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Changes in Adipose Tissue Cellular Composition During Obesity and Aging as a Cause of Metabolic Dysregulation

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Abstract

Adipose tissue represents complex endocrine organ containing several different cellular populations including adipocytes, pre-adipocytes, mesenchymal stem cells, macrophages and lymphocytes. It is well establishing that these populations are not static but alter during obesity and aging. Changes in cellular populations alter inflammatory status and other common metabolic complications arise, therefore adipose tissue cellular composition helps dictate its endocrine and regulatory function. During excessive weight gain in obese individuals and as we age there is shift towards increase populations of inflammatory macrophages with a decrease of regulatory T cell. This altered cellular composition promote chronic low grade inflammation negatively affecting mesenchymal stem cell progenitor self-renewal, which result in deterioration of adipogenesis and increased cellular stress in adipocytes. All these changes promote metabolic disorders including age- or obese-related insulin resistance leading to type 2 diabetes.

Introduction

As obesity has become a worldwide epidemic, it is not surprising that the interest in adipose tissue has increased over the years to search for a metabolic cause. It is widely accepted that increase in visceral adipose tissue (VAT) is associated with many metabolic diseases including cancer, cardiovascular diseases and most prominently type two diabetes. Originally, adipose tissue was thought to be inert and composed solely of adipocytes. However, over the past several years, other cellular populations have been discovered within the stromal vascular fraction (SVF). The SVF is a collection of non-adipocyte cells including leukocytes, progenitor cells, and other immune cells (Ergen et al., 2013; Lumeng et al., 2011; Zuk et al., 2002). With all these newly discovered cellular populations it has become evident that adipose tissue is not actually inert; rather, it contains both endocrine and immune functions.

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Many similarities in adipose tissue have been identified between obesity and aging. Adipose tissue changes over time as we grow and age like all other tissues. One of the significant changes with age is a general increase in percentage of body fat, particularly in visceral adipose tissue (VAT) (Tchkonia et al., 2010). Importantly, an age- or obesity-related increase in visceral adipose tissue is usually accompanied by low grade chronic inflammation, which has been postulated as a cause of various metabolic diseases. More specifically, it is associated to increased insulin resistance. One of the most studied and powerful intervention known to positively affect adipose tissue, improve metabolic status and extend longevity is calorie restriction (CR) (Bartke and Brown-Borg, 2004; Masternak et al., 2005a, 2005b). However, recent study of long-living growth hormone receptor knock-out (GHRKO) mice indicated that these mice do not benefit from calorie restriction on either longevity or improvement of insulin action (Bonkowski et al., 2006, 2009; Masternak et al., 2009).

Interestingly, growth hormone (GH)-deficient (Ames dwarf mice, $Prop1^{df}$, df/df) as well as GH-resistant (GHRKO) mice have increased visceral adipose tissue compared to their normal (N) siblings as they age (Masternak et al., 2012; Menon et al., 2014). Regardless of this tendency towards increased obesity, both df/df and GHRKO mice live longer and healthier lives than their normal littermates (Bartke and Brown-Borg, 2004; Coschigano et al., 2000). These mutant mice are also characterized by decreased inflammation and improved insulin sensitivity and glucose tolerance (Masternak et al., 2012; Menon et al., 2014). It was previously shown that surgical removal of VAT from rats improves metabolic status and extends longevity (Barzilai et al., 1999). Our group also showed that the same surgical intervention improves insulin sensitivity and glucose tolerance in normal mice (Masternak et al., 2012; Menon et al., 2014). Surprisingly, the same surgical intervention in either df/df or GHRKO mice caused a decrease in insulin sensitivity, glucose intolerance and elevated fasting glucose levels. It was also observed that the VAT from these long-living df/df mice had decreased levels of proinflammatory cytokines. All of these recent findings present direct contradiction to the widely accepted notion that increased VAT negatively impacts metabolic health and longevity, indicating that the same endocrine organ can have different physiological effects on metabolism. However, the detailed mechanism of these different functions of adipose tissue is not yet established.

One common belief is that both aging and obesity cause stress on adipocytes, and that these stressed adipocytes produce and release proinflammatory chemokines to attract immune cells. These immune cells then infiltrate the adipose tissue and enhance the inflammatory status of this tissue. Although it is accepted that the immune cells promote inflammation, it remains controversial as to what initiates the attraction of these cells; as with most mechanisms it is probable that there is more than one factor involved.

It is well accepted that it is not just the increase in VAT that affects metabolic function, but the cellular composition of the VAT also plays an important role in the regulation of its function. Rather than studying VAT as a whole organ, it is necessary to investigate the roles of specific cellular populations that compose VAT, to determine any abnormalities in these cellular populations and as well as to assess their interactions with adipocytes. Changes in quantities of infiltrating macrophages, mesenchymal stem cells to progenitor cells, or

regulatory T cells (T_{regs}) all have a potentially significant influence underlying the negative impacts observed in both obesity and aging.

The self-renewal capability of mesenchymal stem cells (MSC) to produce progenitor cells is key in maintaining the structural characteristics of VAT, such as high plasticity. This requires precise regulation and balance between adipogenesis and progenitor cells self-renewal. There has been evidence showing the decreased proliferative activity in aged MSC from adipose tissue to young MSC (Mareziak et al., 2016). Both aging and obesity cause a decrease in adipogenesis and an increase in insulin resistance and inflammation. The inflammation is mainly caused by a population shift of macrophages towards proinflammatory M1 macrophages and a decrease in T_{regs} in VAT (Cancello et al., 2006; Cipolletta et al., 2012, 2015; Feuerer et al., 2009, 2009; Lumeng et al., 2011). Increase in chronic inflammation then causes a dysregulation in progenitor cells self-renewal, which then causes a decrease in the progenitor cell population and reduces adipogenesis.

Macrophages and Visceral Adipose Tissue

The root cause of inflammation lies in the presence of primary proinflammatory molecules like tumor necrosis factor α (TNF- α), interleukin-6 (IL-6), soluble intercellular adhesion molecule (ICAM). These molecules are overexpressed in obese individuals and are related to the metabolic complications seen with obesity and metabolic syndrome (Weisberg et al., 2003). Although adipocytes express receptors for proinflammatory molecules, they are not typically the primary source of those molecules. Macrophages however, do secrete proinflammatory molecules and are one of the cell types found in the stromal vascular fraction of adipose tissue (Nishimura et al., 2007). Macrophages play a crucial role in the innate immune response by initiating an inflammatory response. They are in fact, the primary source of TNF- α (Weisberg et al., 2003). When determining the cause of an inflammatory response macrophages are recognized as a primary candidate, and therefore, it was not surprising when an increased infiltration of macrophages was observed in adipose tissue from obese individuals (Cancello et al., 2006; Weisberg et al., 2003). Further, macrophage accumulation was shown to be directly proportional to adiposity in both mouse models and humans (Cancello et al., 2006; Weisberg et al., 2003). It was also observed that the percent increase of macrophages found in VAT is strongly related to age (Cancello et al., 2006). Therefore, it was accepted that an increase in macrophages in both obesity and age lead to an increase in systemic inflammation.

Importantly, studies of diet induced obesity (DIO) in mice indicated that there are actually three distinct populations of macrophages in VAT, distinguished by different cellular markers (Zeyda et al., 2010). The most studied, M1 macrophages represent proinflammatory macrophages that express genes and cytokines that are involved in obesity complications (Zeyda et al., 2010). M1 macrophages were identified with the CD11c marker (Lumeng et al., 2011; Zeyda et al., 2010). M2 macrophages are identified with the mannose receptor (MR) marker also called the CD206 marker and they are recognized as anti-inflammatory macrophages (Lumeng et al., 2011; Zeyda et al., 2010). The third group represent double negative (DN) macrophages that do not have either the CD11c or CD206 markers (Lumeng et al., 2011; Zeyda et al., 2010). Some studies have shown that there is no difference

between macrophage populations when comparing VAT from lean mice to VAT of obese mice, but there is a phenotypic switch between M1, M2 and DN populations (Lumeng et al., 2011; Zeyda et al., 2010).

Overall, these studies showed that obesity and age are associated with population shifts that lead to an increase in the presence of proinflammatory macrophages. In obese VAT there is an increase in M1 proinflammatory macrophages with a greater increase in DN, while anti-inflammatory M2 macrophages have a tendency to decrease; a similar trend was also detected in aged mouse models (Lumeng et al., 2011; Zeyda et al., 2010). Different studies showed that throughout aging, the total macrophage infiltration did not increase but there was a shift indicating decrease of M2 macrophages, while DN populations increased without observed alterations of M1 macrophages (Lumeng et al., 2011). Interestingly, regardless of lack of similar cellular markers between DN and M1 or M2 macrophages there is a high similarity in the gene expression profile between these populations (Zeyda et al., 2010). Originally, it was thought that these populations had beneficial anti-inflammatory effects like the M2 macrophages however, they also express the proinflammatory cytokine IL-1 β (Lumeng et al., 2011; Zeyda et al., 2010). The proinflammatory capability along with a significant increase in the DN macrophage population in both obese and aged mice lead to the conclusion that in these conditions, DN macrophages are a significant cause of inflammation in both obesity and aging. Present studies suggest that an increase of M1 macrophage is not enough to be the sole cause of inflammation especially because the increase is only observed in obese mice and not aged mice. Yet, it remains to be answered what exactly causes DN macrophages to have a proinflammatory effect over an anti-inflammatory one.

Lymphocytes and Visceral Adipose Tissue

Lymphocytes represent important cellular populations that regulate immune responses in adipose tissue. Both aging and obesity have been shown to affect the distribution of these cells in VAT. T cells represent the main population of cells involved in regulating inflammatory responses. Originally, CD8⁺ and CD4⁺ T cells were of the greatest focus in adipose tissue research. The presence of CD8⁺ populations in obese adipose tissue was shown to precede the infiltration of macrophages (Nishimura et al., 2009). Studies of VAT from diet-induced obese mice indicated an increase of CD8⁺ cells with a concomitant decrease of CD4⁺ cells (Nishimura et al., 2009). Neutralization of CD8⁺ cells with an anti-CD8 antibody caused a decrease in M1 proinflammatory macrophages without any changes in M2 anti-inflammatory macrophages in VAT (Nishimura et al., 2009). This finding strongly suggests that the increase of CD8⁺ T cells triggers the cascade of macrophage-dependent inflammation in VAT. Importantly, it has also been reported that there is also an age-related increase in both CD8⁺ and CD4⁺ T cells (Lumeng et al., 2011). It is likely that this deregulation of CD8⁺ and CD4⁺ T cells during aging represents one of the main mechanisms of age-dependent chronic inflammation.

The other cell population found in VAT that regulates T cell immune response are regulatory T cells (T_{regs}); previously lumped together in the CD4⁺ group of lymphocytes, these cells have since been proven to have their own important role in the immune system. T_{regs} can be

identified in tissue with expression of Foxp3 in CD3⁺ CD4⁺ cells (Ramos-Ramírez et al., 2016). Regulatory T cells have been deemed a crucial part of the immune system due to their aid in the control of autoimmunity and allergies (Feuerer et al., 2009). Many autoimmune responses, like allergies, have an inflammatory response, leading to the suggestion that T_{regs} have a role in other inflammatory pathways. T_{regs} control, suppress, or stop immune responses initiated by other immune cells- particularly the responses initiated by CD8⁺ and CD4⁺ cells. T_{regs} can be found in a variety of tissues besides VAT including the peripheral lymphoid organs.

T_{regs} found in visceral adipose tissue (VAT) have some differences compared to those found within the spleen and lymph nodes, notably the genetic expression profile (Cipolletta et al., 2012; Feuerer et al., 2009). T_{regs} found in VAT have different genes actively being transcribed and expressed compared to the T_{regs} found in the spleen and lymph nodes. VAT T_{regs} have been reported to have higher IL-10 transcript levels and transcript-encoding molecules involved in leukocyte migration and extravasion (Feuerer et al., 2009). IL-10 is a cytokine that has anti-inflammatory effects (Couper et al., 2008). Higher IL-10 production is consistent with the regulatory role of T_{regs} as it suppressed any inflammatory response initiated by other immune cells like CD8⁺ cells or macrophages (Cipolletta, 2014; Cipolletta et al., 2011). Importantly, VAT T_{regs} also have a different T cell receptor (TCR) repertoire compared to other T cells (Cipolletta et al., 2012; Feuerer et al., 2009). This correlates with the divergent responses between T_{regs} and CD8⁺ and CD4⁺ T cells. T_{regs} instead of inducing an immune response like CD4⁺ and CD8⁺ T cells are known to suppress or end immune responses. In order to be able to have this function they need the ability to bind to a different set of ligands. Having increased T_{regs} in VAT would be beneficial for controlling and reducing any inflammation induced by other cells.

Regulatory T Cell Population Varies with Obesity and Age

The VAT T_{reg} population varies depending on age and obesity. T_{regs} have been shown to increase in lymphoid organs in mice with respect to age (Raynor et al., 2012). The T_{reg} population seems to be more variable in VAT. It has been noted that there are low levels of T_{regs} seen at birth and then over time these cells accumulate in VAT but not in subcutaneous adipose tissue (Cipolletta et al., 2015; Feuerer et al., 2009; Lumeng et al., 2011). However, those studies did not indicate if this was over a shorter period of time or over the lifespan of the mice.

In general, it is accepted that VAT T_{regs} decrease with obesity and age. Feuerer and his colleagues reported that there was a decrease in T_{regs} in old *ob/ob* mice as compared to younger ones (Feuerer et al., 2009). In this study they looked at both leptin-deficient (*ob/ob*) mice, a subset genetically altered towards development of obesity, as well as a subset of high fat diet (HFD) fed mice. They observed that aged *ob/ob* mice and HFD mice had a decrease of T_{regs} when compared to their heterozygous littermates, while young *ob/ob* mice had an increase of T_{regs} compared to their heterozygous littermates (Feuerer et al., 2009). It was also noted that the spleen and lymph nodes had relatively normal levels of T_{regs}, therefore, the decrease was only observed in the VAT (Feuerer et al., 2009). However, they did not compare the young *ob/ob* to the old *ob/ob* mice; it would be interesting to see overtime how

the T_{reg} population decreased. It was also noted that as the T_{reg} population decreased there was an increase in insulin resistance (Feuerer et al., 2009). As insulin resistance is one of the major problems associated with obesity that leads to type 2 diabetes this is crucial relationship to explore further.

Predominantly, it has been shown that obese VAT has a decrease in T_{regs} while lean VAT has an increased amount of T_{regs} (Cipolletta et al., 2012, 2015; Deiluiis et al., 2011; Feuerer et al., 2009). However, there are some contradicting works that show an increase of VAT T_{regs} with obesity (Lumeng et al., 2011). Combining the contradicting reports of chemokine attraction of macrophages with the contradicting number of T_{regs} in obese VAT, it is possible that different stages of obesity have a different distribution of cellular populations. Instead of assessing adipose tissue as a whole organ, the functions of the different cellular populations, like T_{regs} and macrophages, need to be assessed overtime when considering the effect on obesity or aging. It is possible that adipose tissue is in a constant flux with changing cellular populations, and something like obesity can tip the scale towards the accumulation of certain populations and the dysregulation of others, like progenitor cells.

Obesity is a progressive disease. Whether it is due to genetics or a high fat diet, excessive weight gain occurs over time. In the early stages of obesity, when weight is first starting to accumulate, will have a different amount and composition of VAT with different cell populations of macrophages, T cells and a normal composition of progenitor cells. As obesity progresses or as we age and cellular senescence begins the decrease of T_{regs} and increase of proinflammatory macrophages causes a dysregulation of progenitor cell self-renewal resulting in decreased adipogenesis and increased inflammation.

Adipogenesis Decreases with Obesity and Age

Adipogenesis is a process when preadipocytes or progenitor cells differentiate into adipocytes. This process occurs as we grow and age but becomes dysregulated with senescence and obesity. This is evident by assessing the different cellular clusters in different mouse models. There are more adipogenic/angiogenic clusters in young mice, while there are more “crown-like” structures observed in old mice (Nishimura et al., 2007).

Angiogenesis is essential for adipogenesis to supply nutrients and blood to the newly formed adipocytes. “Crown-like” clusters identify differently than adipogenic/angiogenic clusters by using the HAM56 marker (Cancello et al., 2006). The HAM56 marker identified macrophages that assemble in a “crown” formation around dead-like adipocytes (Cancello et al., 2006; Zeyda et al., 2010). As the adipocytes become stressed through senescence or obesity, the macrophages are recruited to those areas. The macrophages found in “crown-like” structures are positive for the CD11c marker, indicating that they are M1 proinflammatory macrophages (Wentworth et al., 2010; Zeyda et al., 2010). This is evidence supporting the macrophage population shift from anti-inflammatory to proinflammatory.

“Crown-like” structures are not solely composed of macrophages, but also have CD8+ T cells within the structures, while some T_{regs} have been identified near them (Feuerer et al., 2009; Nishimura et al., 2009). T_{regs} have not completely disappeared from VAT during

obesity and aging but have diminished enough that they cannot combat the combined inflammatory response generated by both proinflammatory macrophages and CD8⁺ T cells.

Obesity does not immediately result in the generation of “crown-like” structures; it takes time for them to develop. There is an initial increase of adipogenic/angiogenic clusters observed in the early stages of obesity and then more “crown-like” structures in the later stages (Cancello et al., 2006; Nishimura et al., 2007). The increase of adipogenic/angiogenic clusters is due to the increase of progenitor cell differentiation, thereby creating more adipocytes. The ability of progenitor cells to differentiate and self-renew is limited however, and essentially once the cells are depleted and adipocytes cannot be formed, there is an increase in “crown-like” structures and a decrease in angiogenesis. Active angiogenesis has also been observed differently in obese tissue than in lean tissue; obese VAT is growing and therefore has a need for more blood vessel formation to accommodate the increase in adipose tissue (Cancello et al., 2006; Nishimura et al., 2007).

The adipogenic structures were not observed in older mice, even in obese models, because at this older age weight gain had already plateaued and new adipocytes are not being formed (Nishimura et al., 2007). It is possible that the weight gain plateau is due to a decrease in progenitor cells. If there were an increased number of progenitor cells continually generating adipocytes and self-renewing, weight gain would not plateau and adipogenesis would continue. Obesity appears to mimic what naturally occurs with age: the increased generation of adipocytes from progenitor cells seems to diminish the progenitor cell population.

MicroRNAs Regulate Progenitor Cell Differentiation

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression. Several miRNAs have been identified in regulating progenitor cells or preadipocytes found in the SVF to adipocytes (Fei et al., 2013; Iacomino et al., 2016). It has been made clear that miRNA play a role in adipogenesis regulation, and evidence shows that dysregulation of miRNA expression is causative or indicative of disease states (Iacomino et al., 2016; Villageois et al., 2011). Several miRNAs, specifically miR-143, have been identified to regulate the ERK5-PPAR γ pathway during preadipocyte differentiation, and that this dysregulation promotes adipogenesis and obesity with increased age (Fei et al., 2013). Another preliminary study identified circulating miRNAs miR-31-5p, miR-2355-5p and miR-206 as potentially associated with early obesity (Iacomino et al., 2016). They defined early obesity as childhood obesity and compared overweight samples to normal weight samples and identified three main circulating miRNAs that were differentially expressed (Iacomino et al., 2016). The study focused on preadipocyte differentiation, indicating that miRNAs are altered by obesity most likely affecting progenitor cells differentiation or self-renewal.

MicroRNAs are still a relatively novel area of study but have the potential to have a significant impact on the regulation of many immune functions. The inflammation caused by the population shift of macrophages and the decrease in T_{regs} causes dysregulation of miRNA. If the miRNAs that regulate progenitor cell self-renewal are not at optimal level of

function, this would cause the progenitor cells to have limited self-renewal ability. If new progenitor cells are not being produced, a decrease in progenitor cell population can lead to diminished adipogenesis and an increase in inflammation. Since there have been discrepancies in the signaling induced by age and obesity between cellular populations, miRNAs are a new and interesting area of research that may provide a bridge between the different theories behind inflammation.

Conclusion

The similarities between obesity and aging on adipose tissue cellular metabolism are striking; it appears that both states affect distribution of cellular populations and more importantly progenitor cell self-renewal. The most consistent similarity is the overall increase in systemic inflammation. However, there is strong evidence that VAT plays an important role in the regulation of inflammatory status during aging and obesity. One considered cause of inflammation is a shift in the distribution of cellular populations in VAT. While adipose tissue consists of loose connective tissue that is mostly composed of adipocytes, all the other cells including lymphocytes, macrophages, and mesenchymal stem cells (MSC) play important endocrine and regulatory roles. It is known that pluripotent MSC plays important regenerative role in different organs including adipose tissue. While there is a great interest in using these pluripotent stem cells in regenerative medicine to target wide spectrum of different diseases, these cells are also crucial for well-functioning adipogenesis. High plasticity of VAT and constant changes during lifespan, i.e. gaining/losing weight, require precise mechanisms to maintain the balance between adipogenesis and fat progenitor cell self-renewal. This process depends on several different genetic, hormonal and cellular factors including more recent interest in the role of miRNA in the process of progenitor cell self-renewal. On the other hand, some inflammatory factors produced by pro-inflammatory macrophages are known to inhibit the self-renewal process causing inhibition of adipogenesis, increased adipocyte size and further negative alterations in the metabolic status of VAT. Adipose tissue-residing T_{reg} cells play important roles in maintaining a healthy immune balance and research indicates that these regulatory cells prevent infiltration of VAT by inflammatory macrophages. Based on this knowledge, we could speculate that quality not quantity, of VAT regulates insulin sensitivity, systemic inflammation, and promotes healthy aging or causes age-dependent metabolic disorders.

Summarizing, healthy function of adipose tissue during aging or obesity depends on well-functioning adipogenesis, indicating the importance of the balance between differentiation into adipocytes and self-renewal of progenitor cells throughout one's the lifespan. High self-renewal of fat progenitor cells stimulates production of new well-functioning adipocytes through enhanced adipogenesis, rather than expanding the size of adipocytes with concomitant loss of function.

To maintain this process undisrupted, there are necessary precise mechanisms involving genetic stability. However, the environment in VAT is known to also play an important role in this process. Increased population of T_{reg} cells is crucial for maintaining healthy immune balance and low inflammation by preventing infiltration of VAT by M1 inflammatory macrophages. On the other hand, depletion of T_{regs} and further increase of inflammatory

macrophages increases cellular stress causing mobilization of regenerative MSC and finally disruption of the self-renewal process. With time there is a devastating depletion of progenitor cells (Figure 1).

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Highlights

- Obesity and aging alter the cellular composition of adipose tissue.
- Regulatory T cells maintain healthy inflammatory status in adipose tissue.
- Balanced adipocytes differentiation and MSC self-renewal promote healthy fat.

Figure 1.

Left Side: Young or lean have high populations of mesenchymal stem cells (MSC), increase capacity for adipogenesis promoting high insulin sensitivity and low inflammation. *Right Side:* Old or obese causes depletion of MSC and inhibition of adipogenesis, insulin resistance and high inflammation. Decrease of Tregs and increase of inflammatory M1 macrophages stimulate high inflammation and further inhibits self-renewal of MSC.