

Chapter 1

Introduction

1.1 Obesity: A global health concern

Obesity is indeed a significant global health issue and is often described as an epidemic due to its widespread prevalence and its impact on individuals and societies. The World Health Organisation (WHO) defines obesity as the abnormal or excessive accumulation of fat that presents a risk to health. Obesity is a complex and multifaceted condition influenced by a myriad of factors, including dietary patterns, physical activity levels, genetic predispositions, and environmental influences (WHO, 2000). Over the past few decades, the prevalence of obesity has risen at an alarming rate, posing significant public health concerns and challenging healthcare infrastructure globally (Ng et al., 2014).

Due to the widespread distribution of adipose tissue throughout the body, it is not possible to measure obesity directly. Simply assessing body weight fails to provide any understanding of fat accumulation. Instead, range of metrics such as body mass index (BMI), waist circumference, waist-to-hip ratio, skin fold thickness, and bioimpedance are utilized to evaluate obesity and overweight status. The BMI is often used to determine the prevalence of overweight and obesity. BMI is calculated by dividing a person's weight in kilograms by the square of their height in metres (kg/m^2). BMI is categorised based on the standards established by the National Institute of Health in the United States and is endorsed by the WHO. Generally, BMI below $18.5 \text{ kg}/\text{m}^2$ is classified as underweight, whereas a BMI between 18.5 and $24.9 \text{ kg}/\text{m}^2$ is considered to be within the normal or healthy weight range. A BMI equal to or greater than $25 \text{ kg}/\text{m}^2$ denotes overweight, while a BMI equal to or greater than $30 \text{ kg}/\text{m}^2$ signifies obesity. The following table presents the details of the global classification for adult underweight, overweight, and obesity, which is determined by BMI (Table 1.1).

Several factors contribute to the obesity epidemic, include:

Unhealthy diets: High consumption of calorie rich foods, often high in fats and sugars.

Lack of physical activity: Sedentary lifestyles and decreased physical activity levels contribute to weight gain. In a study, it is demonstrated that participating in physical exercise, which includes both aerobic and resistance training, plays a crucial role in managing obesity. A curvilinear dose-response relationship was established between physical activity and health from being inactive to moderately active (Raiman et al., 2023).

Table 1.1 BMI-based classification of individuals as underweight, overweight or obese

Classification	BMI (kg/m ²)
Underweight	< 18.50
Normal Weight	18.50 - 24.99
Overweight	25.00 - 29.99
Obese Class I	30.00 - 34.99
Obese Class II	35.00 - 39.99
Obese Class III	≥40.00

Genetic factors: Some individuals are genetically predisposed to obesity. Genome-wide scans have effectively identified more than one hundred loci associated with the common (polygenic) form of obesity. Although genetic factors certainly contribute to an individual's propensity to gain weight, obesity linked Single Nucleotide Polymorphisms (SNPs) identified so far only account for less than 3% of the inherited tendency to develop obesity. The percentage mentioned is significantly lower compared to the heritability estimates of BMI, which vary between 47% and 80%. It is apparent that there are other genomic locations, including alleles with low or rare frequencies, or other genetic changes like copy number variations that probably contribute to making individuals more prone to weight gain. In addition to genetic variables, epigenetic factors such as variations in histone modifications (including methylation, acetylation, phosphorylation), miRNAs and changes in gut flora are also important players in determining susceptibility to obesity (Albuquerque et al., 2017).

A population-based study genotyped 12 SNPs linked to obesity and found that the association with BMI was more pronounced in sedentary individuals compared to those who were physically active. Maintaining a physically active lifestyle significantly decreased (upto 40%) the hereditary predisposition towards obesity (Li et al., 2010).

Environmental factors: Obesity prevalence are significantly associated with variables such as gender, race, ethnicity, and socioeconomic status. The links between each of these traits form complex and varied relationships. The easy accessibility to cheaper food options that are high in calories and energy, combined with reduced physical activity due to changes in

job and transportation, might result in a long-term state of having more energy intake than expenditure. Apart from the availability and quality of food, the changes in the type, quantity, and pricing of food are also important factors that contribute to the obesity epidemic. Sedentary lifestyles, reduced physical activity, sleep time, and stress levels are all independently associated with weight growth. Obesity is not just caused by an imbalance between calorie intake and energy expenditure, but is also influenced by environmental, genetic, and psychosocial factors (Panuganti et al., 2023; Yadav & Jawahar, 2023). Obesity increases the chances of developing several comorbid conditions, such as type 2 diabetes, cardiovascular disease, chronic renal disease, site specific malignancies, musculoskeletal issues, and infections. Weight loss can lead to a substantial decrease in risk for most of these comorbid diseases (Pi-Sunyer, 2009).

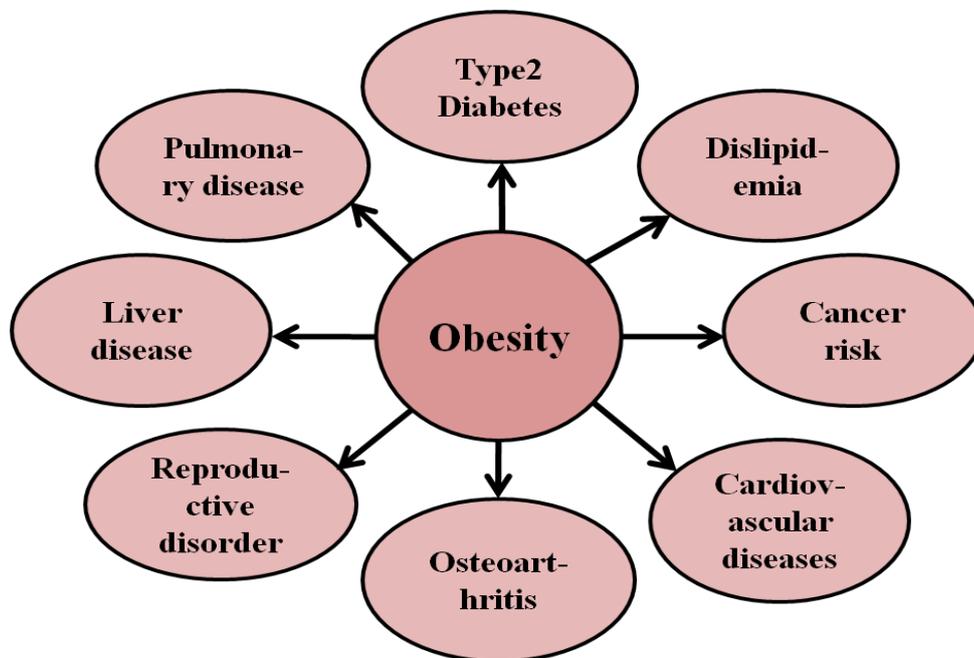


Figure 1.1: Obesity and its associated co-morbidities

Data from two Finnish cohort and the UK Biobank cohort were compared and analysed for simultaneous assessment of 78 health outcomes. This comparative study provides a detailed understanding of the health consequences associated with obesity. The study revealed a strong association between obesity and 21 separate diseases that affect different organ systems, with no overlap among them. Individuals with obesity had a significantly higher risk of both simple and complex multi-morbidity compared to those with a healthy weight. The risk of simple multi-morbidity was five times higher, while the risk of complex multi-morbidity was more than 12 times higher. Participants who developed obesity before the

age of 50 had a higher risk of complicated multi-morbidity compared to those who developed obesity at older ages. Implementing healthier environments to reduce the risk factors that lead to obesity is an economical straightforward approach to decrease the prevalence of multiple health conditions at the population level. Meanwhile, interventions targeting obesity, including lifestyle changes, medications, and surgical procedures, have the potential to prevent the development of multiple health conditions in individuals (Kivimäki et al., 2022).

1.2 Obesity in India: A growing health concern

India, once known for its high prevalence of undernutrition and related underweight, is witnessing a significant shift in its health scenario due to increasing burden of obesity. Rapid urbanization, changing dietary habits, and sedentary lifestyles contribute to the increasing prevalence of obesity across diverse age groups in the country. This shift presents India with a complex health challenge due to the coexistence of undernutrition and obesity, which creates a dual burden of malnutrition.

BMI serves as an indirect measure of body fatness, primarily quantifying excessive weight rather than specifically targeting excess body fat. This approach may not be ideal for assessing body composition in the South Asian Indian population. A considerable portion of the South Asian population may exhibit elevated body fat levels despite falling within the normal range of BMI. This phenomenon is known as normal weight obesity. Besides BMI, alternative indicators for evaluating obesity include waist circumference, waist-hip ratio, neck circumference, waist-height ratio, and body fat estimation (Table 1.2). In addition to these indicators, more advanced imaging techniques can be utilized to estimate visceral fat. Visceral fat is widely considered the most reliable marker of obesity, offering precise assessment of the underlying cardio-metabolic risk factors (Kalra et al., 2023).

Despite (nearly) 12% of the population being affected by obesity, the prevalence of abdominal obesity was notably high across all BMI subcategories (Gupta et al., 2023). Several factors were found to increase the risk of both obesity and abdominal obesity, including older age, female gender, higher educational attainment, increased wealth index, marital status, and urban residency. Moreover, North Indian population and their alcohol consumption were associated with an elevated risk of abdominal obesity. While southern India was associated with an increased risk of obesity (Gupta et al., 2023). The NFHS5 survey conducted between 2019 and 2021 evaluated abdominal obesity by measuring waist

circumference (NFHS-5., 2019). A study based on NFHS-5, highlighted that the rate of abdominal obesity in the country is 40% among women and 12% among males. The results indicate that 50-60% of women aged 30-49 are suffering from abdominal obesity. Abdominal obesity in women is more strongly associated with older age groups, urban inhabitants, higher socioeconomic status, and junk food diets. The pervasiveness of abdominal obesity is increasing in rural regions and is affecting individuals from lower and middle socio-economic backgrounds (NCD Risk Factor Collaboration (NCD-RisC), 2024).

Table 1.2 Other indicators for evaluating obesity

Markers	Measures in South Asians	Measures in Western Population
BMI	Females $\geq 23 \text{ kg/m}^2$	Females $\geq 25 \text{ kg/m}^2$
	Males $\geq 23 \text{ kg/m}^2$	Males $\geq 25 \text{ kg/m}^2$
Waist circumference	Females $\geq 80 \text{ cm}$	Females $\geq 88 \text{ cm}$
	Males $\geq 90 \text{ cm}$	Males $\geq 102 \text{ cm}$
Waist to Hip ratio	Females ≥ 0.8	Females ≥ 0.8
	Males ≥ 0.9	Males ≥ 0.9
Body fat percentage	Females $\geq 33\%$	Females $\geq 35\%$
	Males $\geq 20\%$	Males $\geq 20\%$

A meta-analysis consisting of 21 studies conducted between 2003 and 2023, involving 186,901 children in India, revealed significant rise in childhood obesity. The combined prevalence of childhood obesity was found to be 8.4 %, while the prevalence of childhood overweight was determined to be 12.4%. Male children were more inclined towards obesity in comparison to female children. In addition, children who have a family predisposition to obesity have a higher susceptibility of developing obesity (Singh et al., 2023).

The problem of overweight and obesity places a considerable financial strain on the Indian economy. India is ranked third worldwide in terms of estimated economic expenditures linked to overweight and obesity, after China and the USA. Based on a 2017 report from the World Obesity Federation, it is estimated that India will provide a yearly budget of US\$13 million by 2025 for the treatment of ailments associated with obesity. In addition to direct

medical expenses, which make about 32% of the overall spending on obesity, there are also significant indirect expenditures involved. These indirect costs include expenditures associated with seeking medical treatment, economic losses resulting from early death, absence from work, and the adverse effect on work efficiency. Collectively, indirect expenses account for 68% of the overall cost of obesity, highlighting the complex economic consequences of the obesity crisis in India (Kalra et al., 2023).

Obesity is a significant and concerning threat to public health in India, requiring an urgent action at individual, community, and policy levels. By understanding the complex interplay of social, environmental, and behavioural factors contributing to obesity, focused treatments can be developed to reduce its prevalence and alleviate its negative health effects.

1.3 Adipose Tissue Dysfunction in Obesity

Obesity arises as a result of an imbalance between energy intake and expenditure. Adipose tissues (AT) are reservoirs of energy, which is stored in the form of triglycerides. AT has a crucial role in maintaining energy balance. There are two forms of adipose tissue, Brown Adipose Tissue (BAT) and White Adipose Tissue (WAT). BAT is involved in non-shivering thermogenesis, while WAT is responsible for maintaining metabolic homeostasis. Additionally, inducible BAT, also known as beige adipocytes, has gained attention for its thermogenic properties in response to external stimuli such as cold, exercise, prolonged exposure to PPAR γ agonists, β 3-adrenergic receptor activation, and tissue injury (Giralt & Villarroya, 2013). AT expansion in a non-neoplastic manner is an important feature of obesity. AT formed at distinct areas as specific depots, commonly classified as subcutaneous and visceral fat. The visceral fat is well linked with metabolic disorders (Salazar et al., 2014).

Furthermore, adipose tissue also serves as a vital endocrine organ, secreting a variety of adipokines. Apart from adipocytes, immune cells and endothelial cells residing within AT are also significant sources of adipokine production (Luo & Liu, 2016). Obesity results in increase in both the number of adipocytes, known as hyperplasia and their volume, known as hypertrophy, consequently leading to an augmentation of adipose mass (Spalding et al., 2008).

The abnormal expansion of the AT induces alterations in the composition of immune cells within the AT. This abnormal expansion of adipocytes also leads to functional impairment

of AT due to lipotoxicity, endoplasmic reticulum (ER) stress, hypoxia, and inflammation. These processes are implicated in the development of insulin resistance (IR) (Wondmkun, 2020). Metabolic stress and dysfunction in AT establish a close connection between obesity and inflammation. Inflammation originating from within the adipose tissue is recognized as a significant contributing factor to systemic inflammation, further compromising the insulin signalling pathway (Ellulu et al., 2017; Rehman & Akash, 2016).

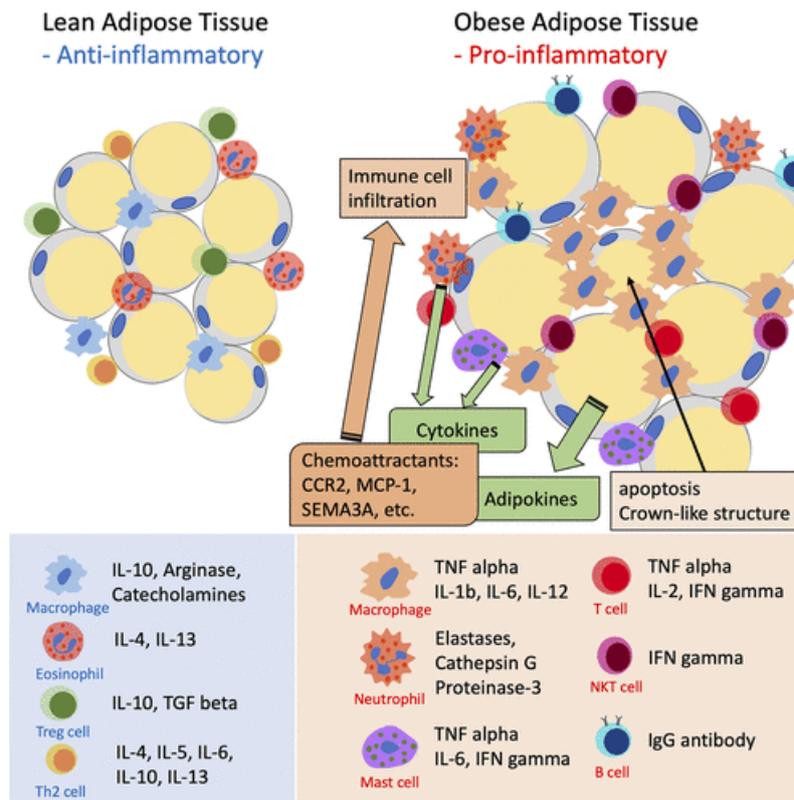


Figure 1.2 AT Remodelling during obesity adapted from (Kawai et al., 2021)

1.3.1 Inflammation: As mentioned above, obesity is marked by the low grade sterile inflammation, known as meta-inflammation. Inflammation in obesity is a complex process involving dysregulation of the immune system and chronic low-grade inflammation throughout the body. Adipose tissue, once thought to be just a storage site for excess energy, is also an endocrine organ that secretes various pro-inflammatory molecules. Several adipokines, including adiponectin, leptin, resistin, visfatin, chemerin, TNF α , IL1 β , IL6, IL8, IL10, plasminogen activator inhibitor 1, monocyte chemoattractant protein-1 (MCP1), and retinol binding protein-4, have a role in regulating insulin resistance (IR).

In obesity, adipose tissue undergoes significant changes, including an increase in adipocyte size (hypertrophy) and an influx of immune cells such as macrophages, T cells and neutrophils. These immune cells, along with adipocytes, produce pro-inflammatory cytokines. This chronic low-grade inflammation can disrupt normal metabolic processes and contribute to the development of insulin resistance, type 2 diabetes, cardiovascular diseases, and other metabolic disorders. Furthermore, obesity-associated inflammation can also affect other tissues and organs in the body, such as the liver, skeletal muscle and brain, contributing to systemic metabolic dysfunction.

TNF α : TNF α is recognised as a significant immune response cytokine in obesity, as well as in development of T2DM. The first observation of elevated levels of TNF α in the adipose tissue of various genetically obese and diabetic animal models by Hotamisligil G established a connection between immune mediators and metabolic disorders (Hotamisligil et al., 1993). The same group confirmed it from fat tissues in obese Caucasian women and also established the mechanism of TNF α affecting the insulin signalling pathway (Hofmann et al., 1994; Hotamisligil et al., 1995). Various studies also showed augmented TNF α in AT and plasma in obese and T2DM patients. TNF α levels were correlated with BMI and fasting blood glucose, FBG (Fontana et al., 2007; Patel et al., 2019).

It has been suggested that TNF α has a role in the development of insulin resistance in obese individuals. Chronic exposure of TNF α to 3T3-L1 resulted in downregulation of GLUT4 and C/EBP (CCAT/enhancer binding protein), suggesting its role in IR development (Stephens & Pekala, 1991). Similarly, prolonged exposure to a low dose of TNF α interfered with insulin stimulated glucose uptake by inhibiting Tyr phosphorylation of IRS-1 (Hotamisligil et al., 1994). Furthermore, the phosphorylation of IRS1 has been observed to be impaired in both obese and non-insulin-dependent diabetes mellitus (NIDDM) Zucker rat models. Neutralizing TNF α increased Tyr phosphorylation of insulin receptors and IRS-1 in fat and muscle tissues and restored glucose, FFA, and insulin levels (Hotamisligil et al., 1994). Thus, TNF α disrupts insulin signalling and leads to systemic IR. Moreover, TNF α exposure also regulates the secretome and phosphoproteome of adipocytes. Upregulation of NF- κ B pathway and proteins associated with leukocyte migration while downregulating Galectins and Galectin binding proteins aids in maintaining the inflammatory state in AT (Mohallem & Aryal, 2020). Galectins are glycol-conjugates and regulate inflammation and

immune cell activation (F.-T. Liu & Stowell, 2023). Galectins are glycol-conjugates and regulate inflammation and immune cell activation (F.-T. Liu & Stowell, 2023).

IL6: Increased circulating levels of IL6 has been noted in obese and T2D individuals. Mohamed Ali et al. (1997) demonstrated the release of IL-6 in subcutaneous AT (SAT) as well as in the circulation, with its levels correlating to BMI in Caucasians (Mohamed-Ali et al., 1997). IL-6 levels are notably higher in Visceral AT (VAT) compared to SAT (Fain et al., 2004). The promoter polymorphism at -174 G>C in IL-6 gene has been linked to the Metabolic syndrome (MetS) (Zafar et al., 2019). IL-6 exhibits a paradoxical role in obesity and IR. It is secreted by various cell types within adipose tissue, including adipocytes, macrophages, and T cells (de Jong et al., 2018). Skeletal muscles release IL6 during exercise, which is important in maintaining glucose homeostasis (Febbraio et al., 2004). IL6 also regulates fatty acid oxidation (FAO) in humans. Infusing rIL6 into healthy men increases lipolysis without causing hypertriacylglycerdemia (van Hall et al., 2003). HFD fed mice lacking adipocyte specific IL6 demonstrate reduced body weight, macrophage accumulation, and inflammation while increasing energy expenditure and improving glucose tolerance. On the other hand, myeloid specific deletion of IL6 induced obesity, hyperglycemia and hyperinsulinemia. Similarly, IL6 expression in muscles also suppress ATM accumulation (Han et al., 2020). Thus, IL6 differentially regulates metabolic homeostasis and more work is required to fully understand its role

IL1 family: The IL1 family of cytokines, which includes IL1 α , IL1 β , IL18, IL33, among others, is significant in the pathogenesis of obesity and diabetes. Overnutrition causes an elevated level of free fatty acids, which are ligands for Toll like receptors (TLRs) (2 and 4), that trigger inflammation and release of several cytokines, including the IL1 family (Jialal et al., 2014). The activation of IL1 β and IL18 requires cleavage by caspase1, which is regulated by the inflammasome (Guo et al., 2015). Differentiated adipocytes exhibit elevated caspase-1 activity, and inhibiting this activity leads to upregulation of PPAR γ , GLUT4, and Adiponectin expression. Administering a caspase1 inhibitor to obese mice resulted in a significant decrease in IL1 β levels and an improvement in insulin sensitivity (Stienstra et al., 2010). Administering an IL-1 antagonist to individuals with T2D for 13 weeks resulted in improved IR and β -cell function (Larsen et al., 2007). Eliminating IL-1Ra in mice resulted in reduced body weight and adipogenesis, while increasing energy expenditure (Somm et al., 2005).

IL1 β : IL1 β is an important player in obesity and T2D. Multiple studies have noticed elevated circulatory levels of IL1 β in obese and diabetic individuals (Randeria et al., 2019; van der Zalm et al., 2020). The NLRP3 inflammasome increases IL1 β production in obese VAT via caspase-1 activation. IL1 β and NLRP3 expressions correlated with IR (Esser et al., 2013). IL1 β impairs insulin sensitivity by modulating PPAR γ (Lagathu et al., 2006) mediated by caspase-1 (He et al., 2008). High glucose levels induce the production of IL1 β in human islets, which subsequently activates NF- κ B and upregulates FAS, leading to β -cell apoptosis. IL1R antagonist improves β -cell function due to glucotoxicity mediated by IL1 β (Maedler et al., 2002). Intraperitoneal administration of IL1 β antibody in mice on HFD ameliorated glucose intolerance and IR. Neutralising IL1 β reduced serum levels of glycated Hb, proinsulin, and insulin, as well as islet size (Osborn et al., 2008).

IL10: IL10 is an anti-inflammatory cytokine that signals through the JAK/STAT pathway. It plays a role in promoting wound healing and maintaining tissue homeostasis (Riley et al., 1999). It displays anti-inflammatory properties by suppressing the secretion of TNF α and stimulating the release of TNF receptor from the surface of macrophages (Joyce et al., 1994). It enhances the activation of macrophages towards the M2 or alternative phenotype and regulates the recruitment of eosinophils (Chuang et al., 2016; Makita et al., 2015). It promotes metabolic reprogramming in macrophages that involves the inhibition of glycolytic flow by reducing mTOR activity, while simultaneously encouraging OXPHOS (Ip et al., 2017). The expression of IL10 is inversely correlated with BMI in SAT (Nishimura et al., 2013). There are strong evidences linking IL10 to T2D. Over-expressing IL10 in mice muscles showed reduced expression of MCP1, resulting in a decreased infiltration of macrophages (Hong et al., 2009). Elevated glucose levels interferes with the activation of STAT3 in macrophages, thus affecting the ability of IL10 to suppress the release of TNF α (Barry et al., 2016). This suggests that IL10 plays a function in suppressing inflammation in AT.

IL13 & IL4: IL13 and IL4 are anti-inflammatory cytokines, like IL10, that induce macrophages to undergo alternate activation, but through different mechanisms (Bhattacharjee et al., 2013). IL13 regulates glucose homeostasis in the liver as well. IL13 null mice exhibited elevated blood glucose levels and hepatic IR. IL13 inhibits the expression of genes involved in gluconeogenesis in the liver through the activation of STAT3 (Stanya et al., 2013). The decreased levels of IL13 in the serum and myotubes of

individuals with T2D suggest that IL13 plays a significant role in regulating glucose metabolism in skeletal muscle cells (L. Q. Jiang et al., 2013).

IL4 attenuates adipogenesis by downregulating the transcription of PPAR γ and C/EBP α . It also enhances Hormone Sensitive Lipase (HSL) activity to promote lipolysis. Thus, it decreases the lipid content in adipocytes (Tsao et al., 2014). IL4 improves insulin sensitivity and glucose tolerance by enhancing Akt phosphorylation while reducing GSK3- β activity. IL4 overexpression reduces lipid accumulation in fat tissues via STAT6 (Chang et al., 2012). IL4/STAT6 signalling improves insulin action by inhibiting PPAR α pathway. STAT6 null mice had impaired insulin action (Ricardo-Gonzalez et al., 2010). Although, it has been observed that diabetic individuals have increased levels of IL4, in addition to IL13 (Randeria et al., 2019).

TGF β : The TGF β family comprises pleiotropic cytokines that play a role in regulating diverse biological processes, including fibrosis, development and tissue homeostasis. The process of signal transduction is facilitated by activating Smad (Suppressor of mothers against decapentaplegic; latent cytoplasmic transcriptional factor) through phosphorylation (Tzavlaki & Moustakas, 2020). It increases the production of proinflammatory cytokines (IL1 β , TNF α & IL6), which in turn decreases leptin expression in 3T3-L1 cells (Granowitz, 1997). Increased levels of TGF β were found in AT from obese mice, resulting in an upregulation of TNF α expression (Samad et al., 1997). TGF β downregulates adiponectin in differentiated adipocytes while it increases expressions of Collagen IV in preadipocytes for ECM synthesis (Maharjan et al., 2022). hASCs (Adipose derived stem cells, humans) from visceral WAT of obese human undergoes ECM remodelling which subsequently triggers mitochondrial dysfunction through TGF β signalling. This ultimately leads to senescence of the hASCs (Han et al., 2023). Blocking TGF β improves the metabolic dysfunction but not obesity (Xu et al., 2024). Although, Yadav H et al. demonstrated that mice lacking Smad3 displayed enhanced mitochondrial biogenesis, decreased cytokines, and reduced adiposity, resulting in protection against DIO and diabetes (H. Yadav et al., 2011). Similarly, the release of TGF β is increased in human AT during obesity (Corica et al., 1997; Fain et al., 2005) as well as in the circulation (Fain et al., 2005; Yadav et al., 2011). Additionally, it is linked to BMI (Corica et al., 1997), adiposity, and VO $_2$ consumption, while being inversely correlated with HOMA-IR (Yadav et al., 2011).

Adiponectin: Adiponectin is an adipokine produced from adipocytes. The protein, first named in 1995, as Acrp30 (Adipocyte Complement Related Protein of 30kD, due to its structural resemblance to complement factor C1q). It was observed upregulated in differentiated 3T3-L1 adipocytes and involved in maintaining metabolic homeostasis (Scherer et al., 1995). Simultaneously, it was referred to by other researchers as apM1 (Adipose Most Abundant Gene Transcript1) (Maeda et al., 1996), GBP28 (Gelatin Binding Protein of 28kDa) (Nakano et al., 1996), and AdipoQ (Hu et al., 1996). These groups noted that it is secreted exclusively by adipocytes. AdipoQ is analogous to Acrp30 and its expression is markedly reduced in the adipose tissues of obese mice, rats, and humans (Hu et al., 1996). In 1999, Arita, et al. demonstrated a paradoxical reduction in plasma adiponectin levels and their inverse relationship with BMI in the Japanese population. This study also found that males had lower levels compared to females, regardless of whether they were fat or non-obese (Arita et al., 1999). Moreover, the over-expression of adiponectin increases glucose uptake and thus protect against IR via AMPK activation. Additionally, it reduces inflammation by blocking the NF- κ B pathway (Achari & Jain, 2017; Nigro et al., 2014).

Leptin: In 1994, Leptin was identified as a peptide hormone produced by AT. It has a crucial role in regulating food intake and energy expenditure, making it a key factor in the development of obesity (Zhang et al., 1994). The administration of recombinant leptin in genetically obese mice resulted in a decrease in adiposity and hyperphagia, while simultaneously increasing metabolic rate and energy expenditure (Halaas et al., 1995; Pelleymounter et al., 1995). In contrast, obese individuals exhibit reduced responsiveness to naturally occurring leptin. Its mRNA expression in AT and serum content are significantly elevated in obese humans (Considine et al., 1996). Leptin is also a cytokine, with its structural and functional similarity to IL6 and signals via JAK/STAT pathway. Leptin resistance can represent a connection between metabolic dysregulation and inflammation in the development of obesity, its associated co-morbidities like diabetes and hypertension, athero-thrombosis and Cardiovascular Diseases (CVD) (Martin et al., 2008). Elevated levels of leptin are linked to IR, regardless of BMI (Askari et al., 2010; Segal et al., 1996). High levels of leptin induce IR in muscles by inhibiting insulin stimulated p38 MAPK transduction and its downstream effector, CREB. Even GLUT4 translocation remains unaffected (Sweeney et al., 2001). Leptin resensitization is linked to the amelioration of

endocrine and metabolic disturbances observed during obesity and has been suggested as a strategy for weight management (Izquierdo et al., 2019).

Resistin: Resistin is an adipokine that is synthesised by adipocytes. Resistin regulates the breakdown of fatty acids through the inhibition of PGC1 α , while PPAR γ is recognised for its ability to decrease resistin levels (Steppan et al., 2001). It is also secreted by macrophages (Jung et al., 2006; Patel et al., 2003). Resistin, produced from human macrophages, induced inflammation in WAT and developed IR in mice (Qatanani et al., 2009). High resistin levels in obese population are correlated with decreased Insulin sensitivity (Nieva-Vazquez et al., 2014).

Chemokines: Chemokines are cytokines that belong to the chemotactic family. They play a crucial role in the trafficking of immune cells, such as macrophages, and are therefore an essential component in the process of inflammation. In 1994, Salaman and Borkoski proposed that the microinfusion of MCP1 (CCL2) and RANTES (CCL5) resulted in a decrease in short-term food consumption in Wistar rats (Plata-Salamán & Borkoski, 1994). Huber et al. (2008) noted the altered chemokine levels in obese individuals (Huber et al., 2008). MCP1 is recognized as a critical chemokine in obesity and diabetes. Deletion of *Mcp1* or its receptor CCR2 in mice reduces the infiltration of ATM and improves insulin sensitivity when they are fed a high-fat diet (Weisberg et al., 2006). MCP1 promotor SNP is also associated with diabetic nephropathy (Raina et al., 2021). CCL5 plays a crucial role as an inflammatory mediator in both obesity and diabetes. Expression of CCL5 is increased in WAT and CCR5⁺ macrophages are accumulated with obesity. Ablating CCR5 decreases the recruitment of macrophages, alters the macrophage phenotype to M2, and enhances glucose homeostasis (Kitade et al., 2012). Although the precise mechanism by which chemokines influence macrophage polarisation remains unclear.

Overall, understanding the role of inflammation in obesity is crucial for developing effective therapeutic strategies to mitigate the metabolic complications associated with obesity and related diseases.

1.3.2 Cellular alterations in AT: The AT contains innate immune cells, including macrophages, dendritic cells, neutrophils, natural killer cells, eosinophils, and innate lymphoid cells (ILCs), as well as adaptive immune cells like B and T cells. During the lean state, immune cells associated with type 2 immunity, such as alternatively activated M2-like

macrophages, eosinophils, and ILCs, play a significant role in preserving metabolic homeostasis in AT and promoting insulin sensitivity by secreting cytokines such as IL4, IL13, IL5, and IL33 (Gao et al., 2024). Conversely, with obesity, the immune cell makeup and behaviour of the adipose tissue undergoes a shift towards a more pro-inflammatory state, mostly dominated by M1 type macrophages, mast cells, CD8⁺ T cells, and neutrophils. Pro-inflammatory mediators released by adipocytes, such as MCP1, IL6, TNF α , leptin, chemerin, and resistin promote the activation and recruitment of immune cells. This shift contributes to metabolic dysfunction and insulin resistance in AT (Ferrante, 2013; Gao et al., 2024).

Macrophages: Macrophages represent a prevalent cell population within adipose tissue, and their numbers expand during obesity. This increase occurs due to recruitment, proliferation, and retention. During obesity, macrophages accumulate in AT via both monocyte recruitment and in situ proliferation, driven by factors like monocyte chemoattractant protein1 (MCP1) (Lumeng et al., 2008). Their proportion increases from 10% of the stromal vascular fraction (SVF) in normal physiological conditions to 40-50% of the SVF in cases of obesity (Weisberg et al., 2003). ATMs undergo a phenotypic transition from the M2, alternatively activated phenotype in the normal state to the pro-inflammatory M1 classically activated phenotype in obese adipose tissue. Macrophages with M1-like characteristics in the adipose tissue of obese individuals release pro-inflammatory cytokines, including TNF α and IL1 β (Lumeng, DeYoung, Bodzin, et al., 2007). These cytokines directly affect adipocytes and hinder insulin signalling in these cells (Lumeng, DeYoung, & Saltiel, 2007). Despite displaying pro-inflammatory characteristics, macrophages found in obese adipose tissue exhibit clear distinctions from M1 macrophages, which typically arise during acute inflammation. Instead, ATMs become metabolically activated during obesity (Kratz et al., 2014). Similarly, lipid-associated macrophages (LAMs) expressing a unique lipid receptor, Trem2, identified from obese mice and humans AT localised in CLS. Trem2⁺ LAMs demonstrate increased transcription of genes associated with phagocytosis and endocytosis, lysosome function, PPAR γ signalling, and oxidative phosphorylation (Hill et al., 2018). Thus, ATMs display heterogeneity and engage in a variety of functions, such as lipid scavenging, phagocytosis, and cytokine synthesis.

Neutrophils: Similar to the classical immune response, where neutrophils are the first cells recruited to the site of acute inflammation. These also infiltrate adipose tissues early on, before macrophages. In mice, this infiltration starts early in response to a high-fat diet, peaks

between 3-7 days in duration, and then starts to recede (Elgazar-Carmon et al., 2008). The functions of neutrophils undergo alterations during obesity. Peripheral blood neutrophils from obese patients exhibit increased levels of superoxide emission and chemotactic activity. The correlation between basal superoxide generation and BMI, suggests that obesity is linked to low grade inflammation and oxidative stress. Nevertheless, the adhesion and phagocytic activity of neutrophils remain unaffected (Brotfain et al., 2015). Increased secretion of elastase by neutrophils, while decreased serum levels of the Neutrophil elastase (NE) inhibitor, α 1antitrypsin (A1AT, SerpinA1) which are involved in sterile inflammation, have been reported in both mice and humans (Mansuy-Aubert et al., 2013). Genetic ablation of neutrophil-specific elastase in mice led to resistance to obesity caused by a high-fat diet. It also resulted in reduced inflammation, improved glucose tolerance and insulin sensitivity, increased fatty acid oxidation in the liver, and decreased infiltration of macrophages (Mansuy-Aubert et al., 2013; Talukdar et al., 2012). Similarly, Wang Q et al have demonstrated elevated levels and heightened activity of myeloperoxidase (MPO), released from recruited neutrophils, in the adipose tissue of obese mice. HFD fed MPO deficient mice demonstrated reduced body weight and IR while increasing expressions of UCP1 in BAT (Wang et al., 2014). Therefore, the recruitment of neutrophils to the AT during the initial phase of obesity plays a crucial role in the development of adipose tissue inflammation and IR.

Eosinophils: Eosinophils are mainly involved in allergic reactions, helminth immunity and maintaining anti-inflammatory responses. The decreased proportions of eosinophils in adipose tissue is associated with obesity in mice. These cells secrete IL4 and IL13 and this aids macrophages towards maintaining the M2 phenotype (Ricardo-Gonzalez et al., 2010), thereby providing protection against obesity and IR. The absence of eosinophils in HFD mice led to increased adiposity and impaired glucose tolerance. While infecting mice with helminth resulted in eosinophilia and improved insulin sensitivity (Ricardo-Gonzalez et al., 2010). Weight loss can reinstate eosinophil numbers and thus rehabilitating cellular homeostasis in AT (Bolus et al., 2018). In contrast, a study has demonstrated that there is an elevated localization of eosinophils in AT during obesity to counteract metabolic dysfunction in AT. Lack of eosinophils in mice resulted in reduced adiposity, impaired adipocyte maturation, and glucose intolerance. However, they had higher numbers of macrophages, B cells, and CD4+T cells (Lee et al., 2018).

ILC2: Similar to eosinophils, innate lymphoid cells type 2 (ILC2s) also contribute to type 2 immunity and significantly influence adipose tissue function and metabolic homeostasis. Both humans and mice exhibit dysregulated numbers of ILC2s in WAT during obesity. IL33 mediates the maintenance of ILC2s in WAT. ILC2s can also activate beige adipogenesis in WAT by promoting UCP1 expression (Brestoff et al., 2015). ILC2s residing in visceral AT secrete IL5 and IL13, which promote eosinophil accumulation and thereby sustain the M2 phenotype of macrophages. Further, helminth infection or IL33 administration promotes ILC2 activation (Molofsky et al., 2013). Thus, the ILC2-eosinophil-M2 axis is important in lean physiology.

NK cells: Natural Killer (NK) cells are effector cells that provide defence against pathogens and tumors. HFD in mice led to an increase in the number and activation of NK cells in AT. NK cells also regulate macrophage activation. Genetic eliminating of NK cells in obese mice resulted in a reduced inflammation, decreased proportion of macrophages, and improved systemic insulin resistance (Lee et al., 2016). Similarly, NK cell accumulation in the VAT of obese humans contributes to low grade inflammation and IR. NK cells in VAT were associated with BMI, circulating levels of TNF α , the M1/M2 ratio and whole body glucose disposal (Wouters et al., 2020). NK cells exhibit altered metabolism during obesity, resulting in reduced glycolytic capacity and OXPHOS, while increased lipid uptake occurs via PPAR α/δ that leads to functional impairment. The lipid overload or lipotoxic obese environment impairs the cytotoxic and anti-tumor responses of NK cells (Michelet et al., 2018).

Mast Cells: Mast cells are important in immune response against infection and in allergic hypersensitivity. WAT from obese humans and mice displays a higher number of mast cells compared to WAT from subjects who are not obese (Liu et al., 2009). Although there was no significant difference in the number of mast cells in subcutaneous WAT between obese and lean mice, the number of mast cells in visceral WAT increased considerably in obese mice compared to lean mice. Additionally, obese visceral WAT exhibited a higher prevalence of mast cell containing crown-like structures (Altintas et al., 2011). Diabetes patients with obesity showed an accumulation of mast cells in their adipose tissue. Mast cell number was linked to fibrosis, macrophages, and clinical parameters like fasting glucose and HbA1c. In addition to their increased density, a significant proportion was activated, releasing their granular enzymes tryptase and chymase (Divoux et al., 2012). Mast cells also release preformed TNF α upon degranulation (Altintas et al., 2011). The number of mast

cells in the adipose tissue of American adults with metabolic syndrome is positively associated with waist circumference, glucose levels, HOMA-IR, leptin, inflammation markers (IL6, IL1 β), and circulating macrophages (Gurung et al., 2019). Genetic deletion or pharmacological stabilization of mast cells resulted in reduced obesity and inflammation in the WAT and serum of obese mice (J. Liu et al., 2009). The secretion of 15-deoxy- δ PGJ2 (a prostaglandin) from mast cells cultivated in high glucose stimulates adipocyte development by activating PPAR γ (Tanaka et al., 2011). Additionally, treating these cells with a high concentration of leptin leads to a reduced responsiveness of mast cells to leptin (Żelechowska et al., 2018). Leptin-deficient mast cells predominate in lean human and mouse adipose tissues and are involved in facilitating the polarisation of M2 macrophages, thereby attenuating weight gain and diabetes (Zhou et al., 2015). Thus, mast cells are a potential contributor to adipose homeostasis.

Dendritic Cells: Dendritic cells (DCs) are specialised antigen-presenting cells and play a crucial role in AT inflammation. Stefanovic-Racic et al. showed that the abundance of CD11c⁺ DCs significantly increases in the liver and AT of obese mice, and their numbers correlate with crown like structures. The mice lacking DC displayed reduced macrophage trafficking, weight gain, IR, and liver steatosis (Stefanovic-Racic et al., 2012). Simultaneously, in a study by Bertola et al. (2012), DCs in mice and humans were characterised as CD11c^{high} F4/80^{Low} and CD11c⁺CD1c⁺, respectively. These were characterised as inflammatory and associated with BMI and insulin resistance. HOMA-IR showed a strong relationship with the expression of CD1c⁺ cells (Bertola et al., 2012). Since F4/80 is also a marker for ATMs in mice, Cho et al, described CD11c⁺CD64⁻ as ATDC, distinguishing it from ATMs, which are identified as CD45⁺ CD64⁺ in both mice and humans. This study also clarified that DCs, residing in AT independently contribute to inflammation during obesity. CCR7 regulates the recruitment of DCs while ATM accumulation is dependent on CCR2 (Cho et al., 2016). AT-derived chemerin, a chemoattractant chemokine believed to stimulate DC recruitment to the VAT in obesity, induces Type I IFN response, resulting in meta-inflammation (Ghosh et al., 2016). In addition, CD11c⁺ dendritic cells also produce IL17, IL6, TGF β , and IL23, and stimulate TH₁₇ response (Bertola et al., 2012; Chen et al., 2014). Therefore, DCs are important regulators of AT inflammation and are involved in linking innate immune responses to adaptive responses during obesity.

T Cells: Alterations in the T-lymphocyte numbers in AT have been observed during obesity and are associated with inflammation and hypoxia (Rausch et al., 2008). Increased infiltration of CD8⁺ effector cells in the AT of obese mice promotes macrophage accumulation and thus contributes to local inflammation. On the other hand, CD4⁺ T cell and regulatory T cell (T_{reg}) numbers are low. CD8⁺T cell depletion mitigates inflammation and macrophage infiltration while improving glucose homeostasis and insulin sensitivity (Nishimura et al., 2009). The diminished number of CD4⁺FOX3⁺T_{reg} cells in obese mice drives the secretion of several chemokines including IFN γ and metabolic dysfunction. Reduced expression of FOX3P is linked to BMI in humans (Feuerer et al., 2009). Further, other studies also demonstrated that T_{H2} CD4⁺ cells are replaced by those of T_{H1} sub-lineage in the VAT of obese mice. VAT in lean mice had a high proportion of CD4⁺ FOX3⁺ T cells, while VAT in obese mice consisted of IFN γ -secreting T_{H1} cells (Strissel et al., 2010; S. Winer et al., 2009). Similarly, BMI in humans correlates with the T_{H1} to FOX3⁺ ratio. T cells orchestrate the invasion of CD11c⁺ ATMs. T_{H2}-primed CD4⁺ FOX3⁺ T cells limit inflammation by secreting IL10. This maintains the macrophages in the M2 state, thus preserving insulin sensitivity. On the other hand, T_{H1} T cells contribute to inflammation and glucose intolerance (Winer et al., 2009). Thus, T-cell phenotype alterations in AT have significant metabolic influences.

B cells: B cells are lymphocytes involved in humoral immunity. B cells provoke inflammation, adipocyte hypertrophy, glucose intolerance, and IR (DeFuria et al., 2013). HFD in mice led to an increase in class-switched IgG⁺ B cells in VAT. B-cell null mice exhibited a reduced number of CD8⁺ cells and Th1 CD4⁺ T cells in VAT (Winer et al., 2011). B cells promote T cell mediated inflammation and IR in both mice and humans (DeFuria et al., 2013; D. A. Winer et al., 2011). Distinct IgG profiles were characterised in IR humans, suggesting IgG mediates IR (Winer et al., 2011). However, Nishimura et al. demonstrated that B_{reg} cells maintain metabolic homeostasis in AT, which predominates during a lean state. These cells produce IL-10, suppress inflammation, and maintain glucose homeostasis. B cell-specific IL10 deletion in obese mice also triggers the activation of CD8⁺ T cells. Similarly, B-cell markers were inversely correlated to BMI and IL10 in humans subcutaneous AT (Nishimura et al., 2013). These studies demonstrated the importance of adaptive immune responses in IR.

Therefore, immunological dysregulation in individuals with obesity leads to a persistent, mild inflammation. Furthermore, the variation in the distribution of immune cells in adipose

tissue is a significant factor to consider in order to have a deeper understanding of their impact on metabolic dysfunction (Dam et al., 2016; Lenz et al., 2020).

1.3.3 Hypoxia in AT

Obesity causes adipose tissue to expand faster than blood vessels can grow, resulting in specific areas of lower oxygen levels within the adipose tissue, which is referred to as adipose tissue hypoxia.

Although there is expansion of adipose tissues during obesity, but it does not result in an increase in the blood flow to the tissues. Obese subjects have reduced basal abdominal blood flow compared to lean individuals and it increases with weight loss (Blaak et al., 1995). AT blood flow control partial pressure of oxygen (pO_2) in AT. The pO_2 decreases as body weight increases in mice (Yin et al., 2009). Impaired blood flow is associated with IR. Post meal blood flow to AT increases in lean individuals but is impaired in the obese (Karpe et al., 2002). There is strong correlation between a decrease in AT pO_2 and the percent body fat (Pasarica et al., 2009). Therefore, AT in obese individuals are also distinguished by a decrease in capillary density as well as lowered oxygenation (Pasarica et al., 2010). However, in a contrasting study it is reported that individuals with obesity have reduced blood flow in their AT, but higher pO_2 or hyperoxia. Consequently, this causes decrease in O_2 consumption. This is accompanied by reduced capillarization, inflammation and IR in AT (Goossens et al., 2011). A microarray analysis on differentiated SGBS cells (human preadipocyte cell strain for adipogenic differentiation) examined the genes regulated when adipocytes are exposed to low oxygen (1%). Hypoxia affected different cellular processes like glycolysis, response to nutrient levels and regulation of cell motility (Geiger et al., 2011).

Hypoxia-inducible factor-1 alpha ($Hif1\alpha$) is a key operator during hypoxia. Low pO_2 stabilizes $Hif1\alpha$ to form $Hif1\alpha$ and $Hif1\beta$ dimer which leads to alterations in metabolism, angiogenesis, determines cellular survival or death and encourages invasion or metastasis (Brahimi-Horn & Pouyssegur, 2007). $Hif1\alpha$ is transcription factor activated during hypoxic condition in AT as well. It is key protein that regulates various cellular responses during low pO_2 in AT, such as adipogenesis, IR, inflammation and metabolism. Hypoxia stabilizes $Hif1\alpha$ in 3T3-L1 and suppress adiponectin expressions. H_2O_2 treatment downregulates $PPAR\gamma$ and $C/EBP\alpha$ (Chen et al., 2006). It also stimulates AT fibrosis which is also linked

to local inflammation (Halberg et al., 2009). Low O₂ exposure and treatment with chemical mimetic of hypoxia (CoCl₂) enhanced Hif1 α protein accumulation in adipocyte nuclei (Lolmède et al., 2003). Hypoxia, and Hif1 α activity is also linked to adiposity in mice and humans (He et al., 2011; Wang et al., 2007). Hif1 α activity is induced during adipocyte differentiation (3T3-L1) by insulin, and increases adipogenesis, hyperinsulinemia and hypoxia (He et al., 2011).

Hypoxia is one of the key contributing factor to the cause of chronic inflammation in AT during obesity by dysregulating the adipokine secretion. Low pO₂ (5%) and CoCl₂ (chemical mimetic) upregulated the expression of leptin, Vascular Endothelial Growth Factor (VEGF) and matrix Metalloproteases or MMPs (2 and 9) (Lolmède et al., 2003). In humans, AT pO₂ correlated with expressions of macrophage markers (CD68 and CD163) while inversely related to secretion of Macrophage inflammatory Protein (MIP)1 α (Pasarica et al., 2009). Hypoxia suppress AdipoQ and C/EBP α transcription in 3T3-L1 by stabilising Hif1 α (Chen et al., 2006). Hypoxia also destabilizes AdipoQ mRNA, indicating a post-transcriptional regulation (Hosogai et al., 2007). Moreover, Hif1 α upregulates TLR4 expressions in macrophages. Hypoxia and CoCl₂ exposure to Raw 264.7 cells (murine monocyte derived cell line) resulted in enhanced transcripts of COX2, IL6 and activation of T cells. Ablating Hif1 α inhibits hypoxia/ CoCl₂ induced TLR4 expressions (Kim et al., 2010).

Several metabolic genes are also regulated in response to hypoxia. Increased rates of glucose uptake have been noted as a result of hypoxia. GLUT1 mRNA and protein levels remarkably increased in adipocytes in mice and human, when exposed to hypoxia (Hosogai et al., 2007; Wang et al., 2007; Wood et al., 2007). It is under the control of Hif1 α . Mice over-expressing Hif1 α in AT exhibit weight gain, impaired glucose tolerance, accumulation of macrophages and inflammation (Halberg et al., 2009). Increased glucose uptake also releases lactate. Lactate activates GPR81 (orphan GPCR) expression in fat cells in mouse, rat and human (Liu et al., 2009). Lactate inhibits IRS-1 and IRS-2 via PI3K, thus suppress insulin stimulated glucose uptake (Choi et al., 2002). Hif1 α also induces PI3K and mTOR which further influences adipogenesis and insulin sensitivity (He et al., 2011). Reduced pO₂ during weight gain blocks Akt phosphorylation (Ser 473) resulting in inhibited insulin pathway. Altered insulin signalling in response to hypoxia thus affect FFA uptake by inhibiting transcription of PPAR γ and C/EBP α , which promotes the expression of FATP1 and CD36 (Yin et al., 2009). Moreover it is demonstrated that dysregulated response to hypoxia in AT

during obesity (Hong et al., 2011), induces altered adipokine secretion mediated by ER stress (Hosogai et al., 2007). Short term intermittent hypoxia improved glucose tolerance in both lean and obese mice lacking leptin, while long term intermittent hypoxia exacerbated glucose intolerance (Polotsky et al., 2003).

Therefore, hypoxia in adipose tissue, caused by its increased size, plays a crucial role in potentially addressing the consequences of obesity-related conditions by affecting AT functions. This is manifested by chronic inflammation, vascularisation, IR and ER stress. Reversing hypoxia or attenuating the O₂-signaling mechanisms, such as the HIFs or other regulatory elements, presents novel opportunities in improving metabolic dysfunction.

1.3.4 Obesity induces ER stress

ER is involved in maintaining vital cellular and metabolic functions that include protein folding and maturation, calcium homeostasis and lipid synthesis. ER malfunctioning is linked to metabolic and immune alterations in the tissues (Rutkowski & Kaufman, 2004). ER stress triggers Unfolded Protein Response (UPR) which alters cellular functioning. The UPR involves three main branches controlled by different proteins: Inositol-requiring enzyme (IRE)-1, Protein kinase like ER kinase (PERK) and Activating Transcription Factor6 (ATF6).

IRE-1 pathway: The IRE-1 pathway is the most conserved branch of UPR. It is activated early in the process. IRE-1 is functionally an endoribonuclease which upon activation splice XBP1 mRNA. Spliced XBP1 gets translocated to nucleus and further activates the genes associated with Endoplasmic-reticulum-associated protein degradation (ERAD), protein folding and lipid biosynthesis. Thus this pathway is primarily involved in adaptive phase of UPR. However, prolonged IRE1 α signalling promotes apoptosis by associating to Bax and Bak proteins.

PERK pathway: The ER stress leads to PERK dimerization and phosphorylation thus activating it. Activated PERK further phosphorylates eIF2 α , which attenuates global protein translation. However, translation of ATF4 is increased by PERK, which further regulates oxidative stress response and amino acid transport. PERK activation also regulates expressions of proapoptotic genes CHOP and GADD34.

ATF6 pathway: ATF6 belongs to family of transcription factors located within the membrane of ER. It is translocated to golgi in response to ER stress, where it undergoes proteolytic cleavage. This releases its cytosolic domain, ATF6 (N), enters the nucleus and regulates the expression of ER chaperons and ERAD proteins.

Over-nutrition in AT disrupts metabolic homeostasis within the cells that results in inflammation, ROS, oxidative stress as well as ER stress. Excessive lipids in AT thus affect the ER functioning leading to UPR that results in inflammation, alter lipid and glucose metabolism, and insulin signalling in AT. ER stress markers during obesity are correlated with BMI (Sharma et al., 2008). Weight loss improved the ER stress signals in obese subjects (Gregor et al., 2009). Upregulated ER stress proteins induces JNK activation which promotes inflammation and induces IR in tissues of obese subjects (Boden et al., 2008).

ER stress and Inflammation: ER is demonstrated to affect metabolic homeostasis by promoting secretion of pro-inflammatory adipokines. Tunicamycin treatment in 3T3-L1 induced CHOP while downregulating adiponectin and PPAR γ (Hosogai et al., 2007). ER stress in adipocytes is correlated with the levels of IL6 and TNF α (Song et al., 2016) while inhibiting ER stress resulted in reduced levels of IL6 (Fernández-Sánchez et al., 2011). ER stress reduces the expression of adiponectin and leptin while increasing IL6 mRNA. ER stress suppresses IRS1 by regulating adipokine secretion and thus reducing insulin signalling, however prolonged ER stress did not change the glucose transport and lipolysis (Xu et al., 2010).

Mechanisms by which ER stress leads to inflammation is through activation of NF- κ B, MAPK or JNK. During ER stress, phosphorylated IRE1 α forms complex with TRAF2, which recruits IKK, leading to I κ B degradation by phosphorylating it, thus promoting NF- κ B translocation to nucleus. In addition, IRE1 α -TRAF2 complex also activates JNK. JNK activation induces transcription of inflammatory genes by phosphorylating AP1 (transcription factor, Activating Protein 1) (Zhang & Kaufman, 2008). Similarly, PERK-eIF2 α also promote NF- κ B activation, by inhibiting translation of I κ B, which reduces the I κ B levels, thus releasing free NF- κ B to be translocated to the nucleus (Zhang & Kaufman, 2008). ATF6 also activates NF- κ B via Akt phosphorylation and consequently augmenting inflammation (Yamazaki et al., 2009).

ER stress modulates metabolism: ER is very important organelle in regulating metabolic response. UPR modifying immune signalling also impact metabolism. ER stress molecules influence several pathways including lipogenesis and gluconeogenesis which are disrupted in obesity.

Glucose metabolism: UPR mediators are crucial in maintaining glucose homeostasis. Chronic high glucose caused IRE1 α hyperactivation that resulted in insulin mRNA degradation (Lipson et al., 2008). IRE1 α deficiency led to hypoinsulinemia, hyperglycemia and low weight in mice by disrupting pancreatic histology (Iwawaki et al., 2010). Moreover, mice with XBP1 haplo-sufficiency when fed on HFD exhibited IR, probably due to JNK activation which inhibits insulin signalling (Ozcan et al., 2004). Treating genetically obese mice (ob/ob) with chemical chaperones (TUDCA) repressed the PERK activation, phosphorylation of eIF2 α and JNK activation in liver and AT. PBA treatment resulted in reduction in hyperglycemia and hyperinsulinemia and improved insulin sensitivity in peripheral tissues (Ozcan et al., 2006). Phosphorylation of eIF2 α is crucial for regulating stress response and glucose homeostasis. Mice deficit in phosphorylated eIF2 α showed defect in gluconeogenesis in liver and pancreatic β -cells deficiency (Scheuner et al., 2001). Loss of PERK function resulted in hyperglycemia in mouse. PERK deficiency also affected β -cell development and β -cell death (Gao et al., 2012). On the contrary, GLP1 agonist, Liraglutide, improved insulin sensitivity of mouse adipocytes and AT of obese mice by reducing the expression of CHOP and ATF4 which suppresses insulin stimulated Akt phosphorylation (Jiang et al., 2018). Similarly, upregulated CHOP in adipocytes in AT of HFD mice encourages M1 polarization which further promotes IR in AT (Suzuki et al., 2017). CHOP deficiency in HFD mice improves Glucose infusion rate (Suzuki et al., 2017).

Lipid metabolism: Obesity induced ER stress stimulates lipolysis by enhancing production of cAMP and PKA (Protein Kinase A) activity in adipocytes (Deng et al., 2012). IRE1 α -XBP1 is essential for adipogenesis. XBP1 promotes C/EBP α by binding to its promotor (Sha et al., 2009). Enforcing dephosphorylation of eIF2 α reduced adipogenesis, downregulated PPAR γ and C/EBP α in liver of HFD mice, thus diminishing hepatic steatosis and improving glucose tolerance (Oyadomari et al., 2008). Further, CHOP induces lipogenesis in liver by

interacting with FoxO6 (Forhead box O6) by stimulating the expression of PPAR γ (Kim et al., 2020). ATF4 deficiency increases lipolysis and UCP2 in WAT and thus helps in weight loss in HFD fed mice (Wang et al., 2010). Therefore, three pathways of UPR function differently in response to nutritional stress in different tissues.

1.4 Macrophages in Obesity

Macrophages have a significant impact on the development of adipose tissue malfunction and metabolic problems in obesity. Macrophages constitute upto 40% of all cells in adipose tissue of obese mice, whereas they make up only 10% in lean mice. It has been conclusively demonstrated that these macrophages play a pivotal role in the development of obesity and insulin resistance (Castoldi et al., 2015).

1.4.1 Macrophage polarization

Macrophages are important cells of the immune system that are formed in response to an infection or accumulating damaged or dead cells. Based on the inflammatory state, macrophages are classified as classically activated, M1, known to be pro-inflammatory or alternatively activated, M2 that are anti-inflammatory (Fig 1.3).

M1 Macrophages: M1 macrophages are typically activated in response to microbial products (e.g., lipopolysaccharide, LPS) or pro-inflammatory cytokines (e.g., IFN γ) and produce proinflammatory cytokines including IL1 β , TNF α , IL12, IL18 and IL23. These cells also secrete chemokines like CXCL9 and CXCL10 (Mosser & Edwards, 2008). This facilitates the antigen specific Th1 and Th17 response mediated inflammation by upregulating IRF5 (Krausgruber et al., 2011). M1 macrophages express major histocompatibility complex class II (MHC II), CD68 and CD86 (Mosser & Edwards, 2008). LPS and TLR4 interaction activates STAT1 and upregulates intracellular protein suppressor of cytokine signalling 3 (SOCS3) which further induces NF- κ B pathway to promote inflammation (Arnold et al., 2014). Additionally, activation of iNOS (NOS2) is also an important characteristic of M1 in supporting inflammation. iNOS is an enzyme that produces NO from L-arginine. NO is an important pro-inflammatory mediator (Xue et al., 2018). M1 macrophages show higher glycolysis facilitating rapid energy production to support their robust pro-inflammatory functions. These cells also have higher level of lipid synthesis to meet the need of increased prostaglandin synthesis as inflammatory mediators. Thus, M1

are implicated in initiation and amplification of inflammatory responses (Kelly & O'Neill, 2015).

M2 Macrophages: M2 macrophages are activated in response to anti-inflammatory cytokines IL4; IL13 or signals associated with tissue repair and remodelling. M2 activation is also induced by apoptotic cells, fungal and helminth infection. These signals provoke secretion of anti-inflammatory cytokines IL4, IL10, while suppresses IL12, IL23, IL1 β and IL2. These cells express mannose receptor (CD206) and scavenger receptors (CD163). Unlike M1, M2 utilises arginine to produce ornithine and polyamine, which promotes growth (Mosser & Edwards, 2008).

Based on the stimuli, phenotype and functional characters, M2 are further categorised as M2a, M2b, M2c and M2d. M2a are stimulated by IL4 or IL13 and produce IL10, IL1Ra, CXCL9 and CXCL10. M2b are elicited by immune complexes and LPS which promote Th2 response and secretion of IL10, IL1 β , TNF α and CCL1. M2c are activated by IL10 (or glucocorticoids). These cells control the deactivation of inflammation and promote tissue remodelling by secreting TGF β (Mantovani et al., 2004). M2d are induced by IL6 and adenosine, release VEGF and promote angiogenesis and tumour progression. Overall, M2 macrophages play a role in resolving inflammation, tissue repair, and remodelling. They contribute to the clearance of apoptotic cells, the resolution of inflammation, and the promotion of tissue healing and regeneration (Mosser & Edwards, 2008; Röszer, 2015).

Table 1.3 Types of M2 macrophages based on stimulations and their phenotype

	M2a	M2b	M2c	M2d
Stimulations	IL4,IL13, Fungal/ Helminth Infection	ICs	IL10, TGF β , GCs	IL6, Adenosine
Markers	CD163, CD209, IL1R	CD86, MHC-II	CD163, CD206, TLR8	IL10R, IL12R
Cytokines/ Chemokines	IL10, IL1R, CCL17, CCL22	IL10, TNF α , IL1 β , CCL1	IL10, TGF β , CCL16, CCL18	IL10, TGF β , VEGF

Macrophage Phenotype and Metabolism: Metabolism and macrophage polarization are intricately intertwined processes, and different macrophage subtypes exhibit their distinct

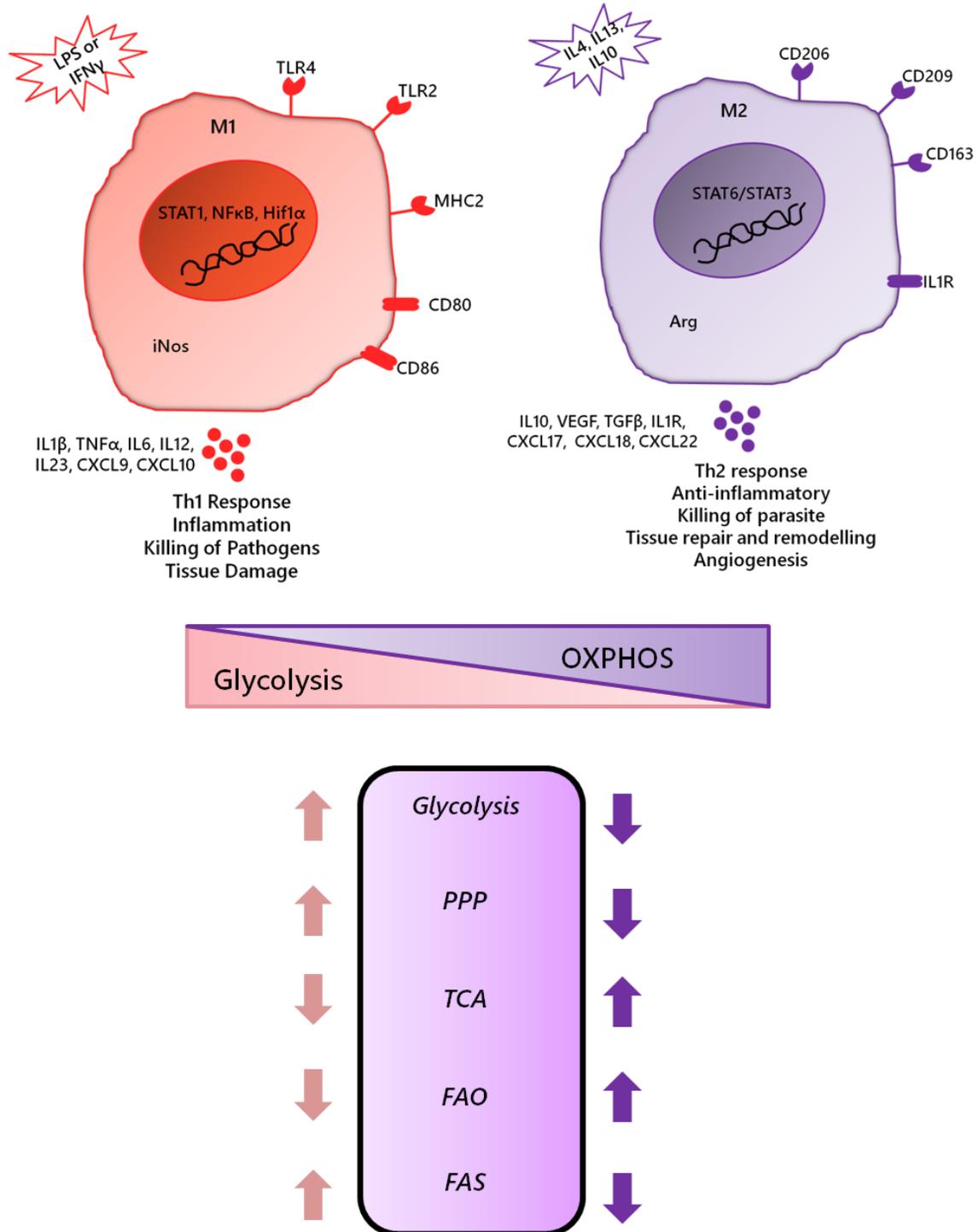


Figure 1.3 Phenotypic and metabolic characters of M1 and M2 macrophages

metabolic characteristics (Fig 1.3). In a study, LPS stimulation was found to shift metabolism towards glycolysis, increasing mitochondrial membrane potential and ROS through succinate dehydrogenase (Mills et al., 2016). The glycolytic pathway enables quick ATP generation, crucial for the production of pro-inflammatory cytokines and ROS during immune responses. Studies have shown that the metabolic reprogramming towards glycolysis in M1 macrophages is orchestrated by specific signalling pathways, including the activation of AMPK and HIF-1 α (Kelly & O'Neill, 2015). Treatment with 2-deoxy-D-glucose (2DG) affected the production of IL1 β and IL10.

Metabolically, M2 macrophages rely more on oxidative phosphorylation and fatty acid oxidation. IL4 induced activation of STAT6 and PGC1 β is crucial for promoting FAO in M2 macrophages (Odegaard et al., 2008; Vats et al., 2006). This metabolic preference aligns with their roles in tissue repair, immunoregulation and resolution of inflammation. Multiple studies have also highlighted the critical role of glycolysis in M2 macrophage activation (Huang et al., 2016; Wang et al., 2018), while FAO is redundant for M2 polarization (Nomura et al., 2016).

However, interfering with metabolic pathways in ATMs from obese mice has been shown to impact cytokine release. This study suggested that etomoxir, an inhibitor of CPT1 α did not significantly impact cytokine expression. In contrast, 2DG reduced the expression of cytokines and concurrently increased the production of lactate (Boutens et al., 2018).

1.4.2 Adipose Tissue Macrophages

ATMs are a critical contributing factor in the development of AT inflammation and metabolic dysfunction associated with obesity. Macrophage infiltration is an important event for these functional and phenotypic changes in AT. Macrophages accumulate around the dead adipocytes in obese mice and humans, forming a "crown like structure" (CLS). Macrophage recruitment to AT is facilitated by dead and dying adipocytes. The frequency of adipocyte death increases in obesity and is associated with WAT inflammation in both mice and humans. Macrophages forming syncytia or CLS around the dead adipocytes scavenge the lipids from residual adipocytes and result in chronic inflammation. This induces PLIN2 expression in macrophages (Cinti et al., 2005). FFAs, such as palmitic acid, are reported to cause IKK activation and I κ B phosphorylation through the activation of TLR4 and subsequent inflammatory signalling (Strissel et al., 2007). FFAs have been

identified as crucial adipocyte-derived paracrine molecules triggering inflammation in macrophages *in vitro* (Suganami et al., 2005).

Lumeng et al suggested that macrophages recruited to AT upon HFD in mice showed upregulated expressions of genes associated with inflammation and migration that included IL6, iNOS and CCR2. These macrophages had increased lipid content and overexpression of genes associated with lipid metabolism, including PPAR γ , PLIN2 and Srebp4 (Lumeng, DeYoung, Bodzin, et al., 2007). Further, the same group demonstrated that resident macrophages possess M2a phenotype and remain localised to interstitial spaces between adipocytes while in diet induced obesity (DIO), AT recruits macrophages to form clusters around dead adipocytes and switch their phenotype towards M1 activation (Lumeng, Bodzin, et al., 2007). This creates an inflammatory environment in AT by recruiting macrophages from circulation but not by converting resident M2 macrophages (Lumeng et al., 2008; Lumeng, Bodzin, et al., 2007). At the same time, Zeyda et al., proposed that ATMs from human WAT exhibit expressions of CD206 and CD163 on their surface and possess endocytic capacity which are characteristic features of M2 macrophages but also have a capability to produce high amount of inflammatory cytokines (Zeyda et al., 2007).

Bourlier et al., suggested that most of the ATMs in humans are CD14⁺ CD206⁺ CD16⁻ and their numbers are correlated with BMI. These ATMs affect markers of both M1 (TNF α , IL6, IL23, MCP-1, IL8) and M2 (TGF β , IL10, CCL18, COX1). ATMs from obese AT affect adipogenesis while promoting the migration of endothelial cells in humans (Bourlier et al., 2008).

In line with these studies, Xu et al., demonstrated that ATMs in HFD mice are proinflammatory but accumulate lipids and activate lysosomal biogenesis to reduce lipid load. These properties distinguish ATMs from classically activated M1 macrophages (Xu et al., 2013). Similarly, lipid filled macrophages similar to foam cells have been reported from SC and Omental (Om) AT. The lipid content in these cells from Om fat was associated with BMI and fasting insulin. These cells surrounded the dead adipocytes to form CLS (Shapiro et al., 2013).

Kratz et al., suggested that ATMs from humans and mice do not express M1 markers like CD38, CD274 and CD319. They demonstrated that "metabolic activation" of blood derived monocytes using palmitate, glucose and insulin closely mirrors the characters of obese ATMs *in vivo*, beyond the M1/M2 dichotomy. They called these macrophages as

"Metabolically Activated Macrophages" or "MMe", unlike M1 macrophages, MMe and ATMs express CD36, PLIN2 and ABCA1 on their surface. CD36 and ABCA1 expression in ATMs were correlated with BMI (Kratz et al., 2014). MMe are able to produce inflammatory cytokines. These macrophages accumulate around the dead adipocytes and clear apoptotic adipocytes via lysosomal exocytosis, thus aiding in restoring adipose tissue health. PPAR γ and p62 also limit the inflammation due to metabolic activation, thus lowering its intensity (Coats et al., 2017; Kratz et al., 2014). Thus, these possess both damaging and protective characteristics within the context of obesity, depending on the duration of HFD in mice (Coats et al., 2017).

1.4.3 Mitochondrial Dysfunction

Mitochondria known as "powerhouse of the cell", are the primary sites for ATP production. However, these are important in regulating immune responses. Mitochondria are susceptible to the surroundings signals and can be damaged easily. Mitochondrial integrity and functions within ATMs is critically important for its phenotype and inflammatory state. Mitochondrial dysfunction contributes to chronic inflammation, metabolic alterations, aggravates IR and thus leads to MetS.

Impaired mitochondrial biogenesis in ATMs leads to decrease in mitochondria number and thus affects its function. FFA induces mitochondria fragmentation by promoting DRP1 (Dynamic related protein-1) oligomerization. Mitochondrial fragmentation is important in mitigating inflammation (Zezina et al., 2018). Mitochondrial OXPHOS, mitochondrial biogenesis and mitochondrial DNA metabolism is impaired in SAT in obese humans. Three CpG sites in *PGC1 α* gene are hypermethylated that reduce its expression in obese SAT (Heinonen et al., 2015).

Obesity inhibits adipocyte to macrophages mitochondrial transfer in mice mediated by long chain fatty acids present in high fat diet (Borcherding et al., 2022). High FFA or hyperglycemia increases levels of Drp1 while downregulates PGC1 α and PGC1 β in 3T3-L1 adipocytes. This was accompanied by decrease in mitochondrial membrane potential and ROS accumulation (Gao et al., 2010). Palmitate induces FABP4 expressions in human macrophages which downregulates PGC1 α , thus inhibits mitochondrial biogenesis. FABP4 inhibition reduces ROS production, improved mitochondrial membrane potential and

complex II & IV activity, thus impeding mitochondrial dysfunction (Li et al., 2018). Human VAT from obese patient displayed higher density of mitochondria in comparison to SAT and thus possess higher OXPHOS activity. However, mitochondrial respiration per cell per mitochondria is less in VAT comparing to SAT (Kraunsøe et al., 2010). Mitophagy is also important in maintaining healthy mitochondrial pool. Impaired mitophagy promotes inflammation in macrophages and IR in adipocytes (Wu et al., 2019).

Deletion of complex I decreases efferocytosis in macrophages thus lowering the expressions of anti-inflammatory cytokines (Cai et al., 2023). Ablating Sirt3 (NAD⁺ dependent mitochondrial deacetylase) in HFD mice aggravate obesity and VAT inflammation. Deleting Sirt3 from macrophages cause mitochondrial dysfunction, accelerates inflammation through succinate accumulation and promotes macrophage polarization towards M1 (Zhou et al., 2023). Overall, overnutrition causes AT expansion that leads to elevated FFAs. This induces mitochondrial functional impairment. This results in altered metabolism and inflammation in ATMs. Impaired mitochondria in adipocytes along with macrophages contribute to inflammation in AT and promotes obesity related co-morbidities (Wang et al., 2021). Modulating mitochondrial function in macrophages and adipocyte can affect AT composition and function. Therefore, important in pathophysiology of obesity and MetS.

1.4.4 Macrophage Adipocyte interaction:

Over nutrition results in adipocyte hypertrophy with increased cellular stress. Further it leads to adipocyte death. Necrotic- like adipocyte death is characteristic of obesity and its frequency is correlated with adipocyte size in mice and humans. Adipocyte death further encourages macrophage accumulation at the site of death which forms a characteristic crown like structure in obese mice and human. This promotes adipose and systemic inflammation. Free lipid droplets of dead adipocyte act as site for macrophage fusion and lipid uptake (Cinti et al., 2005). Macrophage does efferocytosis, i.e. it forms hydrolytic extracellular compartments at the contact site for degrading adipocyte fragments. This leads to macrophage foam cell formation which alters metabolic characters of macrophages (Haka et al., 2016). Similarly, *in vitro* co-culture of macrophages and differentiated adipocytes (using primary preadipocytes or SGBS cell line) has revealed that macrophage phagocytosed differentiated adipocytes which resulted in IL6 secretion via NF-κB pathway (Sárvári et al., 2015).

Adipocytes from obese mice release lipid filled vesicles and AdExos, which transport neutral lipids, taken up by macrophages and this modulates macrophage polarization. This leads to lipid accumulation, increases lysosomal biogenesis and upregulates genes associated with lipid metabolism in macrophages (Flaherty et al., 2019). Further miR34a released from adipocytes also alters macrophage polarization and interferes with insulin sensitivity. miR34a expression from obese VAT have been associated with HOMA-IR and cytokines (Pan et al., 2019).

Intrestingly, Brestoff et al., has demonstrated that mitochondrial transfer from adipocyte to neighbouring macrophages is important in maintaining metabolic homeostasis *in vivo*. This mitochondria transfer is decreased during obesity which further fosters the macrophage polarization towards M1 (Brestoff et al., 2021). HFD mice demonstrated upregulation of CHOP in adipocytes, which alters the macrophage polarization and thus impacts the insulin sensitivity (Suzuki et al., 2017). Further, adipocyte conditioned media modulates macrophages phenotype in AT of obese human by secreting soluble factors. Lipid mediators (palmitic acid being the most abundant) are the major players in immunomodulatory effects and also display relative concentrations along the BMI (Klein-Wieringa et al., 2013).

In vitro co-culture of differentiated 3T3-L1 and Raw264 has resulted in upregulation of MCP-1, IL6 and TNF α while decreased expression of adiponectin along with increase in the release of FFAs. Conditioned media from Raw264 cells promote MCP1, IL6 and TNF α and similarly conditioned media from 3T3-L1 induces IL6 and TNF α in Raw264 cells. Thus, FFAs released from adipocyte induces TNF α in macrophages and TNF α from macrophages increases the release of FFAs from 3T3-L1, thereby making a paracrine loop in AT mediating inflammation during obesity (Suganami et al., 2005). Further, 3T3-L1 co-cultured with macrophages also demonstrated decreased expression of GLUT4. This alters the macrophage phenotype. TNF α , IL1 β and IL6 block the Akt phosphorylation and impact adipocyte differentiation and therefore decrease in lipid accumulation (Xie et al., 2010). Macrophage secreted factors block insulin action by repressing GLUT4 and IRS1 expression thereby repressing Akt phosphorylation in mouse and human (Gao et al., 2014; Lumeng, DeYoung, & Saltiel, 2007). Neutralising TNF α partially reversed the IR caused by exposure to conditioned media of LPS stimulated macrophages (Lumeng, DeYoung, & Saltiel, 2007). In addition, IL1 β downregulates GLUT4, GSK3 β , PPAR α and PPAR γ expressions while inducing expressions of inflammatory genes that include IL6, CCL5 and NF- κ B. IL1 β also

alters adipocyte metabolism causing reduction in glucose consumption while enhancing lipolysis (Gao et al., 2014).

Cytokines released from macrophages affect mitochondrial biogenesis. IL6 and IL1 β also affect the maximal respiratory capacity of 3T3-L1. Similarly, TNF α increases proton leak and decreases membrane potential (Hahn et al., 2014). Activated macrophages modifies complex I and complex III activity in adipocyte mitochondria. Treating adipocytes with conditioned media of IL10/TGF β activated macrophages decreased the expression of UQCRC2 (component of complex III) and NDUFB8 (component of complex I), thus shifting adipocytes bioenergetics towards glycolysis from OXPHOS while LPS/IFN γ activated macrophages increased OXPHOS in human adipocytes (Keuper et al., 2017).

Thus, the crosstalk between macrophage and adipocytes in AT is crucial in maintaining energy homeostasis. Their interaction in AT microenvironment influences local and systemic inflammation, macrophage recruitment and polarization, adipocyte differentiation and energy metabolism and ultimately insulin sensitivity.

1.5 Macrophages in other metabolic diseases

Macrophages are versatile immune cells that are crucial players in preserving the structure and function of tissues, as well as promoting metabolic homeostasis. Since, macrophages in AT are important in the development of metabolic diseases. The interaction between macrophages and metabolic tissues has developed to regulate insulin signalling and nutrients handling.

Diabetes: Macrophages are significant player in the pathophysiology of both Type1 and Type2 diabetes. Macrophage infiltrates into insulin sensitive tissues that are AT, liver and skeletal muscles.

As mentioned above, ATM accumulation leads to secretion of several proinflammatory cytokines which affects the insulin signalling. On the other hand phenotypic shift in ATMs decrease anti-inflammatory mediators which are important in maintaining insulin sensitivity (Fujisaka et al., 2009). In addition, increased resident macrophages or Kupffer cells (KCs) population is also evident in liver which undergoes phenotypic switch from M2 to M1. During high fat diet KCs are activated and produce cytokines which affect the hepatic insulin sensitivity (Odegaard et al., 2008). Further skeletal muscles are also important site for IR and glucose disposal. Increased CD68⁺ macrophages in skeletal muscles have been linked

to BMI while inversely related to insulin sensitivity. Like ATMs and KCs, macrophages in muscles also switch their phenotype towards inflammatory type (Liu et al., 2018). Exposing conditioned media from palmitate treated macrophages induced IR in L6 myoblasts (Varma et al., 2009).

β -cell dysfunction is also a key player in development of T2D. The presence of amyloid deposits, fibrosis, increased β -cell death, macrophage infiltration and augmented cytokine secretion are evidences of inflammation in the pancreatic islets of T2D patients (Potter et al., 2014). IL1 β and TNF α production by M1 macrophages leads to β -cell dysfunction. Further secretion of chemokines from β -cells and cytokines from macrophages creates a domino effect that intensifies the inflammation (Eguchi & Nagai, 2017).

Cardiovascular Diseases (CVD): Macrophages are important mediators of inflammation in CVD e.g. atherosclerosis and myocardial infarction (MI). Later, macrophages change their phenotype and develop into foam cells. Polarization into different macrophage subtypes are involved in development and stabilisation of plaque. Initially, lipid plaque leads to accumulation of proinflammatory macrophages that express cytokines, chemokines and cell adhesion molecule. However, proliferation of M2 macrophages is important in plaque stabilisation. Similar to atherosclerosis, during the early stages of MI, M1 macrophages are predominant. This M1 activation results in local inflammation. Further, M2 macrophages assume control at later stages to suppress inflammation and promote tissue repair (Nasser et al., 2020; Schelemei et al., 2024).

Liver Diseases: Macrophage accumulation and inflammation in humans during Non-alcoholic fatty liver disease, NAFLD (or non-alcoholic steatohepatitis, NASH). Liver resident macrophage or KCs, which are of embryonic origin, are reduced during NAFLD progression while population of monocyte derived macrophages is increased. This recruitment is through CCR2 and thus attain proinflammatory phenotype (Morinaga et al., 2015; Tran et al., 2020). In addition, ATMs in obesity are not only important in AT inflammation and IR but also involved in NAFLD progression. Transplanting VAT from obese mice into NASH mice led to increase in neutrophil and macrophages in liver. ATMs from VAT induced neutrophil chemotactic proteins and thus contributing liver damage. Similarly, macrophages in VAT correlated with liver macrophage and neutrophil markers in obese patients with NASH (Bijnen et al., 2018).

It is clear that, macrophages play a crucial role in maintaining immune homeostasis, tissue remodelling, and inflammatory responses across various physiological and pathological conditions. In metabolic diseases like obesity, adipose tissue macrophages (ATMs) are key regulators of inflammation and insulin resistance. Despite the importance of ATMs in metabolic diseases, studying them remains challenging. Unlike general M1/M2 macrophage models, which are widely used to study inflammatory versus anti-inflammatory responses, there are no well-established models specifically designed to mimic ATMs. Most research on ATMs relies on adipose tissue biopsies from humans or macrophages isolated from animal models, such as obese mice. These models add variability and are also cumbersome at times. To overcome these challenges, there is a growing need to develop an *in vitro* ATM models that can mimic the functional characteristics of macrophages in obese adipose tissue. While existing macrophage models, such as THP-1 and RAW 264.7 cell lines, allow for M1/M2 differentiation, they do not fully capture the unique environment and plasticity of ATMs. ATMs can be better understood by creating more physiologically relevant stimulations. A deeper understanding of macrophages, particularly ATMs in obesity and insulin resistance, is essential for understanding the molecular pathophysiology better and identifying novel therapeutic targets. We are using THP-1 to develop an *in vitro* model for MMe by employing a cost-effective approach with lower concentrations of stimulants. Unlike conventional methods that rely on recombinant colony-stimulating factors (like GM-CSF), as commonly reported in most of the literature, we utilized phorbol 12-myristate-13-acetate (PMA). This alternative method not only reduces costs but also minimizes batch variability, ensuring greater consistency in experimental outcomes.