CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Obesity as a Global Epidemic

Obesity is a complex, multifactorial, and largely preventable disease that results from an imbalance between energy intake and expenditure (Panuganti et al., 2019). The World Health Organization defines obesity as abnormal or excessive fat accumulation that may impair health (World Health Organisation, 2018). Obesity is assessed by determining the body mass index (BMI) of the individual wherein the weight in kilograms is divided by the square of the height in meters (kg/m^2). A BMI value greater than or equal to 25 is considered overweight, and a BMI value greater than or equal to 30 indicates obesity. Although measuring BMI is useful as a screening tool since it applies to all ages of adults and both sexes, it does not measure body fat directly and should only be considered as a rough guide (World Health Organisation, 2018).

As a multifactorial disease, obesity results from a myriad of genetic, social, or cultural factors. The fundamental cause of obesity is a chronic energy imbalance as a consequence of excessive calories consumption and insufficient calories expenditure, resulting in excessive weight gain (Blüher, 2019). A sedentary lifestyle and nutritional habits depict major risk factors for the aetiology of obesity (Hruby et al., 2016). Women who increased their low physical activity (< 30min/day) to high levels (≥ 30 min/day) has significant weight reduction. In contrast, those whose physical activities remained low or fell from high to low had elevated weight gain risk (Mekary et al., 2009). Meanwhile, individuals with a high intake of processed meat, potatoes, sweets, and sugar-sweetened beverages have gained more weight in 8 years than those with high intakes of fruits, vegetables, fish, and poultry (Schulze et al., 2006). Aside from dietary patterns and physical activity, the energy imbalance is partially attributed to individual's physiology and behaviour shaped by economic and environmental changes, including eating culture, stress, smoking, recreational drug use, policies, and workplace (Blüher, 2019). Genetic

predisposition, such as mutations in genes coding for leptin, leptin receptor, proopiomelanocortin, melanocortin 4 receptor, and others, have also shown to cause obesity, thus highlighting the significance of biological factors in obesity pathogenesis (Montague et al., 1997; Clément et al., 1998; Krude et al., 1998; Farooqi et al., 2000).

Obesity has progressively worsened for the past 50 years (Panuganti et al., 2019). The worldwide prevalence of overweight and obesity has doubled since 1980. The prevalence of obese men increased from 5% in 1980 to 10.1% in 2015, and from 8.9% to 14.8% in women (Chooi et al., 2019). In low-income countries, obesity is mostly higher among middle-aged adults from wealthy environments, while obesity affects both sexes and all ages in high-income countries (Swinburn et al., 2011). In most cases, the prevalence of obesity is higher in women than men in all sociodemographic levels (GBD 2015 Obesity Collaborators, 2017). Despite being very common in the world, its condition is often underestimated (Ofei, 2005). If secular trends continue, it is estimated that 20% of the world's adult population will be obese by 2030 (Hruby & Hu, 2014).

Obesity is considered hazardous as it is correlating strongly with chronic diseases, including hypertension, dyslipidaemia, non-alcoholic fatty liver, insulin-dependent diabetes mellitus, and cardiovascular diseases, all of which are associated to dysfunctional changes in the adipose tissue. The disease is also an established risk factor for many other complications, including arthritis, cancers, varicose veins, pulmonary disease, and gall-bladder disease (Tortora & Derrickson, 2017; Klop et al., 2013). The increase in adiposity correlates with increased adipokine synthesis, lipid production, the activity of the sympathetic nervous system and renin-angiotensin-aldosterone system, which contribute to the development of type 2 diabetes, steatohepatitis, congestive heart failure, and more. Mechanical stress from increased adiposity also contributes to the development of osteoarthritis, obstructive sleep apnoea, and oesophageal adenocarcinoma (Heymsfield & Wadden, 2017).

1.2 Adipose Tissue

Adipose tissue is an essential metabolic organ that represents the primary site for energy storage. Briefly, the adipose tissue is a mass of loose connective tissue that is made up of adipocytes and stromal vascular cells, including the fibroblasts, resident immune cells, preadipocytes, endothelial cells, and mesenchymal stem cells (Figure 1.1) (Henriques et al., 2019). The tissue is located throughout the body, making about 18% body weight of an average person (Tortora & Derrickson, 2017). There are two major types of adipose tissues, namely the white adipose tissue (WAT) and the brown adipose tissue (BAT). WAT has two major depots, the subcutaneous WAT and visceral WAT around internal organs. The brown adipose tissue (BAT) is localised in the supraclavicular and paravertebral areas (Figure 1.2). Rodents have similar adipose tissue localisation as humans. However, they have epididymal WAT that does not exist in humans but is often studied as a visceral WAT model (Choe et al., 2016).

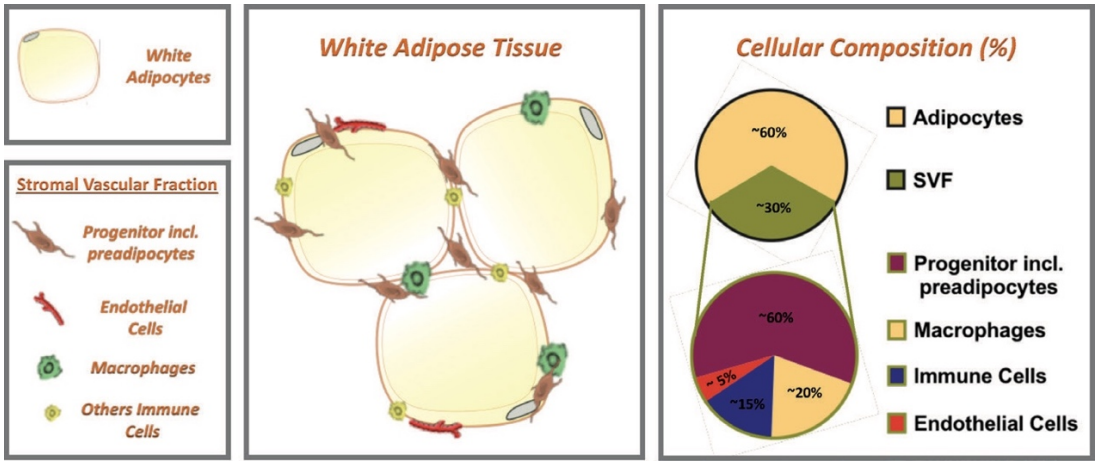

Figure 1.1 Adipose tissue cellularity. The majority of the adipose tissue is comprised of adipocytes (approximately 60%), while other cell types in the tissue are called the stromal vascular fraction (SVF). Mesenchymal stem cells, preadipocytes, macrophages, other immune cells, and endothelial cells make up the stromal vascular fraction (Henriques et al., 2019).

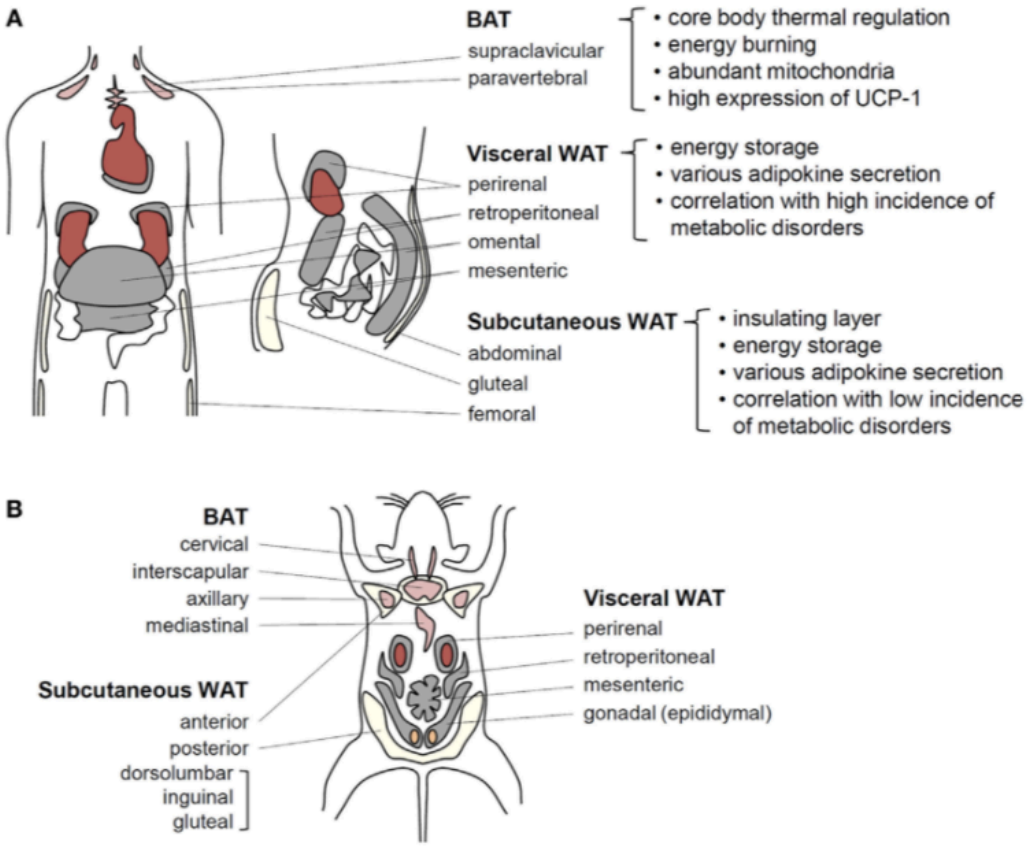

Figure 1.2 Adipose depots. (A) In humans, BAT is located around the shoulders. The brown adipocytes express UCP-1 and have abundant mitochondria, which contributes to thermogenesis. Subcutaneous WAT is localised beneath the skin and well-distributed around the body while visceral WAT encapsulates intra-abdominal organs. Both subcutaneous and visceral WAT are capable of storing energy. The visceral WAT is often correlated with obesity-induced metabolic disorders. (B) In adult mice, BAT is more easily observed than those of adult humans. The gonadal WAT depots located around the testes in males and the ovaries in females are generally utilized as visceral WAT models, although those depots do not exist in humans (Choe et al., 2016).

Both WAT and BAT play significant roles in the body. BAT has a distinct darker colour attributed to its rich blood supply and high mitochondrial density, which are essential for aerobic cellular respiration (Tortora & Derrickson, 2017). BAT produces heat to maintain body temperature, which occurs primarily on the back of babies who cannot produce body heat by shivering (Marieb & Hoehn, 2013). Meanwhile, WAT mainly functions as a storage for excess

energy in the form of lipids, particularly intracellular triglycerides droplets (Tortora & Derrickson, 2017). A single, large triglyceride droplet, coated mainly by Perilipin A, fills up the cell and pushes the cytoplasm and nucleus to the periphery (Tortora & Derrickson, 2017). (Matafome & Seiça, 2017). In times when energy is needed, lipolysis occurs, which is the hydrolysis of triglycerides into free fatty acids and glycerol that are released to the circulation (Matafome & Seiça, 2017). Certain WAT depots, in response to appropriate stimuli, may undergo a browning process in which uncoupling protein 1 (*UCP1*), a gene responsible for thermogenesis, is induced (Lo & Sun, 2013). Subcutaneous WATs are more susceptible to browning than visceral WATs (Seale et al., 2011). These adipocytes, termed as brite (brown in white) or beige adipocytes, possess a degree of thermogenic activity and are shown to regulate glucose and energy homeostasis (Sidossis & Kajimura, 2015).

WAT also has paracrine and endocrine functions in which the tissue secretes 600 different factors such as chemokines, cytokines, and coagulation factors, all collectively known as adipokines. An example of adipokine is leptin, which is one of the most well-studied adipokines. Leptin is released in response to food intake and suppresses the appetite by regulating the neural circuits in the hypothalamus (Choe et al., 2016). Another well-known adipocyte-secreted adipokine is adiponectin, which is abundant in the blood. Adiponectin regulates glucose production, alleviates insulin resistance, and enhances fatty acid oxidation (Z. V. Wang & Scherer, 2016). Altogether, the adipokines are known to regulate various physiological processes, including appetite and inflammation, as well as metabolic homeostasis, such as glucose and lipid metabolism, of adjacent and remote organs (Matafome & Seiça, 2017). A dysregulation in the secretion of these factors may occur during abnormal adipose tissue remodelling, resulting in impaired inter-organ communications and, eventually, the development of a broad spectrum of metabolic disorders (Juge-Aubry et al., 2005).

1.3 Adipose Tissue Expansion and Remodelling

1.3.1 Adipose Plasticity

Adipose tissues are highly plastic in nature to maintain metabolic homeostasis in response to nutrient excess or deficiency. Excessive calories in diets are generally converted to triglycerides and stored in adipocytes. In the presence of excess energy, the mature adipocytes enlarge and undergo cellular hypertrophy to accommodate more triglycerides for storage (Tortora & Derrickson, 2017). Cellular hypertrophy triggers subsequent adipocyte proliferation, resulting in increased cell number (hyperplasia) (Tortora & Derrickson, 2017). Large adipocytes also release hormones and cytokines, facilitating recruitment of surrounding preadipocytes to differentiate into fully mature adipocytes (Figure 1.3) (Martinez-Santibañez, 2015).

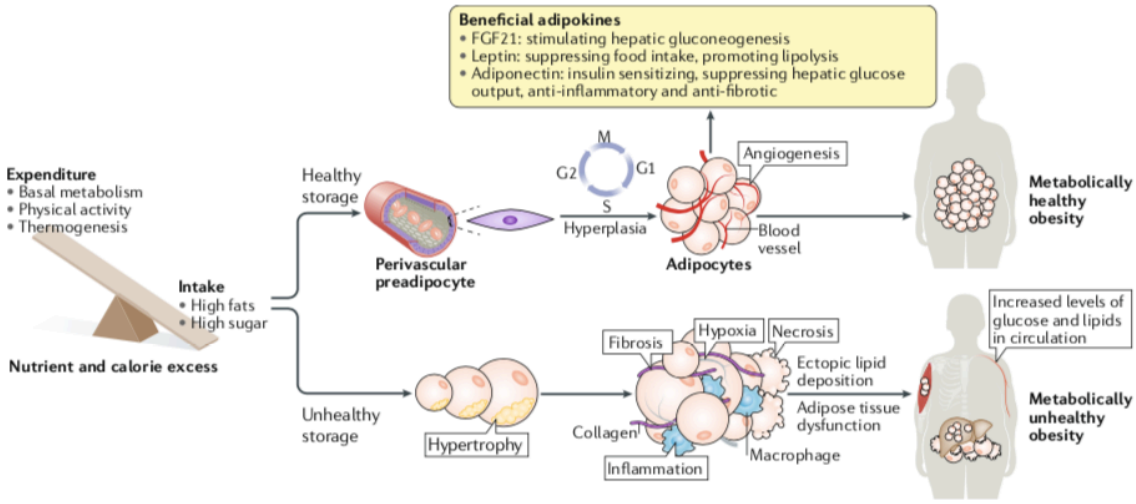

Figure 1.3 Mechanism of adipose tissue expansion. Adipose tissue expands during states of overnutrition to store caloric excess. The expansion can be done through either hyperplasia or hypertrophy, wherein the former is considered as healthy while the latter is deemed unhealthy. Contrary to proper angiogenesis and beneficial adipokine secretion observed in hyperplasia, hypertrophic adipocytes experience hypoxia that is insufficient to promote new blood formation. As a result, the hypoxic environment induces fibrosis, cell death and inflammation, altogether causing pathological adipose tissue remodelling and metabolic dysfunction (Ghaben & Scherer, 2019).

1.3.2 Adaptive Remodelling

A “healthy” adipose tissue remodelling is an acute response to excessive nutrient availability, in which adipose tissue expansion and increased lipid storage is short-lived and can be reversed readily (Crewe et al., 2017). During early adipose tissue expansion, an increase in dietary lipids induces the adipose tissue to be in a state of mild hypoxia as the rapid adipocyte hypertrophy maximizes the diffusional limit of oxygen (Crewe et al., 2017). To re-establish tissue homeostasis, the mild hypoxia induces stress signalling that stimulates the secretion of angiogenic factors. The hypoxia-inducible factor-1-alpha (HIF-1α) transcription factor is a major mediator of the hypoxia response (Palazon et al., 2014). Consequently, angiogenic factors are secreted to induce new blood vessel formation. Among these factors, the vascular endothelial growth factor A/vascular endothelial growth factor receptor 2 (VEGFA/VEGFR2) signalling pathway is known to be importantly involved in angiogenesis (Crewe et al., 2017). Aside from the angiogenic factors, the adipose tissue also releases essential adipokines and angiogenic inhibitors, such as adiponectin, leptin and plasminogen activator inhibitor, to create a balance of blood vessel formation and promote a healthy angiogenic cascade (H. P. Lee et al., 2015; Cao et al., 2001; Scroyen et al., 2009).

Besides vasculature, immune cells are imperative in the adipose tissue environment as the crosstalk between resident leukocytes and adipocytes allows a harmonized system in regulating energy storage. Both innate and adaptive immune cells are identified in adipose tissue, but macrophages make up the largest population of resident immune cells (Schipper et al., 2012). Adipose tissue macrophages, initially discovered in murine adipose depots by expression of the macrophage protein, F4/80, are dispersed throughout WAT and has an alternatively activated (M2) anti-inflammatory phenotype (Lumeng et al., 2007). They secrete interleukin (IL)-10 to promote adipocyte insulin sensitivity (Exley et al., 2014). Eosinophils are also present in adipocytes, being the main source of IL-4, a cytokine that mediates M2 activation

of macrophages (Wu et al., 2011). Moreover, resident adipose B cells are required for immunity against infection, while Tregs are required for maintaining a steady anti-inflammatory environment in lean adipose tissues (Exley et al., 2014; Feuerer et al., 2009).

Extracellular matrix (ECM) is a fundamental component in adipose tissue for its various roles. It acts as a scaffold to maintain cellular and structural integrity, while also serving as a medium for cell-cell and cell-ECM crosstalk, thus allowing cells to sense and adapt to the demands of the dynamic environment (Martinez-Santibañez, 2015). ECM, mainly collagen, is deposited during early adipocyte expansion. When expansion continues, ECM remodelling occurs by degrading existing ECM and producing new ECM components to accommodate changes in shape (Divoux & Clément, 2011). Components such as osteopontin, hyaluronan, thrombospondins, matrix metalloproteases (MMPs), and tissue inhibitor of metalloproteinases (TIMPs) are involved in ECM remodelling (Ruiz-Ojeda et al., 2019). It is this strict regulation of ECM remodelling enzymes that is crucial for a proper adipose tissue remodelling, and that an imbalance between ECM deposition and degradation is observed in obesity. For instance, a study with mice subjected to one week of a high-fat diet, MMP14, a gene involved in collagen breakdown, was reported to be significantly upregulated. Consistently, mice with deletion of MMP14 demonstrated inappropriate ECM remodelling (Chun et al., 2010). In contrast, collagen VI-deficient mice exhibited unrestricted expansion (Khan et al., 2008). Hence, there is ECM degradation signalling in early adipose tissue expansion to accommodate adipocyte growth and preserve metabolic homeostasis.

1.3.3 Maladaptive Remodelling

Expansion in adipocytes eventually reaches an upper volume limit. Severe hypoxia and stress signalling are present in overly expanded adipocytes, which later translates into injury signalling, dysfunctional adipocytes and consequent cell death, leading to the loss of functional

adipocytes (Figure 1.3) (Crewe et al., 2017; Matafome & Seiça, 2017). The process also triggers immune cell infiltration wherein monocytes are recruited and undergoes differentiation into pro-inflammatory macrophages that encircle necrotic adipocytes, forming a "crown-like structure" (Cinti et al., 2005; Trayhurn et al., 2008). These macrophages produce cytokines, including tumor necrosis factor-alpha (TNF-α), IL-6, IL-1β and monocyte chemoattractant protein-1 (MCP-1), that propagate the inflammatory response and further aggravates adipocyte dysfunction (Hotamisligil et al., 1993; Rotter et al., 2003; Longo et al., 2019; Weisberg et al., 2006). During this process, neutrophils and macrophages produce toxic reactive oxygen species (ROS) and reactive nitrogen species (RNS) which adds on to the injury (Marseglia et al., 2014).

Adipose tissue depots are highly vascularized to support tissue expansion. Vasculature provides the oxygen, nutrients, hormones and growth factors, as well as helps with the removal of metabolic waste products (Crewe et al., 2017). In view of obesity and pathological adipose tissue expansion, progressive enlargement of adipocyte sizes would increase the inter-capillary distance, resulting in a relative reduction in blood perfusion to each adipocyte, causing a net decrease of adipose oxygen tension. Eventually, the adipose perfusion becomes incompatible with the expansion of the adipose tissue. The onset of hypoxia inhibits the differentiation of preadipocytes, causes adipocytokine dysregulation and enhances the pro-inflammation responses.

Fibrosis here is defined as an excessive accumulation of ECM components that causes an imbalance between increased production of fibrillar components and reduced degradation of those components. Fibrosis development was previously reported to be a result of continuous HIF-1α activation that induces synthesis of ECM components (Matafome & Seiça, 2017). Moreover, infiltrated macrophages and adipocytes in the inflammatory environment secrete collagens which further stimulates fibrosis (Keophiphath et al., 2009). Although fibrosis has been continuously linked to pathological adipose tissue remodelling and metabolic

dysfunction, recent studies have shown this to be debatable. Fibrosis may be beneficial as it improves metabolic homeostasis by providing rigid ECM, thus preventing abnormal adipocyte enlargement (Datta et al., 2018). Conversely, excessive rigidness exerts shear stress on expanding adipocytes, leading to cell death, inflammation, and metabolic derangement (Lin, Chun & Kang, 2016). The conflicting reports may be due to variations in fat depots as results are dependent on the adipose depot affected. For instance, interferon regulatory factor 5 (IRF5)-deficient mice on a high-fat diet limits visceral WAT expansion but enhances subcutaneous WAT expansion (Dalmas et al., 2015). The differences among studies may also be due to the dissimilarity of fat pad distribution between human and murine subjects.

To sum up, complex events occur in the adipose tissue in order to rapidly remodel and adapt to calorie intake and maintaining metabolic homeostasis. Nevertheless, chronic overnutrition will lead to an integrated response among the three factors, namely impaired angiogenesis, unresolved inflammation, and ECM dysfunction and fibrosis, which ultimately result into a pathological adipose tissue expansion and obesity-associated metabolic consequences.

1.4 Challenges in Conventional Management of Obesity

As dietary habits and sedentary lifestyle represent major risks for obesity, the principal management of the disease is diet therapy and lifestyle modifications. Calorie restrictions are enforced by consuming very-low-calorie diets (200-800kcal/day) or low-calorie diets (800-1600kcal/day) (Ruban et al., 2019). Behaviour modification programs are also available for obese people to alter eating behaviour into more nutritious ones and initiate exercise activities (Tortora & Derrickson, 2017). However, these strategies are difficult to achieve over the long term and were observed to result in weight re-gain.

When diet and exercise alone could not control the body weight effectively, pharmacological treatment is recommended. These treatments aim to lower lipid absorption, limit food intake or increase energy expenditure. An example is Orlistat which inhibits the release of lipase to the gastrointestinal tract to reduce triglyceride absorption. Another example is Liraglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist of the GLP-1 hormone acting on peripheral and central receptor pathways that affects satiety, food intake and glucose homeostasis (Ruban et al., 2019; Srivastava & Apovian, 2017). These weight lowering pills, however, may cause unfavourable side effects and carries a risk of further injury (Ruban et al., 2019; Tortora & Derrickson, 2017).

If all other interventions failed, bariatric surgery poses as an alternative to treat obesity. Procedures may involve gastric bypass or gastroplasty which reduces stomach size, biliopancreatic division which rearranges the gastrointestinal tract, and liposuction which suctions fat deposits and reshaping the body (Tortora & Derrickson, 2017; Marieb & Hoehn, 2013). Although the effect on weight loss is superior to non-surgical interventions, surgeries have raised concerns about safety and potential long-term complications. Despite the importance of all these conventional therapies, effective management of obesity remains a significant unmet medical need. Considering that obesity is a multifactorial disease, drugs that target multiple pathological processes contributing to the development and progression of obesity may offer a better treatment outcome.

1.5 Novel Therapeutic Targets for Obesity

The currently available pharmacotherapy for obesity is mostly focused on regulating central nervous system pathways that either enhance satiety or reduce appetite. However, the association of ‘unhealthy’ WAT expansion with hypoxia, inflammation, and fibrosis presents potential novel therapeutic avenues (Kusminski et al., 2016).

The use of anti-inflammatory drugs to treat obesity is under investigation. Antagonizing compounds or neutralizing antibodies targeting pro-inflammatory signalling have shown beneficial outcomes. Monoclonal antibodies against TNF have significantly lowered systemic inflammatory markers and fasting glucose levels in obese patients, while also improving insulin resistance in rheumatoid arthritis patients (Stanley et al., 2011; Dominguez et al., 2005; Solomon, 2011). Another promising approach is blockage of macrophage chemotaxis into WAT. C-C chemokine receptor 2 (CCR2) is crucial for macrophage migration. Phase II clinical trials of a CCR2 antagonist, CCX140-B, have improved glycaemic parameters in diabetic patients (Di Prospero et al., 2014).

Novel potential strategies in targeting fibrosis and hypoxia have also emerged. Endotrophin is a potent WAT fibrosis co-stimulator. Adipocyte-specific overexpression of endotrophin enhanced fibrosis and insulin resistance, while the inhibition by neutralizing antibodies reverses fibrosis and improves insulin sensitivity (Sun et al., 2014). Additionally, enhancing platelet-derived growth factor receptor-alpha (PDGFR-α) signalling also promotes WAT fibrosis by transforming perivascular cells into ECM-producing pro-fibrotic cells (Iwayama et al., 2015). Future studies on PDGFR-α signalling inhibition should prove to be interesting. Meanwhile targeting HIF-1α to alleviate obesity possesses a great potential. Studies on diet-induced obesity mice with PX-478 treatment, a HIF-1α inhibitor, effectively elevated energy expenditure, reduced body weight gain and mitigated inflammation and fibrosis (K. Sun et al., 2013). However, adipose tissue specificity is imperative to avoid adverse side effects. Thus, HIF-1α inhibitors have not been used in human clinical trials for obesity and type 2 diabetes.

Another alternative emerging strategy is to trigger BAT activation and browning process in WAT. Since the cold temperature has shown to do so, pharmacological agents that mimic the effects of cold exposure may aid in BAT activation and WAT browning (Cannon & Nedergaard, 2004). Constant β3-adrenergic stimulation promoted WAT browning, high energy expenditure,

and ameliorated obesity in rodents (Ghorbani & Himms-Hagen, 1997). β3-adrenergic receptor agonists, therefore, harbours the therapeutic potential for drug intervention.

The peroxisome proliferator-activated receptors (PPARs) are members of nuclear receptor superfamily of transcription factors that are fundamentally important for regulating lipid metabolism, adipokine production and adipogenesis (Evans et al., 2004). PPARγ is highly expressed in WAT and BAT depots, predominantly regulating of adipogenesis, lipid pathways and insulin sensitivity (Evans et al., 2004). Phosphorylation of PPARγ is identified in adipose tissue depots of mice subjected to high-fat diet, resulting in dysregulation of genes essential for metabolic homeostasis (Choi et al., 2010). Moreover, extracellular signal-regulated kinase (ERK)-mediated PPARγ phosphorylation leads to insulin resistance in mice models. Inhibiting the ERK kinase, mitogen-activated protein kinase kinase (MEK), demonstrated improved insulin sensitivity in the diabetic *ob/ob* mice (Banks et al., 2014). Hence, MEK inhibitor is a potential insulin-sensitizing agent that signals through PPARγ in adipose tissues.

1.6 Leucine-rich Alpha-2-Glycoprotein 1

Leucine-rich alpha-2-glycoprotein 1 (LRG1) is a member of the leucine-rich repeat (LRR) family that contains the LRR domain and a C terminal capping domain (Ng et al., 2010). The LRRs are sequence motifs present in over 2000 proteins (Enkhbayar et al., 2003). They have been identified in a large number of prokaryotic and eukaryotic proteins such as hormone receptors, cell-adhesion molecules, enzymes, extracellular matrix-binding glycoproteins, and more. The primary function of these motifs was known to provide a framework for protein-protein interactions, which includes signal transduction, cell adhesion, DNA repair, and apoptosis (Enkhbayar et al., 2003). Mutations or polymorphisms in LRR proteins have been associated with diseases, in particular, class II transactivator (CIITA) in rheumatoid arthritis and nucleotide-binding oligomerization domain-containing protein 2 (NOD2) in Crohn disease (Ng et al., 2010).

LRG1 was first isolated in 1977. It was identified by Haupt and Baudner to be an inflammatory protein in human serum (Haupt & Baudner, 1977). However, its functional role remained undetermined until 2013 when Wang and colleagues first reported LRG1 as a novel pro-angiogenic factor. LRG1 is specifically required for pathological neovascularisation in the eye and it exerts its function by switching endothelial transforming growth factor-beta-1 (TGF-β1) signalling from the angiostatic activin receptor-like kinase (ALK)-5-Smad 2/3 axis towards the angiogenic ALK1-Smad1/5/8 cascade in the presence of endoglin (Figure 1.4) (X. Wang et al., 2013). Furthermore, inhibition of LRG1 via antibody blockade attenuates angiogenic signalling and abnormal blood vessel formation *in vivo* (X. Wang et al., 2013).

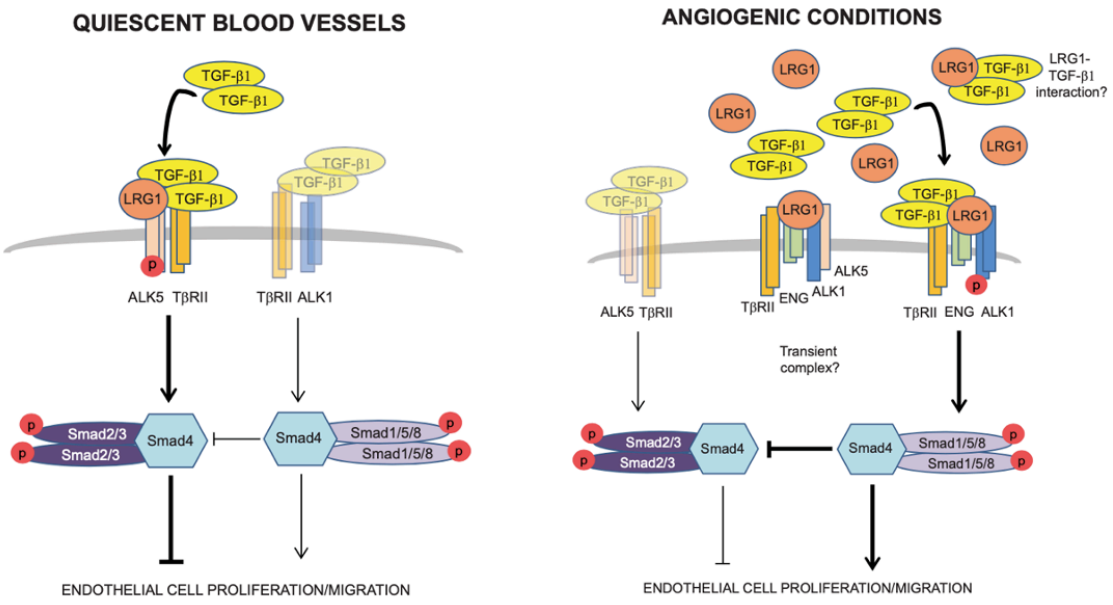

Figure 1.4 Proposed model of LRG1 mediated TGF-β1 endothelial cell signalling. In resting conditions, vascular homeostasis is maintained predominantly by the TβRII-ALK5-Smad2/3 pathway of the TGF-β1 signalling. As for pathological angiogenesis conditions, there is elevated LRG1 and ENG production. LRG1 and TGF-β1 interaction promote efficient ENG/TβRII/ALK1 receptor complex formation, leading to a switch in TGF-β1 signalling to the ALK1-Smad1/5/8 pathway and a pro-angiogenic transcriptional response. There is the possible formation of a transient LRG1/ENG/TβRII/ALK1/ALK5 complex that enables ALK1 phosphorylation, but in the presence of TGFβ1, LRG1/ENG/ALK1/TβRII is more prominent (ENG: endoglin; TβRII: TGF-β type II receptor) (X. Wang et al., 2013).

Since then, many studies on LRG1 and its role in various diseases have followed. LRG1 levels have been shown to be increased in the plasma of several inflammatory diseases. Serum LRG1 was found to be elevated in systemic juvenile idiopathic arthritis, acute appendicitis, and ulcerative colitis patients, while LRG1 levels in cerebrospinal fluid are also increased in bacterial meningitis patients (Shimizu, Inoue, Mizuta, Nakagishi & Yachie, 2019; Rainer et al., 2017; Shinzaki et al., 2016; Chong et al., 2018) Thus, LRG1 is noted to be a novel biomarker for such diseases. Furthermore, a study reported that LRG1 promotes proliferation and differentiation of T helper 17 cells, and is involved in the progression of collagen-induced arthritis, a mouse model of rheumatoid arthritis, through TGF-β-Smad2 signalling enhancement (Urushima et al., 2017).

There have also been studies of LRG1 in tissue fibrosis. TGF-β has an important role in fibrotic diseases, including idiopathic pulmonary fibrosis. Remembering the fact that LRG1 was identified as a modulator of TGF-β signalling in angiogenesis, LRG1 level was escalated in the lungs of wild-type (WT) mice 21 days after bleomycin administration prior to fibrosis development, thus indicating that LRG1 may have a role in the pathogenesis of lung fibrosis (Honda et al., 2017). Contradicting to the results observed in the lung fibrosis study, a study in skin fibrosis showed that LRG1-deficiency in fibroblasts increased alpha-smooth muscle actin (α-SMA) expression. At the same time, treatment with recombinant LRG1 protein decreased expression of α-SMA. The results observed suggest that fibroblast activation is associated with LRG1-deficiency condition, and that increased LRG1 levels suppress fibroblast activity of the skin (Sng et al., 2018). Another study stated the significance of LRG1 in cardiac fibrosis wherein LRG1 expression inversely correlates with the expression of fibrotic genes. Moreover, as it is known that TGF-β1 is a master regulator in fibrotic cardiac remodelling, it is observed that cardiac fibroblasts (CFs) isolated from LRG1 KO mice were more migratory, contractile, and expressed higher fibrotic proteins than WT CFs in response to TGF-β1 stimulation. The data hence suggested that LRG1 has a suppressive role in TGF-β1-induced CF activation (C. Liu et al., 2019).

Additionally, LRG1 has been reported to be upregulated in multiple malignancies, including hepatocellular carcinoma, leukaemia, retinoblastoma, thyroid carcinoma and pancreatic ductal adenocarcinoma (PDAC) (Xiao & Zhu, 2018; Amer, Tiosano & Pe'er, 2018; Ban, He, Tang, Zhang & Xu, 2019; Xie, Zhang, Jin, Mao & Fu, 2019). In thyroid carcinoma, knockdown of LRG1 inhibited cell migration, invasion, and epithelial-to-mesenchymal transition, but not proliferation and apoptosis (Ban, He, Tang, Zhang & Xu, 2019). In PDACs, LRG1 demonstrated promotion of survival, proliferation, migration and invasion of PDAC cells *in vitro*, and tumour growth *in vivo* (Xie, Zhang, Jin, Mao & Fu, 2019). Both studies found that the bioactivities of LRG1 are a result of the enhancement of the mitogen-activated protein kinase (MAPK)/p38 signalling pathway (Ban, He, Tang, Zhang & Xu, 2019; Xie, Zhang, Jin, Mao & Fu, 2019). Therefore, LRG1 is noted to play a detrimental role in multiple malignancies, which are also associated with pathological angiogenesis, inflammation and fibrosis.

1.7 Potential Role of LRG1 in Obesity

As mentioned previously, studies have suggested the involvement of LRG1 in angiogenesis, inflammation and tissue fibrosis. Moreover, LRG1 acts as a direct downstream target gene of PPARβ/δ, a transcription factor that critically regulates energy homeostasis and adipogenesis (C. Liu et al., 2019; Ravnskjaer et al., 2009). Additionally, Pek and colleagues reported that plasma LRG1 level is increased in obese patients, suggesting LRG1 as a biomarker for obesity (Pek et al., 2018). Given all of the above preliminary indications, we hypothesise that LRG1 may exert a role in mitigating adipose tissue remodelling in obesity through modulation of adipogenesis, angiogenesis, ECM remodelling and inflammatory signalling. To gain insights into LRG1's role in these pathological processes contributing to obesity, we will subject LRG1-deficient (*Lrg*$^{-/-}$) and WT male littermates to diet-induced obesity using a proprietary LIDPAD diet for 26-weeks and follow-up with a series of blood biochemical analysis, histopathological

staining and molecular study of excised adipose tissues. This is the first known work to explore the role of LRG1 in regulating systemic metabolic health.

The information extracted from this study would allow us to gain valuable insights into the general systemic metabolic changes associated with LRG1-deficiency and the corresponding adipose depot-specific changes. Results from histological staining would enable us to understand the cell-type-specific changes while the molecular data would help us to gain preliminary understanding to unravel mechanism governing the systemic and cellular changes. The information from this study would pave the way for future projects to design adipose-centric strategies to mitigate the global fight against obesity more effectively. Apart from obesity development and progression, changes in inflammation, angiogenesis and fibrosis are also reflected in other fibrovascular complications such as cancer, atherosclerosis, various blinding eye diseases and rheumatoid arthritis (Aguilar-Cazares et al., 2019; Camaré et al., 2017; Friedlander, 2007; Campa et al., 2010; MacDonald et al., 2018). Therefore, the outcome of this study may provide not only useful insights into obesity pathophysiology but also other fibrovascular diseases in general. Moreover, the information extracted from this study may aid the ongoing development and application of LRG1-mediated therapeutics.

1.8 Research Objectives

To test our hypothesis, the specific objectives of this study are:

1. To establish the expression pattern of *Lrg1* transcripts in different adipose depots
2. To characterize the gross anatomical changes in *Lrg1*-null and wild-type littermates subjected to diet-induced obesity
3. To assess the histological and molecular changes in the epididymal WAT from *Lrg1*-null and wild-type littermates subjected to diet-induced obesity