Chapter-1

INTRODUCTION

Obesity, a nutritional pandemic [Egger et al. 1997], is a response to a plethora of unhealthy calories and decreased energy expenditure. It is a chronic, complex, and multifactorial disorder that advances due to the interaction of numerous factors in varying degrees, including fats and sugar-loaded unhealthy diet, sedentary lifestyle, hormonal imbalance, genetics, and environment. The repercussions of obesity and its complications constitute a salient source of morbidity and jeopardized the quality of life together, significantly affecting life expectancy [Prieto-Hontoria et al. 2011]. The factsheet of the World Health Organization (WHO) suggests that global obesity occurrence has tripled between the years 1975 and 2016. The prevalence of overweight and obesity among children and adolescents was 4% in 1975, whereas in 2016, it reached 18% [World Health Organization, https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight]. World Obesity Federation has predicted that by 2025, 2.7 million adults will be overweight or obese from 1.9 billion in 2016 [Mojto et al. 2019]. The USA, followed by China and India were ranked among the top three nations, together comprising more than 50% of the obese population with 23%, 20%, and 14%, respectively [López et al. 2017; Karri et al. 2019]. Due to its progressive increase over the past few decades, it presents a growing health threat in developed and developing countries and has turned out to be a mammoth challenge in the 21st century for public health.

Obesity is defined as the accumulation of excess or unhealthy fat reserves, which promotes a persistent positive energy balance inside the body, inducing multiple health risks. The fundamental concept behind energy balance is the maintenance of equilibrium between calorie intake and utilization (Fig. 1.1). Prolonged increase in energy intake eventually augments fat accumulation in visceral and subcutaneous adipose depots.

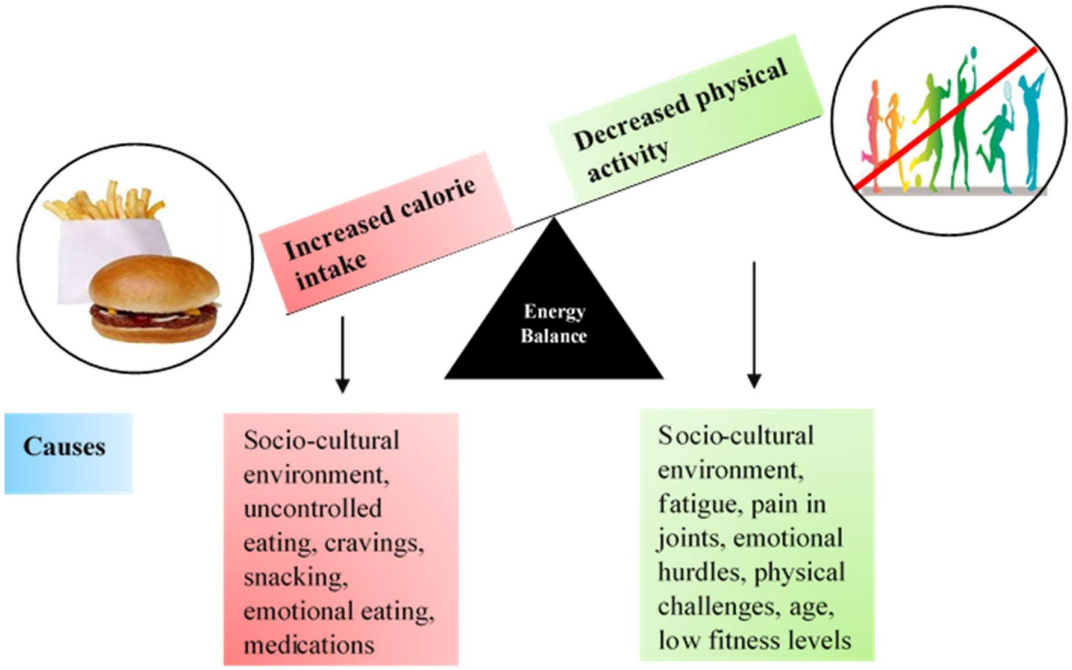

Figure 1.1: Energy balance in Obesity [Sharma et al. 2010]

Adipose tissue is functionally and morphologically distinguished into white and brown types [Trujillo et al. 2006]:

- White adipose tissue (WAT): ample, white coloured with few mitochondria containing unilocular lipid droplets
- Brown adipose tissue (BAT): scarce, brown coloured with abundant mitochondria containing multilocular lipid droplets

WAT reserves surplus calories in positive energy states and releases them in conditions of nutritional demand. Besides this, WAT serves as a dynamic endocrine organ releasing hundreds of adipokines, including adiponectin, apelin, chemerin, leptin, etc., that regulate energy homeostasis. In contrast to WAT, BAT acts as a heat-producing organ concerned with the maintenance of constant body temperature by regulating non-shivering thermogenesis [Timmons et al. 2007].

Obesity is an alarming indicator for the development of metabolic syndrome and associated comorbidities (Fig. 1.2) [Polyzos et al. 2019]. According to the definition of metabolic syndrome, three among five of the following features must be present to diagnose metabolic syndrome: central obesity, increased triglyceride level, decreased high-density lipoprotein-cholesterol (HDL-C), increased blood pressure, and impaired glucose homeostasis [Aguilera-Mendez et al. 2018].

Figure1.2: Complications of Obesity [Blüher 2019]

The primary storage depot of triglycerides is WAT, but in states of increased energy intake, ectopic lipid deposition occurs in the liver, skeletal muscles, and pancreas, which hampers the regular function of these organs [Cusi 2009]. WAT stores excess triglycerides through lipoprotein lipase enzyme, and releases them in states of nutritional states, through the enzyme hormone-sensitive lipase. The lipoprotein lipase activity increases in high-calorie intake incorporating more fatty acids in WAT, subsequently expanding the adipocytes through hypertrophy or hyperplasia or both, together with an increase in immune cell infiltration [Sun et al. 2011; Reccia et al. 2017]. WAT remodeling is accompanied by chronic low-grade inflammation compromising its endocrine function affecting pro-inflammatory mediators and adipokines secretion along with elevated oxidative stress [Xu et al. 2003; Hauner 2005]. This creates susceptible conditions for the increased infiltration of macrophages inducing the release of pro-inflammatory cytokines such as IL-6, TNF-α, and MCP-1, which inhibit insulin action in insulin-responsive tissues through paracrine and endocrine mechanisms, inducing metabolic complications [Yoshida et al. 2014]. Insulin regulates the fatty acid release from WAT by inhibiting lipolysis and maintaining glucose homeostasis through glucose transporter-4 (GLUT-4). However, in insulin resistance, lipolysis is enhanced with the increased efflux of free fatty acids in the systemic circulation and impaired re-esterification along with reduced glucose uptake. This increases the pool of free fatty acids in the systemic circulation over spilling it to non-adipose locations, i.e., skeletal muscle, liver, and pancreas [Garg 2011].

Non-alcoholic fatty liver disease (NAFLD), a chronic hepatic manifestation of obesity, is one of the frequent causes of liver associated morbidity and fatality

worldwide, affecting 25-30% of the general population [Ahmed et al. 2015; Wu et al. 2018]. The increasing prevalence of obesity-driven NAFLD is predictive of its strong relationship with obesity and metabolic syndrome [Rayyan et al. 2018]. According to the multiple-hit hypotheses, nutritional, environmental and genetic factors are among the key insults of NAFLD. They promote hepatic fat accumulation due to adipose tissue dysfunction, insulin resistance, and impaired adipokine and pro-inflammatory cytokines secretion (Fig. 1.3) [Calzadilla Bertot et al. 2016]. This increases the free fatty acid influx and oxidative stress in the liver, facilitating an environment for the progression of NAFLD [Buzzetti et al. 2016].

De-novo lipogenesis is stimulated in obesity and NAFLD, along with reduced fatty acid oxidation [Morigny et al. 2016]. The circulating free fatty acids are responsible for 59% of intrahepatic triglycerides accumulation, whereas 26% and 14% are contributed by *de novo* lipogenesis and obesogenic diet, respectively [Donnelly et al. 2005]. *De novo* lipogenesis is increased by more than three folds in states of fatty liver or obesity [Ameer et al. 2014]. The fatty acid reservoir in the liver precipitates oxidative stress and mitochondrial dysfunction due to the production of toxic metabolites viz., ceramides and diacylglycerol and inflammatory cytokines, which induces insulin resistance. On other ectopic sites such as skeletal muscle, triglyceride deposition prevents insulin signaling whereas in the pancreas, it prevents the insulin secretion capacity of β-cells, both eventually contributing to the development of insulin resistance [Guebre-Egziabher et al. 2013]. Obesity and physical inactivity are interlinked risk factors for the development of DM, and both are implicated in NAFLD development. *De novo* lipogenesis, a fundamental metabolic pathway occurring

predominantly in the liver, converts excess carbohydrates or fats, reaching the liver into fatty acids that are stored as triglycerides after re-esterification. The accumulation of lipid is a first and pivotal step in hepatic steatosis, and suppressing it improves hepatic steatosis along with increased insulin sensitivity [Caputo et al. 2017].

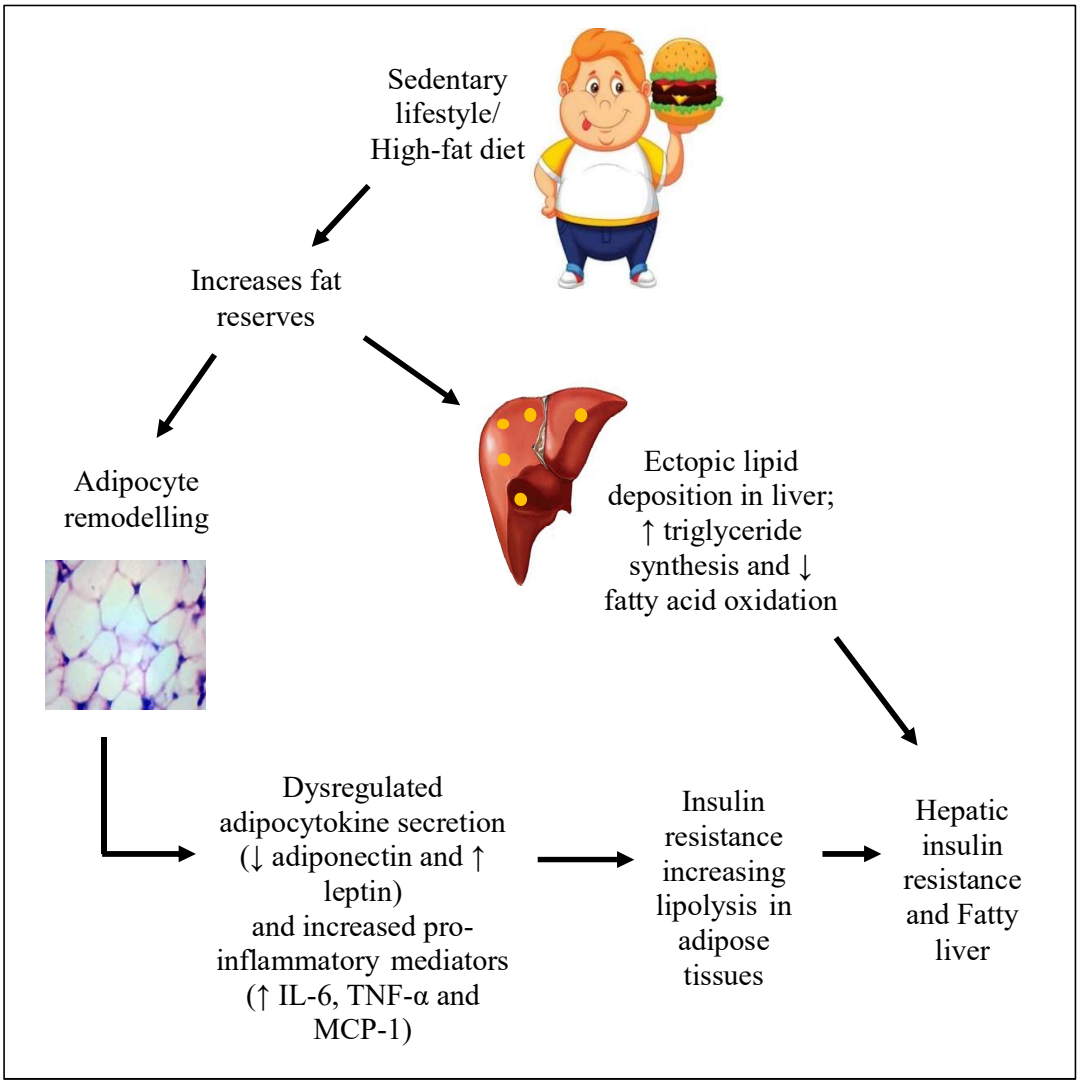

Figure 1.3: Effect of High-fat diet on the adipose tissue and the liver [Cooke et al. 2016]

5’ adenosine monophosphate-activated protein kinase (AMPK), a serine-threonine kinase, and a heterotrimeric complex is an endogenous and central metabolic sensor which maintains the cellular energy status of the cell by regulating metabolic pathways [Kim et al. 2016], oxidative stress, endoplasmic reticulum stress, and inflammation [Cool et al. 2006]. The AMPK phosphorylation switches the cells from anabolic to catabolic state catalyzing ATP generation while inhibiting anabolic processes, hence decreasing lipogenesis and increasing fatty acid oxidation [Fruhbeck et al. 2001]. It regulates critical metabolic enzymes involved in *de novo* lipogenesis and fatty acid oxidation; acetyl co-a carboxylase (ACC), fatty acid synthase (FASN), carnitine palmitoyltransferase-1A/1B (CPT-1A/1B) and peroxisome-proliferator-activated receptor α (PPARα) [Guo et al. 2010]. In obesity, NAFLD and chronic low-grade inflammation, structural and molecular alteration of AMPK occurs in various tissues, including liver, skeletal muscle, and adipose tissues [Mottillo et al. 2016]. The mitochondrion is responsible for carrying out many important biological functions essential for metabolic homeostasis. Being the powerhouse of the cell, they are involved in ATP production, intracellular Ca^{+2} regulation, and reactive oxygen species generation. In early NAFLD, mitochondrial activity increases to protect the hepatocytes from free fatty acid deposition, but in the long run, mitochondrial adaption decreases to prevent lipotoxicity. The mitochondrial remodeling also occurs in adipocytes during the condition of obesity [Fernández Vázquez et al. 2018]. AMPK governs normal cellular function by promoting mitochondrial biogenesis through regulating master regulator PGC-1α which further signals downstream targets; nuclear respiratory factor-1 (NRF-1) and nuclear respiratory factor-2 (NRF-2), which activates mitochondrial transcription

factor A (TFAM) ultimately increasing the mitochondrial abundance [Giampieri et al. 2017]. The low adiponectin level impairs AMPK-PGC-1α signaling pathway hindering mitochondrial biogenesis [Yan et al. 2013].

AMPK activation has a protective role against obesity, fatty liver, and metabolic complications. It occurs through pathophysiological situations, metabolic hormones, and pharmacological agents [Rasineni et al. 2012; Hardie 2015]. US FDA approved anti-obesity drugs for long term use include orlistat, lorcaserin, phentermine-topiramate, naltrexone-bupropion, and liraglutide [Daneschvar et al. 2016]. However, these drugs work either by altering the appetite or decreasing the calorie absorption, which cannot be an ideal strategy for treating obesity. For NAFLD, no treatment is approved by regulatory agencies at present, but search for effective drugs is in the process together with diet and lifestyle modification [Vilar-Gomez et al. 2015]. As both the metabolic complications are multifaceted and inter-dependent disorders, factors such as adipose tissue dysfunction, insulin resistance, mitochondrial dysfunction, inflammation, and altered metabolic profile, should be considered in the intervention strategy [Moctezuma-Velázquez 2018].

WAT derived endocrine hormones leptin and adiponectin regulate appetite, metabolic homeostasis, inflammation, insulin signaling, and hepato-protection. Leptin resistance is the hallmark of obesity inducing insulin resistance. It sends the peripheral signal of nutritional status to the central nervous system by regulating energy intake and expenditure, but in obesity, it facilitates hyperphagia [Li et al. 2016]. Adiponectin is inversely associated with adiposity, insulin resistance, and DM [Ghadge et al. 2018]. There is a considerable decline in adiponectin levels in metabolic syndrome and a

reversal of this can ameliorate metabolic complications. Adiponectin exerts its effect by binding with the adiponectin receptors: AdipoR1 and AdipoR2. AdipoR1 activates AMPK, which is involved in glucose utilization and fatty-acid combustion while AdipoR2 increases the expression of PPARα ligands inducing increased fatty acid combustion and energy consumption. Due to decreased adiponectin levels in metabolic complications, adiponectin signaling is disrupted. However, AdipoR1/AMPK and AdipoR2/PPARα are reinstated when adiponectin levels are restored [Yamauchi et al. 2014].

In obesity, the free fatty acid release gets intensified from over-accumulated fat in WAT inducing nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in adipose cells generating superoxide [Furukawa et al. 2017]. Moreover, increased β-oxidation of free fatty acids escalates superoxide generation in adipocytes, which further represses the release and mRNA expression of the adiponectin [Brownlee 2005]. BAT dissipates heat from fat by generating ATP through uncoupling mitochondrial oxidation by uncoupling protein 1 (UCP1), expressed selectively in BAT. For thermogenesis, increased mitochondrial biogenesis is required, which is regulated by PGC-1α. However, mitochondrial biogenesis is reduced in high-fat diet-induced obesity in organs with high metabolic activity [Valerio et al. 2006]. The repercussion of fatty diet on BAT is similar to WAT, increased tissue mass, mitochondrial malfunctioning, and oxidative stress.

The drug selected for the present work is US-FDA approved dipeptidyl peptidase-4 (DPP-4) inhibitor, Sitagliptin. DPP-4 enzymes are membrane-bound peptidases expressed at multiple tissues and organs involved in rapidly degrading

incretin, the metabolic hormones in the gastrointestinal tract. Following food ingestion, there is a sharp increase in glucose levels, which prompts the release of incretins, glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic peptide (GIP) by endocrine cells of the intestine. GLP-1 and GIP eventually lower blood glucose by causing glucose-dependent insulin release [Green et al. 2006; Coskun et al. 2017].

The present work attempts to investigate the effect of sitagliptin on body weight reduction, visceral adipose tissue inflammation, adipokine levels, metabolic syndrome, and fatty liver in high-fat diet-induced obese mice models with attention on adiponectin/AMPK signaling. The evaluation of oxidative stress and mitochondrial biogenesis markers in brown and white adipose tissues is also envisaged. The 60% high-fat diet and 20% Fructose Water (HFFW) was fed to the animals for sixteen weeks, and treatment was given for the last eight weeks. The selection of diet based on fat and fructose was done to mimic the metabolic abnormalities along with obesity, as observed in the phenotype of human metabolic syndrome. Body weight gain, cumulative food intake, and organ weight index were determined. The following biochemical parameters were evaluated; fasting blood glucose (FBG), fasting serum insulin (FSI), oral glucose tolerance, insulin tolerance, lipid levels (TC, TG, LDL-C, HDL-C), adipokine levels, uric acid, GLP-1 levels, and liver marker enzymes ALT and AST. Insulin sensitivity was calculated from the HOMA-IR formula. The level of hepatic fat content was estimated by measuring the hepatic TG and TC levels in the liver. For oxidative stress, superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione (GSH) were determined in the respective tissues. Histopathological staining was performed with Hematoxylin & Eosin (H&E) dye. The expression of P-AMPK and

P-ACC proteins were determined by western blotting with respect to β-actin, while reverse transcriptase-polymerase chain reaction (RT-PCR) was used to determine mRNA levels of AdipoQ, CPT-1A, FASN, PPARα, PGC-1α, NRF-1, TFAM, and UCP-1 relative to ACTB gene. Statistical analysis of results was done by GraphPad Prism 5-software, USA. One-way Analysis of variance (ANOVA) gave differences among the group means followed by Tukey's multiple comparison post hoc test for finding the statistical significance, unless otherwise stated. The *p*-value was set at <0.05.