Adipose Tissue Inflammation in Aging

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Abstract

Adipose tissue has traditionally been viewed as an organ of interest within studies of obesity and diet-associated metabolic disorders. However, as studies reveal the role white adipose tissue plays as an energy storage, a lipid metabolism site, and an adipokine secretor, it has become recognized as an organ of importance for metabolic health in both the young obese and the old obese. Within the realms of aging research, the pursuit of senolytics have taken the field’s spotlight, where the clearance of senescent cells have shown to attenuate aspects of age-related disorders. More interestingly, these senolytics have also revealed that these senescent cells, specifically p16<sup>Ink4a</sup> cells, accumulate within adipose tissue, skeletal muscles, and eye. These results implicate the importance of adipose tissue inflammation in aging and widens the discussion on how senescent cells among other immune and non-immune cells cross paths to influence an organism’s lifespan and healthspan.

Introduction

The Centers for Disease Control and Prevention (CDC) estimates that by year 2030, 1 in 5 Americans will be 65 years or older. This statistic would implicate two challenges. This proportion of older adults will be unprecedented in the United States, increasing both geriatric healthcare costs and the volume of age-associated diseases. Medical research is challenged with a shift from focusing on infectious diseases and acute illnesses towards chronic and degenerative diseases. Chronic and degenerative diseases cause a decrease in the quality of life of elderlies, driving interest in research to investigate both healthspan and lifespan.

The past century has seen an impressive increase in human life expectancy and prevalence of obesity. Body weight and body mass index (BMI) in humans reach their peak at the 6th decade of life. The National Health and Nutrition Examination Survey (NHANES) estimated that 37% of 60 years and older adults are obese, with BMI ≥30. However, body fat percentage (the total mass of fat divided by total body mass) does not reach its maximum until the 7th and 8th decades. Women generally have a higher percentage of body fat than men, in part as a response to the demand of pregnancies. There is also a change in fat distribution with aging, including a relative increase in intra-abdominal fat and ectopic fat.
deposition. Importantly, obesity in aging has been linked to many aging-associated chronic diseases. Excess weight in old age also contributes to the decline in physical function, loss of independence, and the development of frailty.

**The Seven Pillars of Aging**

Targeting chronic diseases is a major challenge, as they are often intertwined. Nonetheless, clinicians and researchers alike have accepted that age-related diseases and dysfunction stems from similar pathways, mechanisms, and even biological bases—therefore, underlying processes can be targeted to impede aging or revert age defects. The ‘Seven Pillars of Aging’ serves as a visual model to depict the interconnectedness of aging processes and link them to chronic pathologies. The seven pillars consist of: metabolism, macromolecular damage, epigenetics, inflammation, adaptation to stress, proteostasis, stem cells and regeneration.

Inflammation is of particular interest, as it is a ubiquitous trait of aging tissues and is present in most, if not all, age-associated chronic diseases.

**Inflammaging**

Human aging is in part, characterized by a chronic, low-grade inflammation that develops in various aging tissues. This phenomena is termed ‘inflammaging’. The health detriments and benefits of inflammaging are inconclusive. While super centenarians boast a higher inflammatory cytokine profile, inflammatory mediators are critical to the pathogenesis of age-related diseases such as arthritis, diabetes, sarcopenia, cardiovascular disease (CVD), cancer, and dementia. While sources of inflammaging remain under investigation, some prominent conversations include immunosenescence, self-debris, senescent cells, mitochondria dysfunction, microbiome, and adipose tissue.

This review aims to highlight and discuss adipose tissue inflammation in aging, not only as a component of inflammaging but also as an independent dialogue of how adipose tissue inflammation affects health in old age.

**Adipose Tissue Inflammation in Aging and Obesity**

Adipose, or fat tissue, is the largest endocrine organ in humans and in other cases, can be the largest organ in an obese individual. Adipose tissue plays a pivotal role in age-related metabolic dysfunction and longevity. With old age, fat distribution shifts from subcutaneous to visceral fat depots, while triglycerides ectopically deposit on liver, muscle, bone marrow, and heart. These changes are associated to the development and progression of a variety of age-associated diseases.

Obesity accelerates the onset of these age-associated diseases, further emphasizing the level of impact adipose tissue plays in aging. Similar to inflammaging, obesity is linked to a systemic, chronic, low-grade inflammation. Adipose tissue inflammation in obesity is also termed ‘metaflammation’. While most recent studies focus on how adipose tissue dysfunction progresses in diet-induced obesity, much less efforts have been devoted to understanding the pathogenic mechanisms of old-age obesity. Whether inflammaging and metaflammation share common inflammatory pathways or have similar sources of...
inflammation, including the role of different fat depots, are important questions. It is likely there are fundamental differences between diet- versus age-dependent obesity, given the widespread immunological and physiological changes that are known to occur in old age.

The majority of interventions that extend lifespan function through nutrient sensing and processing pathways, and they have important effects on adipose tissue formation and function. Growth hormone deficient mice, in addition to improved lifespan, have less ectopic fat deposition, improved adipocyte progenitor cell function, and reduction in cellular senescence. A number of single-gene mutations are known to extend lifespan in lower organisms, and similar lifespan extension is observed even if the mutations are restricted to adipose tissue.

**Obesity Paradox and Aging**

The obesity paradox refers to a collection of unexpected findings, where several chronic diseases, including cardiovascular, have lower all-cause mortality rates in elevated BMI patients. The “obese healthy” implicates that having a higher BMI can be protective and that the “lean” BMI is in actuality, not the lowest mortality group. For example, obese (BMI >30) and severely obese (BMI >40) patients after coronary artery bypass grafting are at lower risk for postoperative complications than patients with a lower but “normal” (18.5 < BMI <25) categorization. It has been controversial as to how this can occur—and many of these observations were thought to be particularly true in elderly patients. It is reasonable to postulate that, in some circumstances, adiposity may confer a degree of biological resiliency that enhances recovery after a stressful life event.

Studies described the mortality risks as a ‘U-shaped curve’ for the elderly while the younger individuals have a ‘check-mark’ or ‘j’ shape mortality curve. Where increased BMI in the overweight and even obesity ranges have exhibited protective effects on patients, although true in the datasets, also held many caveats in the design study. When body composition was measured using a different method, it seems BMI frequently misclassifies body fat status, and it was argued that BMI is a better predictor of lean body mass than of adiposity. Therefore, patients with higher BMI are protected by higher lean body mass and not body fat.

There are many other prominent theories summarized—with some arguing that lean individuals plagued with chronic conditions could also be suffering ‘malnutrition-inflammation complex syndrome’ which would be worse than carrying a high BMI. It was also hypothesized that young obese with abdominal obesity die earlier and those who survive towards higher age categories like those of the obesity paradox studies actually carry greater degree of lower-body obesity. Alternate theories suggest muscle quality determined by muscle mass and grip strength would also be important indicators of health. Others suggest genetic predisposition contributes to the formation of these obese healthy or metabolically obese phenotypes observed.
Diet-induced Adipose Tissue Inflammation

Adipose Tissue Immunological Profile During Diet-induced Obesity

In obesity, adipose tissue homeostasis is perturbed: the balance between energy intake and expenditure is pushed towards the former. In aging, there is also a reduction in resting metabolic rate and lower total daily energy expenditure, particularly in frail individuals\textsuperscript{31}. With excessive accumulation and expansion of adipose tissue in obesity, there is an increased likelihood of metabolic syndrome, cardiovascular disease and type 2 diabetes mellitus\textsuperscript{32}. Adipose tissue inflammation, specifically white visceral-gonadal adipose tissue, is a major contributor to metaflammation and insulin resistance. Compared to lean individuals, white adipose tissue (WAT) from obese adults secretes higher levels of tumor necrosis factor α (TNFα), an inflammatory cytokine capable of interfering insulin signaling. When TNFα is inhibited, glucose tolerance and insulin sensitivity is improved\textsuperscript{33}. Pro-inflammatory cytokines and chemokines such as interleukin 1b (IL-1b), monocyte chemoattractant protein (MCP-1), and interleukin 6 (IL-6) are also secreted at elevated levels from obese adipose tissue macrophages\textsuperscript{34}.

Adipose Tissue Macrophages, Inflammation, and Organelle Stress

Aging is associated with important changes in the innate immune system. Macrophages perform important innate immune functions including phagocytic clearance of dying cells. Depending on the stimuli, macrophages can become polarized into “M1” or “M2” subsets. Classically-activated or M1 “killer” macrophages and alternatively activated M2 “healing” macrophages are convenient terms that describe the plasticity of macrophage subsets and function. However, it is important to note that macrophages may not form clear-cut activation subsets nor expand clonally\textsuperscript{35}. Studies aimed to elucidate the source of inflammation in obesity have found that macrophages accumulate in WAT\textsuperscript{36} and exhibit a predominantly proinflammatory “M1” profile, as compared to the less or anti-inflammatory “M2” in lean healthy control adipose tissue\textsuperscript{37,38}. Hypertrophy and hypoxia also leads to the formation of crown-like structures where macrophages surround a dead or dying adipocyte\textsuperscript{39}. This very characteristic crown-like structure is used as one means of quantifying levels of inflammation in adipose tissue and has been shown to persist in the tissue even with weight loss in mice\textsuperscript{40}.

In obesity, adipose tissue macrophages are burdened with an increase in adipocyte death, leading to inflammasome activation, and formation of crown-like structures in the adipose. Diet-induced obesity has also been shown to increase endoplasmic reticulum (ER) stress in mice, involving all 3 major branches of the unfolded protein response (UPR)—BIP, CHOP, and ATF4\textsuperscript{41,42}. The higher ER stress responses involving IRE1α and CHOP has recently been shown to directly lead to stress-induced polarization of macrophages\textsuperscript{43,44} away from the M2 state, contributing to the domination of M1 polarization in adipose tissue.

Outside of macrophages, the immune cell profile of obese WAT undergoes a multitude of other changes. CD4+ and CD8+ effector T cell ratios also shift towards CD8+ T cells—a significant number of CD8+ T cells infiltrate the adipose tissue while CD4+ T cell numbers remain low\textsuperscript{45} during diet-induced obesity. It is also suggested that this is how T cells can
initiate adipose tissue inflammation via recruitment and stimulation of resident ATMs.\textsuperscript{45} Of note, regulatory T cells (Tregs) in the adipose tissue are also reported to be diminished in lean animals when compared to obese.\textsuperscript{46} Other studies have also reported on how eosinophils promote adipose tissue inflammation via supplying ATMs with a steady source of IL-4 in the tissue.\textsuperscript{47} While similar to CD8+ T cells, B cells during obesity can accumulate in the adipose tissue and secrete pathogenic IgG, spurring further inflammation, insulin resistance, and glucose intolerance.\textsuperscript{48} Mast cells have been shown to also contribute to inflammation and insulin resistance via production of IL-6 and IFN$\gamma$.\textsuperscript{49} The immune profile of obese WAT all contribute to adipose tissue inflammation and sustenance of proinflammatory polarization of ATMs.

Non-immune Players in Adipose Tissue Inflammation

As previously mentioned, adipose tissue is an endocrine organ. Adipocytes can by themselves secrete adipokines which are chemokines and cytokines, some more unique to the adipose tissue and metabolism (e.g., leptin, adiponectin) while others are familiar to the immune system (IL-6, TNF$\alpha$).\textsuperscript{50} During obesity, adipocytes increase proinflammatory cytokine and chemokine secretion of IL-6 and TNF$\alpha$, where TNF$\alpha$ has been shown to increase insulin resistance in diet-induced obese mice.\textsuperscript{51} Apart from immune cell infiltration, adipose tissue dysfunction in obesity is complicated by abnormalities of lipid metabolism.\textsuperscript{52,53} While adipose dysfunction is not solely reliant on immune cell-driven inflammation, adipocyte function also cross-talks with immune cells and independently contributes inflammatory signals. For instance, higher levels of circulating free fatty acids in obese conditions that undergo lipolysis generate saturated fatty acids (SFAs), proinflammatory lipid compounds that stimulate macrophage, adipocyte, myocyte, and hepatocyte inflammation, leading to insulin resistance.\textsuperscript{58–60} SFAs activate macrophages via Toll-like receptor 4 (TLR4), but they are also precursors for ceramide biosynthesis which can directly decrease insulin sensitivity or indirectly affect insulin signaling through the production of Fetuin-A (FetA).\textsuperscript{61} Interestingly, FetA null mice have protection against insulin resistance in an obesity and aging model as well,\textsuperscript{62} and we recently report that TLR4 KO mice have a reduction in adipose tissue inflammation in aging.\textsuperscript{63} These findings indicate that while adipose tissue inflammation is primarily attributed to ATMs in literature, there are other non-immune players that similarly contribute and overlap in perpetuation of the inflammatory cycle in the adipose tissue.

Aging Adipose Tissue Inflammation

Loss of Basic Adipose Function

With old age, adipose tissue distribution shifts towards visceral fat storage.\textsuperscript{23} Patients with human immunodeficiency virus (HIV) also suffer loss of subcutaneous fat with an increase in visceral fat. (HIV)-associated lipodystrophy leads to increased risk of CVD and diabetes, stressing the importance of subcutaneous-visceral adipose tissue distribution and metabolic health.\textsuperscript{64} Ectopic deposition of triglycerides also occurs on other organs such as liver,
skeletal muscle, and even bone marrow. This trend is associated to higher risk of cardiovascular and metabolic disorders.

**Aging Adipose Tissue and Immunological Profile**

We have previously examined and characterized immune changes in WAT in old mice and found that in aging adipose tissue, similar to obesity, macrophages exhibit a shift away from M2 and towards double negative (CD206-CD11c-) expression. In this study, young and old mice adipose tissue was fractioned into ATMs (CD11b+), adipose tissue stromal cells (ATSCs) (CD11b–), and adipocytes. These fractions were then individually analyzed for cytokine and chemokine production. While all 3 fractions excreted IL-6 and MCP-1 in vitro, ATSCs and ATMs produced substantially higher levels, implicating that the major contributors to adipose tissue inflammation in aging are the resident fat immune cells and not adipocytes.

In separate study, we investigated the role endoplasmic reticulum (ER) stress plays on macrophage inflammation in old adipose tissue. Similar to obese ATMs, old ATMs also display elevated ER stress and inflammation. Alleviating the ER stress in old murine ATMs in vitro and in vivo via a chemical chaperone decreases the production of inflammatory cytokines and chemokines. Another study similarly employed chemical chaperones to reduce ER stress in the adipose tissue of diet-induced obese mice and succeeded decreasing adipose tissue inflammation. In a later investigation, we observed that old mice also possess perturbed autophagy function within their SVF, contributing to adipose tissue ER stress and inflammation. Furthermore, blocking autophagy function in the SVF increased ER stress marker CHOP and pro-inflammatory markers IL-6 and MCP-1. These results emphasize the importance of adipose tissue homeostasis and metabolism, particularly at how large of a role non-adipocytes can play.

In old adipose tissue, there was also a higher total stromal vascular cells (SVF) per gram of WAT, showing that adipose tissue accumulates resident cells over time. The increase in SVF was observed in T cells (CD3+), particularly CD4+ T cells and a minor increase in CD8+ T cells. A three-fold increase of regulatory T cells (Tregs) was present in old WAT. Aging has been reported to be associated with increased Treg function secondary to age-related epigenetic drift and T cell DNA hypomethylation. Excessive Tregs activity may contribute to age-related susceptibility to infection, neurodegeneration, and cancer. The origin of the Tregs in aging adipose tissue is not defined but is presumably derived from peripheral naïve CD4 T cells responding to the local aging microenvironment. Nevertheless, the results were unexpected as others have shown that visceral adipose tissue of lean animals have higher number of Tregs compared to their obese cohort. The adipose tissue Tregs presumably playing a protective role in metabolic disorder, suppresses the production of local inflammatory mediators and improves insulin resistance. Importantly, a recent study also showed that the depletion of these fat-specific Tregs prevent age-associated insulin resistance in mice, supporting the notion that distinct immune cell subpopulations all play an inter-connecting role in age-associated inflammation and diseases.
**Senescent Cells in Aging Adipose Tissue**

Senescence serves as the brakes to tissue renewal and cancer cell development in organisms. More specifically, senescence is a process where cells become irreversibly arrested in cell-cycle to prevent further replication in response to stress\(^\text{74}\). In aging, environmental stresses compound overtime: DNA damage, telomere attrition, mitogenic signals, and chromatin dysfunction lead to the accumulation of senescent cells in various tissues\(^\text{74}\). While senescent cells become non-replicative, they also undergo phenotypic changes, including tumor-suppressor and secretome switches\(^\text{75}\), that enable them to affect the tissue microenvironment, influencing neighboring cell structure and function.

Two primary tumor-suppressor pathways, involving p53 and pRB, are attributed with regulating the senescent states of cells. These transcriptional regulators are nestled in the center of the pathways, with stress response proteins upstream and downstream effectors that promote and maintain senescent states. Loss of p53 function in studies have shown to diminish replicative senescence in human cells\(^\text{76}\) while inactivation of p21, a cell cycle inhibitor that is also a p53 target, allows cells to bypass telomere attrition-dependent senescence\(^\text{77}\). The pRB pathway contains p16, a cell cycle inhibitor that responds to stress.

Senescence occurs with either pathway or both; however, downstream of these pathways, upregulated genes include secreted proteins that include cytokines and chemokines\(^\text{78}\).

Senescent cells have been reported to secrete various proinflammatory cytokines such as IL-6, IL-8, and TNF-a, collectively known as the senescence-associated secretory phenotype (SASP)\(^\text{13}\). These proinflammatory factors accumulate with aging, as the old immune system becomes less efficient with the clearance of senescent cells\(^\text{74}\),\(^\text{78}\). They become a potential source of inflammation in inflamming. Recently, studies indicate that visceral and inguinal adipose tissue in mice harbor large quantities of senescent cells that are p16\(^{\text{Ink4a}}\) positive\(^\text{1}\). Tissue accumulation of p16, p21, and β-galactosidase are markers of senescence, and senolytics are designed for the clearance of senescent cells that fail to be naturally cleared by the host immune system. Recent senolytics studies successfully depleted p16\(^{\text{Ink4a}}\) via a novel transgene, \textit{INK-ATTAC}, and it was demonstrated that age-related disorders can be attenuated in the BubR1 progeroid mouse model\(^\text{1,79}\).

**Conclusion**

Aging research that blossomed in the last decade bring promising lifespan extension, but improving healthspan in parallel is crucial for reducing chronic disease burden. Alleviating this burden will be essential for managing the projected geriatric demographics’ growth as the prevention of one chronic condition can reduce the risk of developing other chronic conditions, and prospectively, this will reduce comorbidity and mortality in the elderlies. Much of the current research on white adipose tissue focuses on its role in obesity, specifically in young or diet-induced cases. However, recent studies show there is still much to be understood with the role adipose tissue plays as an organ that influences metabolic health outside of diet-induced obesity. Obese adipose tissue distribution and function displays accelerated aging, emphasizing the impact adipose tissue has on longevity.
Aging studies that focus on improving lifespan and healthspan have seen immense progress with tackling sources of inflammaging. Adipose tissue inflammation, while only a part of inflammaging, has captured more attention as white adipose tissue in the inguinal depot contains one of the highest accumulation of p16\(^{\text{ink4a}}\) positive cells in the most recent senolytics performed on aging mice. It is now clear that, with the multitude of changes that occur in the immune system during aging (immune senescence), there are fundamental cellular and molecular differences between diet-induced obesity and age-associated obesity. Potentially, as the field of senolytics expand, more aging or senescent markers will surface to better elucidate whether white adipose tissue is the primary reservoir of senescent cells or if old adipose tissue loses its ability to clear the cells prior to other aging organs.

**Acknowledgments**

This project has been funded in part by NIH grants AG020628, AG028268, HL58984, AG-024824, T32-AI007413, T32-AG000114 and Geriatrics Research, Education and Clinical Care Center (GRECC) of the VA Ann Arbor Healthcare System. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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