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Systemic and white adipose tissue inflammation in obesity and insulin resistance

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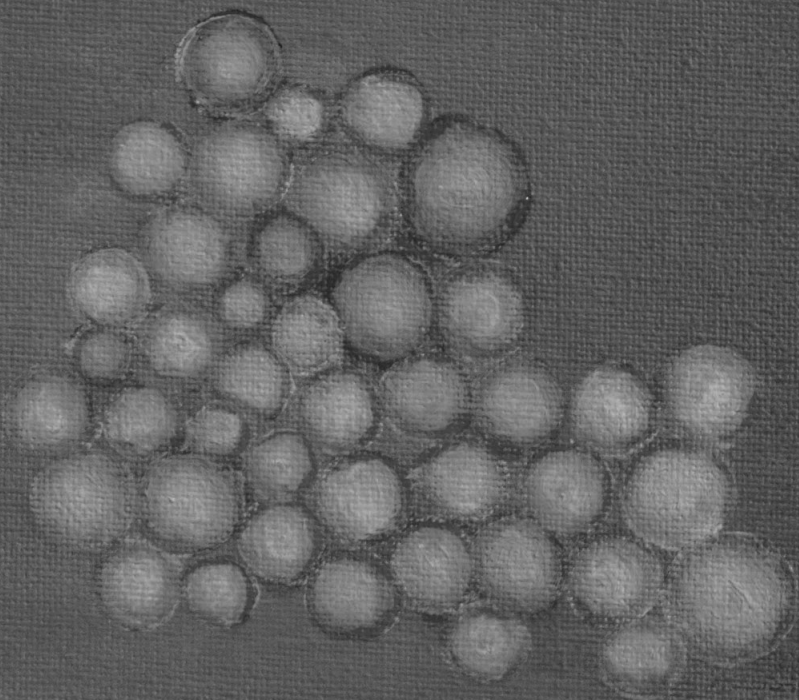
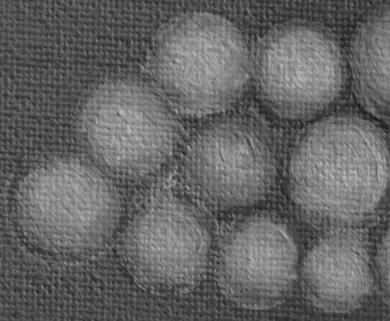
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General introduction



Introduction

Obesity and metabolic disorders

Obesity is defined as abnormal or excessive fat accumulation and presents a risk to health. It is a major risk factor for the development of various chronic diseases, like diabetes mellitus, cardiovascular disease and several cancers (1), and is one of the leading preventable causes of morbidity and mortality (2-4). As obesity is reaching epidemic proportions and the prevalence is still increasing not only in western societies but also in low- and middle-income countries, it is a cause for worldwide concern (5).

When energy intake exceeds energy expenditure this will lead to weight gain and eventually to obesity. Excess energy will be stored as triglycerides (TG) primarily in the white adipose tissue (WAT), leading to WAT expansion. One TG molecule is composed of one glycerol and three fatty acid (FA) molecules. TG are common lipids in our diet and the main energy source for the body. FA derived from TG can be used for ATP production by the muscle and for generation of heat by brown adipose tissue (BAT) (6, 7). However, if TG are not being used for energy production or properly stored in WAT, plasma lipid levels may increase resulting in hyperlipidemia. TG can also be stored in organs other than WAT, like liver or muscle which is known as ectopic fat storage. Ectopic fat storage can contribute to the development of metabolic disorders, which is discussed later on.

Obesity is the major cause of the development of the metabolic syndrome, which is characterized by the co-occurrence of several cardio-metabolic risk factors, including central obesity, insulin resistance, hypertension, and dyslipidemia (8). The metabolic syndrome represents a major risk for the development of type 2 diabetes and cardiovascular disease. However, about 20 percent of the obese population seem to remain relatively insulin sensitive and metabolically healthy. The reason why these people are protected from the development of metabolic disorders has not been extensively characterized yet.

White adipose tissue

For a long time it was thought that WAT did little else than storing and releasing energy and to function as cushioning and insulation of the body. It is now known that WAT also is an endocrine and inflammatory organ (9). Adipocytes (or fat cells) release hormones like leptin and adiponectin that regulate satiety and metabolic processes, as well as cytokines which are all known as adipokines (10). Recently, it was found that adipocytes also have infection-protective properties via the production of antimicrobial peptides (11). Furthermore, adipocytes show similarities to immune cells; in addition to the capacity to produce all kinds of cytokines, they have antigen-presenting cell (APC) properties and express MHCII molecules on their cell surface (12, 13).

WAT is distributed in different depots throughout the body. A subcutaneous depot (sWAT) is located underneath the skin, and visceral depots (vWAT) are situated around the abdominal organs. vWAT is known to be more metabolically active and pro-inflammatory, and to exert more negative effects on health as compared with sWAT (14-16). WAT is composed of adipocytes and a number of different cell types which are compositely known as the stromal vascular fraction (SVF). The SVF

contains vascular endothelial cells, fibroblasts, pre-adipocytes, and several types of immune cells (17). Adipocytes mature from pre-adipocytes that originate from the mesenchymal cell lineage (18).

The effect of obesity on white adipose tissue

During the development of obesity, WAT expands by increase in size (hypertrophy) and/or number (hyperplasia) of adipocytes (18, 19). A typical human adipocyte has a size of 0.1 mm in diameter, however, adipocytes can expand to more than twice that size during obesity. Generally, hypertrophy occurs prior to hyperplasia to increase the fat storage capacity of WAT during the development of obesity (20). In humans, adipocyte hyperplasia is a matter of some debate. According to Arner et al. (21), the number of adipocytes is determined during childhood and remains stable during life. This is supported by others that show hypertrophy rather than hyperplasia during weight gain in humans (22). A recent study did, however, show increased numbers of newly generated adipocytes in sWAT of healthy volunteers after overfeeding (23). Also in some individuals with morbidly obesity, hyperplasia of the adipocytes has been demonstrated once hypertrophy of the adipocytes was limited (24).

During the development of obesity, hypertrophic adipocytes release increased levels of FA and adipokines that both have immune modulatory activities (25, 26). Pro-inflammatory adipokines, including leptin, TNF α , and IL6, stimulate the influx of pro-inflammatory immune cells into the obese WAT. Obesity-induced WAT inflammation is associated with metabolic dysfunction and is hypothesized to contribute to the development of insulin resistance, as discussed later on in the section “Impact of inflammation on insulin signalling”.

WAT expansion requires tissue remodelling, which includes extracellular matrix breakdown and resynthesis as well as angiogenesis to maintain nutrient and oxygen supply (27). It has been shown that the inflammatory response induced by expanding adipocytes is essential during this process, as these signals drive healthy adipose tissue expansion and remodelling (28). However, when expansion of adipocytes is not associated with appropriate remodelling and angiogenesis, oxygen may become deficient in WAT, leading to hypoxia. Adipose tissue hypoxia leads to adipocyte stress and cell death, characterized by dysregulation of the production of cytokines, as well as altered FA fluxes (29) which contributes to the development of WAT dysfunction in obesity (30). However, WAT hyperoxia during obesity has also been associated with adipose tissue dysfunction and insulin resistance (31). Obese subjects seem to have higher oxygen tension despite lower adipose tissue blood flow, which could be explained by a lower oxygen consumption of obese adipose tissue.

Obesity-induced inflammation

The body induces an immune response to eliminate pathogens or damaged cells. Two general types of immune responses are recognized: the innate and the adaptive immune response. The innate immune response is characterized by a relatively non-specific and rapid response of the body to fight infections, and is in evolutionary terms the oldest. It is known as a first line of defence mechanism, and the cellular component includes macrophages, granulocytes (neutrophils, eosinophils, and basophils),

mast cells, dendritic cells, and natural killer cells. The evolutionary more recent adaptive immune response is antigen-specific and includes T and B lymphocytes that express variable T-cell receptors and produce antibodies, respectively. Antibodies undergo a process called affinity maturation to increase the specificity of the antigen recognition. This process takes time and upon first exposure, the adaptive immune response is relatively slow. However, T and B lymphocytes create immunological memory after initial response to a pathogen, which ensures a rapid response upon repeated exposure.

WAT contains several types of immune cells that in the lean state are mainly considered as anti-inflammatory immune cells (Figure 1). These cells are presumably involved in immune surveillance and adipose tissue remodelling (32). During the development of obesity, the expanding adipocytes release adipokines, like leptin, MCP1, TNF α , and IL6, which attract and activate pro-inflammatory immune cells into the WAT (Figure 1). The immune cells themselves also release pro-inflammatory cytokines and chemokines, which lead to additional infiltration of immune cells into WAT during WAT expansion (33). WAT inflammation eventually causes chronic low-grade systemic inflammation, characterized by increased levels of cytokines and other inflammatory markers in the circulation which are thought to contribute to the development of insulin resistance in peripheral organs (34). The immune cells playing an important role in obesity-induced inflammation and studied in this thesis are discussed below.

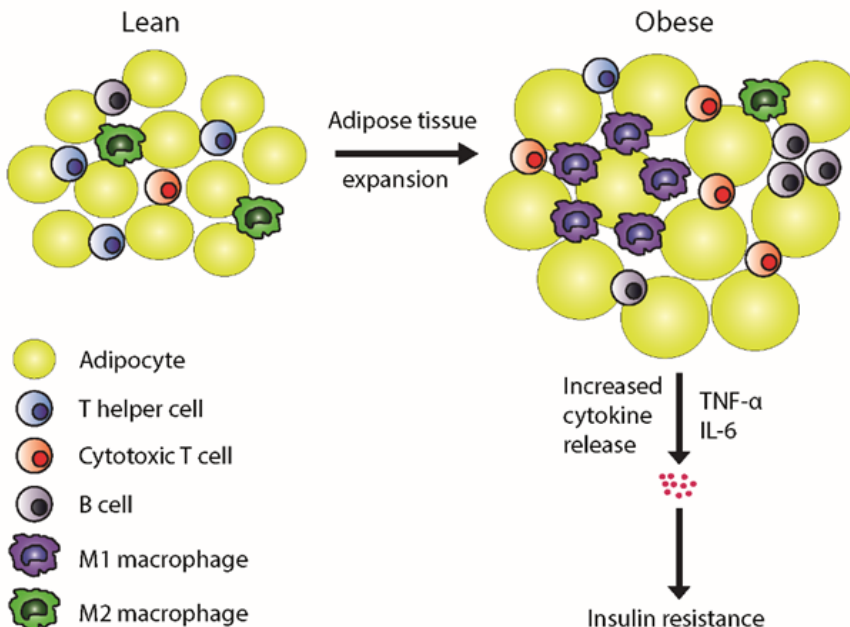


Figure 1. Schematic overview of adipose tissue inflammation during the lean and obese state. Lean adipose tissue mainly contains anti-inflammatory immune cells. During the development of obesity adipocytes expand and pro-inflammatory immune cells infiltrate into the adipose tissue. Adipocytes and immune cells in the obese adipose tissue release increased levels of pro-inflammatory cytokines, which are thought to contribute to the development of insulin resistance. Adapted from Kalupahana et al (33).

Role of monocytes and macrophages

Monocytes are circulating immune cells that are able to migrate into tissue in response to inflammatory signals. These inflammatory signals include increased expression of chemokines, like monocyte chemoattractant protein 1 (MCP1) which is specifically known to recruit monocytes to the site of inflammation (35). Monocytes differentiate into macrophages or dendritic cells once they enter the tissue. Macrophages are phagocytic cells, specialized in the removal of pathogens, dead cells and cellular debris by engulfment and digestion (36). In the adipose tissue this can be recognized by the presence of crown-like structures (CLS) that exist of macrophages surrounding dying and dead adipocytes (37). After digestion of pathogenic material, the macrophage presents pathogen-derived antigens via its MHC molecules to corresponding T cells to facilitate further effective pathogen clearance and to initiate immune memory. Tissue resident macrophages, that are presumably involved in immune surveillance, are termed alternatively activated macrophages (M2 type) and have an anti-inflammatory phenotype, characterized by release of anti-inflammatory cytokines such as IL10 and TGF β (38, 39). Pro-inflammatory macrophages are termed classically activated or M1 type macrophages and secrete pro-inflammatory cytokines like IL1 β , IL6, IL12, and TNF α (38, 39).

Numbers of monocytes are increased in the circulation of obese human subjects and diet-induced obese mice compared to lean (40, 41). Furthermore, cytokine levels in the circulation of obese subjects are increased as compared to lean, including IL6 and TNF α which are known to be secreted, amongst others, by monocytes and macrophages (42). IL6 and TNF α are both pro-inflammatory cytokines, indicative for systemic inflammation and may contribute to the development of metabolic disorders (43).

Obese WAT is known to contain higher numbers of macrophages and CLS as compared to lean WAT (44). The presence of CLS in the adipose tissue has been linked with adipose tissue dysfunction and the development of metabolic disorders (37, 45). Recently, it has been shown that in addition to monocyte recruitment, macrophages are also able to proliferate locally in WAT to increase the number of macrophages during obesity (46). Lean WAT primarily contains M2 types macrophages, whereas obesity induces a phenotypic switch leading to increased numbers of pro-inflammatory M1 types macrophages (47). Macrophages thus do seem to play an important role in the development of obesity induced inflammation.

Role of T cells

T cells are lymphocytes characterized by the expression of the T cell receptor on their cell surface. Several subtypes of T cells are recognized, including T helper and cytotoxic T cells, characterized by expression of the cell surface markers CD4 and CD8, respectively. The main function of T helper cells is to assist other immune cells during immune responses. Antigen presenting cells (APCs), such as macrophages, can present antigens via their MHCII molecules to T helper cells. In this way T helper cells can become activated, which triggers them to produce cytokines that regulate immunological processes including B cell maturation, monocyte recruitment, and cytotoxic T cell activation (48).

There are different subtypes of T helper cells, including Th1, Th2, Th17, and regulatory T cells. Th1 and Th17 T cells are generally considered pro-inflammatory, whereas Th2 and regulatory T cell are considered as anti-inflammatory cell types (49). Cytotoxic T cells are also known as killer cells, as they can directly destroy for example infected cells by releasing cytotoxins that induce apoptosis of the target cell. The infected cells present antigens via their MHC I molecules, which are expressed by nearly all nucleated cells. The presented antigens are recognized by the antigen specific T cell receptor on the cytotoxic T cell. After recognition, the antigen-specific T cells undergo clonal expansion to eliminate the antigen-positive target cells. In addition, memory T cells are formed in order to induce a quick and efficient immune response after re-exposure to the same pathogen.

T cells originate from precursors in the bone marrow which mature and differentiate in the thymus before they end up in the circulation. Thymus size, as well as numbers of thymocytes are increased in diet-induced obese mice (40). Also circulating T cell numbers are elevated by obesity, which is primarily caused by an increase of T helper cells (50, 51). The activation status of T cells in the circulation is also increased with obesity (52). Although the type and activation status of circulating lymphocyte subsets in relation with obesity have been studied extensively in humans (41, 50, 52), lymphocyte subsets in relation with obesity in the mouse systemic circulation have been poorly characterized (53).

Numbers of T cells are increased in obese WAT of both humans and mice (54, 55). Obese WAT is characterized by increased numbers of pro-inflammatory Th1 and cytotoxic T cells, whereas the number of regulatory T cells is decreased (55). Both Th1 and cytotoxic T cells have been associated with increased insulin resistance (56, 57). T cell infiltration into the WAT seems to be a primary event in WAT inflammation (58). Several studies have shown that Th1 and cytotoxic T cells help recruit macrophages into the expanding WAT and stimulate macrophage polarization towards the pro-inflammatory M1 subtype (56, 57, 59). Thereby, this clearly implicates T cell mediated inflammation in the pro-inflammatory phenotype of obesity.

Role of B cells and immunoglobulins

The primary function of B cells is to produce antibodies against specific antigens. Furthermore, they can function as antigen presenting cells and release cytokines that regulate immune responses (60). Similar to T cells, B cells can form an immune memory pool after first exposure, to enable a quicker and stronger response after re-exposure to the same antigen. After antigen exposure, plasma B cells are formed that produce large amounts of antibodies, also termed immunoglobulins (Ig). An antibody consists of a variable region containing a specific antigen binding site and a constant Fc-region to communicate with and activate other immune components (61), the Fc-region determines the antibody isotype. Upon binding of antigens, the different antibody isotypes bind to isotype-specific Fc-receptors (e.g. IgG binds to Fc γ -receptors), thereby inducing specific immune responses. Fc-receptors are expressed by a variety of cells, including macrophages, dendritic cells, B cells and mast cells which can be activated by antibody-antigen immune complexes (62). Binding to Fc-receptors can induce phagocytosis of the immune complex, cytokine production, and cell death of the target cell (61, 63).

Furthermore, immune complexes are able to bind to C1q, the recognition component of the classical complement pathway. Binding of C1q activates the complement system and initiates a cascade of reactions that finally cleaves the central complement component C3, inducing phagocytosis and/or lysis of the pathogen (64). There are distinct differences between the human and mouse Fc-receptor biology (65). The human IgG Fc-receptor family consists of six receptors, whereas in mice only four Fc γ -receptors have been identified. Human and mouse Fc γ -receptors both include several activating receptors and one inhibitory receptor. Two of the human and all mouse activating Fc γ -receptors contain a γ -subunit, which is necessary for signalling and cell surface expression of the receptors. For the IgG subclasses there are also differences between humans and mice. Human IgG nomenclature is given by order of abundance in plasma (IgG1-4), which does not account for mice (IgG1,2A/B,3). Moreover, the human and mouse IgG subtypes differ in function and receptor affinity (65).

Total leukocyte counts as well as B cell numbers are increased in the circulation of obese women compared to non-obese women (41). Circulating B cells show a pro-inflammatory cytokine profile (increased IL6 and TNF α , and decreased IL10 secretion) in diabetic patients, as well as in spleens from obese mice (66, 67). This is thought to promote pro-inflammatory T cell functioning and to regulate inflammation in T2DM (67). Obese children have elevated total plasma IgG levels as compared with lean children, which is associated with a less favourable metabolic phenotype (68). In the mouse circulation IgG3 is primarily present, however IgG2c is the only subtype that is increased by HFD intervention in the circulation and in WAT (69).

Shortly after high fat diet (HFD) feeding in mice, numbers of B cells increase in WAT (55), the accumulation of B cells in the obese WAT contributes to the development of insulin resistance by the production of pathogenic IgG antibodies (Figure 2) (69, 70). Transfer of IgG from obese mice to HFD-fed B-null mice induced rapid local and systemic changes in the inflammatory cytokine production and a phenotypical conversion of the WAT macrophages to a pro-inflammatory M1 phenotype (69). Obesity related antigens, against which B cells produce antibodies have not been identified yet. However, as IgG antibodies were found to be located in CLS, it is possible that dead adipocytes are a source of the antigens (70). B cells and their antibodies may thus be important regulators during the development of obesity related insulin resistance.

Impact of inflammation on insulin signalling

Insulin is produced by beta cells in the pancreas and regulates postprandial glucose metabolism, via inducing glucose uptake from the circulation by muscle and adipose tissue, and by inhibition of the glucose production by the liver (71). Obesity can lead to the development of insulin resistance, a condition where organs and tissues like muscle, liver, and adipose tissue do not respond properly to insulin anymore and higher levels of insulin than normal are required to maintain glucose levels. Insulin resistance can lead to disturbed insulin mediated glucose uptake by the muscle and adipose tissue (72). Furthermore, hepatic glucose production may not be efficiently repressed by insulin (72). When the condition proceeds, more and more insulin is needed eventually leading to pancreatic

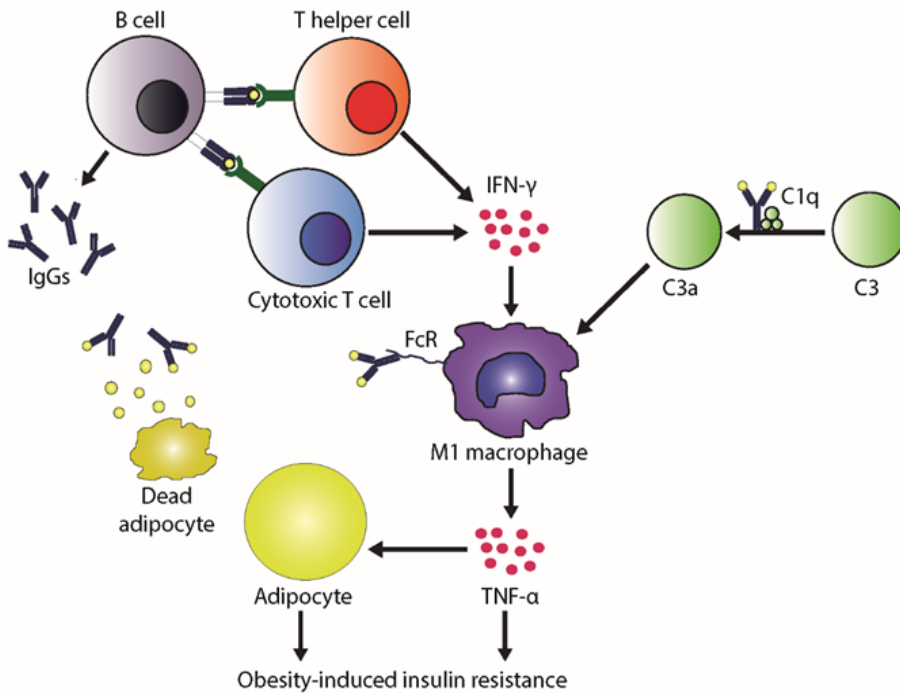


Figure 2. Schematic overview of the role of B cells and IgG antibodies in obesity-induced adipose tissue inflammation and related insulin resistance. Obesity leads to B cell activation possibly by antigens from dead adipocytes. B cells mediate MHC-dependent antigen presentation to T helper and cytotoxic T cells, which leads to an immune response. Activated B cells produce antigen specific IgG antibodies which form immune complexes. These immune complexes are able to activate immune cells via Fc-receptors and to induce complement activation via binding to C1q. This leads to immune responses including pro-inflammatory cytokine release, which contributes to the development of obesity-induced insulin resistance. Adapted from Mallat et al (70).

beta cell exhaustion and failure of the pancreatic beta cells to secrete the required levels of insulin. Pancreatic beta cell failure is a direct cause for type 2 diabetes mellitus.

As discussed in previous sections, obesity-induced adipose tissue and systemic inflammation are thought to contribute to the development of insulin resistance. Pro-inflammatory mediators like IL1 β , IL6, and TNF α are secreted by immune cells in the adipose tissue during obesity (73). These pro-inflammatory cytokines are able to directly interfere with the insulin signalling pathway, thereby inducing insulin resistance. Obesity-induced inflammation is thought to activate the Jun N-terminal kinase (JNK) and I κ B kinase- β (IKK β)/nuclear factor- κ B (NF- κ B) pathways in muscle, liver, and fat cells (74, 75). Pro-inflammatory cytokines can activate these pathways via classical receptor-mediated mechanisms (e.g. TNF- and IL1-receptors). Other mechanism that activate these pathways are toll-like receptor (TLR) ligand binding and cellular stress factors including reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress. JNK has been shown to induce insulin resistance via serine phosphorylation of insulin receptor substrate-1 (IRS-1) (76, 77). IKK β induces transcriptional activation

of NF- κ B that leads to increased expression of inflammatory markers and mediators which lead to the development of insulin resistance (78). The IKK β inhibitor salicylate promotes insulin sensitivity and improves glucose tolerance in obese mice and diabetic patients (79, 80). Furthermore, JNK and IKK β knock-out mouse models are protected against the development of HFD-induced insulin resistance (76, 79).

Pro-inflammatory cytokines have direct effects on adipocytes. TNF α inhibits GLUT4 mediated glucose uptake, attenuates PPAR γ and lipoprotein lipase (LPL) activity and thus affects FA esterification. Furthermore, TNF α increases cAMP that leads to hormone-sensitive lipase (HSL) activation (75). Thereby, obesity-induced inflammation increases lipolysis and decreases TG synthesis in adipocytes. These actions result in increased circulating FFA levels that may induce ectopic fat storage in muscle and liver (81). Impaired insulin responsiveness of skeletal muscle is a precursor of the development of T2DM. Ectopic lipids decrease the expression of genes involved in mitochondrial functioning, such as PPAR γ co-activator-1 (PGC-1). Additionally, endogenous lipids are thought to activate TLRs during obesity, which is supported by the finding that saturated FA can activate TLR2 and TLR4 and induce pro-inflammatory responses (82-84). Thus, obesity-induced inflammation likely contributes to the development of insulin resistance of muscle, liver, and adipose tissue through several pathways. This may therefore be promising targets to treat obesity-induced insulin resistance.

Outline of the thesis

Obesity induced adipose tissue inflammation is thought to play a key role in the development of insulin resistance and other metabolic disorders. If specific inflammatory pathways are causal, they present promising targets in the treatment of metabolic disorders. However, this requires extensive knowledge on the triggers for adipose tissue inflammation and subsequent inflammatory pathways and their effect on metabolic functioning. The research described in this thesis aims to gain more insight in the development of obesity-associated adipose tissue and systemic inflammation and the contribution thereof to metabolic disorders.

In **chapter 2** we studied systemic as well as adipose tissue inflammation in a human cohort of lean women, obese women with normal glucose tolerance, and obese women with type 2 diabetes mellitus. We determined to what extent differences in metabolic health are associated with differences in inflammatory phenotype and found increased systemic and WAT inflammation in obese women with T2DM compared to obese women with normal glucose tolerance. In **chapter 3** we determined adipose tissue depot specific differences in expandability and immune cell influx during the development of obesity in mice. We characterized adipocyte size and functionality of different adipose tissue depots, as well as extent of inflammation as a function of body weight. We observed significant differences in WAT depot expandability and immune cell composition. Furthermore, we found that gonadal WAT seems to primarily expand during the initial development of obesity in mice, after which the expansion tapered off and CLS formation, liver steatosis, and insulin resistance progressed. **Chapter 4** describes the composition of immune cells in the circulation and WAT of obese humans and mice. A comparison

of the WAT depots indicated major differences of the composition of immune cells between obese humans and mice. The composition of immune cells in the circulation was also significantly different between humans and mice, however the effect of obesity on circulating immune cells shows similarities.

B cells and their immunoglobulins have been shown to contribute to the development of obesity related insulin resistance (69, 70). Immunoglobulins can induce immune responses by immune cell activation via Fc-receptors, or via complement activation. **Chapter 5** describes the role of the FcR γ -chain in the development of HFD-induced obesity and related metabolic disorders. We studied FcR γ $-/-$ mice, which lack the signal transducing γ -chain of the Fc-receptors, leading to non-functional Fc γ RI, III, and IV and Fc ϵ RI, and therefore have diminished IgG and IgE antibody mediated cellular responses. Mice that lack the FcR γ -chain are protected against HFD-induced obesity and related disorders. To further identify the effector pathway by which obesity-induced IgG antibodies contribute to the development of insulin resistance, we studied Fc γ R1234 $-/-$, Fc γ R2b $-/-$, and complement C3 $-/-$ mice during HFD-induced obesity in **chapter 6**. We showed that Fc γ R or C3 deficiency does not result in decreased WAT inflammation or insulin resistance. This suggests that if obesity-induced IgG antibodies play a role in insulin resistance, this is not limited by deletion of Fc γ R or complement mediated pathways. **Chapter 7** provides an overall summary and discussion of the results described in this thesis.

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