Chapter-2

LITERATURE REVIEW

2.1 Obesity

WHO defines obesity as abnormal or excessive fat accumulation, to the extent that it impairs health. It has become one of the major challenges for public health in the 21st century. Obesity is measured by the Body Mass Index (BMI), which is simply the ratio of weight (kg) of a person to the square of his height (m^2). According to WHO, BMI ≥ 25 represents overweight, and BMI ≥ 30 represents obesity [World Health Organization, https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight] . Though BMI is the most widely used criteria for diagnosing obesity, recent studies have suggested BMI only to be a surrogate and not an exact measurement of body fat. Therefore, other indicators are proposed to determine obesity, where body fat percentage is the most useful criterion. Thus, body fat levels between 10%–20% for men and 20%–30% in women are considered to be healthy in adults, and above this indicates overweight and obesity [Gómez-Ambrosi et al. 2011; Blundell et al. 2014].

2.2 Epidemiology of Obesity

The rates of obesity have almost tripled across all the age groups. According to the factsheet of WHO, 1.9 billion adults, i.e., 39% were overweight (39%-men & 40%-women), among which 650 million, i.e., 13%, were found to be obese (11%-men & 15%-women) in 2016. Among the children and adolescents between 5 to 19 years of age, above 340 million (18%) were overweight or obese in 2016. Below the age of 5

years, 38 million children were found to be overweight or obese [World Health Organization, https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight]. According to the World Obesity Federation, 2.7 billion adults and 268 million school-age children will be overweight or obese by 2025 [Mojto et al. 2019]. 2.8 million people across the globe die each year due to overweight or obesity. WHO has adopted "Global action plan on physical activity 2018–2030: more active people for a healthier world", which provides a solution to tackle obesity by increasing physical activity. Also, the 2030 Agenda for Sustainable Development recognizes non-communicable disease as a serious challenge for sustainable development. The Sustainable Development Goal 2 deals with improving nutrition, health, and ending malnutrition in children and adults [Dukhi 2019]. There is a drastic rise in obesity over the past few decades due to inclination towards the increased usage of sugar-sweetened beverages, processed foods, and increased use of edible oils accompanied by decreased physical activity leading to a sedentary lifestyle [Popkin et al. 2012].

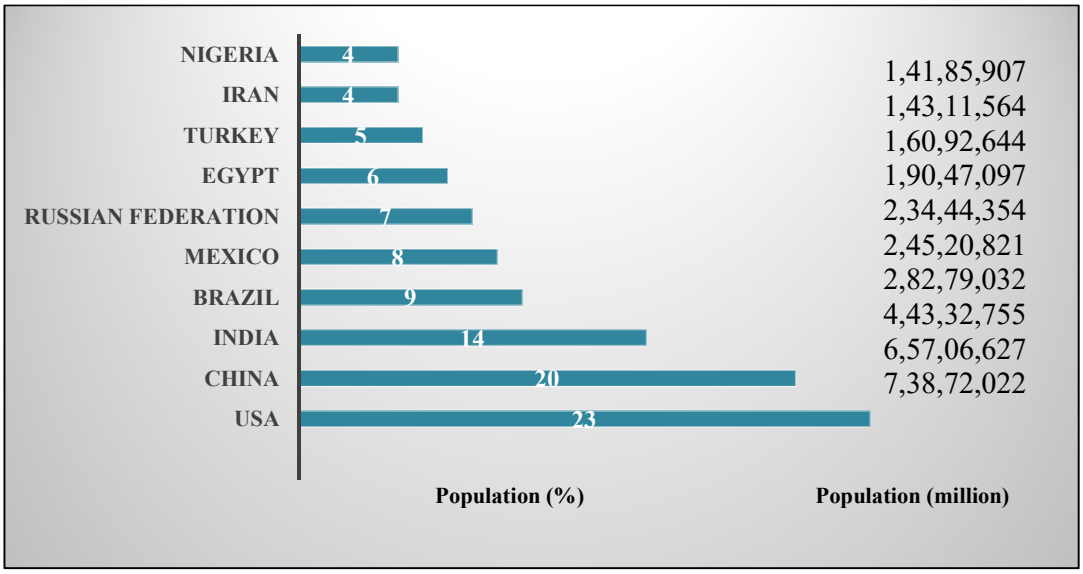


Figure 2.1: Global Obesity prevalence of the top ten countries in 2017 with percentage and population [Karri et al. 2019]

The Global Action Plan for the Prevention and Control of Non-Communicable Diseases of WHO aims to reduce premature mortality from non-communicable diseases by 25% up to 2025. It targets seven major risk factors, which include insufficient physical activity, intake of salt or sodium, diabetes, and obesity, among others [World Health Organization, https://www.who.int/nmh/events/ncd_action_plan/en/]. For the prevention of nutrition-related non-communicable diseases, be they obesity, diabetes, or fatty liver, we must look for ways to improve the dietary patterns around the globe. To prevent obesity and its associated other non-communicable diseases, the WHO has given the following suggestions:

- To make a healthier food choice and engage in regular physical activity (60 min a day and 150 min a week for children and adults respectively);
- By limiting the intake of fats and sugars; and switch to unsaturated fats from saturated fats;
- By increasing the consumption of fruits, vegetables, legumes, whole grains and, nuts;
- The food industry can also contribute to promoting healthy diets by:
    - ✓ decreasing the fats, sugars, and salts in processed foods;
    - ✓ ensuring healthy and nutritious choices to all consumers;
    - ✓ restricting marketing of sugar, salt and fat-enriched foods, especially foods that are aimed at children; and
    - ✓ ensuring healthy food choice options along with regular physical activity at workplaces

Most of the world's population lives in countries where morbidity and premature mortality due to increased weight are more than those underweight. It presents an ever-increasing threat to global health [Blüher 2019]. The literature suggests that obesity is no longer a concern only for developed countries. However, it includes many developing countries, even though the prevalence is higher in economically developed regions than the developing nations. **Fig. 2.1** represents the top ten countries having the most obese population. Ironically, the developing countries, which had the burden of communicable diseases and under-nutrition for generations, are now facing an upsurge of obesity and its adverse health consequences [khan Afridi et al. 2004; Boutayeb 2010]. The adaption of western lifestyle and economic modernization is leading to a rapid increase in the development of obesity. The pandemic of obesity is so great that it has even created a new word, 'globesity'[Speakman et al. 2003; Costa-Font et al. 2016]. **Fig. 2.2** represents the estimated obesity prevalence in 2025.

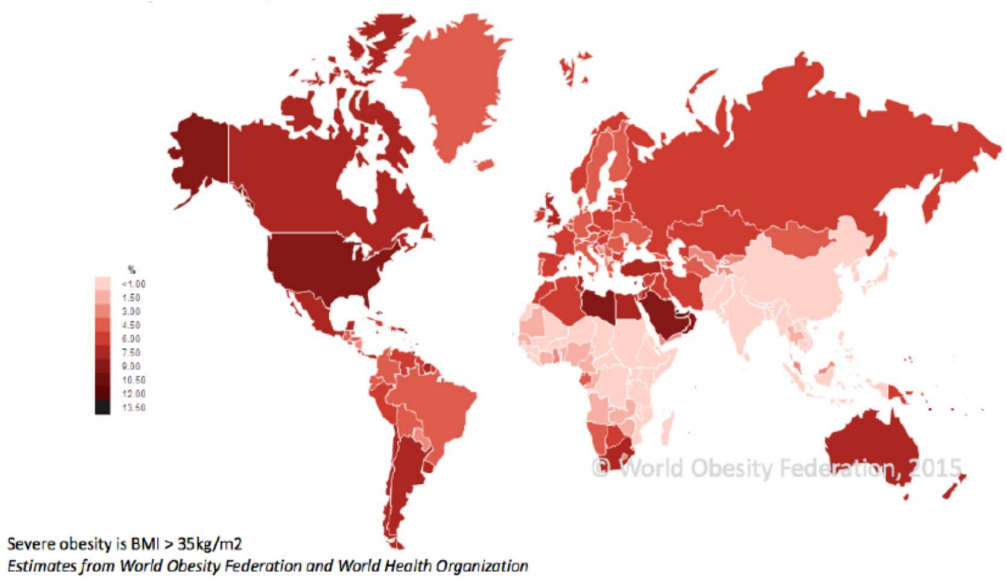


Figure 2.2: Estimated obesity prevalence in 2025 according to the World Obesity Federation (Adapted from World Obesity Federation, Obesity Atlas, https://www.worldobesitydata.org/map/overview

2.3 Pathogenesis of Obesity

Obesity is a complex multifactorial disorder developing from the interactions of multiple genes with environmental factors. The favorable environment for developing obesity refers to the availability of excess calories with decreased expenditure leading to long term energy imbalance, which favors an unhealthy increase in body weight. Other factors are genetic, psychological, physiological, social, and economic [Aronne et al. 2009]. The phenotypic variability of obesity ranges from mild overweight to morbidly obese. Even though the obesogenic environment drives the increase in body weight, an individual's genetic susceptibility also facilitates increased weight gain [Lake et al. 2006]. It is believed that both genes and everyday life activities mediating more food intake and reducing domestic and living work activities are involved in the pandemic of obesity [Bray 2004; Marti et al. 2004]. Correlation between heredity and fat also exists according to some studies. Although rapid globalization of the western lifestyle is responsible for the substantial rise in obesity cases, it also results from a complex interplay of environmental and genetic factors, as shown by numerous epidemiological studies in the different population [Stunkard et al. 1990; Kopelman 2000]. The contributing factors to the obesity epidemic include diet, physical inactivity, sleep-debt, drug-induced weight gain, genetics, hormonal, and socio-economic status. Different studies have shown that in obesity, food craving results from disturbed signaling in the hypothalamus, whereas appetite and satiety are regulated by adipose tissue, gut, or liver hormones [Farooqi 2014; Heymsfield et al. 2017]. The Ob gene (encodes for leptin hormone) mutation induces obesity in rodents, reflecting that energy homeostasis works by integrating signals from the peripheral tissues, such as adipose tissues [Lahlou et al.

2002; Farooqi et al. 2014]. Mutation in other genes encoding for leptin receptor, melanocortin-4 receptor, and pro-opiomelanocortin are also responsible for obesity. Some studies also suggest obesity to be an inherited dysfunction of energy imbalance [Blüher 2019].

2.4 Management of Obesity

The treatment of obesity should not only be focussed on the severity of overweight, but also the associated coexisting complications. The prevention and treatment options for obesity are not successful in the long run. However, the strategies include lifestyle and behavioral intervention, pharmacotherapy, medical devices, and bariatric surgery [Heymsfield et al. 2017; Gadde et al. 2018].

- **Lifestyle and behavioral interventions** are the cornerstones of obesity treatment. It is the first option for managing weight gain due to low cost and minimal risks [Cardiology et al. 2014]. It is concerned with limiting calorie intake and increasing calorie expenditure. Patients find limited effects in achieving long-lasting benefits due to the compliance issues along with the complex hormonal, metabolic, and neurochemical responses that protect against the weight loss and induce weight to regain [Blüher 2019].
- **Pharmacotherapy** is recommended with lifestyle and behavioral modifications [Garvey et al. 2016]. The five US-FDA approved drugs for long term weight management and their mode of action are represented in **Table 2.1**. Physicians do not suggest the use of these medications to the extent that one can expect. It's for two reasons, first being the patient's disappointment by moderate weight

loss, and second is the cost, which eventually leads to discontinuation from the long-term use [Yanovski et al. 2014].

- **Medical devices** approved by US-FDA for short- and long-term use are intragastric balloons, electrical stimulation systems, and gastric emptying systems [Lee et al. 2017].
- **Bariatric surgery** is recommended for patients either with a BMI of ≥ 40 kg/m^2 or with a BMI of ≥ 35 kg/m^2 and weight-related complications. It gives effective and sustained weight loss results. However, due to its high cost and small risk, it is suitable for patients with severe obesity. The procedures include sleeve gastrectomy, Roux-en-Y gastric bypass, laparoscopic adjustable gastric band, and biliopancreatic diversion with duodenal switch [Lee et al. 2017].

Table 2.1: US-FDA approved drugs for the treatment of Obesity [Kusminski et al. 2016; Heymsfield et al. 2017]

Drug	Approved year	Mode of action	Side effects
Orlistat (Xenical)	1999	Pancreatic and gastric lipase inhibitor; Effects fat absorption reducing energy intake	Oily spotting, fecal urgency, oily evacuation, fecal incontinence, liver failure, regain in body weight
Liraglutide (Saxenda)	2010	GLP-1 agonist; Delays gastric emptying	Constipation, hypoglycemia, vomiting, increased lipase levels, gallstones
Lorcaserin (Belviq)	2012	$5HT_{2C}$ receptor agonist; Promotes satiety	Constipation, fatigue, dry mouth, respiratory tract infection, hypoglycemia (in diabetics)

Phentermine/ Topiramate (Qsymia)	2012	Norepinephrine releasing agent/GABA receptor modulator; Decreases appetite	Insomnia, dry mouth, paresthesias, dysgeusia, glaucoma, kidney stones
Naltrexone/ Bupropion (Contrave)	2014	Opioid antagonist/ Dopamine and norepinephrine reuptake inhibitor; reduces food intake	Constipation, insomnia, dry mouth, diarrhea, seizures, glaucoma, cardiovascular disease risks

2.5 Complications of Obesity

Obesity eventually increases the risk of metabolic complications such as DM and fatty liver; cardiovascular disorders such as hypertension, myocardial infarction, and stroke; musculoskeletal complications such as osteoarthritis; depression and Alzheimer's disease; and cancer of colon, breast, kidney, prostate, ovaries, and liver tissues. It also impacts the quality of life, lower productivity, unemployment, and social disadvantages [Blüher 2019]. Metabolic syndrome, a complex multifactorial endocrine syndrome, is a consequence of obesity and insulin resistance [Ford et al. 2002; Fulop et al. 2006], and is directly related to the severity of obesity (**Fig 2.3**). It is highly prevalent and is of grave concern because of high morbidity and mortality rates. It is a combination of various cardiometabolic risk factors, that includes impaired glucose homeostasis, decreased insulin sensitivity, hyperinsulinemia, dyslipidemia, atherosclerosis, increased blood pressure, pro-inflammatory status, oxidative stress, and NAFLD [Bruce et al. 2009; Aydin et al. 2014; Aguilera-Mendez et al. 2018; Polyzos et al. 2019]. Excessive body fat accumulation is the most common reason for metabolic syndrome. However, more specifically, ectopic fat accumulation is the primary determinant in metabolic syndrome, which is often characterized as a

pathophysiological condition, adiposopathy. It is explained as the pathogenic adipose tissue which develops due to a persistent positive energy balance, inducing adipocyte hypertrophy, fat distribution at ectopic sites, and immune disturbances [Bays et al. 2008; Bays 2009]. Abdominal or visceral obesity is associated with insulin resistance, hyperinsulinemia, increased free fatty acids, and dyslipidemia [Grundy et al. 2004; Bruce et al. 2009]. Even though obesity is considered to be the central determinant in metabolic syndrome, but it is also observed that all obese individuals do not suffer from impaired metabolic complications [Handelsman 2009].

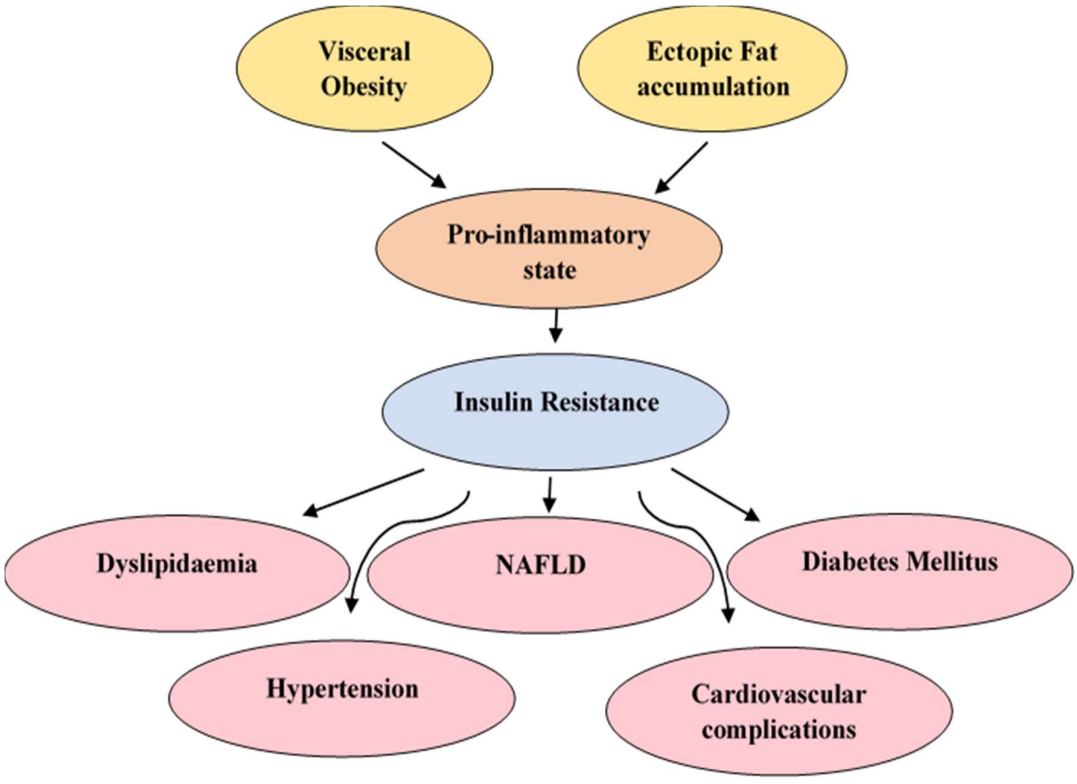


Figure 2.3: Components of the Metabolic syndrome [Fulop et al. 2006; Bruce et al. 2009]

2.6 Adipose tissues

It was identified by Conrad Gessner in 1551 as a loose connective tissue [Cannon et al. 2008]. Traditionally, adipose tissue was considered only a passive storage organ for excess calories in the fed state, which gets released in the fasting state [Rosen et al. 2014]. However, recently adipose tissue has been found as a dynamic and active tissue and one of the largest endocrine organs in the body, playing an essential part in the maintenance of energy homeostasis through various signaling pathways and biologically active molecules [Rodríguez et al. 2007; Ibrahim 2010]. Two types of adipose tissue are found based on their origin and diverse functional and morphological characteristics; White adipose tissue (WAT) and Brown adipose tissue (BAT). The former stores energy in excess calorie supply conditions and the latter generates body heat via thermogenesis [Trujillo et al. 2006; Frühbeck et al. 2009; Sethi et al. 2011]. The presence of a third type of adipose tissue has also been proposed, i.e., Beige or Brite adipose tissue, which evolves from the sustained thermogenic activation of WAT, leading to the formation of brown adipocyte-like cells in WAT [Wu et al. 2013].

2.6.1 WAT

It is a heterogeneous tissue and is composed of adipocytes endowed with the function of lipid storage. Based on its different locations, it regulates energy homeostasis and induces metabolic complications. It is either subcutaneous, i.e., beneath the skin or visceral, i.e., around the visceral organs, such as the pancreas, kidneys, and heart [Cannon et al. 2008; Sethi et al. 2011]. It gained recognition as an endocrine organ secreting adipocytokines and inflammatory molecules that assist in mediating the metabolic networks [Hotamisligil et al. 1993; Fantuzzi 2005]. The

characteristic feature of WAT in represented in **Table 2.2**. Besides maintaining energy homeostasis, it controls numerous functions such as inflammation, immune regulation, lipid, and glucose homeostasis, regulation of the metabolic process, and food intake [Falcão-Pires et al. 2012]. WAT's pleiotropic role is attributed to the synthesis and release of hormones, cytokines, enzymes, growth factors, matrix proteins, and complement factors, collectively termed as adipokines, which mediate insulin sensitivity, energy regulation and inflammatory process [Frühbeck et al. 2001; Frühbeck 2008].

2.6.2 BAT

Initially, it was believed that the presence of BAT in humans is limited to neonates and young children. However, recent studies with positron emission tomography technique, have confirmed the presence of BAT in adult humans also, at different locations [Frühbeck et al. 2009; Schulz et al. 2011] (**Table 2.2**). BAT is mainly responsible for inducing energy expenditure and also non-shivering and diet-induced thermogenesis [Cannon et al. 2004; Feng et al. 2013; Villarroya et al. 2017]. Studies have shown that BAT is inversely correlated with basal metabolic index [Cypess et al. 2009]. Brown adipocytes regulate thermogenesis by transferring the energy from food into heat. They have abundant UCP-1, a key regulator of thermogenesis, which is responsible for adenosine triphosphate (ATP) production through oxidative phosphorylation [Wu et al. 2012]. The thermogenesis process gets stimulated in BAT during energy intake, cold exposure, and sympathetic stimulation. They are mitochondria enriched tissues that help in heat dissipation [Frühbeck et al. 2009; Park et al. 2014]. The manipulation of UCP-1 in animal models led to obese animals, along

with a more than 50% reduction in BAT mass. Reduced BAT activity leads to obesity development in rodent models and vice-versa [Hansen et al. 2006; Kozak et al. 2008].

Table 2.2: Characteristics of WAT and BAT [Frühbeck et al. 2009]

Characteristic	WAT	BAT
Macroscopic features	White in colour, which changes from a light ivory to yellow. The depot locations are subcutaneous, retroperitoneal, epididymal, inguinal, and perirenal. The vascularization is adequate.	Brown in colour, in between to pink and reddish tone. Present at the intercapsular, axillary, paravertebral and perirenal locations. Profusely vascularized.
Microscopic features	The shape of adipocyte is polyhedral to spherical and size ranges from 25µm to 200µm, where it can expand upto 1000 folds. The peripheral nucleus is present occupying only 2-3% of cell volume. Unilocular and single large lipid droplet occupying 90% of cell volume. Mitochondria are few in number.	The shape of adipocyte is polygonal and the size is from 15µm to 60µm. The nucleus is centrally placed. Multilocular and plentiful lipid droplets. occupying 90% of cell volume. Mitochondria are abundantly present.

2.7 Adipokines

Adipose tissue secretes adipocyte-specific factors or an array of hormones, adipocytokines (or adipokines), which are cell-signaling proteins responsible for mediating the crosstalk between adipose tissue and other key metabolic organs. These

polypeptide cytokines exert autocrine, paracrine, and endocrine functions [Polyzos et al. 2015]. With the discovery of leptin in 1994, WAT was recognized as an endocrine organ [Zhang et al. 1994]. One-third of WAT is composed of adipocytes, and the remaining is of monocytes, macrophages stromal cells, and fibroblasts [de Oliveira Leal et al. 2013]. The functional status of adipose tissue is mediated by adipokines to the targets in the brain, liver, pancreas, immune system, vasculature, muscle, and other tissues [Blüher 2014; Blüher et al. 2015]. These bioactive peptides are released from the adipocytes, endothelial and immune cells, macrophages, fibroblasts, neutrophils, foam cells, lymphocytes, and others. More than 600 bioactive molecules have been identified through the proteonomic profiling approaches from the adipose tissue [Fasshauer et al. 2015]. They influence a variety of biological processes, including appetite, satiety regulation, energy expenditure, fat distribution, glucose and lipid metabolism, insulin sensitivity, endothelial function, immune responses, inflammation, myocardial contractility, etc. Besides being involved in metabolic thermogenesis, BAT exerts endocrine functions also, through releasing various bioactive molecules, which function in autocrine or paracrine manner [Villarroya et al. 2017]. The adipokines secreted by WAT and BAT are listed in **Table 2.3** and **Table 2.4**, respectively**.**

Table 2.3: List of some WAT secreted Adipokines and their functions [Fasshauer et al. 2015; Unamuno et al. 2018]

Adipokines	Important functions
Leptin	Satiety signal; regulates appetite, energy expenditure, food intake, locomotor activity, fertility, etc.
Adiponectin	Ameliorates insulin sensitivity and has anti-diabetic, anti-atherogenic, anti-inflammatory effects.

Visfatin/ Nicotinamide phosphorribosyl transferase (NAMPT)	NAMPT is responsible for mediating NAD biosynthesis, which is important for β cell function
Vaspin	It is a serine protease inhibitor, and involved in reducing food intake, and improving hyperglycemia
Retinol Binding protein-4 (RBP4)	Regulates insulin sensitivity, dyslipidemia, and visceral fat distribution
Apelin	Inhibits insulin secretion, involved in the regulation of blood pressure, cardiac contractility, and lipolysis
Omentin	Involved in insulin sensitivity and has anti-inflammatory property
Lipocalin 2	Responsible for insulin resistance and has anti-inflammatory action
TNF-α	It is a pro-inflammatory molecule and induces IR in obesity
IL-6	It is a pro-inflammatory molecule involved in acute-phase response
IL-1β	Involved in pro-inflammation
MCP-1	It is a chemoattractant protein and regulates adipose tissue inflammation
Resistin	Involved in obesity, insulin resistance and adipose tissue inflammation
Progranulin	It is a chemoattractant protein, and is involved in neurodegenerative disease and adipose tissue inflammation
Plasminogen activator Inhibitor-1 (PAI-1)	It is a fibrinolysis inhibitor and involved in the development of atherosclerotic plaques
Tissue inhibitor of matrix metalloproteinase-1	It reduces adipogenesis and reduces glucose tolerance
Adipsin	Activates the alternative complement pathway

Fibroblast growth factor-21 (FGF-21)	It regulates thermogenesis, fat utilization, energy expenditure, glucose and lipid metabolism, and glucose uptake in the adipocytes
Fetuin-A	It is involved in lipid-induced inflammation, insulin resistance and progression of cancer
DPP-4	Responsible for GIP and GLP-1 breakdown
Nesfatin-1	Glucose mediated insulinotropic effect on β-cells
WNT5A	Mediates adipogenesis in adipose tissues

Table 2.4: List of some BAT secreted Batokines and their functions [Unamuno et al. 2018]

Adipokines	Important functions
Adiponectin	Induces proliferation of M2 macrophages.
Endothelin-1	Inhibits thermogenesis.
Fibroblast growth factor-21 (FGF-21)	Increases glucose waste in the adipose tissues, and promotes lipidemia and glycemia
Fibroblast growth factor-2 (FGF-2)	Controls the preadipocyte levels in the BAT
Interleukin-1a	Regulates thermogenesis in BAT
Interleukin-6	Regulates thermogenesis in BAT
Nerve growth factor	Controls preadipocyte numbers in the BAT
Chemerin	Responsible for lipid deposition
Triiodothyronine	It induces the expression of UCP-1

2.8 Dysfunctions induced in adipose tissue in Obesity

Adipose tissue becomes severely impaired and fails to fulfill its primary function of storing the excessive calories in obesity, which migrate to ectopic sites, negatively affecting the metabolic homeostasis and inducing obesity-associated metabolic

complications such as insulin resistance, DM, and many more, as discussed in section 2.5. Along with the primary function, its secondary function of secreting the adipokines is also impaired in obesity which further promotes the precipitation of the several metabolic complications, worsening the situation [Moore 2010; Landsberg et al. 2013; Trayhurn 2013; Picon-Ruiz et al. 2017; Unamuno et al. 2018]. The typical symptoms of dysfunctional adipose tissue include fat accumulation in the visceral and ectopic sites, elevated immune cells in the adipose tissues, hypertrophy of the adipocytes along with an increase in the autophagy and apoptosis of adipocytes, alteration in the cellular and intracellular matrix composition and fibrosis of the adipose tissues and change in the mRNA and protein expression profiles of the key molecules in the adipose tissues [Blüher 2013] (**Fig. 2.4**).

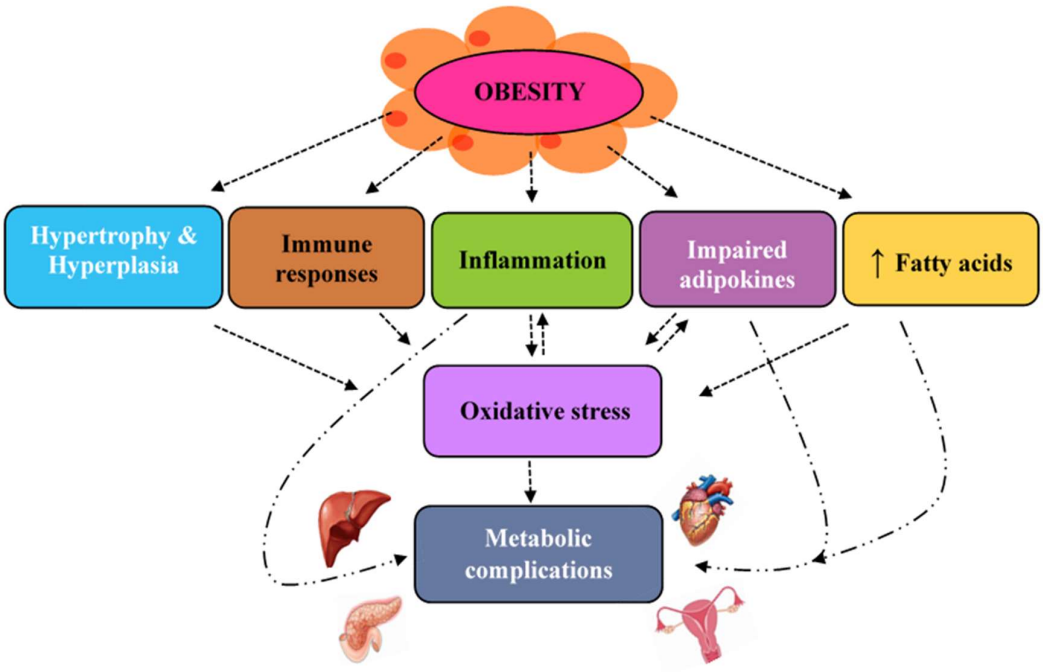


Figure 2.4: Dysfunctions in the adipose tissue in obesity, further inducing the metabolic complications [Matsuda et al. 2013]

2.8.1 Adipose tissues expansion

Adipocytes in adipose tissues remain under control with the synthesis of new adipocytes and the death of old ones through apoptosis. There is a slight increase in the number of adipocytes in adulthood, suggesting that the number of adipocytes is set in the individuals, but obesity results in an increase in the adipocyte numbers [Arner et al. 2011; Cooke et al. 2016]. During obesity, adipocytes increase in size or number or both, i.e., hypertrophy and hyperplasia through peroxisome proliferator-activated receptor-γ, contributing to the expansion of adipose tissues, resulting in an increase in autophagy, in respect to the programmed cell death [Frühbeck 2008; Sun et al. 2011; Kosacka et al. 2015]. Due to the adipose tissue expansion, adipocyte biology is significantly affected, and it further leads to dysfunctional adipose tissue and also develops a physiological response to the change so occurred in the adipose tissue. This further promotes chronic stress and hypoxia in adipose tissue and fat deposition at ectopic sites [Fasshauer et al. 2015].

2.8.2 Adipose tissues inflammation

A state of chronic low-grade inflammation develops in obesity, especially in visceral obesity [Wellen et al. 2003], following the interaction between the immune cells and adipocytes, which further provides the signals for driving a persistent pro-inflammatory immune response [Fantuzzi 2005]. This induces alteration in adipose tissue function, affecting the adipokines release also [Nishimura et al. 2009]. The level of inflammatory markers, such as C-reactive protein (CRP), IL-6, TNF-α, is elevated in obesity, which is also correlated with insulin resistance [Cottam et al. 2004]. Adipose tissue is characterized by the presence of different types of immune cells such as mast

cells, macrophages, neutrophils, eosinophils, lymphocytes, and foam cells, located in the stromal vascular fraction (SVF) of the adipose tissues, which interact with the adipocytes inducing inflammation [Winer et al. 2011; Rodríguez et al. 2015]. There is an increase in the population of activated macrophages with the expansion of the adipose tissues in obesity [Fantuzzi 2005]. In non-obese states, prominent cells in adipose tissues are anti-inflammatory T regulatory (Treg) and M2 macrophages, whereas, the immune cells serve the housekeeping function [Schipper et al. 2012]. With the WAT expansion in obesity, immune cell infiltration occurs within the tissue, with the formation of crown-like structures around the necrotic adipocytes, for clearing the cellular debris. WAT activates CD8 (+) T-cells, which promote the macrophages recruitment and activation, thereby playing a critical role in the initiation and propagation of inflammation in the adipose tissues [Nishimura et al. 2009]. Also, the demand for the immune cells increases with the expansion of WAT. The free fatty acids arising from the expanded adipose tissue activates different intracellular signaling cascades; c-Jun N-terminal protein kinases (JNK), protein kinase R (PKR), and inhibitor of nuclear factor kappa-B kinase subunit beta (IKK-β), which are also involved in the regulation of inflammatory response, through different cytokines, such as TNF-α and IL-1β [Hirosumi et al. 2002; Nakamura et al. 2010]. MCP-1 also induces the migration of monocytes and macrophages, which is increased with obesity [Sartipy et al. 2003; Ito et al. 2008]. Free fatty acids in the adipocytes promote the production of TNFα in the macrophages, facilitating the inflammation process [Suganami et al. 2005].

2.8.3 Disturbed adipokine profile

As discussed above, the endocrine nature of adipose tissue, the adipokines so released, regulate a plethora of pleiotropic biological functions of the body, as listed in Table **2.3** and **2.4**, respectively. However, their synthesis and release are significantly altered in obesity, disrupting the multiple physiological processes. [Fantuzzi 2005; Jung et al. 2014]. The synthesis and secretion rate of the adipokines is largely influenced by the amount of adipose tissue [Tchkonia et al. 2013]. Significantly increased pro-inflammatory mediators in the expanded adipose tissues hamper the synthesis and secretion of adipokines [Klöting et al. 2014].

The adipokine, **Leptin**, is a 16 kDa secreted protein and is produced mainly by the adipocytes [Zhang et al. 1994]. Leptin levels are found to be directly correlated with adipose tissue mass, i.e., high in obesity [Yang et al. 2001]. Leptin regulates satiety, food intake, energy expenditure, locomotor activity, fertility. Its receptors are found in multiple tissues, and hence it exerts a variety of functions from metabolism to wound healing to reproduction to bone remodeling, and cardiovascular functions. [Gautron et al. 2011; Unamuno et al. 2018]. The studies involving ob gene knockout models have shown that they develop obesity and DM [Lindström 2007]. Low levels of adiponectin/leptin ratio are the hallmark of dysfunctional adipose tissue, and it also develops inflammation and oxidative stress [Frühbeck et al. 2017].

The adipokine, **Adiponectin**, is a 30 kDa protein and is expressed mainly in the adipose tissues. The circulating level of this adipokine is considerably high and accounts for 0.01% of total serum protein with 5-10µg/ml in circulation [Combs et al. 2014]. It exerts its effect after binding with its transmembrane receptors expressed in different tissues, AdipoR1, AdipoR2, and T-cadherin. It activates AMPK, PPARα, and

p38 mitogen-activated protein kinase pathways in the tissues [Kadowaki et al. 2005]. Besides having insulin sensitization, anti-diabetic, anti-atherogenic, and anti-inflammatory effects, it also has hepatoprotective activity. The studies have highlighted a reduced hepatic adiponectin expression in obese patients with fatty liver, indicating a protective effect of adiponectin in the development and progression of fatty liver disorder. [Ma et al. 2009; Unamuno et al. 2018]. As there is a direct relationship between leptin levels and obesity, conversely the serum adiponectin levels are found to be reduced in obesity. It is increased with weight loss and anorexia nervosa [Arita et al. 1999; Yang et al. 2001; Pannacciulli et al. 2003]. AdipoR1 receptor is expressed in liver, muscle, hypothalamus, etc., while AdipoR2 is found in liver, WAT, vasculature, etc. [Yamauchi et al. 2003] Adiponectin up-regulates the expression of PPARα ligands through acting on AdipoR2 receptors and enhances fatty-acid combustion and energy consumption [Yamauchi et al. 2003]. It activates the AMPK pathway through binding with the AdipoR1 receptors, further increasing PGC-1α expression in the cells, which is found to be down-regulated together with a decrease in mitochondrial content, with the suppression of AdipoR1 receptors [Iwabu et al. 2010]. Fatty liver is reported to be developed in obese fa/fa Zucker rats given a high-fat/high-cholesterol diet due to a decrease in the expression AdipoR1 and AdipoR2 receptors, which also mediate fatty acid metabolisms in the liver [Matsunami et al. 2011].

Pro-inflammatory cytokine **IL-6** plays an important role in different pathophysiological conditions relating to obesity and IR [Mauer et al. 2015]. Late-onset obesity and insulin resistance are developed in knockout mice, giving evidence in support of its involvement in obesity [Matthews et al. 2010]. It is also directly related to

the low-grade chronic inflammation developed in obesity [Wellen et al. 2005]. M2 polarized adipose tissue macrophages produce IL-6 in obesity, which promotes M2 polarization by upregulating IL-4 receptor-α expression [Braune et al. 2017]. IL-6 inhibits the transcription and translation of adiponectin in the adipocyte cell lines [Fasshauer et al. 2003].

Multifunctional cytokine **TNFα** is responsible for the regulation of different cellular and biological processes, i.e., energy metabolism, insulin resistance, proliferation, survival, cell apoptosis, and inflammation [Ruan et al. 2003; Cawthorn et al. 2008]. Evidence indicates its high expression in obese animals and humans, which were observed to be reduced after weight loss, along with its role in insulin resistance [Tilg et al. 2006; Unamuno et al. 2018]. The macrophages present in the SVF of the adipocytes are a predominant source of adipose tissue-derived TNFα in obesity [Montague et al. 1998; Trzeciak-Ryczek et al. 2011]. The earlier investigations prove that TNFα blockade in fa/fa Zucker rats mitigates insulin resistance [Hotamisligil et al. 1994]. TNFα acts on the adipocytes and regulates the production of pro-inflammatory mediators within the tissues and also inhibits the adiponectin transcription in the adipocyte cell lines [Maeda et al. 2002; Ruan et al. 2002; Cawthorn et al. 2008]. It can be stated that adiponectin levels are negatively related to the circulating TNFα levels [Bruun et al. 2003].

Proinflammatory cytokine **MCP-1 or CC-chemokine ligand 2 (CCL2)**, is also released from the adipocytes. It regulates the macrophage infiltration, and its *in situ* proliferation inside the adipose tissues [Linton et al. 2003; Kanda et al. 2006; Amano et al. 2014]. Its gene expression is found to be significantly up-regulated in the adipocytes,

and its receptor chemokine receptor-2 is expressed mostly in the adipose tissue macrophages [Lumeng et al. 2008; Amano et al. 2014]. In obese subjects, both humans and animals, MCP-1 expression levels are found to be up-regulated [Takahashi et al. 2003; Christiansen et al. 2005; Catalán et al. 2007]. It is also associated with DM because its levels are increased in diabetic patients [Takahashi et al. 2003]. In this regard, studies have shown that it regulates insulin sensitivity in 3T3-L1 adipocytes by downregulating GLUT-4, lipoprotein lipase, and PPARγ expression [Sartipy et al. 2003].

2.8.4 Adipose tissue oxidative stress

The studies in human subjects and obese mice models have shown elevated systemic and adipose tissue oxidative stress, which is directly correlated with the augmented fat stores [Furukawa et al. 2017]. The lipid peroxidation and hydrogen peroxide levels were also increased in the adipose tissues [Shimomura et al. 1996]. Several studies suggest that adipose tissue is a major source of reactive oxygen species, which migrates to the systemic circulation affecting different organs. Increased nicotinamide adenine dinucleotide phosphate (NADPH) oxidase expression in adipose tissues in obesity contributes to the induction of oxidative stress. This mediates increased reactive oxygen species generation, helping in the precipitation of other metabolic complications, following a reduction in the defense system of the adipose tissue, i.e., a decrease in the antioxidant enzymes. The serum adiponectin levels were negatively related to systemic oxidative stress. Elevated reactive oxygen species suppress the expression of adiponectin and elevate that of pro-inflammatory cytokines. Macrophages are linked with the production of reactive oxygen species, and also the induction of NADPH oxidase enzyme in the adipocytes in obesity [Shiose et al. 2001;

Matsuda et al. 2013; Furukawa et al. 2017]. Reactive oxygen species associated lipid peroxidation generates chemoattractant by-products [Curzio et al. 1987]. The NADPH oxidase enzyme converts molecular oxygen to the superoxide radical. The reactive oxygen species generation increases in the adipocytes loaded with free fatty acids, which gets blocked with the NADPH oxidase inhibitor. This signifies the role of NADPH oxidase in the free radical generation in the adipocytes. Increased reactive oxygen species further increases NADPH oxidase mRNA expression and induces increased macrophage infiltration in the adipocytes. This creates a vicious cycle, promoting increased inflammation in the adipose tissue [Furukawa et al. 2017]. When free fatty acid levels are increased in the adipocytes, their increased mitochondrial oxidation generates elevated electron donors, nicotinamide adenine dinucleotide-reduced form (NADH) and flavin adenine dinucleotide-reduced form ($FADH_2$), in the electron transport chain, leading to reactive oxygen species overproduction [Brownlee 2005]. Free fatty acid also stimulates NADPH oxidase via the synthesis of diacylglycerol. Raised free fatty acid levels also activate the NADPH oxidase enzyme in the adipocytes and the remote cells, aggravating reactive oxygen species production [Inoguchi et al. 2000; Matsuda et al. 2013]. Increased oxidative stress in adipose tissues signifies the onset of pathogenic mechanisms in obesity-associated metabolic complications [Furukawa et al. 2017]. Due to the increased mechanical load on adipocytes in obesity, there is an increased consumption of oxygen, leading to the overproduction of reactive oxygen species as superoxide radicals. Also, there occurs a depletion of antioxidant enzymes during prolonged obesity [Khan et al. 2006; Amirkhizi et al. 2010].

2.8.5 Mitochondrial dysfunction

Mitochondria is the primary source of ATP production in all the cells, including the adipocytes. For the differentiation and regulation of the metabolic function of adipocytes, mitochondria play an essential role. In this regard, the mitochondrial content, its biogenesis, and remodeling are all critical. With the increased energy demand, the adipogenesis, oxidative capacity, insulin sensitivity, and lipid homeostasis in adipocytes gets impaired due to mitochondrial dysfunction induced by oxidative damage, lipotoxic effect, and proinflammatory cytokines, further leading to the systemic metabolic disruption [Bournat et al. 2010]. BAT induced thermogenesis is attributed to the mitochondria in the adipocytes, which is increased with the up-regulated mitochondrial number. It is mediated by the UCP-1 protein, which is located in the inner mitochondrial membrane [Madden 2017]. Increase in the mitochondrial thermogenesis in BAT, either through increased activity or biogenesis, or inducing BAT-like properties in WAT for the energy dissipation will help in the treatment of obesity The mitochondrial targeting in WAT and BAT is proposed as a therapeutic strategy for obesity management [Bournat et al. 2010; Cypess et al. 2010]. Overconsumption of the calories increases the mitochondria load which prevents the effective dissipation of the proton gradient, increasing the reactive-oxygen species generation, inducing apoptosis. Increased reactive oxygen species production is observed in the adipocytes in animal models of high-fat diet-induced obesity, further increasing oxidative stress impairing the adipokine release [Talior et al. 2003; Furukawa et al. 2017]. In response to the metabolic challenges, adipocytes alter the mitochondrial number, morphology, or distribution along with a change in the mitochondrial DNA content. The excessive energy substrate is correlated with the overproduction of reactive

oxygen species, which induces dysfunction in the mitochondria, due to the increased peroxidation of lipids and fatty acids in the mitochondrial matrix, thereby amplifying the mitochondrial damage [Roberts et al. 2009; Bournat et al. 2010]. Dysfunction in the mitochondria entails decreased mitochondrial biogenesis and mitochondrial DNA content, negatively affecting the adipogenesis, lipid metabolism, and insulin sensitivity [Lai et al. 2017]. The oxidative capacity of BAT is impaired in the diet-induced obese animal models, affecting the thermogenesis [Feldmann et al. 2009].

2.9 Obesity and Insulin resistance

The dysfunction in the adipose tissues in obesity exacerbates insulin resistance, which is one of the primary components of the metabolic syndrome, leading to the progression of DM [Aguilera-Mendez et al. 2018]. As discussed above, free fatty acid and adipocytokines release is impaired together with increased secretion of pro-inflammatory cytokines from the inflammed adipose tissues in obesity, which are responsible for inducing insulin resistance [Shoelson et al. 2006]. Due to the increased delivery or decreased metabolism of fatty acids, there is an increase in the fatty acid metabolites diacylglycerol, fatty acyl-coenzyme A, and ceramides, which induces serine/threonine phosphorylation of insulin receptor substrates reducing their ability to activate phosphoinositide 3-kinase, further inhibiting insulin signaling mechanism [Shulman 2000]. The insulin-mediated postprandial inhibition of lipolysis is impaired in insulin resistance, leading to increased and persistent free fatty acid flux from the adipose tissue into the circulation [Basu et al. 2001]. Also, the ability to clear free fatty acid after a heavy meal is reduced in insulin resistance. Thus, adipose tissue dysfunction contributes to raised systemic free fatty acids due to increased endogenous free fatty

acid release and reduced ability to clear the exogenous free fatty acid [Lewis et al. 1990; Cooke et al. 2016].

Increased free fatty acid induces JNK, IKK-β, and PKR intracellular signaling pathways, which inhibits the insulin signaling pathway [Hirosumi et al. 2002; Nakamura et al. 2010]. Moreover, JNK and IKK-β activation involve a paracrine inflammatory response through the cytokines, TNF-α, and IL-1β [Aguirre et al. 2002]. The first pro-inflammatory cytokine related to obesity-associated insulin resistance was found to be TNF-α. It activates the serine kinases JNK and IKKβ, leading to the serine phosphorylation of insulin receptor substrate (IRS-1) [Odegaard et al. 2013]. TNF-α and IL-6 increase the release of suppressor of cytokine signaling-3 (SOCS-3) proteins, which impairs insulin signaling after binding with the insulin receptors inhibiting the tyrosine phosphorylation of IRS proteins [Ueki et al. 2004]. The deletion of TNF-α or its receptor TNFR1 partially protects from obesity-induced insulin resistance, suggesting the important role of TNF-α in obesity-induced insulin resistance in mice [Hotamisligil et al. 1993] and humans [Hotamisligil et al. 1995]. The role of IL-6 in the pathophysiological condition of insulin resistance is conflicting from the evidence obtained in animals and humans [Cooke et al. 2016]. IL-6 decreased the glucose uptake in the adipocytes by inhibiting insulin-mediated tyrosine phosphorylation of IRS-1 [Rotter et al. 2003]. However, it enhanced lipolysis in the skeletal muscle but not in adipose tissues [Wolsk et al. 2010]. IL-6 knockout mice developed systemic insulin resistance and also acute treatment with IL-6 in humans increased insulin-mediated whole-body glucose disposal [Carey et al. 2006; Matthews et al. 2010]. WAT derived hormone adiponectin improves insulin sensitivity by improving the glucose and lipid

metabolism in the metabolic tissues in obese mice [Yamauchi et al. 2001]. Insulin resistance was ameliorated in ob/ob mice with the replenishment of recombinant adiponectin at the physiological doses along with an increase in the fatty acid oxidation [Yamauchi et al. 2003]. It also activates ceramidase, which reduces hepatic ceramide levels contributing to improved insulin sensitivity. Conversely, increased hepatic ceramide levels are observed with decrease adiponectin relating to insulin resistance [Holland et al. 2011]. Oxidative stress is also linked with the precipitation of insulin resistance [Meigs et al. 2007; Roberts et al. 2009].

2.10 Obesity and NAFLD

The co-occurrence of NAFLD is very high in obese people and is around 80-90% [Le et al. 2017] therefore, it is marked as a major risk factor for NAFLD occurrence and progression. NAFLD is a chronic hepatic manifestation of obesity and metabolic syndrome, and it commences with lipid storage in the liver followed by inflammation [Calzadilla Bertot et al. 2016; Forlani et al. 2016]. Multiple hit hypotheses of NAFLD suggests diet and environmental factor a vital determinant in the fat accumulation in the liver. Also, impaired adipokine and pro-inflammatory cytokine secretion profile from adipose tissue precipitate insulin resistance affecting the hepatic lipid accumulation. This increases the free fatty acid influx in the liver generating oxidative stress, together progressing towards NAFLD [Buzzetti et al. 2016]. The triglyceride content is excessively increased inside liver cells, which can be defined as 5% or more, leading to the lumpy and scarred liver in around 5–6% of NAFLD patients [Zivkovic et al. 2007; Singh et al. 2015]. Increased fat accumulation induces insulin resistance inside the adipose tissues through the different inflammatory pathways,

which, along with the increased oxidative stress induces fatty liver [Fabbrini et al. 2010].

The lipolysis is enhanced in the adipose tissues due to insulin resistance, increasing free fatty acid release over-spilling it in the circulation, which migrates towards the liver, generating an unfavorable effect [Lomonaco et al. 2012]. 50-90% of free fatty acid in the circulation is from the WAT [Donnelly et al. 2005]. This pool of free fatty acid migrates to the ectopic tissues such as skeletal muscles, liver, pancreas, etc., and when it gets deposited into the liver, it induces hepatic insulin resistance [Nagle et al. 2007; Samuel et al. 2012]. The circulating free fatty acid either activates the cell signaling pathways or its metabolic products diacylglycerol accumulates intracellularly in the liver, both responsible for inducing insulin resistance. Diacylglycerol causes protein kinase C activation, which impedes the tyrosine phosphorylation of IRS proteins precipitating the hepatic insulin resistance [Glass et al. 2012; Jornayvaz et al. 2012]. Free fatty acid-derived ceramides inhibit the protein kinase B activity, which helps in insulin signaling [Powell et al. 2004; Holland et al. 2011]. Hyperinsulinemia further contributes to the increased *de novo* lipogenesis in obesity and NAFLD, both. This further elevates the hepatocellular free fatty acid pool [Roden 2006; Tilg et al. 2017]. As leptin levels are increased in obesity, it further induces proinflammatory cytokines to release, amplifying the process [Day 2002]. Due to the defects in the hepatic metabolism in NAFLD, the production and secretion of VLDL and LDL are elevated, and HDL is decreased, causing dyslipidemia [Avramoglu et al. 2006]. The interplay of other factors, such as oxidative stress, mitochondrial

dysfunction, recruitment of cytokines, also mediates NAFLD progression [Koek et al. 2011].

2.11 AMPK

AMPK, a central metabolic energy sensor, and a serine/threonine-protein kinase, regulates the energy metabolism in various tissues through a counter-regulatory mechanism by switching on the catabolic pathways and switching off the anabolic pathways [Zhang et al. 2009]. AMPK regulates energy balance, both at the cellular level and whole-body level, through the hormonal and nutrient signals in the peripheral tissues and the central nervous system via modulating metabolism, energy expenditure, feeding behavior, cell growth, and autophagy [Hardie 2007; Mihaylova et al. 2011]. It exists as a heterotrimeric enzyme, enclosing a catalytic subunit α and two regulatory subunits β, γ [Davies et al. 1994] (**Fig. 2.5**). It is ubiquitously distributed, with two α isoforms (α1, α2), two β isoforms (β1, β2) and three γ isoforms (γ1, γ2, γ3) [Hardie et al. 2001]. The presence of a glycogen-binding domain in the β subunit acts as a bridge between α and γ subunits [Polekhina et al. 2003]. The γ subunit includes four tandem C-terminal cystathionine beta-synthetase domains, which bind adenosine monophosphate (AMP) and are critical for AMPK regulation [Scott et al. 2004]. Although AMP is involved in allosteric activation of AMPK, it has recently been found that adenosine diphosphate (ADP) also regulates AMPK activity by phosphorylation and dephosphorylation [Oakhill et al. 2011]. AMPK activation by upstream kinases largely depends on phosphorylation of the Thr172 residue of catalytic α subunit [Kemp et al. 2007]. Several upstream kinases such as liver kinase B1 (LKB1) [Sakamoto et al. 2006], calcium/calmodulin-dependent kinase kinase β (CaMKKβ) [Hawley et al. 2005], and

transforming growth factor-β-activated protein kinase-1 (TAK1) [Xie et al. 2006], phosphorylates and regulates the AMPK activity. In contrast, protein phosphatases (PP) such as PP2A and PP2C are involved in AMPK dephosphorylation [Sanders et al. 2007].

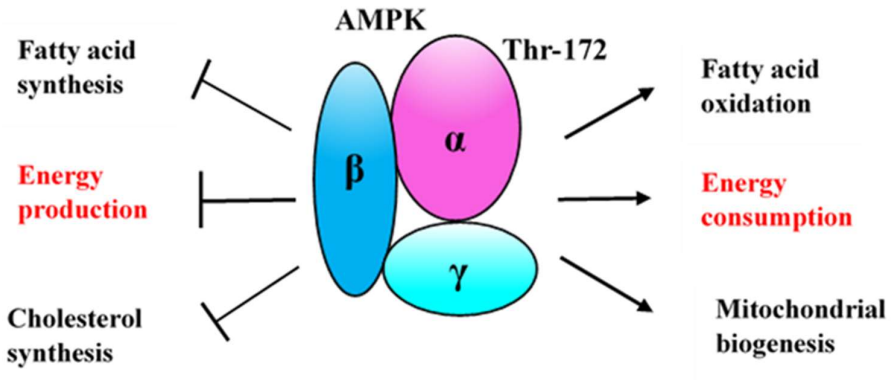


Figure 2.5: Structure of AMPK and its role in energy metabolism [Lage et al. 2008]

Different pharmacological agents and hormones are reported to activate AMPK *in vivo* or through specific treatment of cells and/or tissues. Under conditions of cellular energy demand leading to decreased intracellular ATP and/or increased AMP levels, AMPK gets activated. Thus, physiological stimuli inducing AMPK include exercise, hypoxia, glucose deprivation, ischemia, and heat shock [Carling et al. 2012; Hardie et al. 2012]. Anti-diabetic drug Metformin, thiazolidinedione, and salicylates induce AMPK phosphorylation [Boyle et al. 2010; Foretz et al. 2010; Hawley et al. 2012]. Agents used experimentally for AMPK activation include, 5′-aminoimidazole-4-carboxamide ribonucleoside (AICAR); natural agents such as galgeine, berberine and resveratrol [Lan et al. 2008; Yin et al. 2008; Hawley et al. 2010], and α-lipoic acid [Kim et al. 2004]. Also, the lipid-lowering statin drugs, adipocytokines including leptin,

adiponectin, and ghrelin, activate AMPK in certain tissues [Minokoshi et al. 2002; Yamauchi et al. 2002; Sun et al. 2006]. Also, ciliary neurotrophic factor [Watt et al. 2006], ghrelin, cannabinoids [Kola et al. 2005], and IL-6 [Carey et al. 2006; Lage et al. 2008] are reported to phosphorylate AMPK.

Due to the involvement of AMPK in the regulation of energy homeostasis through the various metabolic pathways, especially the metabolism of glucose and lipid, it is widely recognized as an attractive target for the treatment of obesity and associated complications like DM, metabolic syndrome, and fatty liver [Kahn et al. 2005]. Animal models of high-fat diet-induced obesity has demonstrated diminished AMPK activity in multiple tissues including, muscle [Ruderman et al. 2004], white adipose tissue [Xu et al. 2012; Lindholm et al. 2013], brown adipose tissue [Mottillo et al. 2016], liver [Woo et al. 2014] and skeletal muscle [Bandyopadhyay et al. 2006]. Upon stimulation, AMPK regulates the hepatic lipid metabolism via phosphorylation and inhibition of ACC activity and an increase in malonyl-CoA decarboxylase activity. This results in lower malonyl-CoA levels, which is a critical precursor for fatty acid biosynthesis and a potent inhibitor of mitochondrial fatty acid oxidation. Malonyl-CoA allosterically regulates CPT1, which is responsible for the entry of long-chain fatty acids inside the mitochondria for β-oxidation. This promotes mitochondrial β-oxidation and suppresses fatty acid synthesis. AMPK also regulates the activity of glycerol-3- phosphate acyltransferase enzyme and 3-hydroxy-3-methylglutaryl-coenzyme (HMG-CoA) reductase enzyme, involved in triacylglycerol synthesis and cholesterol synthesis respectively [MUNDAY et al. 1988; Viollet et al. 2009; Woods et al. 2017]. Studies suggest the amelioration of fatty liver complication with Metformin and

thiazolidinediones through hepatic AMPK activation in rodents and humans [Lin et al. 2000; Bajaj et al. 2003]. Chronic ethanol consumption leads to AMPK inhibition in animal models of fatty liver [You et al. 2004]. Transcriptional control via AMPK is proved to be an essential target for the management of fatty liver [Zhou et al. 2001; Foretz et al. 2005]. AMPK phosphorylates PGC-1α, on Thr1722 and Ser538 sites, a key regulator in controlling the expression of transcription factors responsible for mitochondrial biogenesis, resulting in increased mitochondrial biogenesis and mitochondrial content [Jäger et al. 2007; Viollet et al. 2009]. AICAR and catecholamines also induce PGC-1α phosphorylation through the AMPK mediated mechanism, further providing the evidence for the same [Norrbom et al. 2011; Tadaishi et al. 2011].

Evidence shows that targeting of BAT and WAT for the treatment of obesity and associated metabolic complications have gained clinical importance, because of its involvement in the energy expenditure [Saito et al. 2009; Wouter et al. 2009; Bartelt et al. 2014; Wu et al. 2018]. It plays a vital role in the regulation of adipose tissue development, metabolism, and thermogenesis [O'Neill et al. 2013; Day et al. 2017]. AMPK phosphorylation in WAT mediates glucose and lipid metabolism. Although studies suggest inhibition in insulin-mediated glucose uptake by the adipocytes in WAT, conflicting reports are available for lipid metabolism in WAT. AMPK phosphorylates and inactivates ACC in WAT, decreasing fatty acid synthesis and increased its oxidation. AMPK is also reported to suppress proinflammatory signaling in the WAT [Bijland et al. 2013]. Reduced AMPK activity is observed in the adipose tissues of obese and diabetic animal models and also in humans [Ruderman et al. 2010; Gauthier

et al. 2011; Xu et al. 2012]. The adipose tissue-specific deletion of the AMPK subunits β1 and β2 exacerbates high-fat diet-induced hepatic steatosis and insulin resistance due to BAT and WAT dysfunction [Mottillo et al. 2016]. Studies show that AMPKα ablation in mice's adipocytes impairs thermogenesis and energy expenditure predisposing them towards high-fat diet-induced obesity and metabolic dysfunction [Wu et al. 2018]. Moreover, AMPK activation in the adipose tissues is associated with the amelioration of diet-induced obesity in animals [Nguyen et al. 2010; Jiang et al. 2011; Son et al. 2013; Morakinyo et al. 2015]. Berberine promotes thermogenesis in BAT and WAT via the AMPK-PGC1α pathway [Zhang et al. 2014].

Therefore, the pharmacological manipulation of AMPK would be advantageous and a potential target for the amelioration of obesity and its associated metabolic complications.

2.12 Need and scope of repurposing of the US-FDA approved drugs

The research and development of a new drug is a time-consuming process involving enormous efforts, high costs, and above that, the safety and tolerability profile of the drug makes the success rate very low. Drug repurposing or drug repositioning involves identifying an existing and approved drug for a new indication, outside the original indication. It involves low costs and less time, because the clinical trials have already been performed and so the pharmacology, pharmacokinetics, side effects, and drug interactions are known. As the repurposing involves previous research and development efforts, therefore the approval of new therapies by US-FDA could be apace. Aspirin for colorectal cancer, Ketoconazole for cushing's syndrome, and topiramate for obesity are some examples of drug repurposing [Pushpakom et al. 2019].

2.13 Sitagliptin

Sitagliptin (brand name Januvia; Merck Pharmaceuticals), is an oral hypoglycaemic agent approved by the US-FDA in 2006. Its mode of action involves the sustained inhibition of DPP4 enzyme in the gastrointestinal tract, thereby increasing the half-life of incretins, GLP-1 and GIP, released from the enteroendocrine cells of the intestine. The incretins are responsible for increasing the release of insulin from the pancreatic β-cells in response to a meal in a glucose-dependent manner, and decreasing that of glucagon from the pancreatic α-cells during hyperglycemia, decreasing the increased glucose levels [Gallwitz 2007; De Heer et al. 2008] (**Fig. 2.6**). It also reduces glycated hemoglobin (Hb1Ac) levels in diabetic patients [Iwamoto et al. 2010; Kim et al. 2011]. It is either administered as a monotherapy or as add-on therapy with other drugs for the management of DM [Charbonnel et al. 2006; Hermansen et al. 2007; Nauck 2007].

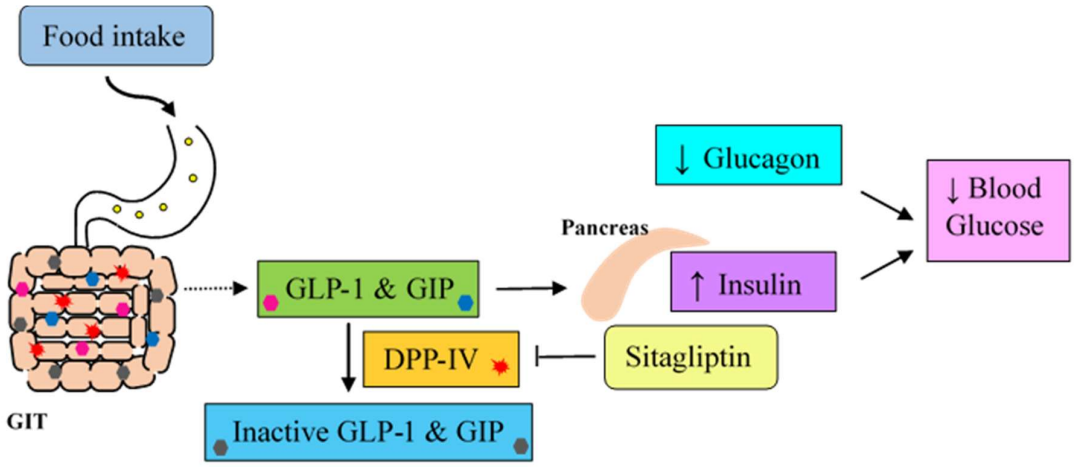
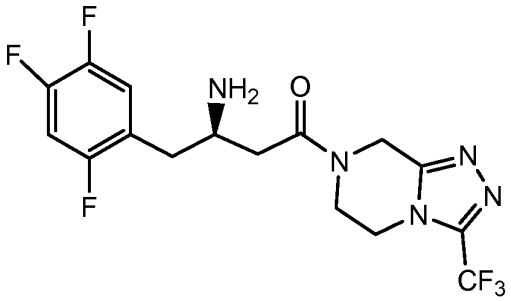


Figure 2.6: Mechanism of action of Sitagliptin [Miller et al. 2006]

Table 2.5: Chemical and Pharmacological Profile of Sitagliptin

Structure		[Gallwitz 2007]
Chemical Formula	$C_{16}H_{15}F_6N_5FO$	[Bennett 2018]
Molecular formula	(2R)-4-Oxo-4-[3-(trifluoromethyl)-5,6 dihydro [1,2,4]triazolo[4,3-a]pyrazin7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine	[Kirn et al. 2005]
Molecular weight	407.32 g/mol	[Bennett 2018]
Dose	100mg once daily	[Lyseng-Williamson 2007]
Bioavailability	87%	[Herman et al. 2007]
Half-life	12-14 hours	[Herman et al. 2006a]
Metabolism	Liver by CYP 3A4 and CYP 2C8 enzymes	[Herman et al. 2006b]
Excretion	Renal; 75% is found unchanged in the urine	[Herman et al. 2006b]
Side effects	Headache, upper respiratory tract infection-nasopharyngitis and headache	[Lyseng-Williamson 2007]

2.13.1 Efficacy of Sitagliptin on the metabolic syndrome parameters from the clinical observations

Katsuyama et al., suggested a significant decrease in body weight in obese patients with DM [Katsuyama et al. 2015] and Soliman et al., suggested a marginal advantage in weight reduction in newly-onset diabetic patients after renal transplant [Soliman et al. 2013]. Sitagliptin improved NAFLD and associated symptoms in diabetic patients [Iwasaki et al. 2011; Yilmaz et al. 2012; Arase et al. 2013]. It reduced intrahepatic lipid content and total body fat in diabetic and obese patients [Kato et al. 2015; Katsuyama et al. 2015]. In DM patients, sitagliptin improved adiponectin levels and exhibited adiponectin dependent anti-atherothrombotic effect [Omoto et al. 2015]. Other studies also reported increased adiponectin levels with sitagliptin [Hibuse et al. 2014; Prayitno et al. 2014]. It significantly improves the endothelial function and inflammation in coronary artery disease in DM patients [Matsubara et al. 2013]. Sitagliptin reduced the TNFα level and increased the IL-10 level in the blood monocytes suggesting its anti-inflammatory role [Satoh-Asahara et al. 2013]. Studies have unveiled the activation of the AMPK pathway in the aorta of high-fat diet-fed apolipoprotein-E-knockout mice [Zheng et al. 2018]. It potentially reduces the infarct size and improves left ventricular function via anti-oxidative stress and cardiac mitochondrial protection mechanisms [McCormick et al. 2014]. Thus no cardiac events are associated with the use of Sitagliptin [Read et al. 2010].

With the above background, Sitagliptin mediated AMPK phosphorylation was proposed, as shown in **Fig. 2.7**.

2.13.2 The dose of Sitagliptin selected for the experimental design

Based on the clinical dose of sitagliptin, its preclinical dose was calculated from the following formula based on the body surface area [Reagan-Shaw et al. 2008]:

Human Effective Dose = Animal Dose (mg/kg) X Animal Km/Human Km

where, Km = Body weight (Kg)/ Body surface area (m^2)

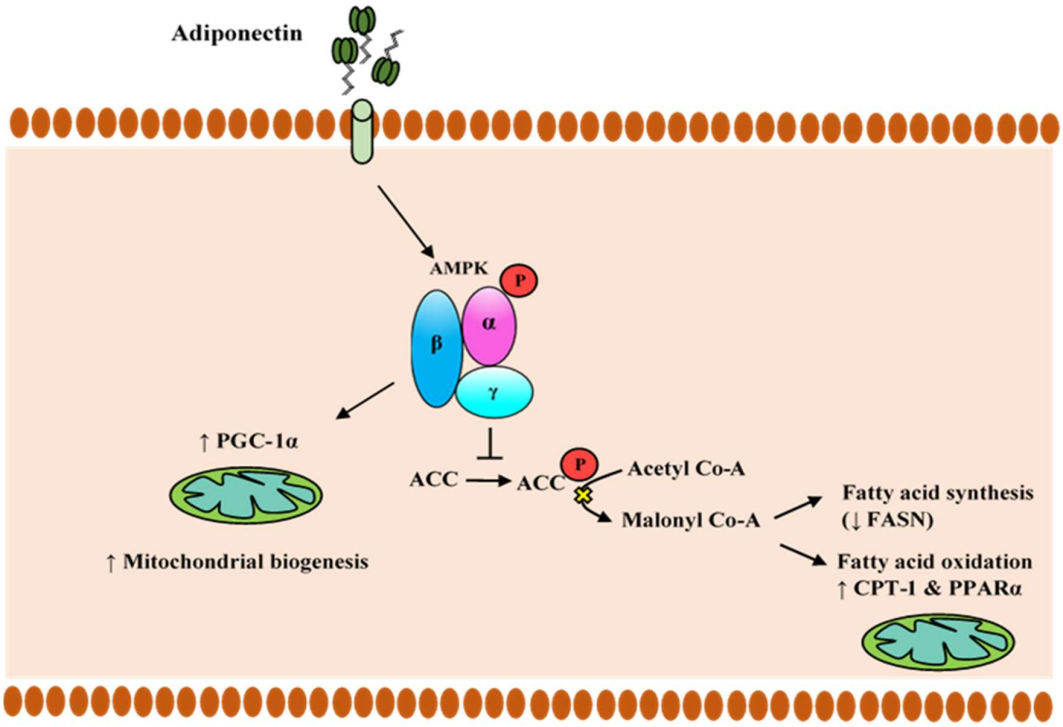


Figure 2.7: Sitagliptin up-regulates adiponectin expression, which induces AMPK phosphorylation in the tissues

2.13.3 Safety of Sitagliptin

Sitagliptin is not associated with the hypoglycemic risk, as observed with other anti-diabetic agents [Bergman et al. 2006]. In a randomized placebo-controlled trial of sitagliptin versus placebo, the trial evaluating cardiovascular outcomes with sitagliptin showed no association of sitagliptin with cardiovascular events or hospitalization due to heart failure [Green et al. 2015]. Engel et al., also reported cardiovascular safety of

sitagliptin in DM patients [Engel et al. 2013]. There have been concerns regarding the use of sitagliptin for pancreatitis. However, the study evaluating the effect of sitagliptin on the pancreas, reported a non-significant difference in the patients developing acute pancreatitis [Drucker 2013].