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Tatiana Gazdová

Regulation of immunometabolism in white adipose tissue

Regulace imunometabolismu v bílé tukové tkáni

Bachelor's thesis

Supervisor: Mgr. Olga Horáková PhD.

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, ……

Tatiana Gazdová

Poděkování:

Ráda bych poděkovala Mgr. Olze Horákové PhD. Za odborné vedení práce a cenné rady, které mi pomohli tuto práci zpracovat.

Abstract

High-fat diet promotes the development of diet-induced obesity, which leads to further complications such as insulin resistance and type II diabetes. The underlying cause for development of obesity associated pathologies is disruption of adipose tissue homeostasis. Excessive lipid accumulation and rapid white adipose tissue expansion stimulate infiltration, proliferation, and activation of immune cells involved in inflammation propagation. Immune cells within white adipose tissue have the ability to modulate adipocyte function as well as whole-body metabolism. These interactions and function modulations are the core topics of immunometabolism, a rapidly developing field of research focused on interpreting how immune system modulates metabolism on cellular as well as systemic level.

In obesity, pro-inflammatory immune cells, for example M1 macrophages and neutrophils, outnumber homeostasis-promoting anti-inflammatory immune cells in white adipose tissue and alter the tissue environment. As a result, pro-inflammatory cytokines prevent adipocytes from adequately responding to extracellular stimuli. The resulting interactions between immune cells and adipocytes maintain inflammation and promote ectopic lipid accumulation.

Experimental studies suggest that white adipose tissue inflammation can be resolved by dietary omega-3 polyunsaturated fatty acid supplementation. This counteracts the effects of omega-6 fatty acids most commonly found in high-fat Western diets and promotes tissue homeostasis.

Key words: white adipose tissue, high-fat diet, diet-induced obesity, inflammation, insulin resistance, immunometabolism, omega-3 polyunsaturated fatty acids.

Abstrakt

Vysokotuková dieta podporuje rozvoj dietou-indukované obezity, což vede k dalším komplikacím jako jsou inzulínová rezistence a diabetes druhého typu. Základní příčina rozvoje s obezitou spojených chorob je narušení homeostáze tukové tkáně. Nadmírná akumulace tuku a rychlý růst bílé tukové tkáně stimulují infiltraci, dělení a aktivaci imunitních buněk zapojených do šíření zánětu. Imunitní buňky v bílé tukové tkáni jsou schopny ovlivňovat funkci adipocytů a zároveň i celotělového metabolismu. Tyto interakce a ovlivňování funkcí jsou hlavním tématem rapidně se rozvíjejícího oboru vědy, imunometabolismu, zaměřeném na interpretaci vlivu imunitního systému na buněčný a systematický metabolismus.

Během obezity pro-zánětlivé imunitní buňky, například M1 makrofágy a neutrofily přečíslují homeostatické proti-zánětlivé imunitní buňky v bílé tukové tkáni a mění tkáňové prostředí. Ve výsledku, pro-zánětlivé cytokiny zabraňují adipocytům, aby přiměřeně odpovídaly na mimobuněčné vjemy. Výsledné interakce mezi imunitními buňkami a adipocyty udržuje zánět a podporuje ukládání tuku mimo tukové tkáně.

Experimentální studie naznačují, že zánět bílé tukové tkáně může být vyřešen doplněním diety omega-3 polynenasycenými mastnými kyselinami. To působí proti efektů omega-6 mastných kyselin, které se často vyskytují ve vysokotukových západních dietách, a podporují tkáňovou homeostázi.

Klíčová slova: bílá tuková tkáň, vysokotuková dieta, dietou-indukovaná obezita, zánět, inzulinová rezistence, imunometabolismus, omega-3 polynenasycené mastné kyseliny.

List of shortcuts used:

HFD - high fat diet

T2D - type II diabetes

BMI - body mass index

DIO - diet-induced obesity

ω-3 - omega -3

PUFA - polyunsaturated fatty acid

TAG - triacylglycerol

WAT - white adipose tissue

BAT - brown adipose tissue

UCP1 - uncoupling protein 1

IR - insulin resistance

LPL - lipoprotein lipase

FFA - free fatty acid

HSL - hormone sensitive lipase

VAT - visceral adipose tissue

SAT - subcutaneous adipose tissue

ROS - reactive oxygen species

ObR - leptin receptor

ob/ob - leptin deficient mice

db/db - leptin receptor deficient mice

TNF-α - tumour necrosis factor α

DC - dendritic cell

ILC - innate lymphoid cell

IL - interleukin

INF - interferon

PPAR - peroxisome proliferator-activated receptor

TNF-α - tumour necrosis factor α

MHC - major histocompatibility complex

Th - helper T cell

NK - natural killer cell

cDC - conventional dendritic cell

pDC - plasmacytoid dendritic cell

Treg - regulatory T cell

Tc - cytotoxic T cell

NKT - natural killer T cell

FoxP3 - forkhead box P3

ATH - adipose tissue hypoxia

HIF-1 - hypoxia induced factor 1

CLS - crown-like structure

MGC - multinucleate giant cell

iNKT - invariant natural killer T cell

EPA - eicosapentaenoic acid

DHA - docosahexaenoic acid

DPA - docosapentaenoic acid

ALA - α-linolenic acid

LA - linolenic acid

ω-6 - omega-6

Outline

1. Introduction

Obesity is a multifactorial disease that is rapidly becoming increasingly prevalent worldwide. As the world develops, people adapt a more sedentary lifestyle, which often entails high-calorie and high-fat diet (HFD) catered by many quick and easy meal options like fast-food and ready-to-eat meals. Consequently, 39% of adults worldwide were obese in 2016. This means that the prevalence of obesity has tripled in the past 45 years. Obesity, however, is not limited to adults. In 2016 more than 340 million children aged 5-19 years as well as around 38 million children under the age of 5 were overweight or obese. (The WHO) High body mass and adiposity are not a disease on their own, however, they potentiate the development of associated diseases like type II diabetes (T2D). (Hruby and Hu, 2015) High body mass index (BMI) is also a risk factor for cardiovascular diseases, which, in 2012, were the leading cause of death. Diet-induced obesity (DIO) and the associated pathologies are preventable. (The WHO) In order to treat and manage them, however, we must better understand what goes on at the cellular level.

Complex crosstalk between adipocytes and immune cells within adipose tissue leads to low-grade inflammation, which is the basis for tissue homeostasis dysregulation. Immunometabolism is a field of study that focuses on the interplay between metabolism and immunity. (Hotamisligil, 2017; Kuda, Rossmeisl and Kopecky, 2018) Research on the topic of immunometabolism has grown rapidly in the recent years, yet there is still a lot more to discover. By being able to understand and manipulate the immunometabolic interface we could create various therapeutic targets and improve local and whole-body metabolism. This has the potential to improve medications for not only obesity-related diseases, such as T2D, but also cancer and sepsis, among others. (Kohlgruber, LaMarche and Lynch, 2016)

The goal of this work is to illustrate the physiological interactions between cells in adipose tissue and the pathological processes that occur if homeostasis is disrupted. Mechanics of a metabolically healthy adipose tissue from lean subjects will be described, so that they can be used as a base line and further contrasted with the situation that occurs in low-grade inflammation. Additionally, I will summarise research into the topic of omega-3 (ω-3) polyunsaturated fatty acid (PUFA) supplementation as an emerging management strategy that aims to renew adipose tissue homeostasis in obese individuals.

2. Adipose tissue

Adipose tissue is a type of highly metabolic connective tissue with the ability to store body's excess energy in form of triacylglycerols (TAG). (Ryu *et al.*, 2019) It can also act as an insulator or heat generator, depending on the needs of the body. (Quesada-López *et al.*, 2019) Despite the fact, that this thesis focuses on a specific adipose tissue type, I believe it is important to note that there are three major types of adipose tissue (Bernasochi *et al.*, 2019):

- White adipose tissue (WAT) – The structure and various functions of WAT are discussed in the following chapters.

- Brown adipose tissue (BAT) – BAT is very mitochondria-rich compared to other adipose tissue types and thus contains large amounts of cytochrome c, which is where its colouring comes from. (Virtanen *et al.*, 2009) It is mostly found in hibernating rodents and human infants, where it aids in body temperature maintenance through non-shivering thermogenesis. (Li *et al.*, 2014) BAT is characterised by its expression of uncoupling protein 1 (UCP1), which is capable of separating respiration from ATP production and thus giving rise to thermogenesis. (Cypess *et al.*, 2009) It has been believed that with age BAT regresses and adult humans have no active BAT. (Yoneshiro *et al.*, 2011) However, new non-invasive studies show that depots of active BAT are present in adult humans. Moreover, it was found that BMI and BAT are inversely proportional, suggesting BAT might play a role in adult human metabolism. (Cypess *et al.*, 2009); Virtanen et al., 2009)

- Beige adipose tissue – Beige adipocytes are UCP1+ cells that arise within white adipose tissue. Additionally, unlike classical BAT, which arises from Myf5 expressing muscle-like cell lineage, (Seale et al., 2008; Kablar, Krastel, Tajbakhsh, & Rudnicki, 2003) beige adipocytes are more similar to WAT. They have low basal levels of UCP1 expression, however, after cold exposure their expression is rapidly promoted. This is a process called browning and it allows beige adipocytes to gain thermogenic function. Furthermore, it was found that transgenic mice lacking beige adipocytes are significantly more prone to obesity, insulin resistance (IR) and liver disease when on HFD. (Cohen *et al.*, 2014) Consequently, even though much is still unknown about beige adipocytes, further research in this field could be beneficial to our overall understanding of adipose tissue and its role in health and disease. (Wu et al. 2012)

The function of a particular WAT depot strongly depends on its localisation and adipocyte composition. It is further enhanced by the presence of other cell types such as preadipocytes (adipocyte precursor cells), nerve cells, fibroblasts and cells of the immune system. (Weisberg *et al.*, 2003) The following chapters of this thesis will focus predominantly on WAT as it is the major site of immune cell localisation and metabolic dysregulation from the above-mentioned tissues.

2.1. Storage and metabolism of white adipose tissue

WAT serves as the main energy storage, which takes up glucose, fatty acids and glycerol in times of excess (Trayhurn and Bing, 2006) and releases them back into circulation when needed. (Fortier *et al.*, 2004) In mammals, WAT is one of the most common tissue types. Lipids are an efficient form of energy storage and can yield more than two times the calories per gram compared to glycogen. Due to their non-polar nature, fatty acids can be stored in nearly anhydrous conditions. (Berg, Tymoczko and Stryer, 2002) In WAT adipocytes, lipids are stored in large, unilocular droplets, as compared to BAT adipocytes, which have multilocular droplets. (DiSpirito and Mathis, 2015)

When energy is present in excess after a meal and blood glucose level rises, lipogenesis takes place. The level of lipogenic enzyme expression is low during fasting, however, after feeding it is upregulated under the influence of insulin. Fatty acid synthase is capable of converting glucose metabolites malonyl-CoA and acetyl-CoA into fatty acids, which are then stored as TAG in adipocytes. (Wong *et al.*, 2009) Lipogenesis can occur in many tissues including WAT. Fatty acids synthesised in liver are then released in form of very low density lipoprotein. (Eissing *et al.*, 2013) Lipoprotein lipase (LPL) is an enzyme capable of hydrolysing TAG within plasma lipoproteins and thus supplying free fatty acids (FFA) to surrounding tissues. (Blomquist *et al.*, 2018) These FFAs can be directly taken up by WAT and consequently stored as TAG. (Koutsari *et al.*, 2012)

Contrastingly, lipolysis refers to the breakdown of TAG within the adipocytes and the release of FFAs and glycerol into the bloodstream. This process is subject to hormonal activation, which starts a cascade of phosphorylation leading up to the TAG breakdown. A key component of this cascade is the translocation of phosphorylated hormone-sensitive lipase (HSL) from cytosol of the adipocyte to the surface of its lipid droplet. This droplet is surrounded by perilipin A, which when phosphorylated, changes its conformation, and allows for HSL interaction with the TAG. During fasting, lipolysis of adipocyte lipid stores is used to supply liver and muscles with fatty acids for lipid oxidation, and to supply liver and kidney cortex with glycerol for gluconeogenesis. (Sztalryd *et al.*, 2003) When low blood glucose levels are detected, glucagon secretion from pancreatic islets of Langerhans is initiated. (Kim *et al.*, 2009) When in bloodstream, glucagon causes rapid production of cyclic AMP by adenylate cyclase. Cyclic AMP then activates protein kinase A, which is capable of phosphorylating HSL and perilipin A, thus triggering lipolysis. (Brasaemle *et al.*, 2000)

2.2. Depots and localisation

As mentioned in Chapter 2., WAT can be found in numerous anatomical locations in both humans and rodents. Their localisation can significantly affect their function. WAT depots in mammals can be categorised depending on their localisation as visceral adipose tissue (VAT) or subcutaneous adipose tissue (SAT) (See Figure 1). (Trayhurn and Bing, 2006) In mammals, overall adiposity plays an

important role in the development of pathologies; however, the differential distribution of fat between these depots is a major factor in onset of obesity-associated diseases. (Kraunsøe *et al.*, 2010)

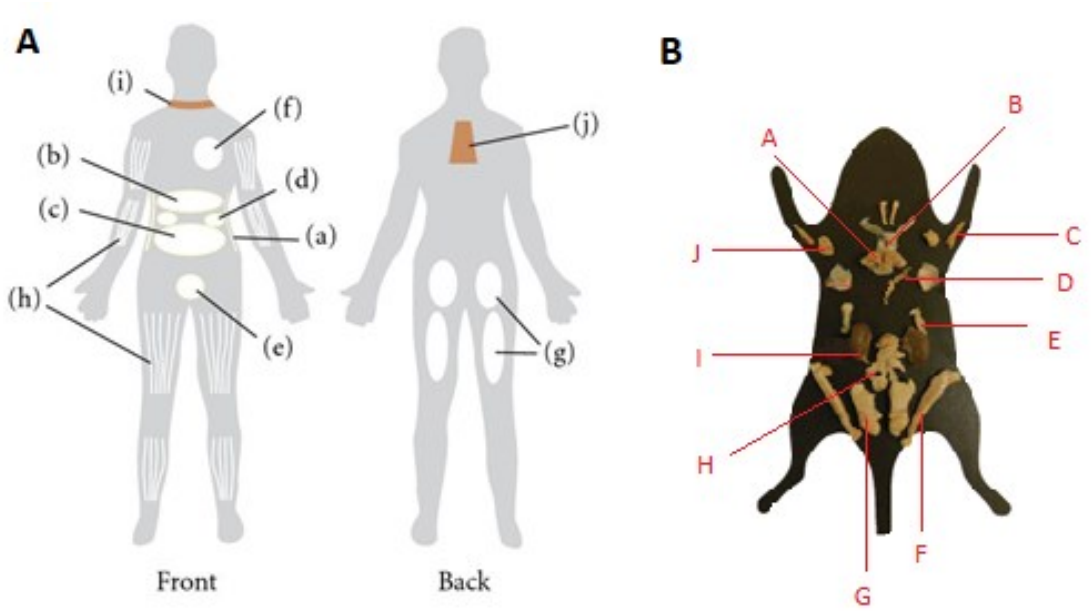

Figure 1: A- Localisation of human adipose tissue depots, adapted from Bjørndal *et al.*, 2011. SAT: abdominal (a), gluteofemoral (g). VAT: omental (b), mesenteric (c), retroperitoneal (d), gonadal (e), pericardial (f), and intramuscular (h). BAT: supraclavicular (i) and subscapular (j). **B-** Localisation of adipose tissue depots in mouse, adapted from de Jong *et al.*, 2015. SAT: interscapular WAT (B), triceps WAT (C), inguinal WAT (F). VAT: cardiac WAT (D), perirenal WAT (E), epididymal WAT (G), mesenteric WAT (H). BAT: interscapular BAT (A), axillary BAT (J), perirenal BAT (I). Numerous human and mouse depots are found in similar locations, eg.: within muscle (h,C), around the gut (c,G), around kidneys (d,E,I), between the scapulae (j, B).

Adipose tissue surrounding internal organs (See b-h in Figure 1A) has been linked to cardiovascular disease when accumulated in excess. This occurs due to its characteristic production of pro-inflammatory cytokines, oxidative damage from reactive oxygen species (ROS) (Kim *et al.*, 2019), and elevated macrophage recruitment. It has also been shown, that visceral obesity is strongly linked to onset of T2D (Cohen *et al.*, 2014), IR, and metabolic syndrome. (Gabriely *et al.*, 2002; Goodpaster *et al.*, 2005) It is important to note, that increased VAT accumulation correlates with higher mortality and disease onset even in patients with normal BMI. (Goodpaster *et al.*, 2003; Pischon *et al.*, 2008)

SAT, on the other hand, plays a smaller part in T2D development. Even though excessive accumulation of both SAT and VAT has been linked to IR and metabolic dysfunction, VAT is much more strongly correlated with adverse effects on metabolism and overall health. (Fox *et al.*, 2007; Preis *et al.*, 2010) Additionally, it has been established, that premenopausal women more readily accumulate SAT, while men are more prone to VAT deposition. (Cohen *et al.*, 2014) Consequently, T2D is more common in males than in females. (Nordström *et al.*, 2016) Furthermore, it has been

found that age is linked to VAT accumulation of both sexes, while it is only linked to SAT increase in females. (Fox *et al.*, 2007)

2.3. Secretory function

The microenvironment within WAT is strongly influenced by the presence of cytokines secreted from adipocytes and immune cells. This thesis will define cytokines as small peptides capable of modulating the behaviour of other cells. In this chapter we will look at adipokines (cytokines secreted from adipocytes) leptin and adiponectin. The effects of adipokines secreted by WAT play a critical role in development of obesity associated diseases. (Antuna-Puente *et al.*, 2008)

Leptin is a polypeptide (16 kDa) that plays a key role in appetite control and energy balance. (Pan *et al.*, 2018) It has been confirmed that plasma leptin levels strongly correlate with fat mass as well as adipocyte size and count. (Mazor *et al.*, 2018) Leptin exhibits its effect by acting on the central nervous system and regulating appetite and metabolic fuel usage via a negative feedback loop. Leptin binds to its receptor, which is widely distributed in both humans and rodents. (Mazor *et al.*, 2018; Pan *et al.*, 2018) When leptin receptor (ObR) is activated by extracellular leptin binding it leads to phosphorylation of Janus kinase 2. Active Janus kinase 2 can then phosphorylate signal transducer and activator of transcription-3. (Pan *et al.*, 2018) Most obese patients, however, show high leptin blood levels alongside with reduced ObR signalling. This state is called leptin resistance and refers to the impaired ability of leptin to exert its anorexigenic effect. (Mazor *et al.*, 2018) The possible mechanisms for leptin resistance are diminished signalling via the ObR and reduced rates of leptin transport across the blood-brain barrier. (Mazor *et al.*, 2018; Kumari, Kumar and Kant, 2019)

Diabetic mouse models with spontaneous mutations are frequently used in research. The two most common types are leptin deficient mice (ob/ob) and leptin receptor deficient mice (db/db). The ob/ob mice have high levels of leptin mRNA, however, a single nonsense mutation leads to premature translation termination and thus these mice have no functional leptin polypeptide. On the other hand, db/db mice have increased levels of leptin but because of ObR mutation lack intracellular leptin response. These mutations have not been identified as a cause of T2D in humans. (Wang, Chandrasekera and Pippin, 2014)

Adiponectin is a polypeptide (30kDa) with anti-inflammatory properties secreted by adipocytes. Expression of the adiponectin gene can by upregulated by transcription factors, for example peroxisome proliferator-activated receptor (PPARγ), that bind to adiponectin promoter. (Deng and Scherer, 2010) Adiponectin is the most abundant adipokine (Esposito *et al.*, 2003) and makes up 0.01% of all plasma proteins. (Ouchi *et al.*, 2001) It serves to lower the rate of liver gluconeogenesis and increase lipid oxidation in skeletal muscle. (Weisberg *et al.*, 2003; Kumari, Kumar and Kant, 2019) Human studies show that hyperadiponectinemia correlates with increased insulin sensitivity, increased lipid oxidation and lesser VAT accumulation, all of which lead to decrease incidence of

T2D development. On the other hand, hypoadiponectinemia has been associated with cardiovascular and metabolic diseases, namely T2D and coronary artery disease. (Kumari, Kumar and Kant, 2019)

Leptin and adiponectin serve opposing functions and thus a balance in their concentrations is critical for adipose tissue homeostasis. Lastly, it is important to note that adipocytes are also able to secrete other cytokines such as resistin, tumour necrosis factor -α (TNF-α), IL-6, and IL-1β (for description see Chapter 4.1.). (Fain, 2006)

2.4. Cell types within white adipose tissue

Adipose tissue contains not only adipocytes but also endothelial cells, neurones, and immune cells. (Kälin *et al.*, 2017) For the purpose of this thesis, I will focus solely on immune cells.

2.4.1. Innate immune cells

Innate immunity is the first line of response in any immunological occurrence and so we will discuss this group of cells first. The most well-researched cell types of innate immune cells are macrophages, mast cells, granulocytes, dendritic cells (DCs), and innate lymphoid cells (ILCs). The following sub-chapters will describe how each cell type functions in healthy, lean individuals in order to set a base line for the rest of this work.

2.4.1.1. Macrophages

Macrophages are immune cells that differentiate from a monocyte precursor. Their main function is to clear cellular debris and pathogens by phagocytosis. (Cinti *et al.*, 2005) In adipose tissue of healthy lean individuals, macrophages make up for 10-15% of all immune cells present and are found in interstitial spaces between the adipocytes. They are attracted and activated via various cytokines. Macrophages can portray two distinct phenotypes: a pro-inflammatory M1 phenotype and an alternative M2 anti-inflammatory phenotype. The type of macrophage activation depends on the secretion profile of WAT. (Mclaughlin *et al.*, 2017)

Tissue resident macrophages in lean individuals are M2 polarised and act to resolve inflammatory responses, aid in tissue repair and angiogenesis. (Kohlgruber, LaMarche and Lynch, 2016) In lean WAT, interleukin (IL) -13 and IL-4 secreted from eosinophils are responsible for M2 macrophage polarisation, which in turn secrete IL-10. On the other hand, M1 macrophages are more prevalent in obesity. They are induced via interferon (INF)-γ and in response produce inflammatory cytokines, including IL-6, IL-1β and TNF-α. (Fujisaka *et al.*, 2013; Mclaughlin *et al.*, 2017) Macrophages have high phenotypic plasticity thus their polarisation can change rapidly in response to external stimuli. (Mclaughlin *et al.*, 2017) For example, adiponectin and IL-4 can directly promote M2 polarisation through their activation via a PPARγ receptor. Additionally, diet composition can have varying effects on macrophage polarisation. While PUFA promote the healthy M2 phenotype, saturated fatty acids

induce M1 polarisation. This can be induced directly or through alterations in adiponectin levels. (Kumari, Heeren and Scheja, 2018)

2.4.1.2. Mast cells

Mast cells are tissue resident haemopoietic cells, which function as the first line of immunological defence. Upon stimulation they have an ability to rapidly degranulate and release pre-synthesised molecules that initiate inflammation. Some of these include lipid mediators and cytokines (TNF-α, IL-1β, IL-6, IL-4, IL-8, IL-10, and granulocyte-macrophage colony-stimulating factor). (Divoux *et al.*, 2012; Mraz and Haluzik, 2014) Only a small number of mast cells are present in adipose tissue of healthy individuals. (Chmelař *et al.*, 2016)

2.4.1.3. Granulocytes

Granulocytes make up 60-70% of blood leukocytes (Talukdar *et al.*, 2012) and are commonly divided into three groups: neutrophils, eosinophils, and basophils. As their name suggests, granulocytes have granule-rich cytoplasm. These granules contain biologically active proteins vital to each cell´s function (Mraz and Haluzik, 2014).

Neutrophils account for more than 90% of all granulocytes (Talukdar *et al.*, 2012). They are the first cell type recruited into the site of acute inflammation. Their mechanism of action includes degranulation of antimicrobial reagents and phagocytosis. Once at the infection site, neutrophils are capable of cytokine and chemokine synthesis, producing TNF-α, IL-1β and IL-8, which in turn promote recruitment of macrophages and their M1 polarisation. (Mraz and Haluzik, 2014).

Eosinophils are white blood cells with characteristic multi-lobe nucleus and granule-rich cytoplasm. They develop in bone marrow and migrate into tissues in response to cytokines and chemokines e.g. IL-5. (Bolus *et al.*, 2018) Traditionally, eosinophils are associated with allergies and immune reactions to parasitic infections. In WAT, however, these tissue resident cells (Vohralik *et al.*, 2020) synthetise numerous lipid mediators and cytokines (IL-4, IL-10, and IL-13). As a result they maintain the anti-inflammatory macrophage profile. (Wu *et al.*, 2011; Mraz and Haluzik, 2014; Bolus *et al.*, 2018)

2.4.1.4. Dendritic cells

DCs are antigen presenting cells that function as the interface between innate and adaptive immunity. Their main function is to activate other immune cells in response to pathogens. They activate T–lymphocytes by presenting them with antigens via major histocompatibility complex (MHC) class II. DCs are also capable of synthetising cytokines such as IL-12, which induces maturation of naïve T cells into T helper (Th) type 1 cells, and IL-15, which aids in proliferation of natural killer (NK) cells and cytotoxic CD8+ T cells. (Mraz and Haluzik, 2014; Mráz et al., 2019) DCs can present antigens

and secrete cytokines once they undergo maturation as a response to damage- and pathogen-associated molecular patter recognition.

Two distinct subtypes of DCs with different morphology and surface markers are recognised: a conventional dendritic cell (cDC) type and a plasmacytoid dendritic cell (pDC) type. (Mráz et al., 2019) cDCs carry a CD11c surface marker and play a role in differentiation of pDCs and Th cells. pDCs express a CD123 marker on their membranes and are the major producers of type I IFN. Type I IFN is a key player in Th1 differentiation, DC maturation and NK cell response. (Hannibal et al., 2017; Mráz et al., 2019) DCs are capable of recruiting and influencing other immune cells, however this is highly dependent on the current metabolic and energetic state of the tissue. In healthy, lean subjects abundant cDCs promote anti-inflammatory phenotype. (Mráz *et al.*, 2019)

2.4.1.5. Innate lymphoid cells

ILCs are a branch of the innate immune system responsible for epithelial protection and tissue homeostasis. They can be found in both lymphoid and non-lymphoid tissues. Currently, ILCs are divided into three groups based on their surface markers: ILC1, ILC2 and ILC3. (O'Sullivan *et al.*, 2016; Saetang and Sangkhathat, 2018; Wang *et al.*, 2019)

Group 1 ILCs can be further divided into two groups: conventional NK cells and non-NK ILC1 cells. Conventional NK cells circulate the body and utilize their cytotoxic abilities to eliminate pathogens. (Everaere *et al.*, 2018; Wang *et al.*, 2019) ILC1 cells have a limited cytotoxicity, however, they can produce numerous cytokines. ILC1s are tissue resident cells, which can usually be found in non-lymphoid tissues, most importantly for this work: WAT. Both types of group 1 ILCs cells are capable of producing TNF-α and INF-γ in response to IL-12, IL-15 and IL-18. (Everaere *et al.*, 2018; Saetang and Sangkhathat, 2018)

Group 2 ILCs are a WAT tissue resident group of cells (Vohralik *et al.*, 2020) characterised by their secretion of IL-4, IL-5, IL-13 and IL-9 and transcription of the RAR-related orphan receptor α. Usually, they are responsible for responses related to parasites and other allergens. (Saetang and Sangkhathat, 2018)

Group 3 ILCs are the least abundant group of ILCs (5%); however, they are the major producer of IL-22. Their mucosal defence function can be triggered via IL-23 or IL-1β, which in turn results in IL-17 and/or IL-22 secretion. (Everaere *et al.*, 2018; Saetang and Sangkhathat, 2018)

2.4.2. Adaptive immune cells

Adaptive immune cells have slower response times than the innate immune cells, however, their actions are more targeted and specific. B- and T- lymphocytes make up the adaptive branch of the immune system. (Herck *et al.*, 2019) Even though they play a big part in disease combat, these lymphocytes also uphold healthy tissue homeostasis.

2.4.2.1. T-cells

T cells are lymphocytes, which originate in the bone-marrow and mature in thymus. They execute their roles by differentiating into numerous different types in response to immunological stimuli. The major subsets are: Th cells, regulatory T cells (Treg), cytotoxic T cells (Tc) and innate immune cells types such as natural killer T cells (NKT). (Mraz and Haluzik, 2014; Herck *et al.*, 2019) T cell function is induced by antigen presenting cells via MHC class I or II. The cells also requires a specialised T-cell receptor and CD3 complex in order to recognise the presented antigens. (Herck *et al.*, 2019)

Th cells regulate inflammatory responses by modulating the responses of Tc cells, phagocytes and B cells. (Herck *et al.*, 2019) They can be further subdivided into subtypes based on their cytokine expression profile. Th1 express IFNγ, Th2 produce IL-4, IL-5, and IL-13, Th17 cells secrete IL-17, IL-21, and IL-22 and lastly, Th22, which also produce IL-22. These cells, along with Tregs, are characterised by CD4 molecular marker expression. (Mraz and Haluzik, 2014) Tregs are also characterised by their expression of the forkhead box P3 (FoxP3) factor. They are vital in immunological self-tolerance, homeostasis, and tissue inflammation suppression. (Ilan *et al.*, 2010; Kälin *et al.*, 2017) In WAT they are a tissue resident cell type. (Vohralik *et al.*, 2020)

On the other hand, Tc cells are recognised by their expression of the CD8 complex. They recognise compromised cells via MHC I and induce their death by cytotoxic agents like granzymes and perforins. These cells originate in the bone marrow and undergo extensive selection in the thymus. (Herck *et al.*, 2019)

It is crucial that the ratios of different T cell subpopulations are kept in check in order to maintain homeostasis and prevent diseases. Anti-inflammatory cells like Tregs must be dominant over pro-inflammatory subtypes (Th1, Th17 and Tc cells) in order to maintain a healthy WAT phenotype (Jagannathan-Bogdan *et al.*, 2011; Mraz and Haluzik, 2014; Herck *et al.*, 2019)

2.4.2.2. B cells

B cells are produced in bone marrow, mature in spleen and lymph nodes, and then reside in target tissues like WAT. Their main function is antibody production, however, they are also able to act as antigen presenting cells. (Mraz and Haluzik, 2014; Lee, Rojas and Gommerman, 2020) Each B cell has a unique B cell receptor. Before being released into circulation B cells are always checked for autocorrectivity to prevent self-damage.

3. Diet-induced obesity

Weight gain is a direct result of the intake/expenditure imbalance in energy. However, Westerterp et al. claim that energy expenditure has not decreased over the past years, yet obesity rates have skyrocketed. This means that diet variation is the most probable explanation. (Westerterp and Speakman, 2008) Dietary composition plays a significant role in body homeostasis. While diets rich in carbohydrates stimulate lipogenesis and adipocyte glucose uptake, high-protein diets downregulate hepatic cholesterol synthesis and adipocyte lipogenesis, while promoting hepatic β-oxidation. (Wensveen *et al.*, 2015)

Furthermore, Hu et al. showed that dietary fat is the only macronutrient strongly associated with increased adiposity. They found out that mice fed HFD overeat and thus have higher caloric intake. This was termed as hedonic over-ride hypothesis. It poses that naturally an individual regulates food intake in relation to energy requirements, thus only inputting the calories needed. However, consumption of sucrose and fat can over-ride natural homeostasis and lead to overconsumption, which result in energy imbalance and obesity. (Hu *et al.*, 2018)

Overnutrition promotes FFA and glucose uptake by adipocytes. The main side effect of this, on cellular level, is the production of ROS in adipocyte mitochondria. Numerous transcription factors, such as NF-κB respond to the rising ROS levels. This in turn correlates with inflammation by promoting pro-inflammatory cytokine secretion (TNF-α, Il-6, IL-1β) from M1 polarised macrophages and adipocytes. (Wensveen *et al.*, 2015)

3.1. Adipose tissue expansion

Adipose tissue possesses a near unlimited ability to expand. Healthy adipose tissue growth entails a coordinated action of immune cells, endothelial cells, neurons, and preadipocytes in WAT. Consequently, various mechanisms are in place to facilitate its growth. (Sun, Kusminski and Scherer, 2011) Adipose tissue hyperplasia, also called adipogenesis, refers to the growth of WAT by preadipocyte differentiation and thus increase in adipocyte numbers. (Li *et al.*, 2019) It is important in maintenance of healthy and functional adipose tissue. Its disruption can lead to metabolic malfunction and inflammation (See Figure 2). (Benchamana *et al.*, 2019) Adipose tissue expansion that progresses through increasing adipocyte volume via lipid-loading is called hypertrophy, and when excessive leads to degradation of adipose tissue health. (Ge *et al.*, 2019) It is believed that adipocyte hypertrophy is the main mechanism for adipose tissue growth in DIO. (Li *et al.*, 2019) Extreme adipose tissue expansion can lead to a plethora of adverse effects such as hypoxia and adipocyte death. (Sun, Kusminski and Scherer, 2011)

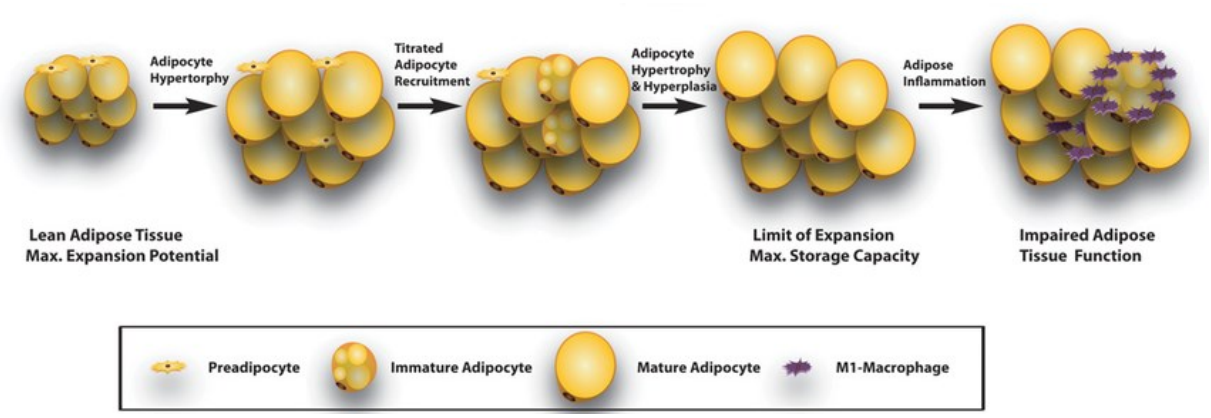

Figure 2: Stages of white adipose tissue expansion, adapted from Sethi and Vidal-Puig, 2010. As the tissue expands, and inevitably reaches its maximum storage capacity, hypertrophy and hyperplasia occur, shortly followed by the development of low-grade inflammation.

3.1.1. Hypoxia

During its rapid growth in times of excess adipose tissue can become hypoxic. This is caused by the inability of vasculature to match the rate of adipose tissue expansion. Consequently, adipocytes reach an oxygen diffusional limit, which is a limiting factor in healthy adipose tissue expansion. Adipose tissue hypertrophy in obesity thus creates microenvironments of hypoxia during the early stages. (Halberg *et al.*, 2009; Sun, Kusminski and Scherer, 2011) While acute hypoxia leads to tissue remodelling, chronic hypoxia results in disruption of physiological tissue function (See Figure 3). (Crewe, An and Scherer, 2017)

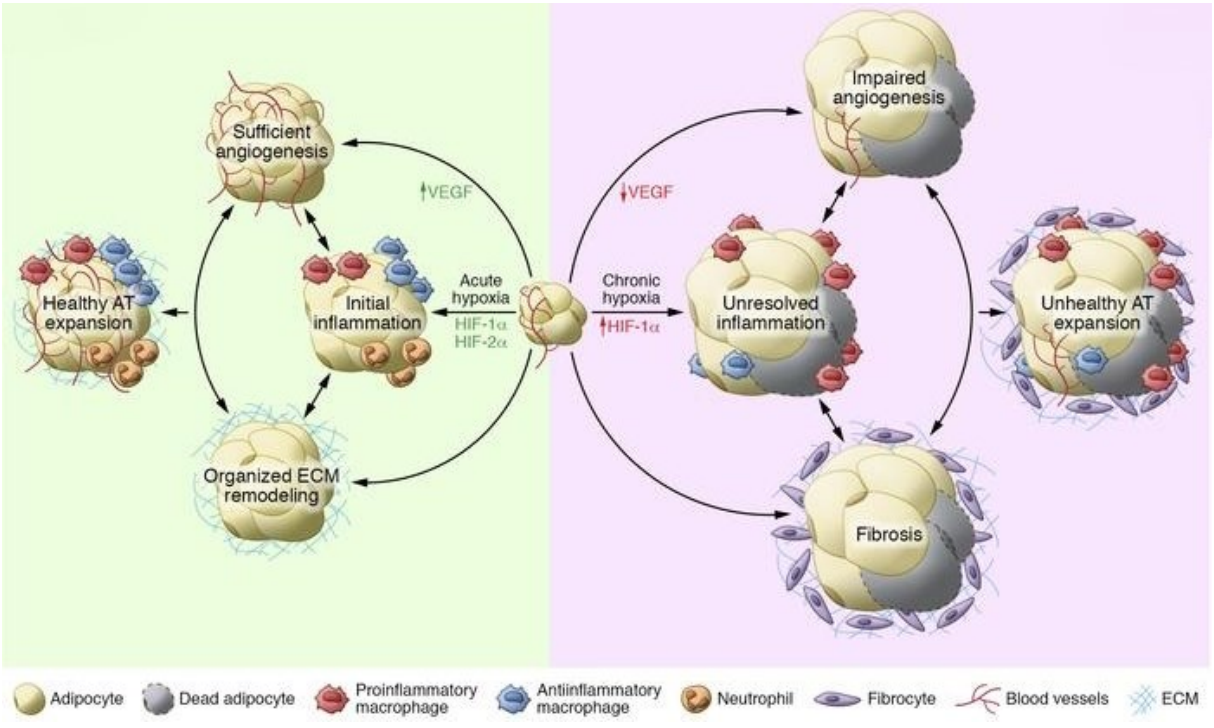

Figure 3: Effect of acute and chronic hypoxia on WAT, adapted from Crewe, An and Scherer, 2017. Shortcuts: HIF- hypoxia induced factor, VEGF- vascular endothelial growth factor, AT- adipose tissue.

Increased expression of hypoxia-induced factor 1 (HIF-1) is typical in adipose tissue hypoxia (ATH). Gene expression of hormones, such as leptin, is directly regulated by this protein. HIF-1 functions as a heterodimer of HIF-1 α and HIF-1 β. HIF-1 β is constitutively expressed while HIF-1 α production is oxygen-dependent. In normoxic conditions degradation of HIF-1 α is triggered, however, in hypoxia its hydroxylation slows down, and the protein accumulates thus taking effect. It has been observed that HIF-1α induces tissue fibrosis, which may be a key factor in stimulation of associated local inflammation. (Halberg *et al.*, 2009; Sun, Kusminski and Scherer, 2011)

Additionally, ATH upregulates adipocyte expression of numerous inflammatory cytokines such as leptin and IL-6. (Sun, Kusminski and Scherer, 2011) ATH was also associated with decreased adiponectin expression. This change in WAT cytokine profile leads to chronic low-grade inflammation by inducing M1 macrophage polarisation, T cell activation and neutrophil infiltration. (Hosogai *et al.*, 2007; Ye *et al.*, 2007)

3.1.2. Adipocyte cell death

It is believed that ATH, oxidative stress and adipocyte hypertrophy are factors that cause adipocyte cell death. (Feng *et al.*, 2011; Kuroda and Sakaue, 2017) In obese mice the rate of adipocyte cell death is increased 30-fold. Adipocyte size has been positively correlated with the rate of adipose cell death and dysregulated metabolism. Additionally, both human and mice adipocytes portray features of necrotic death such as membrane rupture and release of free lipid droplets as well as cell-free DNA. Cell contents that are released into extracellular space trigger immune response and lead to inflammation. (Cinti *et al.*, 2005; Nishimoto *et al.*, 2016) Adipocyte cell death can thus activate a danger-associated molecular pattern initiated pathway, which leads to monocyte, neutrophil, and T cell infiltration (See more in Chapter 3.2.). (Kohlgruber, LaMarche and Lynch, 2016)

3.2. Activation and recruitment of immune cells in obesity

Levels and functions of immune cells rapidly respond to changing conditions such as overnutrition and obesity (See Figure 4). (Kälin *et al.*, 2017) Chapter 2.4. depicts homeostatic situation in lean WAT. Adipocyte stress, such as hypoxia and hypertrophy (See Chapter 3.1.), induce immune cells cytokine secretion. Moreover, stress-ligands are present on adipocyte cell surface, where they serve to activate other cells. (Wensveen *et al.*, 2015) A complex cascade of activation, secretion, and recruitment unfolds when innate immune cells recognise tissue damage signals. The following section of this thesis is dedicated to denoting how the function and population size of immune cells changes in response to DIO.

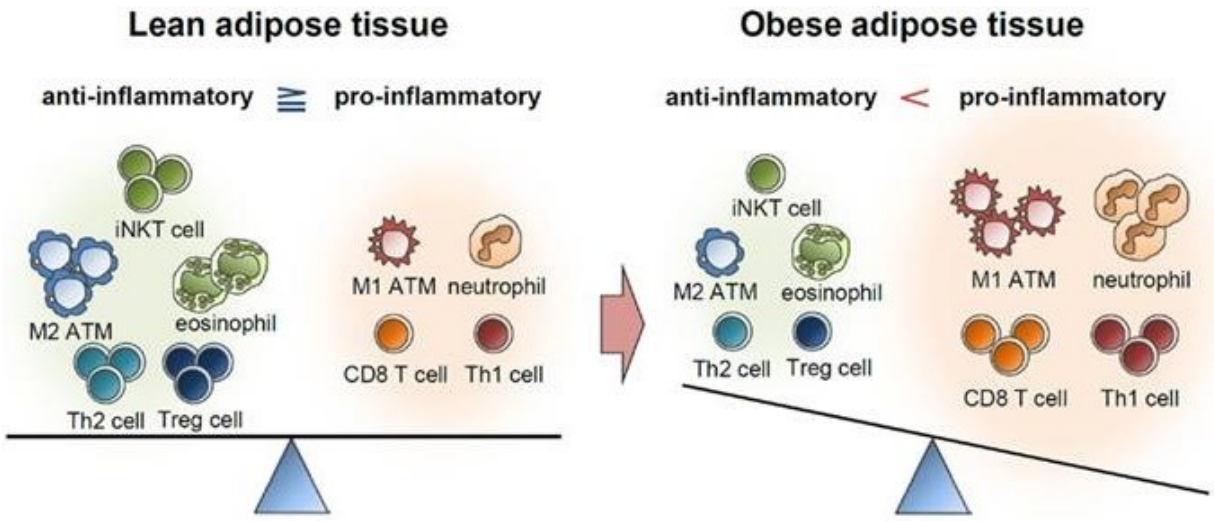

Figure 4: Balance between pro-inflammatory and anti-inflammatory immune cells in WAT of lean (left) and obese (subjects), adapted from Choe *et al.*, 2016. Shortcuts: iNKT- innate natural killer T cell, ATM- adipose tissue macrophage, Th- helper T cell, Treg- regulatory T cell.

3.2.1. Dendritic cells

In WAT of obese subjects the altered adipokine secretion and inflammation promote pDC recruitment. (Ghosh *et al.*, 2016) Accordingly, numerous studies show that in DIO pDC content of adipose tissue is increased, which in turn triggers differentiation of Th17 cells. (Chen *et al.*, 2014; Mraz and Haluzik, 2014; Macdougall *et al.*, 2018) In DIO, DCs secrete pro-inflammatory cytokines and portray an inflammation-promoting phenotype. (Reynolds *et al.*, 2012) Additionally, the number of CD103+ DCs, which are crucial for Treg differentiation, are diminished in obese subjects compared to the lean controls. The reduction in anti-inflammatory Treg cells and increased differentiation of IL-17 producing Th17 cells results in an immunophenotypic shift. Subsequently, this also triggers M1 polarisation of WAT macrophages. (Stefanovic-Racic *et al.*, 2012; Mraz and Haluzik, 2014)

Mice models lacking pDCs were found to have lower numbers of adipose tissue macrophages and appear to be resistant to DIO and associated metabolic complications. (Stefanovic-Racic *et al.*, 2012; Hannibal *et al.*, 2017) Additionally, when all $CD11c^+$ expressing cells (macrophages, granulocytes, DCs) in obese mice have been depleted rapid normalisation of insulin sensitivity and cytokine profile were observed. (Macdougall *et al.*, 2018) Overall, the data suggest that elevated pDC levels, which are induced by HFD, drive pro-inflammatory macrophage phenotype and thus trigger the metabolic consequences of DIO. (Stefanovic-Racic *et al.*, 2012; Mráz *et al.*, 2019)

3.2.2. Mast cells

Mast cell numbers are significantly elevated in adipose tissue of obese individuals. However, whether they play any role in obesity associated inflammation and pathologies remains to be definitively

answered. Opposing views on this topic are present within the scientific society. Some researchers claim that mast cells, which co-localise with fibrotic tissue, stimulate WAT inflammation. Additionally, correlations of WAT mast cell numbers with glucose homeostasis, diabetes and IR have been suggested. (Divoux *et al.*, 2012; Einwallner *et al.*, 2016) Einwallner et al. have observed that mast cell accumulation occurs before macrophage infiltration, which suggest a possible role of mast cells in macrophage recruitment. Furthermore, mast cells synthesise IFN-γ, which promotes a pro-inflammatory state of macrophage activation. Mast cells are also capable of synthetising anti-inflammatory cytokines in the event of inflammation resolution. (Einwallner *et al.*, 2016)

On the other hand, Chmelař et al. claim that mast cells do not play a role in obesity and related disease. A possible explanation for some of the conflicting opinions is the use of two different transgenic mice models, which appear to yield opposing results. (Chmelař *et al.*, 2016)

3.2.3. Macrophages

Macrophages are the most abundant cell type that infiltrates WAT as a response to inflammation. They can make up to 60% of all immune cells within the tissue. (Mclaughlin *et al.*, 2017) In order to prevent lipotoxicity and to remove debris, macrophages form crown-like structures (CLSs) around damaged adipocytes. (Kohlgruber, LaMarche and Lynch, 2016; Mclaughlin *et al.*, 2017) When macrophages remain activated by inflammation they fuse together, which results in the formation of multinucleate giant cells (MGCs). MGCs produce pro-inflammatory cytokines; they are the major producer of TNF-α and also produce significant amounts of IL-6 and nitric oxide within WAT. (Cinti *et al.*, 2005)

There are four major causes for macrophage infiltration and their M1 polarisation: adipocyte death, chemotaxis, fatty acid flux and hypoxia. All these factors are a direct result of rapid tissue expansion (See chapter 3.2.) and contribute to the inflammatory environment within WAT of obese subjects. (Fujisaka *et al.*, 2013)

M1 macrophages can either be recruited into WAT from bloodstream or they can proliferate locally within the tissue. (Kohlgruber, LaMarche and Lynch, 2016) These macrophages can then further activate inflammasome via danger-associated molecular pattern signalling. (Kumari, Heeren and Scheja, 2018)

3.2.4. Granulocytes

Eosinophils secrete IL-4, which promotes anti-inflammatory macrophage polarisation, thus inducing glucose tolerance and promoting adipose tissue health. (Mraz and Haluzik, 2014; Bolus *et al.*, 2018; Lee *et al.*, 2018) However, DIO has been correlated with lower infiltration of eosinophils into adipose tissue. (Bolus, Kennedy and Hasty, 2018) Because eosinophils are the major IL-4 producing WAT cell population, in their absence health of WAT deteriorates, glucose tolerance is impaired, and associated pathologies such as IR and T2D arise. (Wu *et al.*, 2011)

Furthermore, it has been show that simply restoring eosinophil numbers in WAT is not sufficient to improve metabolic properties of the tissue. (Bolus *et al.*, 2018) However, if low fat diet is introduced, eosinophil levels naturally rise and tissue inflammation is reduced. It is still unclear if this occurs due to the effect of eosinophils upon macrophages or vice versa. (Bolus, Kennedy and Hasty, 2018)

Several studies have reported that, compared to lean controls, obese individuals show increased levels of myeloperoxidase and calprotectin (neutrophil-derived factors) and CD66b (neutrophil activation marker). This implies, that obesity affects the activation status of WAT neutrophils. After three days of HFD a 20-fold increase in neutrophil numbers in WAT could be observed (Elgazar-Carmon et al., 2008; Talukdar et al., 2012; Mraz and Haluzik, 2014). The results, however, start to differ regarding the duration of this increased neutrophil activation. While some studies claim, that increased neutrophil numbers can only be observed for about seven days, others have observed this phenomenon for up to 90 days while on HFD (Talukdar *et al.*, 2012; Mraz and Haluzik, 2014). The mechanism of this process is not yet fully understood, however, there has been some research. Nijhuis and the team suggest that increased leptin levels, which are the result of obesity, trigger activation of neutrophils. This occurs via indirect induction of TNF-α secretion from monocytes by leptin (Nijhuis *et al.*, 2009). Furthermore, it was shown that activated neutrophils directly adhere to WAT adipocytes (Elgazar-Carmon *et al.*, 2008). Activated WAT neutrophils also contribute to macrophage infiltration into the tissue (Talukdar et al., 2012).

3.2.5. Innate lymphoid cells

ILC1 and ILC2 cells have opposing responses in tissues of obese individuals. While ILC1s obtain a pro-inflammatory profile, secrete INF-γ, and promote M1 polarization of macrophages, group 2 ILCs secrete anti-inflammatory cytokines and maintain M2 macrophage polarisation, acting to reduce obesity and the related complications. (Saetang and Sangkhathat, 2018)

When looking more in depth at the mechanisms underlying ILC1 pro-inflammatory profile, we discover that these cells promote tissue fibrosis, macrophage activation and glycaemic intolerance in HFD mouse models. These tissue changes are promoted by ILC1 secretion of INF-γ, which is necessary and at the same time sufficient for the pro-inflammatory phenotype switch. ILC1 cell numbers are significantly increased in obese patients. (O'Sullivan *et al.*, 2016; Wang *et al.*, 2019) DIO promotes IL-12 production, which in turn drives the accumulation and proliferation of tissue resident ILC1s. (O'Sullivan *et al.*, 2016) In mice this accumulation can be neutralised using IL-12 neutralizing antibodies. This results in improved glycaemic tolerance and inhibits further tissue fibrosis. In their study, Wang et al., showed that there is a direct link between ILC1 numbers and the occurrence of T2D, IR, and WAT fibrosis. Numbers of circulating ILC1 also have the potential to acts as a marker for T2D and adipose tissue health. In future research, these cells might provide a good target for treatment of T2D and obesity. (Wang *et al.*, 2019)

On the other hand, ILC2s play a critical role in maintaining a healthy phenotype of adipose tissue and preventing obesity. Accordingly, in obesity the numbers of ILC2s are reduced. A critical factor in maintaining ILC2s within WAT is IL-33. (Brestoff *et al.*, 2015) ILC2s function by promoting WAT beiging and thus increasing energy expenditure. They secrete methionine-enkephalins, which can directly stimulate adipocyte beiging or indirectly act on eosinophil and M2 populations, which then secrete IL-4 and norepinephrine, respectively, thus further promoting beiging. (Brestoff *et al.*, 2015; Oldenhove *et al.*, 2018)

3.2.6 T cells

T cells are the second largest immunological cell population in AT. Obesity causes not only increased accumulation of T cells in WAT but also a change in the ratio of the different types. Obesity is characterised by a shift towards the pro-inflammatory Th1, Th17, and Tc cells as well as Treg diminishment. (Jagannathan-Bogdan *et al.*, 2011; Mraz and Haluzik, 2014; Herck *et al.*, 2019)

In obese mice models on HFD, Th1 cells infiltrate the WAT depots, which leads to INF-γ secretion, M1 macrophage polarisation, and inflammation propagation. (Rocha *et al.*, 2008; Mraz and Haluzik, 2014; Herck *et al.*, 2019) High leptin levels aid Th1 cell differentiation as opposed to the anti-inflammatory Th2 phenotype. (Pacifico *et al.*, 2006) Th1 WAT infiltration can be attenuated in knockout mice, which consequently improves glucose metabolism and reduces inflammation. Th1 cells are only involved in the inflammatory response in subject with T2D. They have not been detected in obese individuals without T2D. (Herck *et al.*, 2019)

Th2 cells play an important suppressive role in the propagation of WAT inflammation during obesity. (Mraz and Haluzik, 2014) More research is needed into the mechanism controlling Th2 cell responses, however, it seems that a transfer of healthy Th2 cells into a mouse with DIO reduces inflammation and improves insulin sensitivity. (Herck *et al.*, 2019)

Like Th1 cells, Th17 also promote inflammation of WAT in obesity. They produce IL-17, which can affect many cell populations in adipose tissue such as monocytes and macrophages. As a result, the immune response of innate immune cells is reinforced stimulating inflammation. Th17 cells are enriched in WAT of obese subjects and drive a positive feedback loop, that maintains tissue inflammation. (Hong *et al.*, 2017; Herck *et al.*, 2019)

Th22 cells secrete IL-22, however their production levels are very low and thus unlikely to play a part in the pathology of DIO. (Herck *et al.*, 2019).

Tc cells, also identified by their expression of $CD8^+$ complex, have been found to infiltrate WAT in DIO. The numbers of Tc cells are three to four times higher in obese subjects than in lean controls. (Rausch *et al.*, 2008) More importantly, it is believed that this occurs before macrophage infiltration. (Rausch *et al.*, 2008; Herck *et al.*, 2019) When Tc cells are immunologically depleted in mouse

models it results in decreased macrophage infiltration, inflammation, and improved insulin sensitivity. (Nishimura *et al.*, 2009)

In healthy individuals adipose tissue depots are enriched with Tregs, however, in WAT of obese subject they are severely diminished, which allows for the pro-inflammatory tissue phenotype shift. (Feuerer *et al.*, 2009; Nishimura *et al.*, 2009) Treg complex FoxP3 can inhibit Th17 differentiation thus reducing their propagative effect on inflammation. (Herck *et al.*, 2019) This is not possible in obese WAT, which lacks Tregs, however, in mouse models this can be attenuated by introduction of Tregs from healthy subjects. Increased Treg induction results in lower blood glucose levels and decreases adipose tissue inflammation. (Ilan *et al.*, 2010)

Invariant NKT cells (iNKT) are the most researched subtype of NKT cells. They accumulate in adipose tissue of healthy individuals, where they promote anti-inflammatory environment and produce IL-2 and IL-10. They promote M2 macrophage polarisation and induce Treg proliferation.(Lynch *et al.*, 2015) In obesity, however, iNKTs are severely depleted, which allows for pro-inflammatory macrophage infiltration and inflammation propagation. Importantly, after weight loss, iNKT population is restored. (Lynch *et al.*, 2012)

3.2.7 B cells

In DIO B lymphocytes accumulate in adipose tissue, where they promote pro-inflammatory environment via cytokine secretion. They induce M1 macrophage polarisation, pro-inflammatory T cell accumulation and secrete pathogenic IgG antibodies. Mice models without B cell are still obese, however, they do not show tissue inflammation and IR. This is associated with higher Treg WAT infiltration when B cells are not present. Taken together, these results show that B cells are responsible for Treg diminishment and inflammation propagation, however, they do not have an effect on lipid accumulation in WAT. Additionally, a transfer of IgG antibodies from mice on HFD into wild-type models is enough to induce IR and lower glucose tolerance. (Winer *et al.*, 2011; DeFuria *et al.*, 2013)

4. Immunometabolism

Immunometabolism is a rapidly expanding field of study that has recently received a lot of attention. The exact definition of this word varies from researcher to researcher; however, in this work we will use the following: immunometabolism refers to the interactions and resulting function modulation between immune cells and the surrounding, metabolically active adipocytes. Hormones, metabolites, and other molecules produced by adipocytes affect immune cells, which in turn release signals to initiate a feedback loop back to adipocytes and associated cells. Any disruptions to this highly ordered immune-metabolic interface will result in pathologies. These are brought on by specific cell recruitment and activation as well as an altered secretory profile of WAT and cells within. (Hotamisligil, 2017; Kuda, Rossmeisl and Kopecky, 2018)

At the core of homeostatic immunometabolism is a healthy adipocyte (See Figure 5A). This is an adipocyte capable of adequately responding to extracellular stimuli, storing lipids in times of excess, and releasing fuel during fasting or exercise. A carefully controlled equilibrium of extracellular stimuli keeps fuel uptake and release from adipocytes balanced. A healthy adipocyte can be found in WAT of lean individuals and is maintained by anti-inflammatory immune cells. During HFD, however, WAT undergoes numerous changes (See Chapters 3.1. and 3.2.) and the resulting microenvironment (See Chapter 4.1) no longer preserves the healthy adipocyte phenotype. As a result, metabolic control and signalling of the adipocytes are unbalanced and fuel management is no longer in check. Cellular and whole-body metabolic dysregulation follows (See Figure 5B). (Vegiopoulos, Rohm and Herzig, 2017)

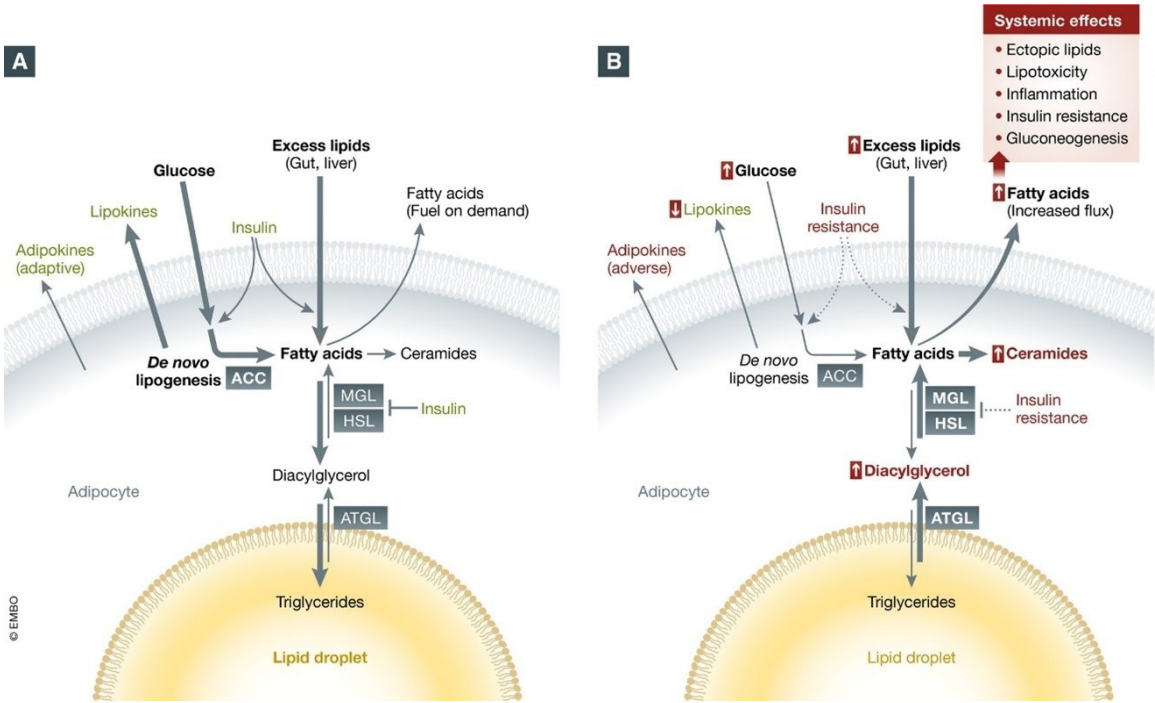

Figure 5: Comparative graphic representation of metabolic process rates in healthy (A) and overloaded (B) adipocytes, adapted from Vegiopoulos, Rohm and Herzig, 2017. Shortcuts: HSL- hormone sensitive lipase, ATGL- adipose triglyceride lipase, MGL- monoacylglycerol lipase, ACC- acetyl CoA carboxylase.

4.1. WAT specific consequences of low-grade inflammation

Immune cell infiltration into WAT, and the consequential changes in tissue homeostasis, leads to an alteration of its cytokine profile. Generally, in obesity, the levels of pro-inflammatory adipokines are increased. This group includes leptin, TNF-α, IL-6, and resistin. On the other hand, adiponectin is an anti-inflammatory adipokine. (Wensveen *et al.*, 2015) Its expression is usually diminished in association with DIO. In this chapter we look at the mechanisms underlying the regulation of expression and effector function of the above mentioned adipokines as well as inflammasome, a potent secretion activator.

Before looking at the effects of specific cytokines, it is important to note that ATH, cytokines (IL-6, INF-γ) and excess circulating FFA also modulate fuel preferences in immune cells and adipocytes (See Figure 6). This significantly alters the rate of many metabolic processes such as lipolysis, lipogenesis, glycolysis, and β-oxidation. In health, protective anti-inflammatory immune cells utilise lipids as fuel and promote homeostasis. Contrastingly, in DIO, immune cell infiltration and pro-inflammatory cytokine secretion promotes glycolysis instead of β-oxidation in immune cells and dysregulates adipocyte response to stimuli. As a result, adipocytes are unable to correctly respond to hormones such as leptin and insulin (store lipids in energy surplus, release FFA and glycogen during fasting) and the healthy adipocyte phenotype is lost. (Kohlgruber, LaMarche and Lynch, 2016)

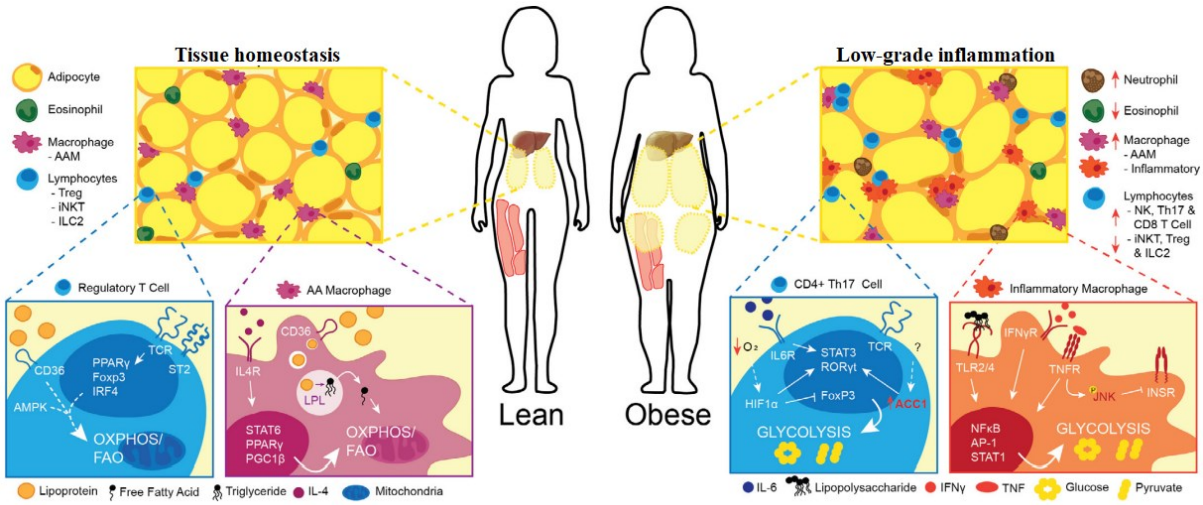

Figure 6: Effects of low-grade inflammation on immune cell fuel usage, adapted from Kohlgruber, LaMarche and Lynch, 2016. Shortcuts: AAM- adipose tissue macrophage, Treg- regulatory T cell, iNKT- innate natural killer T cell, ILC- innate lymphoid cell, NK- natural killer cell, Th- helper T cell, PPARγ- peroxisome proliferator-activated receptor γ, FOXP3- forkhead box protein 3, IRF4- interferon regulatory factor 4, TCR- T cell receptor, AMPK- AMP activated protein kinase, IL4R- interleukin 4 receptor, IL6R- interleukin 6 receptor, STAT- signal transducer and activator of transcription, LPL- lipoprotein lipase, OXPHOS- oxidative phosphorylation, FAO- fatty acid oxidation, HIF- hypoxia induced factor, RORγt- RAR related orphan receptor γ, ACC1- acetyl-CoA carboxylase 1, TLR- toll-like receptor, TNFR- TNF receptor superfamily, JNK- c-Jun N-terminal kinase, INSR- insulin receptor, AP-1- activator protein 1, INFγ- interferon γ, INFγR- interferon γ receptor, TNF- tumour necrosis factor, IL-6- interleukin 6.

We will begin by looking at the pro-inflammatory adipokines, which are present in higher concentrations during DIO. Firstly, leptin levels are proportional to adiposity and thus its plasma concentration is elevated in obese individuals. (Fain *et al.*, 2004) Consequently, when exposed to high leptin concentrations for a long period of time many cells and peripheral tissues become unresponsive, which leads to leptin resistance. (Sáinz *et al.*, 2015) Additionally, leptin can directly induce immune cell expression of TNF-α and IL-6. It also promotes proliferation of activated T cells (DiSpirito and Mathis, 2015), macrophages, DCs, NK cells, and granulocytes (Wensveen *et al.*, 2015), while drastically impairing Treg numbers. (Kohlgruber, LaMarche and Lynch, 2016) This feeds a vicious cycle of immune cell proliferation, cytokine secretion, and inflammation, which is further enhanced by T cell leptin secretion. (De Rosa *et al.*, 2015)

Secondly, TNF-α restricts glucose uptake of adipocytes by downregulating the expression of GLUT4 transporter. (Kaser *et al.*, 2003) This occurs as TNF-α signals via NF-κB to lower the expression of PPARγ, which is responsible for fuel uptake and lipid accumulation. This mechanism also suppresses insulin receptor gene transcription thus decreasing insulin sensitivity of the cells. Another consequence of TNF-α signalling is the upregulation of adipocyte lipolysis due to increased expression of various lipase genes. As a result, over time these lipids accumulate in peripheral tissues and result in metabolic dysfunction (Kohlgruber, LaMarche and Lynch, 2016), which is further enhance by the ability of TNF-α to suppress adiponectin production. (DiSpirito and Mathis, 2015)

Next adipokine from the pro-inflammatory group is IL-6. It modulates tissue microenvironment by promoting TAG secretion from liver while lowering adipocyte lipid accumulation by decreasing its LPL activity. (Kaser *et al.*, 2003) Additionally, Il-6 has a slightly controversial role in WAT. It induces FFA and leptin secretion from adipocytes. The FFA have a negative impact on liver insulin sensitivity. On the other hand, leptin released form adipocytes in response to IL-6 induces insulin secretion and thus improves glucose tolerance. These two distinct effects of IL-6 are also spatially separated. While, in mice, FFA release is triggered in mesenteric WAT depot, leptin is predominantly released from epididymal adipocytes. (Wueest and Konrad, 2018)

As the last pro-inflammatory adipokine I will mention resistin. In humans, resistin is secreted mostly from macrophages, however, in rodents, its major producers are adipocytes. It is thought that the main role of resistin in promoting inflammation is its ability to stimulate TNF-α and IL-6 production. (DiSpirito and Mathis, 2015; Wensveen *et al.*, 2015)

On the other hand, anti-inflammatory adipokines, which serve to maintain tissue homeostasis are severely lacking in DIO. The most researched one is adiponectin. Its plasma levels are inversely proportional to leptin levels and thus are low in obese patients. (Matsubara, Maruoka and Katayose, 2002; Fain *et al.*, 2004) Adiponectin serves to prevent gluconeogenesis in liver and to promote fuel uptake in WAT. In obesity, however, when adiponectin levels are diminished, ectopic lipid

accumulation occurs, accompanied by IR. Adiponectin signals via receptors, which are commonly found on numerous tissues, particularly on M2 macrophages. It acts to inhibit the NF-κB pathway and thus limits immune cell activation. (Wensveen *et al.*, 2015)

The term inflammasome refers to an array of multimeric protein complexes found within cells. In response to pathogen-associated molecular pattern molecules and damage-associated molecular pattern molecules inflammasomes activate immune response cascades. When a stimulus occurs, these protein complexes oligomerize and gain the ability to activate caspase-1, an enzyme capable of converting pro-interleukin-1β into IL-1β. (Kelley *et al.*, 2019) This is significant as IL-1β is capable of further stimulating TNF-α release from WAT macrophages and inhibiting expression of genes such as GLUT4 and PPARγ, which leads to an insulin desensitizing effect. Increased FFA in DIO are also capable of inflammasome induction thus triggering IL-1β secretion by macrophages and adipocytes. (Kohlgruber, LaMarche and Lynch, 2016)

4.2. Whole-body consequences of WAT inflammation

The consequences of low-grade WAT inflammation do not only affect local cell populations but rather interplay with other factors that can affect the whole body (See Figure 7). A great example of this is insulin, a peptide secreted from pancreatic β cells, which helps regulate blood sugar levels. Insulin secretion occurs after a meal and functions to stimulate glucose uptake while inhibiting lipolysis and gluconeogenesis. (Wilcox, 2005) Binding of insulin to its receptor, which belongs to the tyrosine kinase group, initiates intracellular transduction pathway. (Wang, Chandrasekera and Pippin, 2014) In DIO, IR develops and tissues are no longer capable of properly responding to the changing levels of insulin. (Yamamoto *et al.*, 2020) Additionally, insulin secretion increases to counteract the decrease in peripheral tissue response to insulin. (Burke *et al.*, 2017)

It has been established that multiple factors contribute to the development of IR, some of which are ectopic lipid accumulation and chronic low-grade inflammation of WAT. (Yang *et al.*, 2021) In fact, many of the factors responsible for WAT inflammation have been directly linked to IR. Numerous studies report association between IR and lipid overload (Schipper *et al.*, 2012), IgG levels (Winer *et al.*, 2011), macrophage infiltration, IL-1β, IL-6, TNF-α (Winer *et al.*, 2012) and IFN-γ. Specifically, IL-6 plays a role in development of IR by increasing plasma FFA content. It has also been observed that the key depot for the development of IR is VAT. (Wensveen *et al.*, 2015) Excess lipid accumulation in VAT has been linked to higher levels of inflammation markers even in subjects with healthy BMI, which is the usual obesity determinant. (Benites-Zapata *et al.*, 2019) Due to hyperinsulinemia, which is a direct result of IR, adiponectin secretion is downregulated and a vicious cycle between insulin and adiponectin is initiated. Insulin levels increase because of IR, high insulin concentration suppresses adiponectin, insulin sensitivity further decreases. (Deng and Scherer, 2010)

T2D is a multifactorial disease that arises as a result of IR, hyperinsulinemia, pancreatic β-cell dysfunction and impaired glucose tolerance. (Wang, Chandrasekera and Pippin, 2014; Yang *et al.*, 2021) 60-90% of patients with T2D are obese and the numbers keep rising. (Wang *et al.*, 2019) It is difficult to diagnose T2D as it develops gradually and many individuals are asymptomatic for a long time. (Wang, Chandrasekera and Pippin, 2014)

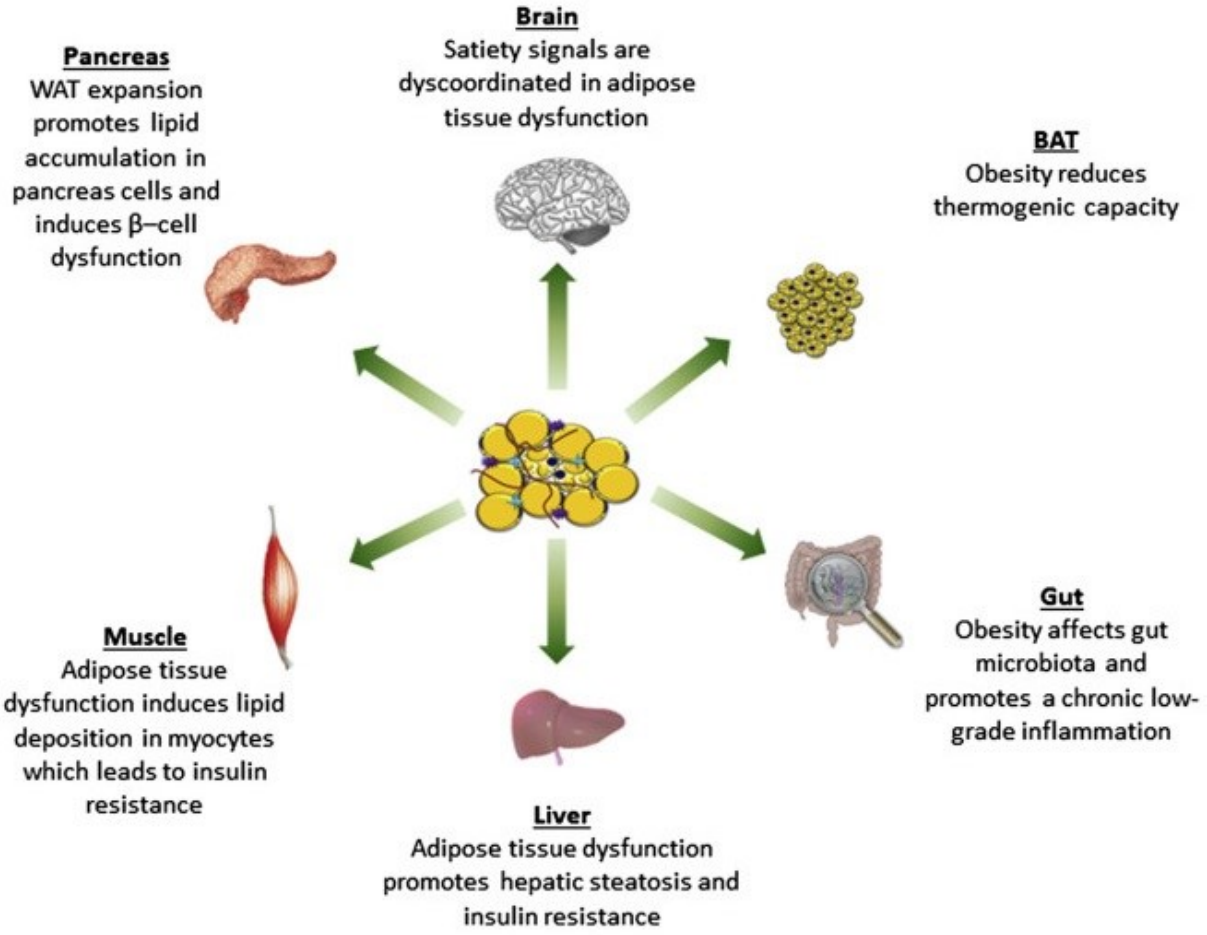

Figure 7: Whole-body consequences of low-grade inflammation in DIO, adapted from Martínez-Fernández *et al.*, 2018. Shortcuts: BAT- brown adipose tissue.

5. Omega-3 polyunsaturated fatty acids

Western diet, which is more common around the world nowadays, is rich in saturated fats and severely lacks ω-3 fatty acids. This might be a reason as to why HFD leads to DIO. ω-3 are PUFAs with a double bond at the third position from their methyl end. Humans are unable to synthetise this bond and so ω-3 PUFAs are an essential part of human diet. The best sources of ω-3 PUFAs are marine fish and phytoplankton, which are enriched in eicosapentaenoic acid (EPA, 20:5), docosahexaenoic acid (DHA, 22:6) and docosapentaenoic acid (DPA, 22:5). (Flachs, Rossmeisl and Kopecky, 2014; Rossmeisl *et al.*, 2014) It has been shown that PUFAs in form of phospholipids are superior in terms of efficacy compared to TAG. (Rossmeisl *et al.*, 2014; Sjövall *et al.*, 2015) α-linolenic acid (ALA, 18:3 ω-3) can be used as a precursor for ω-3 PUFA synthesis in liver, however, here it competes with linolenic acid (LA, 18:2 ω-6) for a crucial enzyme called Δ6 desaturase. Consequently, the ratio between LA and ALA can influence the rate of EPA and DHA synthesis. Despite the competition of LA and ALA, DHA and EPA synthesis is rather inefficient and thus ω-3 long-chain PUFA supplementation is advised. (Kopecky *et al.*, 2009)

The recommended intake is 0,5g of EPA and DHA per day. (Flachs, Rossmeisl and Kopecky, 2014) The therapeutic effects of ω-3 further depend on the ratio of dietary omega-6 (ω-6) PUFAs and ω-3 PUFAs, which was low in the diet of hunter-gatherers but is quite high in modern societies. (Kopecky *et al.*, 2009; Rossmeisl *et al.*, 2014)

ω -3 long chain PUFAs operate via oxylipins (oxidation products) and other lipid mediators generated from their phospholipid forms. (Flachs, Rossmeisl and Kopecky, 2014) They exert beneficial effects by gene expression regulation, promotion of adiponectin secretion and altering levels of other mediators. (Rossmeisl *et al.*, 2012, 2014) Transcription modulation by ω-3 PUFAs occurs via PPARs (Rossmeisl *et al.*, 2012), AMP-activated protein kinase (Kopecky *et al.*, 2009), G protein-coupled receptors or other mechanisms. (Kuda, Brezinova, *et al.*, 2016)

5.1. Omega-3 PUFA derived lipid mediators

Eicosanoids, molecules derived from PUFAs, can exhibit pro-inflammatory effects if derived from ω-6 or anti-inflammatory effects when derived from ω-3. (Kopecky et al., 2009) Even a small increase in dietary ω-3 PUFAs can shift the balance of eicosanoid synthesis in favour of anti-inflammatory molecules. This mechanism accounts for some of the beneficial effect of dietary ω-3 PUFA intake such as inflammation resolution and fat cell proliferation. (Flachs, Rossmeisl and Kopecky, 2014)

ω-3 PUFA derived N-acylethanolamines (endocannabinoid-related molecules) promote anti-inflammatory tissue environment. These mediators include N-eicosapentaenoylethanolamine and N-docosahexaenoylethanolamine. Endocannabinoids can also exert pro-inflammatory effects if derived from ω-6 PUFAs, such as arachidonic acid. Their production, however, is drastically reduced by

increased dietary ω-3 PUFAs. (Flachs, Rossmeisl and Kopecky, 2014; Kuda, Rombaldova, *et al.*, 2016)

Finally, resolvins and protectins, mediators derived from EPA and DHA, exert local anti-inflammatory effects. This results in inflammation resolution, M2 macrophage polarisation, and prevents tissue damage. (Kopecky *et al.*, 2009; Kuda, Brezinova, *et al.*, 2016)

5.2. Omega-3 PUFAs in obesity management

ω -3 PUFAs enhance benefits of diet modifications such as caloric restriction. The benefits include increased lipolysis, lower levels of lipogenesis and reduced plasma TAG levels, all of which can be attributed to the upregulation of futile substrate cycle of TAG (See Figure 8). (Flachs, Rossmeisl and Kopecky, 2014; Rossmeisl *et al.*, 2014) The upregulation of lipolysis also decreases accumulation of lipids in ectopic fat depots. Furthermore, it was shown that ω -3 PUFA consumption decreases leptin secretion leading to a decrease in its plasma concentration. (De Rosa *et al.*, 2015)

In rodent studies ω -3 PUFAs can alleviate IR, although, this was not achieved in human trials. (Rossmeisl *et al.*, 2014; Sjövall *et al.*, 2015) It was, however, found that ω -3 PUFA prevent WAT hypertrophy and upregulate mitochondrial biogenesis in adipocytes. (Kopecky *et al.*, 2009; Rossmeisl *et al.*, 2014)

Macrophages are not directly under the influence of ω -3 PUFAs (Adamcova *et al.*, 2018), however, they are affected by the change of microenvironment that results from ω -3 PUFA supplementation. In obesity, excess lipids are released into circulation and macrophages can store them. Prolonged accumulation of lipids in these cells leads to M1 macrophage polarisation and promotes inflammation. ω -3 PUFAs, however, promote adipocyte proliferation, which means the new cells can take up lipids and reduce the strain on macrophages. This allows polarisation of macrophages towards their M2 phenotype and helps resolve inflammation. (Kuda, Rombaldova, *et al.*, 2016)

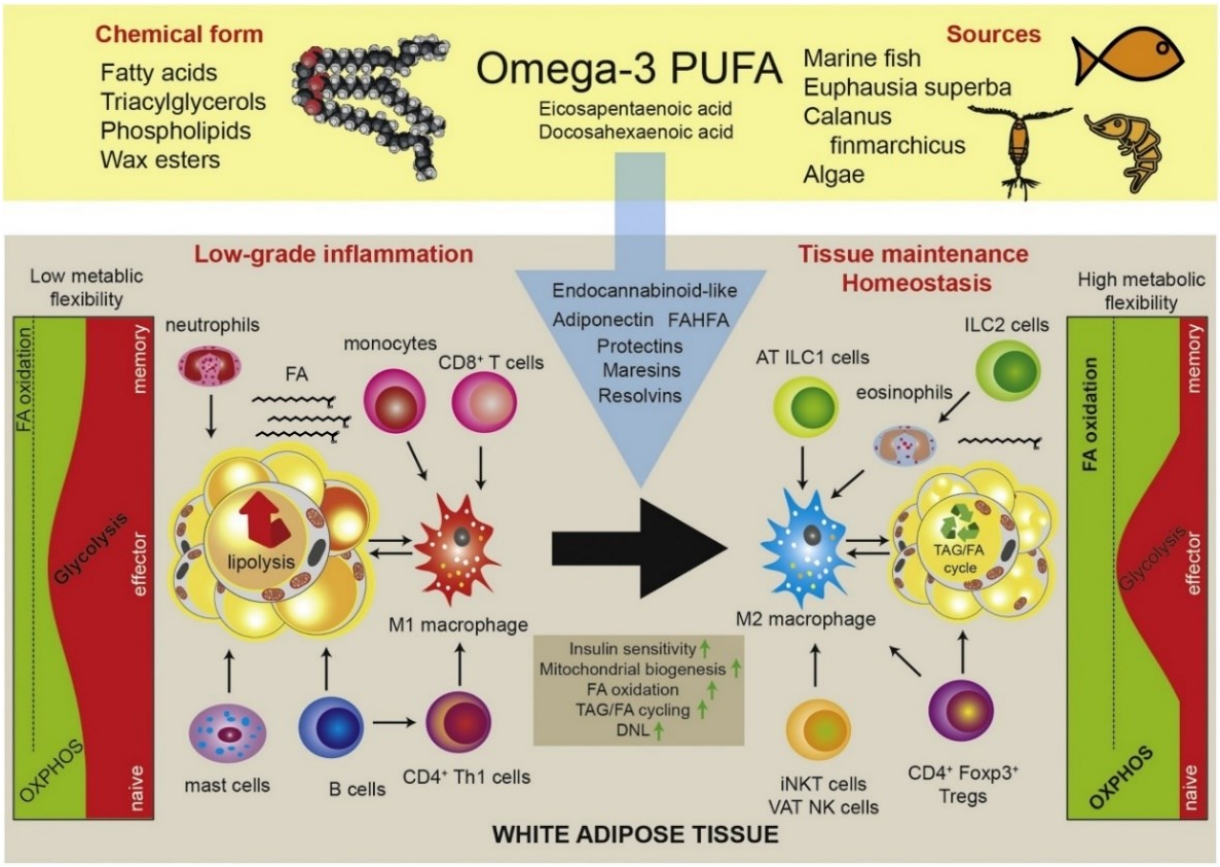

Figure 8: Effects of omega-3 fatty acids on adipose tissue health. (Kuda, Rossmeisl and Kopecky, 2018) The figure portrays how intake of ω-3 PUFAs (top) aids in management and resolution of low-grade inflammation (left), which developed as a direct result of high-fat diet. Omega-3 supplementation leads to adjustment of WAT secretory profile and brings about a micro-environment that allows for tissue homeostasis (right). Shortcuts: FA- fatty acid, Th- helper T cell, FoxP3- forehead box 3 factor, Treg- regulatory T cell, iNKT- invariant natural killer T cell, VAT- visceral adipose tissue, NK- natural killer cell, OXPHOS- oxidative phosphorylation, TAG- triacylglycerol, AT- adipose tissue, ILC- innate lymphoid cells, FAHFA- fatty acid ester of hydroxy fatty acid, DNL- de novo lipogenesis.

6. Conclusion

Low-grade inflammation of adipose tissue is a direct result of WAT hypoxia and hypertrophy, which occur in DIO. Consequently, WAT microenvironment is altered, and the predominant cytokines are leptin, TNF-α, IL-6, and resistin. On the other hand, the presence of anti-inflammatory cytokines is diminished, and adiponectin concentrations fall. The change in secretory profile of WAT is the result as well as the driving force of pro-inflammatory immune cell polarisation and infiltration. pDCs, M1 macrophages, neutrophils, B cells, Th1, Th17 a Tc lymphocytes as well as ILC1 cells become the major WAT populations in DIO. Many of these populations are activated by TNF-α produced by MGCs, which formed because of DIO induced adipocyte death in the first place. A complex interplay between immune cell and adipocytes leads to further degradation of WAT homeostasis and results in the inability of adipocytes to appropriately respond to extracellular stimuli. As lipids keep accumulating due to HFD, leptin levels rise, further expression of TNF-α, IL-6 and other pro-inflammatory cytokines is induced, which only fuels inflammation propagation. As a result, anti-inflammatory immune cell populations degrade and Treg, eosinophil, iNKT, ILC2 and Th2 number in WAT plumet. This leads to whole-body consequences, such as IR and T2D, that are driven by ectopic lipid accumulation, high circulating FFA levels and the inability of adipocytes to regulate fuel storage and release.

ω-3 PUFA supplementation is a promising DIO management strategy. The beneficial effects of ω-3 PUFAs depend on ω-3: ω-6 ratio in the diet. Pro-resolution eicosanoids are synthesised from ω-3 PUFAs and induce anti-inflammatory tissue microenvironment. Inflammation resolution, adipocyte proliferation, increased β oxidation, decreased lipogenesis, and reduced circulating TAG concentration have been associated with increased dietary ω-3content.

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