Parallels in immunometabolic adipose tissue dysfunction with ageing and obesity

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Abstract:
Ageing, like obesity, is often associated with alterations in metabolic and inflammatory processes resulting in morbidity from diseases characterised by poor metabolic control, insulin insensitivity, and inflammation. Ageing populations also exhibit a decline in immune competence referred to as immunosenescence, which contributes to, or might be driven by chronic, low grade inflammation termed ‘inflammageing’. In recent years, animal and human studies have started to uncover a role for immune cells within the stromal fraction of adipose tissue in driving the health complications that come with obesity, but relatively little work has been conducted in the context of immunometabolic adipose function in ageing. It is now clear that aberrant immune function within adipose tissue in obesity—including an accumulation of pro-inflammatory immune cell populations—plays a major part in the development of systemic chronic, low-grade inflammation, as well as limiting the function of adipocytes leading to an impaired fat handling capacity. As a consequence, these changes increase the chance of multi-organ dysfunction and disease onset. Considering the important role of the immune system in obesity-associated metabolic and inflammatory diseases, it is critical to further understand the interplay between immunological processes and adipose tissue function, establishing whether this interaction contributes to age-associated immunometabolic dysfunction and inflammation. Therefore, the purpose of this article is to summarise how the interaction between adipose tissue and the immune system changes with ageing, likely contributing to the age-associated increase in inflammatory activity and loss of metabolic control. To understand the potential mechanisms involved, parallels will be drawn to the current knowledge base derived from investigations in obesity. We will also highlight gaps in research and propose potential future directions based on the current evidence.
1. Introduction

Metabolically-driven inflammation is a hallmark of cardiovascular disease and type-II diabetes mellitus (Hotamisligil, 2017b). Several organs including the liver, skeletal muscle, and the gut, have a role in the generation and progression of these diseases. However, it has become clear that adipose tissue accumulation, sometimes within and around these organs, is a major driving force for metabolic and inflammatory dysfunction (Hotamisligil, 2006; Lumeng and Saltiel, 2011; Hotamisligil, 2017b). Obesity, defined herein as a body mass index (BMI) ≥30 kg/m², leads to extensive adipose tissue deposition and negative health consequences, due to chronic caloric overconsumption and an excessively sedentary lifestyle (de Ferranti and Mozaffarian, 2008; Gonzalez-Muniesa et al., 2017). Globally, over 2.1 billion adults were overweight (BMI ≥25 kg/m² to <30 kg/m²) or obese in 2014 (World Health Organisation, 2015). This number has been steadily rising with global age-standardised mean BMI scores increasing by 0.4 and 0.5 kg/m² each decade in men and women, respectively (Finucane et al., 2011).

Obesity is characterised by poor metabolic control, oxidative stress, mitochondrial dysfunction, impaired immune function, and chronic, low-grade inflammation (Ballou and Kushner, 1997; Paolisso et al., 1998; Bruunsgaard et al., 1999; Bartlett et al., 2012; Pararasa et al., 2015; Pérez et al., 2016). In particular, it is well established that adipose tissue in obesity is a potent contributor to chronic, low-grade systemic inflammation, which can result in multi-organ dysfunction. One of the main factors associated with obesity-induced adipose tissue inflammation is dysregulation of the immune cell populations within the tissue stromal fraction, which contribute to the propagation of a pro-inflammatory microenvironment that spills-over into the circulation and other organs (Hotamisligil, 2017a; Hotamisligil, 2017b).

In contrast to obesity-centric research, the role of immunometabolic adipose tissue dysfunction in human ageing has been largely unexplored. In older people, many biological systems and processes become dysregulated (Lopez-Otin et al., 2013). Indeed, age-associated immunological dysregulation potently modulates systemic inflammation, multi-organ dysfunction, and longevity (Franceschi et al., 2000; Calçada et al., 2014; Arai et al., 2015). Moreover, molecular modulation of adipose tissue inflammatory and metabolic processes influences longevity in animal models, suggesting adipose tissue may play an important role in the ageing process (Picard and Guarante, 2005; Argmann et al., 2009). Further, in humans, an age-associated redistribution of adiposity towards the abdominal cavity occurs independently of obesity (Schwartz et al., 1990; JafariNasabian et al., 2017). However, the role of adipose-resident immune cells and the interplay between metabolic and inflammatory processes in ageing adipose tissue is poorly characterised.

The purpose of this review is to summarise the immunological alterations that take place in adipose tissue with obesity and ageing, with the aim to improve understanding of the role for adipose tissue immunometabolic dysfunction in age- and obesity-associated disease. This review will first evaluate evidence showing obesity-specific immunological alterations in adipose tissue, which will then be used as a basis for understanding the potential role of adipose tissue immunological alterations in the pathophysiology of age-associated diseases.

2. The immune system and inflammation

The immune system protects the host against viruses, bacteria, fungi, parasites, and tumours, through a complex integration of innate and adaptive defences. The innate system
provides a first line of defence, comprising physical barriers such as the skin, and chemical barriers on surfaces exposed to the environment, preventing pathogens entering the body. In addition, the innate immune system has cellular defences, including monocytes, macrophages, natural killer (NK) cells, mast cells, neutrophils, eosinophils, basophils, and dendritic cells. Some cells possess characteristics of both the innate and adaptive immune system. For example, the small population of innate lymphocytes that are cluster of differentiation 1d (CD1d) restricted, referred to as invariant NKT-cell (iNKT) and other T-cells that express natural killer cell associated surface proteins (NKT-like cells). Professional antigen presenting cells link the two arms of the immune system. For example, dendritic cells act as tissue sentinels, and upon antigen encounter, travel from peripheral tissues (e.g., places in contact with the external environment; like the digestive system and dermis) to the lymph nodes where they activate cells of the adaptive immune system: B-cells and T-cells (Huang et al., 2000; Parkin and Cohen, 2001). T-cells comprise 60-75% of all lymphocytes, and originate from bone marrow, maturing in the thymus, and circulate in blood as either antigen-naïve or antigen-experienced cells (Zuniga-Pflucker, 2004). B-cells comprise 5-15% of all lymphocytes and elicit their effector functions, including their memory response, via soluble immunoglobulins, which can neutralise toxins or flag pathogens and target cells for elimination by other cells of the immune system, such as macrophages and NK-cells (Parkin and Cohen, 2001).

In response to injury or infection, a local immune response is initiated, characterised by swelling, heat, and pain. One of the first local changes is an increase in blood flow facilitating an influx of acute phase reactants, such as C-reactive protein (CRP), and an accumulation of innate, and then adaptive immune cells for pathogen elimination and tissue repair. However, alterations to the tissue micro-environment and local stimuli can result in uncontrolled inflammation. Such alterations to the anti-inflammatory or pro-inflammatory milieu can disrupt systemic homeostasis and metabolic demand, perpetuating the inflammatory response which has profound health implications. A degree of inflammation within adipose tissue is central to tissue remodelling, as many of the cells, cytokines, and pro-oxidants produced, at normal levels, regulate tissue homeostasis (Wernstedt Asterholm et al., 2014). However, prolongation of this normally transient and well-controlled process drives chronic, low-grade systemic inflammation that is central to the impaired health with obesity and ageing.

3. Adipose tissue inflammation and metabolic disease

Impairments in adipose tissue function associated with structural and functional changes to the tissue results in the propagation of abnormal, and often pro-inflammatory secretory profiles from adipocytes and cells of the stromal fraction. This association was first understood when murine obesity was linked with increased production of the inflammatory, insulin desensitising cytokine; tumour necrosis factor-α (TNF-α) (Hotamisligil et al., 1993). In the context of obesity, adipose tissue dysfunction is promoted by a chronic positive energy imbalance. Similar metabolic impairments are also observed in other conditions also characterised by adipose tissue dysfunction, including ageing and lipodystrophy. Consequently, the similarities between these conditions allows for comparisons to be made to better understand the processes involved (Tchkonia et al., 2010; Fiorenza et al., 2011; Goossens and Blaak, 2015).

To date, a variety of stimuli for immunometabolic deterioration within adipose tissue have been proposed. These include, increased gut-derived antigens (e.g., lipopolysaccharide),
stimulation of immune cells by dietary or endogenously-derived lipids, adipocyte hypertrophy—
leading to apoptosis, necrosis, fibrosis, and hypoxia—and adipocyte dysfunction from mechanical
stress (Reilly and Saltiel, 2017). Collectively, these alterations impact various aspects of adipose
tissue function, including: changes to local blood flow, which impairs the endocrine potential of
the tissue; changes to the extracellular matrix, which instigates monocyte infiltration to manage
tissue remodelling; and adoption of a pro-inflammatory and pro-oxidative microenvironment,
which act to recruit immune cells driving their pro-inflammatory polarisation (Hosogai et al., 2007;
Bourlier et al., 2008; Lafontan, 2014; Travers et al., 2015). Moreover, the dysfunction of
preadipocytes (adipocyte stem cell precursors) induced by a pro-inflammatory and pro-oxidative
microenvironment inhibits the healthy turnover of adipose tissue, potentiated by, and impacting
upon, impaired endothelial function, which exacerbates local hypoxia (Pasarica et al., 2009;
Lafontan, 2014; Travers et al., 2015). The net result of these disturbances is the aberrant secretion
of adipokines, which, via paracrine and endocrine means, impact appetite, bone health, metabolic
health, and systemic inflammation through the activation of pro-inflammatory signal cascades [i.e.,
nuclear factor κB (NFκB), NLR family pyrin domain containing-3 (NLRP3), and c-Jun N-terminal
kinase (JNK) signalling] (Donath and Shoelson, 2011; Hotamisligil, 2017b). Adipose
tissue immunometabolic dysfunction also impacts the ability of adipocytes to buffer lipids. This
dysfunction leads to the shunting of lipids towards non-adipose tissues, including the liver, skeletal
muscle, and the heart, promoting tissue-specific insulin resistance and inflammation, triggering β-
cell and metabolic dysfunction (Karpe et al., 2011; McQuaid et al., 2011; Hotamisligil, 2017b).

4. Methodological considerations in adipose tissue immunological research

Adipose tissue is an immunometabolically active organ in a constant state of flux
(Thompson et al., 2012a). Assessment of adipose tissue function is therefore a difficult task, and
the nature of sampling tissue means the available data tend to represent a snap-shot of adipose
physiology and function. In the context of the current review, this means that assessment of adipose
cellular composition at a given point in time will not necessarily provide a full and complete picture
of the events that have led to the characterised phenotype. In human research, adipose tissue
samples are often collected under fasting conditions, and very little information is available in the
post-prandial state where much of the day is spent, which potently alters adipose tissue
immunometabolic status (Travers et al., 2017). Furthermore, in human studies, the adipose tissue
sampling methodology (e.g., surgical versus aspiration biopsies) may influence some outcomes
(Mutch et al., 2009). In mice, both the breed and inconsistency in whether animals are fasted or
fed has the potential to influence their metabolic profile (Jensen et al., 2013). Some investigations
have adopted arterio-venous difference blood sampling techniques to sample the blood flowing
across the subcutaneous adipose depots in an attempt to understand the dynamic behaviour of the
tissue (Thompson et al., 2012b). This methodology allows for a greater understanding of how
adipose tissue responds, in real-time, potentially giving a fuller picture of adipose tissue
functionality.

Adipose tissue is distributed throughout the body with site-specific physiological
properties. In humans, abdominal subcutaneous and visceral adipose tissue differ substantially in
function and cellularity, and considerable depot differences are also apparent between
gluteofemoral and abdominal adipose tissues, which is particularly relevant when comparing
males and females due to sex-specific adipose tissue distribution (Karpe and Pinnick, 2016;
Manolopoulos et al., 2010). In mice, different adipose tissue depots also possess unique
physiological properties both between and within specific depots. Although beyond the scope of this review, differences include adipocyte size and expandability in response to high-fat overfeeding, control of lipolysis, and fatty acid and leukocyte composition (Caesar et al., 2010; Cinti, 2012). Most investigations of adipose tissue immunology have been conducted with animal models, with considerably less work with humans. Although differences in adipose tissue biology between humans and animals is beyond the scope of this review, it is important to recognise that human adipose tissue is, indeed, very different to that of animals such as mice in terms of functionality, physiology, distribution, and make-up (i.e., quantities of brown and white adipose tissue, respectively) (Chusyd et al., 2016).

To assess the constituents of the adipose tissue stromal fraction many approaches have been employed, including; immunohistochemistry, gene expression, protein level, and flow cytometry. Each method has benefits and drawbacks, which are summarised in Table 1. For example, it has been shown that immunohistochemistry yields reproducible results, but with flow cytometry, the recovery of macrophages after tissue digestion can be a possible source of variation (Morgan-Bathke et al., 2017). However, there is good agreement between flow cytometric analysis of adipose tissue cellular composition and the expression mRNA for key cell surface markers across a range of adiposities in humans (Figure 1) (Travers et al., 2015). Moreover, differences between studies in the selection of cell markers, coupled with lack of consensus over cell identification strategies, makes direct comparison between studies complex and could feasibly explain conflicting results.

The mode of reporting assessments of cellular composition also requires consideration. Reporting proportional representation of cell types within the stromal fraction versus absolute cell counts can produce different findings. For instance, a proportional decline in certain populations in relation to total stromal cell yields may be a consequence of an expansion of another cell population within the tissue instead of an absolute decline in specific cell numbers. For example, one report showed that, compared to older mice, young mice exhibited around twice as many macrophages in adipose tissue when expressed as a percentage of the total stromal yield. However, these age-related differences were not seen when data were expressed per gram of adipose tissue (Wu et al., 2007a). In summary, when interpreting the results of different studies, it must be considered whether the laboratory methods and data presentation/expression could in-part explain discrepant results, especially when interpreting cell counts and/or proportions within adipose tissue.

5. Traditional concepts in adipose tissue immunology: an inflammatory role for monocytes and macrophages

Macrophages were first discovered within adipose tissue forming multinucleated crown-like structures of ~15 cells clustering around dead or dying adipocytes, in obese C57/BL6J mice (Weisberg et al., 2003). In young obese mice and humans, these structures have been shown to comprise 90% of all adipose tissue macrophages (Cinti et al., 2005). Macrophages are the main leukocyte in the adipose stromal fraction of young, non-obese mice and humans, comprising 4-15% of total stromal cell count (Curat et al., 2004; Harman-Boehm et al., 2007). Macrophage
content increases with adiposity, and the accumulation is greatest in visceral depots in humans (Curat et al., 2006; Harman-Boehm et al., 2007). Adipose tissue macrophages are derived from monocytes, which differentiate in response to growth factors, including; macrophage colony-stimulating factor (M-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) (Geissmann et al., 2010; Wang et al., 2013). Monocytes, defined by their cell surface expression of CD14 and CD16 (see Table 2), circulate in peripheral blood until they migrate into adipose tissue primarily in response to chemokine (C-C motif) ligand-2 (CCL-2)—also known as monocyte chemoattractant protein-1 (MCP-1)—released from adipose tissue (Khan et al., 2009; Geissmann et al., 2010). MCP-1 promotes the local proliferation of adipose-resident macrophages, contributing to the large accumulation of these cells with obesity (Amano et al., 2014). Monocytes also enter the adipose microcirculation in response to adipocyte-derived cell-stress markers, including; CCL-5, also known as RANTES (regulated on activation, normal T-cell expressed and secreted), interleukin-6 (IL-6), interferon-γ (IFN-γ), and TNF-α (Hill et al., 2015). Production of these signals, which enhances macrophage accumulation, also polarises macrophages into highly inflammatory, classically activated cells, referred to herein as M1-like macrophages to be consistent with the reporting of data in the studies we summarise. We acknowledge however that the M1 and M2 classification of macrophages is simplistic and becoming out-dated 1 (see Table 2).

It has been consistently shown that there is a positive relationship between the number of macrophages in adipose tissue and the degree of whole-body insulin resistance in mice and humans (Xu et al., 2003; Wentworth et al., 2010; Travers et al., 2015). Moreover, TNF-α—which is primarily produced by macrophages in adipose tissue—is a potent inhibitor of insulin signalling and an activator of NFκB signalling (Hotamisligil et al., 1993; Schutze et al., 1995). In obese and inflamed adipose tissue, NFκB is activated by the fatty acid chaperone—Fetuin-A—interacting with the lipid-sensing toll-like receptor-4 (TLR-4) on the macrophage cell surface (Suganami et al., 2007; Kopp et al., 2009; Schaeffler et al., 2009). In the commonly studied 3T3-L1 adipocyte cell line, TNF-α has been shown to directly impact insulin signalling by inactivating insulin receptor substrate-1 (IRS-1) through p44/42 mitogen activated protein kinase (MAPK) activation (Engelman et al., 2000). Chronic activation of pro-inflammatory stress sensors and signalling pathways: p44/42, JNK, p38, and MAPK by TNF-α derived from subcutaneous adipose-resident macrophages indirectly stimulates lipolysis in adipocytes, impairing lipid handling capacity (Hauner et al., 1995; Ryden et al., 2002; Lafontan and Langin, 2009). It has also been observed, in vitro, that monocytes incubated with palmitate—an abundant saturated fatty acid—exhibit upregulated IL-6 and TNF-α mRNA (Bunn et al., 2010). Further, upon macrophage-adipocyte co-

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1 Traditionally, macrophages have been categorised as M1 (classically activated, often thought to be pro-inflammatory) and M2 (alternatively activated, often thought to be anti-inflammatory). It is now understood that this is an oversimplification of macrophage phenotype. However, much of the available research in adipose tissue, especially in mice, has maintained the M1 and M2 categorisation. In the present manuscript, for consistency and ease of comparing studies, the terms “M1-like” and “M2-like” will be used. Within adipose tissue it is now accepted that tissue-resident macrophages have a tissue-remodelling and pro-inflammatory phenotype, representing an intermediate population in mice and humans (i.e., cells that display an M2-like surface expression profile whilst demonstrating an M1-like secretory profile and function) (Zeyda et al., 2007; Bourlier et al., 2008; Shaul et al., 2010; Wentworth et al., 2010; Lumeng et al., 2011; Morris et al., 2011; Xu et al., 2013; Kraiz et al., 2014). Therefore, the role of macrophages in expanded and/or dysfunctional adipose tissue is likely to be far more complex than originally envisaged.
...there is a substantial increase in macrophage-derived TNF-α and IL-1β (Suganami et al., 2007). As TNF-α, IL-6, and IL-1β stimulate a feed-forward activation of NFκB and JNK, this leads to IRS-1 phosphorylation at serine residues rather than tyrosine, perpetuating insulin signalling impairments (Chen et al., 2015). Another factor influencing macrophage function in adipose tissue is hypoxia brought about by adipocyte expansion, without concurrent angiogenesis, impairing oxygen supply (Trayhurn, 2013; Muir et al., 2016). Hypoxia potentiates the palmitate-induced pro-inflammatory and pro-oxidative polarisation of human macrophages, whilst hypoxia-inducible factor-1α (HIF-1α) can directly induce pro-inflammatory M1-like macrophage polarisation and pro-inflammatory cytokine productions (Fujisaka et al., 2013; Snodgrass et al., 2016). Macrophage-derived ROS produced as a result of hypoxia may also impair lipid and glucose metabolic control through the S-nitrosylation of the peroxisome proliferator-activated receptor-γ (PPAR-γ) nuclear receptor (Yin et al., 2015).

Macrophages are considered central regulators of fibrosis and may also increase progenitor cell deposition into the extracellular matrix as a means of protecting hypertrophic adipocytes from rupturing (Khan et al., 2009). M2-like macrophages possess a pro-fibrotic potential and are, therefore, recruited into the adipose tissue in response to ‘danger signals’ (i.e., cytokines, acute-phase proteins, and stress signal cascades) released from hypertrophic adipocytes (Morris et al., 2011). However, the exposure of macrophages to hormones, cytokines, chemokines, and fatty acids found within dysfunctional adipose tissue, encourages them to adopt a pro-inflammatory phenotype potentiating metabolic deteriorations and inflammation (Stout and Suttles, 2004; Stout and Suttles, 2005). In human obesity, perhaps counter-intuitively based on simple macrophage phenotyping, it is the M2-like, and not M1-like, macrophages, with a tumour-associated genetic signature and remodelling phenotype, that correlate with obesity in subcutaneous adipose tissue (Bourlier et al., 2008). Another stimulus that might influence macrophage infiltration into adipose tissue is endoplasmic reticulum stress, which promotes a pro-inflammatory microenvironment through the initiation of the unfolded protein response. In mice, increased fatty acid-mediated oxidative stress up-regulates pro-inflammatory cytokine expression which, in humans, is also initiated by elevated TNF-α and IL-1β (Kawasaki et al., 2012; Xue et al., 2005). Endoplasmic reticulum stress responses involving the inositol-requiring enzyme 1α (IRE1α) and CCAAT-enhancer-binding protein homologous protein (CHOP) direct pro-inflammatory, M1-like macrophage polarisation in adipose tissue (Shan et al., 2017; Suzuki et al., 2017). Finally, it is unknown whether macrophages initiate inflammatory responses in adipose tissue or whether other cells present stimulate macrophage accumulation and dysfunction. For example, some studies in mice indicate that macrophages infiltrate adipose tissue in the absence of other leukocytes, whereas other studies show that different leukocyte subsets are central to macrophage accumulation (Duffaut et al., 2009a).

Recent evidence has indicated that a unique adipose-resident macrophage population exists. In mice fed a high-fat diet, a population of sympathetic neuron-associated macrophages accumulate within visceral adipose tissue. This macrophage population regulates adipocyte exposure to norepinephrine by sequestering and degrading norepinephrine released into the adipose tissue interstitium. This process is brought about by the selective expression of the norepinephrine transporter, SLC6A2, and genes controlling norepinephrine-degradation such as monoamine oxidase-A by these macrophages (Pirzgalska et al., 2017). However, unlike other adipose-resident macrophage populations, these sympathetic neuron-associated macrophages do not increase within obese adipose tissue via proliferative mechanisms, but instead appear to infiltrate the tissue selectively (Pirzgalska et al., 2017). Given that catecholamines increase lipolytic rate in adipocytes via adrenergic receptors triggering the downstream hydrolysis of...
triglycerides, selective knock-out of these sympathetic neuron-associated macrophages protects against high-fat diet-induced obesity, in mice. Moreover, the capacity to buffer regional norepinephrine releases, which in healthy adipose tissue may act as a protective mechanism to avoid the dangerous effects of chronic exposure to norepinephrine, is also sufficient to modulate overall sympathetic tone in murine adipose tissue, influencing whole-body metabolism (Czech, 2017).

In summary, a number of factors including adipose tissue cellular composition and secretions plus broader anatomical and physiological characteristics of the tissue, have been found to modulate metabolic and inflammatory processes and influence macrophage phenotype and function. However, due to adipose sampling in humans being a ‘snap-shot’ of the dynamic changes that the tissue undergoes with obesity, further work is required to fully understand these interactions. A summary of the likely interactions between adipocytes and adipose-resident macrophages with obesity is presented in Figure 2.

6. A broader perspective on adipose tissue immunology

Adipose tissue dysfunction is the result of a complex interaction between all cell types within the tissue. Understanding this interplay has been the subject of intensive investigation which will first be discussed in the context of obesity as a foundation for understanding the function of adipose tissue with ageing. The following sections will summarise research examining different immune cell populations in adipose tissue in the context of obesity, considering cells in order of the weight of evidence (most to least) supporting their role within dysfunctional adipose tissue.

6.1. Neutrophils in obesity-associated adipose tissue dysfunction

In mice fed a high-fat diet, the accumulation of neutrophils (identified in mice as Ly6g+CD11b+) occurs in visceral, but not subcutaneous, adipose tissue after three days (Talukdar et al., 2012). Neutrophils are 20-fold more abundant in adipose tissue from mice fed a high-fat diet compared to chow fed mice, accounting for ~2% of total stromal cells (Elgazar-Carmon et al., 2008; Talukdar et al., 2012; Ferrante, 2013). The accumulation of neutrophils in adipose tissue in obese mice leads to elevated elastase production (Talukdar et al., 2012). In vitro experiments have shown that increased exposure to elastase can contribute to IRS-1 downregulation, resulting in impaired glucose tolerance and insulin resistance (Houghton et al., 2010). Further, systemic and intraperitoneal glucose tolerance has been shown to be impaired in mice injected with elastase compared to those treated with an elastase inhibitor. Neutrophil-derived elastase also imparts pro-inflammatory actions on murine intraperitoneal macrophages via TLR-4 signalling, upregulating TNF-α, IL-1β, and IL-6 gene expression, potentially resulting in a feed-forward recruitment of neutrophils and M1-like macrophages into adipose tissue (Talukdar et al., 2012).

In humans, immunohistochemical analyses (CD66b+ staining) have shown neutrophils to accumulate in the microvasculature of adipose tissue with increasing adiposity, correlating with elevated NFκB and cyclooxygenase-2 (COX-2) staining (Shah et al., 2010). Further, it has also been suggested that elevated chemokine (C-X-C motif) ligand-2 (CXCL-2) release from obese
adipose tissue may promote neutrophil infiltration (Rouault et al., 2013). However, in humans at least, several questions remain unanswered. For example, whether neutrophil accumulation is an early step in adipose tissue dysfunction, and to what extent neutrophils modulate ongoing inflammatory responses with adipose tissue.

6.2. NK-cells in obesity-associated adipose tissue dysfunction

Comprehensive investigations in mice have suggested that a population of NK-cells, uniquely expressing the IL-6 receptor alpha and colony stimulating factor 1 receptor (IL6Ra+Csf1r+), induced via IL-6/STAT-3 (signal transducer and activator of transcription-3) signalling, accumulate with obesity within visceral adipose tissue (Theurich et al., 2017). Ablation of IL6Ra+Csf1r+ NK-cells improves insulin and glucose control, and reduces overall adiposity, adipocyte size, macrophage infiltration, and macrophage crown-like structures (Theurich et al., 2017). It has been suggested that IL-15 and aberrant leptin secretion with obesity might instigate an adipose-specific NK-cell expansion and activation in response to high-fat diet overfeeding, stimulating NK-cell IFN-γ, TNF-α, and MCP-1 production, negatively impacting whole body insulin sensitivity (Tian et al., 2002; Wensveen et al., 2015; Lee et al., 2016).

In humans with obesity and type-II diabetes, activated NK-cells accumulate in visceral and subcutaneous abdominal adipose tissue, producing IFN-γ and TNF-α (Henegar et al., 2008; O'Rourke et al., 2009; O’Rourke et al., 2013). Moreover, the increase in IL6Ra+ NK-cells in peripheral blood of people with obesity suggests that obese dysfunctional adipose tissue may cause a pro-inflammatory NK-cell population expansion that spills out into the circulation (Theurich et al., 2017). It has been proposed that adipose-resident macrophages produce CCL-3, CCL-4, and CXCL-10, stimulating the recruitment of NK-cells into adipose tissue, whereupon macrophage-derived IL-15 promotes NK-cell proliferation and activation (Bonamichi and Lee, 2017). Once within adipose tissue, NK-cell modulation of MCP-1 expression could potentiate macrophage recruitment, and proliferation (Amano et al., 2014), and finally, through an upregulation of NK-cell-derived TNF-α and IFN-γ, NK-cells may guide M1-like macrophage polarisation. Therefore, NK-cells may be pivotal in the generation of obesity-associated immunometabolic adipose tissue dysfunction.

6.3. B-cells in obesity-associated adipose tissue dysfunction

In lean murine visceral adipose tissue, B-cells constitute 10% of the stromal fraction, increasing to 20% in response to high-fat overfeeding, and this accumulation has been reported to occur prior to macrophage infiltration (Ying et al., 2017). B-cell localisation around macrophage clusters may be central to their functional relevance within adipose tissue in obesity, suggesting a role in steering macrophage functionality, perhaps via LT-B4/LT-B4R1 (leukotriene-B4 receptor 1) signalling. LTB4/LTB4R1 signalling promotes leukocyte infiltration into tissues, influences cytokine production, and is increased in obese murine visceral adipose tissue B-cells (Ying et al., 2017). In support, B-cells promote visceral adipose tissue macrophage recruitment and TNF-α production, in vivo and in vitro, in mice fed a high-fat diet (Winer et al., 2011). Moreover, B-cells have also been linked with the accumulation and differentiation of IFN-γ-producing CD4+ and CD8+ T-cells within murine visceral adipose tissue (Shirakawa et al., 2016; Ying et al., 2017).
Indeed, CD8+ T-cells have been shown to produce 30% less IFN-γ in B-cell-deficient mice fed a high-fat diet suggesting a role for B-cells in T-cell activation (Winer et al., 2011; Shirakawa et al., 2016). B-cells may also promote the expansion of a senescent population of CD4+CD153+PD-1+ T-cells in murine visceral adipose tissue which, in-turn, secrete osteopontin that promotes B-cell IgG production and suppresses IL-10 secretion (Stromnes and Goverman, 2007; Shirakawa et al., 2016).

In obese human subcutaneous adipose tissue, B-cells comprise <4% of all stromal cells, but localise around macrophage crown-like structures and in the perivascular space (McDonnell et al., 2012). In obese human adipose tissue, a ~3-fold increase in the expression of the B-cell marker B220 mRNA has been reported (Sears et al., 2009), although flow cytometric analysis of human subcutaneous adipose tissue has shown that very few B-cells are present in adipose tissue (Duffaut et al., 2009b). Contradictory findings could be an artefact of methodology, indicating that further work in humans, employing a combination of methodological approaches is warranted. In summary, B-cells may play a role in the early stages of obesity-induced adipose tissue dysfunction, modulating the functions of other cells within the stromal fraction, promoting a pro-inflammatory microenvironment.

6.4. T-cells in obesity-associated adipose tissue dysfunction

Within lean human adipose tissue, T-cells represent ~10% of the stromal fraction (one-third CD8+ T-cells and two-thirds CD4+ T-cells). More than half of the CD4+ T-cell compartment within murine adipose tissue are regulatory T-cells (Feuerer et al., 2009). Studies in humans have shown that T-cells are recruited into adipose tissue in response to adipocyte-derived CCL-20 binding to CCL-6 on the surface of T-cells, as well as CCR-5 to RANTES interactions (Wu et al., 2007b; Kintscher et al., 2008; Duffaut et al., 2009b). Moreover, adipose-resident T-cells display a visceral adipose tissue-specific antigen-driven expansion, suggesting a signal within the tissue promotes their clonal expansion. A potential signal has been suggested to derive from B-cells which may infiltrate or respond to dysfunctional adipose tissue earlier than T-cells (Nishimura et al., 2009; Winer et al., 2009; Shirakawa et al., 2016). T-cells typically exhibit a differentiated or activated phenotype shown by low surface expression of CD62L and high expression of CD25 in murine and human adipose tissue, respectively (Nishimura et al., 2009; Travers et al., 2015).

6.4.1. CD8+ cytotoxic T-cells in obesity-associated adipose tissue dysfunction

In mice fed a high-fat diet, CD8+ T-cells are the predominant T-cell sub-population to accumulate in adipose tissue. The early selective recruitment of CD8+ cells into visceral adipose tissue in mice occurs in parallel with a reduction in blood CD8+ T-cells in the first two weeks of overfeeding (Nishimura et al., 2009). Mouse models also show that adipose-resident CD8+ T-cells have an activated CD62L−CD44+ effector phenotype which is brought about, in situ, by MCP-1 release from adipocytes (Kim et al., 1998; Nishimura et al., 2009). Co-culturing CD8+ T-cells with obese murine adipocytes has been shown to initiate, potentiate, and maintain the inflammatory response by instigating the infiltration of macrophages towards adipocytes, followed by their activation by CD8+ T-cell-derived MCP-1 (Nishimura et al., 2009). Moreover, selective depletion
of CD8+ T-cells in obese mice has been shown to improve metabolic health and reduce local tissue and serum levels of IL-6 and TNF-α (Nishimura et al., 2009).

In obese humans, mRNA levels of CD8A is increased in visceral adipose tissue (Nishimura et al., 2009; Koenen et al., 2011) and in subcutaneous adipose tissue, CD8+ T-cells adopt an activated phenotype compared to blood (Duffaut et al., 2009b; Travers et al., 2015). As serum MCP-1 is substantially elevated in patients with type-II diabetes, and is a potent activator of CD8+ T-cells, MCP-1 may be a cause of elevated adipose tissue CD8+ T-cell activation with obesity or metabolic disease (Kim et al., 1998; Nomura et al., 2000). The stimulus for CD8+ T-cell migration into obese adipose tissue is yet to be confirmed in vivo, in humans, though significantly elevated CCL-20 release by obese adipose tissue has been observed in vitro (Duffaut et al., 2009b). In the circulation, CD8+ T-cells from obese individuals express elevated levels of TNF-α, IFN-γ, and RANTES. Importantly, IFN-γ has been found to inhibit insulin-mediated upregulation of fatty acid synthase (FAS) and lipoprotein lipase (LPL), and was associated with a downregulation of phosphatidylinositol 3-kinase regulatory subunit alpha (PIK3R1) expression (a key component of insulin signalling) (Duffaut et al., 2009b). These observations suggest a modulatory capacity for CD8+ T-cells in adipose tissue metabolic health by influencing insulin-mediated triglyceride storage (Duffaut et al., 2009b).

6.4.2. CD4+ helper T-cells in obesity-associated adipose tissue dysfunction

CD4+ T-cells accumulate within human subcutaneous adipose tissue with obesity, exhibiting an activated CD25+ phenotype (Travers et al., 2015). Recent findings suggest that circulating CD4+ T-cells adopt an effector memory (CXCR-3+CD62L−) phenotype with human obesity in response to a metabolically-driven adaptation within T-cells via the p110δ sub-unit of phosphoinositide 3-kinase (PI3K) (Mauro et al., 2017). Indeed, lipid activation of this sub-unit is also associated with sustained activation of T-cells, indicating that dendritic cell-independent, metabolically-driven activation could drive chronic activation within adipose tissue (Macintyre et al., 2011; Finlay et al., 2012). The helper T-cell balance has the potential to play an important role in adipose tissue inflammatory disorders. For example, in mice, IFN-γ secretion is increased mainly as a consequence of Th-1 cell predominance within visceral adipose tissue upon high-fat diet-overfeeding (Kintscher et al., 2008; Winer et al., 2009). The direct effect of IFN-γ in obesity induced by a high-fat diet includes impaired insulin signalling and the promotion of macrophage infiltration and crown-like formations via MCP-1 (Rocha et al., 2008b). Additionally, IFN-γ from Th-1 cells instigates CXCL-10 release from 3T3-L1 adipocytes, providing a positive feedback recruitment loop (Rocha et al., 2008a). Moreover, IFN-γ secretion, when combined with TNF-α, can lead to chronic activation of NFκB signalling (Cheshire and Baldwin, 1997). However, Th-2 cells could serve to promote M2-like macrophage maturation through their production of IL-4 and IL-13 (Bourlier and Bouloumie, 2009). Human and murine research indicates CD4+ T-cells within both visceral and subcutaneous adipose tissue not only control their own recruitment, activation, and differentiation but are also influenced by other tissue-resident immune cells (i.e., B-cells) and adipocytes. Consequently, CD4+ T-cells have both direct and indirect roles in obesity-associated adipose tissue immunometabolic dysfunction.
6.4.3. Regulatory T-cells in obesity-associated adipose tissue dysfunction

Regulatory T-cells represent more than half of all CD4+ T-cells in lean murine adipose tissue, and in obesity, these cells which accumulate around macrophages, produce IL-4 and IL-10 promoting Th-2 T-cell polarisation (Feuerer et al., 2009). Regulatory T-cells also suppress adipocyte-derived inflammatory markers and restore metabolic health by promoting translocation of glucose transporter-4 (GLUT-4) to the cell membrane, countering the effects of TNF-α. However, adipose-resident regulatory T-cells appear to decline with increasing duration of obesity, in mice (Deng et al., 2017). It is thought that the decline in regulatory T-cell numbers is a result of IFN-γ-induced impairments in proliferative capacity in visceral adipose (Deng et al., 2017). These changes occur in parallel with insulin resistance in mice and humans (Feuerer et al., 2009; Nishimura et al., 2009; Winer et al., 2009). In human obesity—which occurs over a much longer time-span than in mice—it has been shown that there is a substantial increase in regulatory T-cells within subcutaneous adipose tissue and this could be a compensatory mechanism to counter adipose tissue inflammation (Travers et al., 2015). However, a proportional decline in regulatory T-cells has also been reported within the adipose tissue stromal fraction in obesity (Acosta et al., 2016). These observations suggest that regulatory T-cells may limit ongoing pro-inflammatory events that occur with obesity-induced adipose tissue dysfunction. However, it is possible that with chronic local inflammation, regulatory T-cell proliferation is impaired, and numbers decline.

6.4.4. Invariant NKT-cells in obesity-associated adipose tissue dysfunction

Within lean human and murine adipose tissue, iNKT-cells typically express low levels of CD4 and NK1.1 and secrete large amounts of IL-4 and IL-10, demonstrating an anti-inflammatory capacity (van Eijkeren et al., 2017). Indeed, with obesity, iNKT-cells may initially provide an anti-inflammatory function to limit the ongoing pro-inflammatory response driven by other infiltrating leukocytes. However, with obesity iNKT-cells decline substantially, though the reason for this decline is unclear (van Eijkeren et al., 2017). Further, in mice, iNKT-cell accumulation in adipose tissue is limited with adipocyte CD1d knock-out and, in turn, these animals exhibit a poor metabolic profile (Huh et al., 2017). Although it has been suggested that CD1d knock-out models may reduce IFN-γ production, independent of iNKT-cells (Huh et al., 2017). In addition, recent investigations have shown that iNKT-cells have a role in adipocyte browning and modulating regulatory T-cell responses in mice, promoting the anti-inflammatory actions of regulatory T-cells (Lynch et al., 2015; Lynch et al., 2016). In non-obese humans, visceral adipose tissue is also enriched with iNKT-cells and these cells exhibit a distinct Th-2 cytokine profile (Lynch et al., 2009; Lynch et al., 2012). However, with obesity, the proportion of iNKT-cells within the adipose tissue stromal fraction declines substantially, in tandem with the development of metabolic syndrome and inflammation, as observed in obese mice (Lynch et al., 2009; Lynch et al., 2012; Huh et al., 2017). In summary, iNKT-cells likely assist in the regulation of a healthy adipose tissue microenvironment in a similar way to regulatory T-cells and eosinophils (see section 6.6). Therefore, a reduction in iNKT-cells with obesity might contribute to the diminished anti-inflammatory signals within dysfunctional adipose tissue.
6.5. Dendritic cells in obesity-associated adipose tissue dysfunction

Dendritic cells accumulate in adipose tissue of mice fed a high-fat diet and likely contribute to the pro-inflammatory microenvironment via macrophage recruitment and IL-6 production (Stefanovic-Racic et al., 2012; Chen et al., 2014). Myeloid dendritic cells may also promote helper T-cells to adopt a Th-1 phenotype which contribute to IFN-γ production (Pearce and Shen, 2007; Stefanovic-Racic et al., 2012; Martinez and Gordon, 2014; Wang et al., 2014). However, in mice, both the flow cytometric analyses and knock-out models commonly utilised are subject to criticism due to their non-specificity for ‘true’ dendritic cells (Bertola et al., 2012; Stefanovic-Racic et al., 2012).

It has recently been shown that type-I interferon signalling is pivotal to the development of obesity-associated metabolic disease in mice, and therefore plasmacytoid dendritic cells have been implicated in diet-induced obesity (Hannibal et al., 2017). Indeed, in mice fed a high-fat diet, plasmacytoid dendritic cells have been shown to increase ~3-fold, possibly as a result of elevated MCP-1 production and macrophage recruitment/proliferation (Ernst et al., 2010; Goossens et al., 2010; Stefanovic-Racic et al., 2012; Pattison et al., 2013; Amano et al., 2014; Ghosh et al., 2016; Hannibal et al., 2017). In humans, distinct CD1c+ myeloid dendritic cell populations have been identified within subcutaneous, but not visceral adipose tissue, and the number of these cells correlates positively with BMI (Bertola et al., 2012). Moreover, dietary lipids modulate the abundance of dendritic cells within lymphoid structures in adipose tissue and as dendritic cells communicate with other lymphoid cells via lipid-derived molecules, dendritic cells may modulate adipose tissue immunometabolic responses (Morelli and Thomson, 2003; Pond, 2005).

6.6. Eosinophils in obesity-associated adipose tissue dysfunction

Eosinophils in the abdominal adipose tissue of mice have a role in improving insulin sensitivity and stimulating anti-inflammatory responses through the production of IL-4 (Chawla et al., 2011; Wu et al., 2011; Schipper et al., 2012). Mice fed a high-fat diet exhibit a decline in eosinophil numbers within adipose tissue (Wu et al., 2011). In addition, eosinophils have been estimated to produce up to 90% of the IL-4 found within murine adipose tissue, promoting M2-like macrophage polarisation and tyrosine hydroxylase expression. In turn, macrophage tyrosine hydroxylase expression drives the production of catecholamines by macrophages, triggering the expression of uncoupling protein 1 in white adipose tissue (Mohrs et al., 2005; Schipper et al., 2012; Qiu et al., 2014). Moreover, it has been shown that mice fed a high-fat diet and injected with eosinophils intravenously do not accumulate adipose-resident macrophages (Wu et al., 2011).

Further, obese eosinophil knock-out mice demonstrate significantly greater degrees of insulin resistance compared to wild-type mice (Wu et al., 2011).

6.7. Mast cells in obesity-associated adipose tissue dysfunction

Obesity in mice is associated with an accumulation of mast cells in adipose tissue and higher levels of tryptase in serum—a granule released by mast cells (Liu et al., 2009). In mice fed
a high-fat diet, mast cells have been reported to accumulate in visceral adipose tissue and may contribute to the development of obesity through the stimulation of adipocyte protease expression, promoting micro-vessel growth, enabling leukocyte infiltration, and adipose tissue expansion (Liu et al., 2009; Zhou et al., 2015). In humans with obesity and type-II diabetes, pharmacological stabilisation of mast cells to prevent degranulation, leads to improvements in a number of parameters, including glycosylated haemoglobin, fasting blood glucose, total cholesterol, low-density lipoprotein, triglycerides, and high-density lipoprotein, independent of changes to BMI (El-Haggar et al., 2015). Therefore, despite contradictory findings in murine research, work with humans indicates a potential role for mast cell dysregulation in the generation of metabolic diseases. However, whether this is a result of adipose-resident mast cells specifically is yet to be established.

6.8. Summary of the cellular changes associated with obesity-induced adipose tissue dysfunction

Adipose tissue dysfunction with obesity is associated with changes to the numbers and/or function of a variety of immune cells. With this ongoing immunological dysregulation, adipose tissue adopts a pro-inflammatory bias, dramatically impacting the metabolic health of the tissue. Consequently, dysfunctional adipose tissue influences whole-body immunometabolic health contributing to the generation of diseases including type-II diabetes and cardiovascular diseases. A summary of obesity-associated changes to adipose tissue resident immune cell populations is presented in Figure 3.

[INSERT FIGURE 3 HERE]

7. Immunological ageing and adipose tissue

Most physiological systems become dysfunctional with ageing due to accumulations of cellular changes which contribute to the onset of disease. Nine hallmarks of ageing have been proposed characterising age-associated whole-body dysfunction, which include; genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, dysregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intracellular communication (Lopez-Otin et al., 2013). The physiological stresses induced by obesity also cause a range of cellular and whole-body deteriorations that underpin the pathophysiology of adipose tissue accumulation and dysfunction. Many of these obesity-associated changes overlap with the hallmarks of ageing, thus, it has been proposed that obesity could be considered an accelerated model of ageing (Tchkonia et al., 2010; Tzanetakou et al., 2012; Palmer and Kirkland, 2016; Pérez et al., 2016).

Ageing is associated with a decline in immune function, referred to as immunosenescence, which is thought to contribute to chronic, low-grade inflammation or ‘inflammageing’ (Franceschi et al., 2000; Aw et al., 2007; Pawelec, 2012; Shaw et al., 2013; Puzianowska-Kuźnicka et al., 2016). The broader consequences of an ageing immune system are increased susceptibility to infections and cancer in combination with weaker responses to novel antigens, including those
administered by vaccination (Nikolich-Žugich, 2014). Immunosenescence is best characterised and most marked among cells of the adaptive immune system. However, a common general observation is that lymphoid cell numbers decline (along with their proliferative capacity) whereas myeloid cell numbers typically increase, but only some innate immune cells exhibit age-associated dysfunction (Aw et al., 2007). The most robust hallmarks of immunosenescence are alterations to the T-cell pool in peripheral blood. These changes are, in-part, due to thymic involution limiting the output of naïve T-cells, but also due to a lifetime of antigen exposure which drives the differentiation of naïve T-cells into memory T-cells (Nikolich-Žugich, 2014). Thus, ageing is associated with an accumulation of pro-inflammatory effector memory T-cells and a decline in naïve T-cells in peripheral blood (Effros, 2004; Akbar and Fletcher, 2005; Nikolich-Žugich, 2014). Obesity, as with ageing, is associated with increased susceptibility to infections and an inability to mount effective immune responses to novel antigens (Lang et al., 2012; Sheridan et al., 2012; Huttunen and Syrjanen, 2013; Neidich et al., 2017). Thus, obesity might promote immunological decline through several mechanisms, with dysfunctional adipose tissue as a potential driving factor. For example, obesity might exacerbate immunosenescence by promoting activation and differentiation of immune cells passing through the adipose tissue microvasculature, which could have implications for the phenotype and function of cells found in peripheral blood and other tissues. In addition, immune cell accumulation in adipose tissue promotes differentiation into pro-inflammatory cytokine producing cells which likely drives inflammageing. The consequences could influence processes early in the immune response to novel pathogens, such as antigen recognition, processing and presentation, but might also impair the ability to control latent or chronic infections, and undergo processes such as wound healing. Experimental evidence supports some of these ideas, suggesting that adipose tissue dysfunction in obesity promotes immunological ageing (Shirakawa et al., 2016).

Although there is less research specifically examining adipose tissue in the context ageing compared to obesity, a role for adipose tissue in late-onset disease development has been established. Ageing is associated with increased abdominal and ectopic adipose tissue accumulation which is linked with the onset of frailty, and cardiometabolic disease (Mau and Yung, 2017). Moreover, organelle stress and the accumulation of senescent cells (p16INK4a+) within adipose tissue that secrete IL-6, IL-8, and TNF-α, further contribute to a pro-inflammatory, dysfunctional adipose tissue with age (Baker et al., 2011; Tchkonua et al., 2013). However, comparatively little is understood about immune cell populations within aged adipose tissue, especially in humans. Thus, the next sections will review literature examining the accumulation of different immune cells in adipose tissue with ageing, beginning with cells where there is most evidence, followed by cells that have been examined least.

### 7.1. Macrophages in aged adipose tissue

Very few studies have investigated the effects of ageing on adipose tissue macrophage populations to date, however, the available data supports a role for macrophages in age-associated adipose tissue dysfunction. This concept is discussed below and summarised in Figure 4. In visceral adipose tissue from old non-obese mice, it has been shown that the absolute numbers of adipose-resident macrophages do not increase substantially but may decline in proportions given
the expanded stromal vascular fraction with age. However, with age, visceral adipose tissue macrophages have a predominantly pro-inflammatory, so-called “double-negative” (CD11c−CD206−) phenotype producing IL-6 and TNF-α, and down regulating PPAR-γ (Wu et al., 2007a; Lumeng et al., 2011). Furthermore, peritoneal macrophages cultured with adipose-derived conditioned media induces M1-like polarisation, indicating that the products of adipose tissue have a direct impact on immune cells (Lumeng et al., 2011). Age-associated adipocyte-derived inflammation likely comes about by the activation of the NFκB signalling pathway (Wu et al., 2007a). As a result, NFκB activation may act as a stimulus for the pro-inflammatory polarisation of adipose tissue macrophages, supporting the idea that changes within adipose tissue, independent from total adipose tissue mass, drive age-associated adipose tissue inflammation (Starr et al., 2009). These observations in mice likely correspond to those made in obese adipose tissue from humans (Figure 1) but experimental evidence is limited at present. Moreover, another study in middle-aged (11 month old) mice suggests that the proportions of “double-negative” (CD11c−CD206−) and M2-like macrophages are largely unaltered, whereas M1-like macrophages decline slightly when compared to young murine visceral adipose tissue (Bapat et al., 2015). Note that these 11 month old mice are representative of humans aged 30-40 years (Flurkey et al., 2007).

Sympathetic neuron-associated, catecholamine-degrading macrophages which impair lipolysis have also been reported to accumulate in aged murine visceral adipose tissue (Camell et al., 2017). With obesity, these sympathetic neuron-associated macrophages appear to infiltrate the tissue selectively (Pirzgalska et al., 2017). However, with ageing, these cells appear to accumulate in adipose tissue via proliferative mechanisms, in situ (Camell et al., 2017). The metabolic effects of these cells is due to NLRP-3 activation, which upregulates growth differentiation factor 3 (GDF3) expression within macrophages (Camell et al., 2017). GDF3 interacts with adipocytes to inhibit lipolysis and stimulates the expression of enzymes that oxidise and remove catecholamines (Camell et al., 2017; Pirzgalska et al., 2017). Therefore, in the context of ageing, sympathetic neuron-associated macrophages likely regulate whole-body metabolism, inhibiting lipolytic signalling and free-fatty acid mobilisation as with obesity, contributing to immunometabolic dysfunction both locally and systemically.

In humans, only one study has investigated how ageing influences adipose tissue macrophages. Metabolically healthy Pima Indians, 18 to 44 years old, were assessed for insulin sensitivity and subcutaneous adipose tissue macrophage content. Adipose tissue macrophage number increased until ~31-33 years old, plateauing or slightly declining thereafter, but with ageing, adopted an activated phenotype as shown by plasminogen activator inhibitor 1 (PAI-1) and CD11c mRNA expression (Figure 4). The expression of these two macrophage activation markers was also associated with a decline in insulin sensitivity, independent of adiposity. Further, a negative relationship was observed between advancing age and whole-body insulin sensitivity ($r = −0.23$, $P = 0.07$). These findings suggest that although adipose-resident macrophages may decline or plateau in middle age, activation of these cells might impart an insulin desensitising effect (Ortega Martinez de Victoria et al., 2009). Although this study offers the only direct assessment of adipose-resident macrophage populations with advancing age in humans, there are several factors that should be considered. For example, this study provides data up until middle age only, and was conducted in a specific population with unique metabolic, genetic, and lifestyle characteristics. In addition, examining adipose-resident macrophages using gene expression and immunohistochemical analysis has some limitations (see Table 1).

The stimulus for age-associated macrophage pro-inflammatory polarisation is unclear, but endoplasmic reticulum stress, as shown in obese adipose tissue and adipose tissue macrophages, has been implicated. For example, it has been shown in aged murine adipose tissue that
endoplasmic reticulum stress promotes an inflammatory environment, elevating IL-6, MCP-1, and TNF-α production within adipose tissue, potentially in response to altered autophagy (Ghosh et al., 2015; Ghosh et al., 2016). Ageing is associated with decreased vascularisation as a result of impaired angiogenesis, supporting local hypoxia, which might drive adipose tissue macrophage accumulation (Ye et al., 2007; Pasarica et al., 2009; Lahteenvuo and Rosenzweig, 2012; Valli et al., 2015). Ageing is also associated with reductions in the mitochondrial cytochrome C oxidase subunit 5B (COX5B) component of complex IV within adipocytes, which represses HIF-1α (Soro-Arnaiz et al., 2016). Therefore, reductions in COX5B in human visceral adipose tissue with age both elevates HIF-1α and intracellular lipid storage as a result of decreased fatty acid oxidation, promoting adipocyte enlargement, in vitro (Soro-Arnaiz et al., 2016). If this hypertrophic expansion was to continue, stress signals prompting macrophage infiltration could be released, as is observed in obesity (Cinti et al., 2005; Dalmas et al., 2011; Morris et al., 2011).

7.2. Regulatory T-cells in aged adipose tissue

As with obesity, regulatory T-cells increase in the adipose tissue of old compared to young mice (Feuerer et al., 2009; Lumeng et al., 2011; Bapat et al., 2015). The expanded population of these regulatory T-cells is transcriptionally more closely related to adipose-resident conventional T-cells than splenic regulatory T-cells (i.e., selective enrichment for PPAR-γ gene expression, among others), indicating that their characteristics may be driven, in-part, by their location within the adipose tissue (Bapat et al., 2015). However, unlike in obesity, an increase in regulatory T-cells in aged murine adipose tissue is linked with a decline in metabolic function independent of macrophage accumulation (Bapat et al., 2015). These observations indicate that, in aged adipose tissue, a reduction in regulatory T-cells may be protective, because the immune profile of adipose tissue regulatory T-cell knock-out mice is shifted towards those of young mice (Bapat et al., 2015). Moreover, ex vivo adipose tissue basal glucose uptake was nearly twice as high for adipose-resident regulatory T-cell knock-out mice compared to control tissues, supporting a causal association between adipose tissue regulatory T-cells and age-associated insulin resistance (Bapat et al., 2015).

Although it is unclear whether the function of regulatory T-cells with ageing in humans is impaired, studies in aged mice indicate that these cells retain their suppressive capacity (Fessler et al., 2013; Bapat et al., 2015). As a degree of inflammation is essential for the maintenance of healthy adipose tissue, the substantial increase in adipose tissue regulatory T-cells combined with the retention of their suppressive capacity may be an indication of over compensation, impacting the immunometabolic properties of aged murine adipose tissue (Wernstedt Asterholm et al., 2014; Bapat et al., 2015). Whilst the observations concerning regulatory T-cells within aged murine adipose tissue provide evidence that age- and obesity-associated insulin insensitivity may be orchestrated by unique immune cell populations, further work in the adipose tissue of older humans is required to better understand this immunometabolic interplay. Additional work in older mice is also warranted as the animals examined by Bapat et al. (2015) were substantially younger than those examined in other studies investigating age-associated adipose tissue immunological dysfunction (Wu et al., 2007a; Lumeng et al., 2011; Bapat et al., 2015) (see Figure 4). Nonetheless,
recent evidence offers some support for a non-obesity-dependent regulatory T-cell-driven, late-onset metabolic deterioration; coined type-IV diabetes (Bapat et al., 2015).

7.3. B-cells in aged adipose tissue

It has been shown that the visceral adipose tissue of obese, old mice is enriched with a specific B-cell population compared to young, obese mice, and these cells differ from splenic B-cells (Frasca and Blomberg, 2017). Co-culturing splenic B-cells with visceral adipocytes from old, obese mice, caused B-cells to adopt an age-associated phenotype (Frasca and Blomberg, 2017). These B-cells, which had been altered by adipose tissue, had higher levels of basal inflammation and NFκB protein within the nuclear extract, as has been shown with peripheral blood B-cells from older humans (Frasca et al., 2014). Additionally, the visceral adipose tissue of old mice has also been shown to be enriched with IgG2c (a mouse-specific IgG isoform), an observation that has also been made in obese mice (Winer et al., 2011; Frasca and Blomberg, 2017). It was proposed by Frasca and Blomberg (2017) that increased hypoxia and CCL-2 expression, along with increased adipocyte-derived TNF-α, IFN-γ, IL-6, and IL-21, prompted an obesity-driven accumulation of pro-inflammatory B-cells, which is exacerbated with ageing (Frasca and Blomberg, 2017). Nonetheless, it should be noted that older mice in this study were heavier than younger mice and had more visceral adiposity. Thus, it is difficult to tease apart the effects of ageing or obesity in this work. Other evidence suggests that metabolic secretions released from dysfunctional adipose tissue with age, rather than typical pro-inflammatory factors, contributes to B-cell dysfunction. For example, it has been shown that incubation of B-cells from young mice with leptin induces STAT-3 signalling within B-cells, leading to TNF-α and IL-6 production, possibly contributing to the elevated production of these cytokines in aged murine adipose tissue (Wu et al., 2007a; Starr et al., 2009; Frasca et al., 2016).  

7.4. CD4+ helper T-cells and CD8+ cytotoxic T-cells in aged adipose tissue

CD4+ T-cells within the visceral adipose tissue of obese mice display an ‘aged’ phenotype and accumulate 4-fold with obesity (Lumeng et al., 2011; Shirakawa et al., 2016). High-fat overfeeding in mice causes an accumulation of CD4+ T-cells in the visceral adipose tissue expressing markers associated with senescence such as CD153+PD-1+CD44highCD4+ T-cells in visceral adipose tissue. These cells secrete osteopontin, causing local inflammation (Shirakawa et al., 2016). Moreover, CD4+ T-cells expressing markers such as CD153 also accumulate in the circulation with age in mice, perhaps linking changes in blood to changes in adipose tissue, or vice versa (Tahir et al., 2015). Compared to the 4-fold increase in CD4+ T-cells in aged murine visceral adipose tissue, CD8+ T-cells exhibit a 7-fold increase, in parallel with an increase in CCR-5 gene expression, which is involved in the migration and homing of effector T-cells (Crawford et al., 2011; Lumeng et al., 2011). However, whether adipose tissue CD8+ cells impart a pro-inflammatory function with age is unclear. CD8+ T-cells within aged adipose tissue have not been studied in isolation, though when adipose tissue stromal cells from aged mice were cultured ex vivo, no difference was found in the production of IFN-γ compared to young mice (Lumeng et al., 2011). Although there was a greater production of TNF-α from aged murine adipose tissue, this
was attributed to macrophages (Lumeng et al., 2011). It is, therefore, currently unclear what role CD8+ T-cells play in aged adipose tissue. Furthermore, it is unclear what signal promotes this CD8+ T-cell expansion within aged adipose tissue and whether this contributes to, or merely reflects, the increased CD8:CD4 T-cell ratio in the circulation of older individuals (Strindhall et al., 2013).

7.5. Under-investigated cells in aged adipose tissue

Neutrophils, eosinophils, mast cells, dendritic cells, NK-cells, and iNKT-cells have a role in obesity-associated adipose tissue dysfunction. Indeed, obesity is associated with alterations in the numbers, proportions and functions of many cell types, either resulting in a pro-inflammatory change (i.e., neutrophils, mast cells, dendritic cells, NK-cells), or an anti-inflammatory change (i.e., eosinophils and iNKT-cells). These alterations contribute towards, or occur in response to, the immunometabolic dysfunction within adipose tissue caused by obesity. However, there is currently no direct evidence for a similar role for these cells within adipose tissue in an ageing context.

Although no research has specifically examined neutrophils in aged adipose tissue in mice or humans, elevated COX-2 mRNA has been shown in visceral adipose tissue from older mice, which may indicate neutrophil activation in response to elevated basal prostaglandin E2 (PGE$_2$) expression (He et al., 2001; Wu et al., 2007a; Shah et al., 2010; Grainger et al., 2013). Moreover, neutrophils migrate through tissues secreting proteases (e.g., elastase) in response to homing signals including, amongst others, leptin, and aberrant secretion of this adipokine with age may promote neutrophil entry into adipose tissue (Ahren et al., 1997; Li et al., 1997; Sánchez-Rodríguez et al., 2000; Wang et al., 2001). Neutrophils also demonstrate misdirected chemotaxis, with ageing, which, if neutrophils were signalled into adipose tissue in response to aberrant leptin secretions, would potentially exacerbate damage to the tissue, as membrane-bound proteases would be released over a wider area (Sapey et al., 2014). In obese adipose tissue, increased elastase release by tissue-resident neutrophils impacts upon the metabolic health of adipose tissue by interacting with IRS-1 and TLR-4 signalling, impairing insulin sensitivity (Devaney et al., 2003; Pham, 2006; Houghton et al., 2010; Talukdar et al., 2012). Therefore, using obesity as a model, neutrophil degranulation as a result of impaired navigation through the adipose tissue may contribute to age-associated adipose tissue dysfunction.

Mast cell and eosinophil numbers and function in adipose tissue has not been investigated in an ageing context. With eosinophils, considering the number of these cells do not change with ageing in the circulation, it might be speculated that the same is true in adipose tissue (Hazeldine et al., 2015). However, in obesity, the decline in adipose tissue eosinophils in mice might promote a pro-inflammatory macrophage predominance due to less eosinophil-derived IL-4 and IL-10. Subsequently, a loss of eosinophil-derived anti-inflammatory signals could contribute to fewer M2-like macrophages and the increase in M1-like or “double-negative” (CD11c−CD206−) macrophages with ageing in visceral adipose tissue. If mast cells are shown to be present in aged adipose tissue, the reported increase in PGE$_2$ within aged murine adipose tissue may promote mast cell degranulation, considering that PGE$_2$ inhibits the release of mediators that limit mast cell activation (Nguyen et al., 2005; Wu et al., 2007a).

Although no research has examined dendritic cells in adipose tissue with ageing, some evidence suggests that the pro-inflammatory profile of aged murine adipose tissue, as shown by
high gene expression for IL-6, TNF-α, and PGE₂, could influence dendritic cell function (Wu et al., 2007a). PGE₂ augments IL-23 production in aged murine and human dendritic cells, promoting Th-17 responses, whilst a pro-inflammatory aged adipose tissue microenvironment could promote NFκB activation within dendritic cells, as is seen in obese adipose tissue (Agrawal et al., 2009; Myer et al., 2010; Panda et al., 2010; Lee et al., 2011; Bertola et al., 2012; Shi et al., 2015). Dendritic cells from the circulation of older individuals may also have the potential to guide pro-inflammatory macrophage polarisation within adipose tissue due to increased intracellular IL-6 and TNF-α and increased expression of co-stimulatory and activation markers, as is observed in the context of obesity (Agrawal et al., 2007; Stefanovic-Racic et al., 2012; Shaw et al., 2013; Wang et al., 2014). Nonetheless, these observations have yet to be investigated within aged adipose tissue and can, therefore, only represent speculative mechanisms at present.

We are unaware of any research that has investigated the role of NK-cells in aged adipose tissue. However, in mice, aberrant leptin secretions have been suggested to drive specifically-activated CD56<sup>dim</sup> NK-cell polarisation, contributing to elevated TNF-α production and metabolic impairments (Sánchez-Rodriguez et al., 2000; Tian et al., 2002; Theurich et al., 2017). As well as leptin, the elevated TNF-α within aged murine adipose tissue may also serve to recruit NK-cells as TNF-α is essential for NK-cell recruitment into other organs, such as the liver (Pilaro et al., 1994). It remains unclear whether the aberrant secretory profile of aged adipose tissue influences NK-cell function. However, as circulating CD56<sup>dim</sup> NK-cells accumulate in the blood of older people, if these cells were recruited into adipose tissue by TNF-α, leptin, or another yet to be established signal, they may contribute towards pro-inflammatory macrophage polarisation (Krishnaraj, 1997; Borrego et al., 1999; Chidrawar et al., 2006; Hayhoe et al., 2010; Almeida-Oliveira et al., 2011; Bonamichi and Lee, 2017).

Adipose tissue iNKT-cells have not been investigated with ageing, but considering that IL-4 and IL-10 producing NK1.1<sup>+</sup> iNKT-cells decline with obesity (van Eijkeren et al., 2017), it is possible that ageing has similar effects. However, given endogenous lipid antigens derived from adipocytes influence the secretion profile of iNKT-cells (Bai et al., 2009; Birkholz et al., 2015; van Eijkeren et al., 2017), then interactions between adipocytes and iNKT-cells might contribute towards iNKT-cell dysregulation in aged adipose tissue. For example, loading glycolipids into the CD1d receptor on adipocytes is mediated by intracellular ceramides (van Eijkeren et al., 2017) and sphingolipid ceramide content is higher in adipose tissue from old mice compared to young (Wu et al., 2007a). Thus, in principle, enhanced ceramide-mediated glycolipid loading within aged adipocytes might drive over-activation of iNKT-cells with ageing.

8. **Summary, considerations and future directions**

Poor metabolic control, insulin resistance, inflammation, and impaired immune function are hallmarks of ageing and also characteristics of obesity. In addition, as outlined in this review, individuals who are obese, or elderly, exhibit similar immunological profiles in adipose tissue (Table 3A-D). Given the overlap between obesity and ageing, it is likely that changes to the numbers and function of some immune cells within obese and aged adipose tissue—especially macrophages—contribute to dysfunction of this tissue. Moreover, it is possible that alterations to the immune profile of obese adipose tissue accelerates local and whole-body ageing. Finally, changes to the numbers and function of some immune cells within obese, and likely aged adipose
tissue, whether a cause or consequence of adipose tissue dysfunction, may also underpin the development of metabolic diseases, as shown with obesity. However, there is limited work in an ageing context.

Improving our understanding of the interactions between ageing and adipose tissue dysfunction may help with the development of therapeutic and preventative strategies to improve longevity and quality of life in ageing populations. For example, with obesity, immunological dysregulation in adipose tissue is a well-established contributor to type-II diabetes and has consequently been a target for strategies such as caloric restriction, physical activity, and pharmacological manipulation. Caloric restriction imparts beneficial effects on metabolic control and blunts the production of pro-inflammatory cytokines from obese, murine adipose tissue (Kurki et al., 2012; Fabbiano et al., 2016). Caloric restriction in older mice also delays ageing, in-part due to beneficial effects on adipose tissue metabolic health and function (Miller et al., 2017).

Moreover, in obese women, short (1 month) and long-term (6 months) caloric restriction promotes an anti-inflammatory profile in abdominal, subcutaneous adipose tissue, and reduces total adipose tissue macrophage content (Clement et al., 2004; Kovacikova et al., 2011). Physical activity can reduce systemic inflammation, targeting dysregulated chemokine and adipokine secretory patterns in mice and humans (Baturcam et al., 2014; Chen et al., 2017; Thompson et al., 2012a; Yudkin, 2008). In parallel, physical activity also limits the accumulation of immune cells such as CD8+ T-cells—potentially by attenuating RANTES/CCR-5 signalling within human adipose tissue (Baturcam et al., 2014). Moreover, physical activity reduces neutrophil number and elastase expression, as well as shifting macrophages to an M2-like phenotype within the adipose tissue of high-fat over-fed mice (Baturcam et al., 2014; Kawanishi et al., 2010; Kawanishi et al., 2015). In addition, exercise might limit, or delay, immunosenescence by inducing an anti-inflammatory/anti-oxidative microenvironment within aged adipose tissue, limiting non-specific activation of T-cells and propagation of inflammation, by dampening adipose tissue-derived signals (Gleeson et al., 2011; Radak et al., 2008; Turner, 2016). Finally, pharmacological therapies (e.g., the PPAR-γ agonists rosiglitazone) promote M2-like macrophage repolarisation after high-fat overfeeding in mice (Stienstra et al., 2008). Further, PPAR signalling overlaps with the network of longevity genes, and PPAR-γ2 (adipocyte specific) deficient mice have a considerably-reduced lifespan, possibly mediated by the anti-inflammatory effects on adipose tissue macrophages (Argmann et al., 2009; Picard and Guarente, 2005; Stienstra et al., 2008). Therefore, targeting immunological dysregulation in adipose tissue might promote healthy ageing.

In conclusion, obesity is a useful model to develop our understanding of age-associated adipose tissue dysfunction. There is now an improved appreciation of how this metabolically and immunologically active tissue influences ageing and immunometabolic disease(s). The available evidence indicates that age-associated adipose tissue dysfunction occurs irrespective of changes in adipose tissue masses, and this dysfunction appears to play an active role in the pathophysiology of ageing. Moreover, the accumulation of predominantly pro-inflammatory immune cells in obese adipose tissue exacerbates the immunometabolic dysfunction of the tissue and might act as a potent stimulus for accelerating ageing in obesity. Importantly, countermeasures that target inflammation within dysfunctional adipose tissue offer promise to reduce the burden of obesity- and age-associated immunometabolic diseases.
9. References


O'Rourke, R.W., Metcalf, M.D., White, A.E., Madala, A., Winters, B.R., Maizlin, II, et al. (2009). Depot-specific differences in inflammatory mediators and a role for NK cells and IFN-


Dendritic cells promote macrophage infiltration and comprise a substantial proportion of obesity-associated increases in CD11c+ cells in adipose tissue and liver. *Diabetes* 61(9), 2330-2339. doi: 10.2337/db11-1523.


chemokine and chemokine receptor expression. *Int J Obes (Lond)* 34(12), 1684-1694. doi: 10.1038/ijo.2010.103.


Legends to figures

Figure 1. Agreement between RT-PCR and flow cytometric assessment of macrophage abundance in lean, overweight, and obese individuals subcutaneous abdominal adipose tissue. Relative gene expression of CD68 was used to identify macrophages (n=30) and presented as mean 2ΔΔCt ± SEM. Proportions of macrophages in the adipose tissue SVF as a percentage of total cells (n=17). Macrophages were identified as CD45+HLA-DR+CD16+ cells. Presented with permission from Travers et al (2015). CD, Cluster of differentiation; HLA-DR, Human leukocyte antigen-antigen D related; RT-PCR, Reverse transcription polymerase chain reaction; SEM: standard error of the mean.

Figure 2. Adipocyte-macrophage interactions with obesity. With obesity, adipocytes expand promoting a state of pro-inflammation, ER stress and tissue hypoxia, promoting adipocyte apoptosis/necrosis. Upon these changes in obese adipose tissue macrophages are signalled into the tissue by hypoxic, chemokine, and cytokine signals to surround dysfunctional adipocytes. Macrophages in obese adipose tissue release and are exposed to TNF-α and MCP-1 among other factors, including SFAs, which collectively promote the activation of a variety of stress signalling cascades. Consequently, adipose-resident macrophages adopt a pro-inflammatory secretory profile and upregulate their surface expression of CD11c. As a result, adipocytes become exposed to these secreted factors such as TNF-α and MCP-1, exacerbating the pro-inflammatory microenvironment of the tissue, potentiating the activation of stress signal cascades within adipocytes. These interactions promote a feed-forward recruitment of monocytes into the tissue and their pro-inflammatory polarisation as well as impairing tissue insulin sensitivity which potentially promotes the release of FFAs into the microenvironment. CD, Cluster of differentiation; ER, Endoplasmic reticulum; FFAs, Free-fatty acids; HIF-1α, Hypoxia-inducible factor 1 alpha; IKKβ, Inhibitor of nuclear factor kappa B kinase subunit beta; IL, interleukin; IL-1R, Interleukin-1 receptor; iNOS, inducible nitric oxide synthase; IRS-1, Insulin receptor substrate 1; JNK, c-Jun N-terminal kinases; MAPK, Mitogen-activated protein kinases; MCP-1, Monocyte chemoattractant protein 1; NFκB, Nuclear factor kappa B; NLRP3, NLR family pyrin domain containing 3; PPAR-γ, Peroxisome proliferator-activated receptor gamma; ROS, Reactive oxygen species; SFAs, Saturated fatty acids; TNFR, Tumour necrosis factor receptor; TNF-α, Tumour necrosis factor alpha.

Figure 3. A model of obesity-driven immunological changes within adipose tissue. Under a chronic state of positive energy balance, adipose tissue undergoes a multitude of changes that include the hypertrophic expansion of adipocytes without concurrent angiogenic responses. The consequence of these adaptations include local tissue hypoxia as a result of reduced tissue blood flow and impaired oxygen delivery. The rapid expansion of adipocytes also leads to unstable adipocytes prone to rupturing, releasing their lipid contents. This instability is further exacerbated by an exhaustion of the preadipocytes required to facilitate adipose tissue expansion. The net result of these physiological changes to adipocytes is the release of DAMPs and other signalling molecules that initiate stress kinase activation and promote monocyte infiltration to manage the remodelling of the ECM. Neutrophils are the first cells to accumulate in adipose tissue, followed by a range of other immune cells, most notably; B-cells, CD8+ T-cells, and M1-like/intermediate, or “double-negative” (CD11c–CD206–) macrophages that surround unstable adipocytes in crown-
like structures. As a consequence, a Th-1 and M1 cytokine profile predominated and some anti-inflammatory immune cells such as eosinophils and iNKT-cells leave the tissue. Other anti-inflammatory cells, including regulatory T-cells may increase to compensate. Pro-inflammatory cytokines and adipokines dominate the tissue microenvironment potentiating the activation of stress kinases and inflammasomes. Local inflammation reduces adipose tissue insulin sensitivity, impairing metabolic health and promoting ectopic lipid deposition. Pro-inflammatory adipose tissue contributes to the chronic, low-grade inflammation characteristic of obesity and metabolic disease. 

CD, Cluster of differentiation marker; DAMPSs, Damage-associated molecular patterns; ECM, Extracellular matrix; IFN-γ, Interferon gamma; Ig, Immunoglobulin; IL, Interleukin; iNKT-cell, invariant natural killer T cell; MCP-1, Monocyte chemotactic protein-1 (CCL-2); MIP-1α, macrophage inflammatory protein 1 alpha (CCL-3); PAI-1, Plasminogen activator inhibitor 1; RANTES, Regulated on activation, normal T-cell expressed and secreted (CCL-5); Th, Helper T cell; TNF-α, Tumour necrosis factor alpha.

Figure 4. Age-associated changes in adipose tissue macrophages and immunometabolic health in humans (a) and mice (b). This schematic is a theoretical representation based on the limited empirical evidence that is available. With advancing age in humans (a), subcutaneous adipose tissue macrophage content increases steadily until 31-33 years whereupon numbers slightly decline, until 45 years old (Ortega Martinez de Victoria et al., 2009). Although absolute adipose tissue macrophage content declines from around the age 31-33 years old until 45 years old, at least, the presence of more pro-inflammatory activated macrophages [M1-like/ intermediate, or “double-negative” (CD11c−CD206−)] macrophages] increases, which is associated with a decline in insulin sensitivity. With advancing age, insulin sensitivity continues to decline whilst low-grade systemic inflammation begins to steadily increase from middle age into old age. With advancing age in mice (b), an increase in pro-inflammatory macrophages within visceral adipose tissue that produce TNF-α and IL-6 and have downregulated PPAR-γ expression is also observed up to 18-24 months old, at least (Lumeng et al., 2011; Wu et al., 2007a). Further, in middle-aged mice (44 weeks old), “double-negative” (CD11c−CD206−) and M2-like macrophages are largely unchanged, or are modestly elevated, whilst M1-like macrophages exhibit a slight decline (Bapat et al., 2015). Therefore, it is theorised that there is a continued pro-inflammatory macrophage activation/polarisation with advancing age in humans, independent of changes in absolute counts within the adipose tissue. Further, the same pattern of events appears to occur within murine adipose tissue, though the age at which this shift to a pro-inflammatory bias occurs is unknown. The similarities between data from aged mice and humans indicate that macrophages adopt a predominantly pro-inflammatory M1-like/ intermediate, or “double-negative” (CD11c−CD206−) phenotype into old age, contributing to the increased systemic inflammation and impaired metabolic health exhibited by older individuals. CD, Cluster of differentiation marker; IL, Interleukin; PAI-1, Plasminogen activator inhibitor 1; PPAR-γ, Peroxisome proliferator-activated receptor gamma; TNF-α, Tumour necrosis factor alpha.
**Tables**

Table 1. Appraisal of three commonly utilised methods of assessing the cells of the adipose tissue stromal fraction.

<table>
<thead>
<tr>
<th>Method</th>
<th>Benefits</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunohistochemistry</td>
<td>- Small amount of tissue required</td>
<td>- Manual counting is subject to investigator bias</td>
</tr>
<tr>
<td></td>
<td>- Information on cell locality</td>
<td>- Time consuming for manual counting</td>
</tr>
<tr>
<td></td>
<td>- Intact undisturbed tissue sample</td>
<td>- Limited to 2 or 3 antibodies per tissue slice</td>
</tr>
<tr>
<td></td>
<td>- Long-term storage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Some sample remains available for future analysis</td>
<td></td>
</tr>
<tr>
<td>Flow Cytometry</td>
<td>- Commonly 10-12 antibodies can be used in parallel</td>
<td>- Extensive processing required to extract the stromal fraction possibly leading to artefacts</td>
</tr>
<tr>
<td></td>
<td>- Thousands of cells analysed from one sample</td>
<td>- Possibility for selective cell type loss with processing</td>
</tr>
<tr>
<td></td>
<td>- Absolute cell counts can be determined</td>
<td>- Cell auto-fluorescence complicates gating of some populations (i.e., macrophages)</td>
</tr>
<tr>
<td></td>
<td>- Isotype control and fluorescence minus one tubes can be added to an experiment to minimise the influence of subjectivity when gating populations</td>
<td>- Large (&gt;300 mg) tissue sample required</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Small margin for bias when gating cell populations</td>
</tr>
<tr>
<td>Gene and Protein Expression</td>
<td>- Very little tissue required</td>
<td>- Requires isolation of a specific marker not expressed on any other cell type</td>
</tr>
<tr>
<td>(RT-PCR and Western Blot)</td>
<td>- Little processing of the tissue required prior to freezing</td>
<td>- Cannot be multiplexed by examining multiple markers on cell types</td>
</tr>
<tr>
<td></td>
<td>- Long-term storage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Some sample remains available for future analysis</td>
<td></td>
</tr>
</tbody>
</table>

*mg, milligrams; RT-PCR, Reverse-transcription polymerase chain reaction.*
Table 2. Adipose tissue macrophage phenotypic classifications in humans.

<table>
<thead>
<tr>
<th>Type</th>
<th>Cell Surface Expression Profile</th>
<th>Secretion Profile</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1-like</td>
<td>CD14+ CD16++ CD11c+ CD31+ CD44+ CD45+ CD206– HLA-DR+</td>
<td>TNF-α, IL-6, IL-8, IL-1β, NO</td>
<td>Pro-inflammatory, pro-oxidative, antimicrobial, pathogen clearance</td>
</tr>
<tr>
<td>“classically-activated”</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate population</td>
<td>CD14+ CD16+ CD11c+ CD45+ CD206+</td>
<td>TNF-α, IL-6, IL-8</td>
<td>Pro-inflammatory and pro-oxidative, tissue remodeling</td>
</tr>
<tr>
<td>M2-like</td>
<td>CD14++ CD16+ CD45+ CD163+ CD206+</td>
<td>IL-10, TGF-β</td>
<td>Anti-inflammatory, fibrosis, tissue surveillance, angiogenesis</td>
</tr>
<tr>
<td>“alternatively-activated”</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Text in italics and speech marks emphasises other common terms to describe these cells. Intermediate population refers to adipose tissue macrophages expressing M2-like surface markers (e.g., CD206) whilst demonstrating an M1-like pro-inflammatory function. Expression pattern of CD14 and CD16 is best established on monocytes rather than macrophages. It is important to note that macrophage populations within adipose tissue can express markers of alternatively activated cells (e.g., CD206+) whilst also imparting a pro-inflammatory, classically activated function within the tissue, highlighting the spectrum of macrophage polarity within adipose tissue. CD, Cluster of Differentiation; HLA-DR, Human Leukocyte Antigen – antigen D Related; IL, Interleukin; NO, Nitric Oxide; TGF-β, Transforming Growth Factor–beta; TNF–α, Tumour Necrosis Factor–alpha.
Table 3A. A comparison of the change in abundance for select immune cell subsets within adipose tissue with age and obesity compared to young and lean, respectively.

<table>
<thead>
<tr>
<th>Immune Cells Within Adipose Tissue</th>
<th>Ageing</th>
<th>Ageing References</th>
<th>Obesity</th>
<th>Obesity References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8+ T-cells</td>
<td>↑</td>
<td>(Lumeng et al., 2011)</td>
<td>↑</td>
<td>(Nishimura et al., 2009)</td>
</tr>
<tr>
<td>CD4+ T-cells</td>
<td>↑ → ←→</td>
<td>(Lumeng et al., 2011)</td>
<td>↑</td>
<td>(Travers et al., 2015)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Shirakawa et al., 2016)</td>
</tr>
<tr>
<td>Regulatory T-cells</td>
<td>↑ (?)</td>
<td>(Bapat et al., 2015)</td>
<td>↑ / ↓</td>
<td>(Travers et al., 2015)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Deng et al., 2017)</td>
</tr>
<tr>
<td>B-cells</td>
<td>↑ (?)</td>
<td>(Frasca and Blomberg, 2017)</td>
<td>↑</td>
<td>(Winer et al., 2011)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(McDonnell et al., 2012)</td>
</tr>
<tr>
<td>Macrophages</td>
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</tr>
<tr>
<td>M1-like/Intermediate/DN</td>
<td>↑</td>
<td>(Lumeng et al., 2011)</td>
<td>↑</td>
<td>(Wentworth et al., 2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Wu et al., 2007a)</td>
<td></td>
<td>(Pirzgalska et al., 2017)</td>
</tr>
<tr>
<td>M2-like</td>
<td>↓</td>
<td>(Ortega Martinez de Victoria et al., 2009)</td>
<td>↓</td>
<td>(Travers et al., 2015)</td>
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<tr>
<td></td>
<td></td>
<td>(Camell et al., 2017)</td>
<td></td>
<td>(Suganami et al., 2007)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>↑ (?)</td>
<td>(Wu et al., 2011)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mast cells</td>
<td>↑ (?)</td>
<td>(Liu et al., 2009)</td>
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<td></td>
<td></td>
<td>(Zhou et al., 2015)</td>
<td></td>
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<td></td>
<td></td>
<td>(Chmelař et al., 2016)</td>
<td></td>
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<tr>
<td>NK-cells</td>
<td>↑</td>
<td>(Theurich et al., 2017)</td>
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<tr>
<td></td>
<td></td>
<td>(Wensveen et al., 2015)</td>
<td></td>
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<tr>
<td>iNKT-cells</td>
<td>↓</td>
<td>(Huh et al., 2017)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>(Lynch et al., 2012)</td>
<td></td>
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<tr>
<td>Neutrophils</td>
<td>↑</td>
<td>(Talukdar et al., 2012)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>(Shah et al., 2010)</td>
<td></td>
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<tr>
<td>Dendritic cells</td>
<td>↑</td>
<td>(Hannibal et al., 2017)</td>
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<td></td>
<td></td>
<td>(Stefanovic-Racic et al., 2012)</td>
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</tbody>
</table>

↓, decrease; ↑, increase; ←→, little or no change or equivocal data; ?, unclear or no evidence; nd, no data available. CD, Cluster of differentiation marker; DN, Double-negative macrophage (CD11c−CD206−); iNKT-cell, invariant natural killer T cell; NK-cell, Natural killer cell.
<table>
<thead>
<tr>
<th>Broader Tissue Specific Changes</th>
<th>Ageing</th>
<th>Ageing References</th>
<th>Obesity</th>
<th>Obesity References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipocyte size</td>
<td>↑</td>
<td>(Kim et al., 2014)</td>
<td>↑</td>
<td>(Reilly and Saltiel, 2017)</td>
</tr>
<tr>
<td>Endoplasmic reticulum stress</td>
<td>↑</td>
<td>(Ghosh et al., 2015)</td>
<td>↑</td>
<td>(Kawasaki et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>↑</td>
<td>(Ghosh et al., 2016)</td>
<td></td>
<td>(Suzuki et al., 2017)</td>
</tr>
<tr>
<td>Mitochondrial dysfunction</td>
<td>↑</td>
<td>(Soro-Arnaiz et al., 2016)</td>
<td>↑</td>
<td>(Kusminski and Scherer, 2012)</td>
</tr>
<tr>
<td>Stem cell exhaustion</td>
<td>↑</td>
<td>(Pérez et al., 2016)</td>
<td>↑</td>
<td>(Pérez et al., 2016)</td>
</tr>
<tr>
<td>Tissue blood flow</td>
<td>↓</td>
<td>(Valli et al., 2015)</td>
<td>↓</td>
<td>(Pasarica et al., 2009)</td>
</tr>
<tr>
<td>Tissue hypoxia</td>
<td>↑</td>
<td></td>
<td>↑</td>
<td>(Ye et al., 2007)</td>
</tr>
</tbody>
</table>

↓, decrease; ↑, increase.

<table>
<thead>
<tr>
<th>Soluble and Secreted Factors Within Adipose Tissue</th>
<th>Ageing</th>
<th>Ageing References</th>
<th>Obesity</th>
<th>Obesity References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>↑</td>
<td>(Starr et al., 2009)</td>
<td>↑</td>
<td>(Mohamed-Ali et al., 1997)</td>
</tr>
<tr>
<td>MCP-1</td>
<td>↑</td>
<td>(Lumeng et al., 2011)</td>
<td>↑</td>
<td>(Amano et al., 2014)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>↑</td>
<td>(Wu et al., 2007a)</td>
<td>↑</td>
<td>(Hotamisligil et al., 1993)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>←→</td>
<td>(Lumeng et al., 2011)</td>
<td>↑</td>
<td>(Nishimura et al., 2009)</td>
</tr>
<tr>
<td>IL-10</td>
<td>←→</td>
<td>(Lumeng et al., 2011)</td>
<td>↓</td>
<td>(Lumeng et al., 2007)</td>
</tr>
<tr>
<td>IL-1ß</td>
<td>↑</td>
<td>(Wu et al., 2007a)</td>
<td>↑</td>
<td>(Morris et al., 2011)</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>↓←→</td>
<td>(Atzmon et al., 2008)</td>
<td>↓</td>
<td>(Kawano and Arora, 2009)</td>
</tr>
<tr>
<td>Leptin</td>
<td>↑</td>
<td>(Ahren et al., 1997)</td>
<td>↑</td>
<td>(Zhang et al., 1994)</td>
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<tr>
<td></td>
<td></td>
<td>(Li et al., 1997)</td>
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</tbody>
</table>

↓, decrease; ↑, increase; ←→, little or no change or equivocal data. IFN-γ, Interferon gamma; IL, Interleukin; MCP-1, Monocyte chemotactic protein 1; TNF-α, Tumour necrosis factor alpha.
Table 3D. Changes in the expression of proteins within adipose tissue (adipocytes and cells of the stromal fraction) with age and obesity compared to young and lean, respectively.

<table>
<thead>
<tr>
<th>Proteins Expressed Within Adipose Tissue</th>
<th>Ageing</th>
<th>Ageing References</th>
<th>Obesity</th>
<th>Obesity References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR-2</td>
<td>↑</td>
<td>(Lumeng et al., 2011)</td>
<td>↑</td>
<td>(Weisberg et al., 2006)</td>
</tr>
<tr>
<td>CCR-5</td>
<td>↑</td>
<td>(Lumeng et al., 2011)</td>
<td>↑</td>
<td>(Wu et al., 2007b)</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>(Kintscher et al., 2008)</td>
</tr>
<tr>
<td>CCR-7</td>
<td>↓</td>
<td>(Lumeng et al., 2011)</td>
<td>↓</td>
<td>(Zeyda et al., 2010)</td>
</tr>
<tr>
<td>CX3CR-1</td>
<td>↓</td>
<td>(Lumeng et al., 2011)</td>
<td>↑</td>
<td>←→</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Shah et al., 2011)</td>
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<td>(Polyak et al., 2014)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(Morris et al., 2012)</td>
</tr>
<tr>
<td>CXCR-3</td>
<td>↑</td>
<td>(Lumeng et al., 2011)</td>
<td>↑</td>
<td>(Deiuliis et al., 2014)</td>
</tr>
<tr>
<td>PGE$_2$</td>
<td>↑</td>
<td>(Wu et al., 2007a)</td>
<td>↑</td>
<td>(Garcia-Alonso et al., 2016)</td>
</tr>
<tr>
<td>PPAR-γ</td>
<td>↓</td>
<td>(Wu et al., 2007a)</td>
<td>↑</td>
<td>(Vidal-Puig et al., 1997)</td>
</tr>
</tbody>
</table>

↓, decrease; ↑, increase; ←→, little or no change or equivocal data. CCR, (C-C motif) chemokine receptor; CX3CR-1, (C-X3-C motif) chemokine receptor 1; CXCR-3, (C-X-C motif) chemokine receptor 3; PPAR-γ, Peroxisome proliferator activated receptor gamma.
CD68 Relative Gene Expression

CD45+HLA-DR+CD16+ Cells

Relative gene expression

Proportion of Macrophages in SVF (% Total)

Lean Overweight Obese

Lean Overweight Obese

LN(2^(-ΔΔCt)) mean (SEM)
**Key:**
- M1-like/Intermediate or “double negative” Macrophage
- M2-like macrophage

**a.**
Adipose Tissue

(Systemic Insulin Sensitivity vs. Systemic Inflammation)

(Ortega Martinez de Victoria et al., 2009)

Human Age (years)

20 30 40 50 60 ≥70

No data available

**b.**
Adipose Tissue

(Systemic Insulin Sensitivity vs. Systemic Inflammation)

(Bapat et al., 2015) (Lumeng et al., 2011; Wu et al., 2007a)

Mouse Age (months)

≤4 8 12 16 20 24

TNF-α IL-6 PPAR-γ