 2011) and liver (Saranya et al., 2013).

EL enormously demonstrated anti-lipidemic potentials as TG, LDL, VLDL, LDL/HDL and total cholesterol were significantly reduced in cholesterol fed rats (Vasu et al., 2005).

Our lab conducted a clinical trial to elucidate anti-diabetic activity of EL. NIDDM patients were orally fed with EL aqueous extract and were observed for fasting and post prandial blood glucose levels, glycosylated haemoglobin and lipid profile. Subjects had dramatic reduction in all the diabetic parameters like fasting and post-prandial blood glucose and glycosylated haemoglobin. Lipid profile was remarkably improved as LDL, FFA, TG were reduced and HDL was increased which efficiently proved anti-diabetic and anti-hyperlipidemic activity of EL extract (Vasu et al., 2003).

Apart from anti-hyperglycemic, anti-inflammatory, antihyperlipidemic and anti-oxidative activities, efficacy of EL was also examined for diabetes mediated complications. Diabetic nephropathy is one of pertinent complication that arise due to diabetes. EL extract was administered to Charles foster rat that suffered from gentamicin induced nephropathy. EL extract ameliorated mitochondrial dysfunctions generated due to gentamicin toxicity (Niraj Mukundray Bhatt et al., 2009). CVDs are

the major complications associated with insulin resistance and obesity. Cardio-protective potentials of EL were examined in rats suffering from CVD induced through high fructose diet. EL extract administration diminished hypertension, insulin resistance, hyper-triglyceridemia, platelet aggregation, coagulation of blood and vascular dysfunctions in animals suffering from CVD. Further, EL extract was scrutinized for its neuroprotective effects. EL extract was administered for 45D to animals suffering from alloxan induced diabetic neuropathy. Nociceptive effects were reduced in EL extract treated animals. Oxidative stress was reduced with concomitant increase in nerve function as examined by tail flick in hot immersion test (Niraj Mukundray Bhatt et al., 2011). Thus, the results depicted strong evidences of EL extract being an ideal therapeutic candidate for treatment of metabolic syndrome (Niraj M Bhatt et al., 2012).

EL is rich in phytocompounds and two active molecules were isolated from EL namely SM and swertisin (Soni et al, unpublished). SM is a seco-irridoid found abundantly in the plant extract. It ameliorates hyperglycemia, hyperlipidemia and HGP. Our, *in vivo* and *in vitro* studies demonstrated that SM targets PPARγ, lipogenic genes, carbohydrate metabolism regulating enzymes (Patel et al., 2013). It also ameliorated oleic acid induced hepatic steatosis and insulin resistance in adipocytes (Patel et al., 2016) (Patel et al, unpublished). Swertisin is a flavonoid was isolated from EL which demonstrated remarkable anti-hyperglycemic and anti-diabetic effects. Our lab proved its islet neogenic and islet differentiation abilities. Swertisin was used as an differentiating agent to produce insulin producing islet like clusters from NIH-3T3, PANC-1 and mouse pancreatic progenitors which ameliorated diabetes in streptozotocin (STZ) treated mice by reducing blood glucose levels and forming neo-islets in the diabetic animals (Dadheech et al., 2013; Dadheech et al., 2015; Srivastava et al., 2018).

Currently, the field of regenerative medicine has been promising and centre of attraction as stem cell therapy has proven to be promising in treatment of several metabolic diseases (Mahla, 2016).