

The ominous triad of adipose tissue dysfunction: inflammation, fibrosis, and impaired angiogenesis

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There are three dominant contributors to the pathogenesis of dysfunctional adipose tissue (AT) in obesity: unresolved inflammation, inappropriate extracellular matrix (ECM) remodeling and insufficient angiogenic potential. The interactions of these processes during AT expansion reflect both a linear progression as well as feed-forward mechanisms. For example, both inflammation and inadequate angiogenic remodeling can drive fibrosis, which can in turn promote migration of immune cells into adipose depots and impede further angiogenesis. Therefore, the relationship between the members of this triad is complex but important for our understanding of the pathogenesis of obesity. Here we untangle some of these intricacies to highlight the contributions of inflammation, angiogenesis, and the ECM to both “healthy” and “unhealthy” AT expansion.

An old perspective on a new problem

Adipose tissue (AT) has evolved to be a highly dynamic organ, readily remodeling to meet the demands of an ever-changing metabolic landscape, as illustrated by the rapid changes in adipose depots of rodents undergoing various nutritional stresses. It takes as little as one week of high-fat feeding (HFF) in rodents for adipocytes to become enlarged, storing four-fold the amount of triglyceride per cell compared with baseline (1). The mouse visceral AT weight can double within one week of the initiation of a high-fat diet (HFD) (2) but can undergo a dramatic reduction within 24 hours of fasting. It is this plasticity that has made AT an essential organ for maintaining metabolic homeostasis during times when food is either abundant or scarce.

The ability of AT to rapidly remodel is the result of the coordinated response of resident AT cell types: adipocytes, immune cells, endothelial cells, and fibroblasts. When nutrient availability is in excess, adipocytes store various lipid species in lipid droplets, which quickly expand in size and, in the process, reach the diffusional limit of oxygen. The ensuing hypoxia is mild but induces a stress signal that drives angiogenesis and remodeling of the extracellular matrix (ECM) to facilitate further expansion of AT and reduce hypoxia (Figure 1). This acute effect can be thought of as “healthy” AT expansion. But in the evolutionary history of AT, the necessity to expand and store lipids was short lived, with periods of fasting or starvation guaranteeing reversal of weight gain. The current pervasive state of chronic overnutrition results in an increase in unresolved AT hypoxia. At some point during the progression of obesity, adipose depots expand beyond the tissue’s capacity for adequate angiogenesis, resulting in persistent hypoxia, fibrosis, cellular senescence, and necrotic adipocyte death (Figure 1), ultimately leading to unhealthy AT tissue expansion, a major contributor to the systemic metabolic disturbances that are characteristic of obesity and type 2 diabetes.

The adipocyte stress signal: mild hypoxia

Hypoxia is an important driver of the early AT response to increased dietary lipids. Within three days of HFF, rapid adipocyte hypertrophy along with mitochondrial uncoupling increase the demand for oxygen and reduce oxygen availability (3). Adipocytes respond by inducing glycolysis and angiogenesis in an attempt to alleviate the hypoxia (3). The major mediators of the hypoxia response are the hypoxia-inducible factor (HIF) transcription factors. HIFs contain an α subunit that, at normal tissue oxygenation, is hydroxylated by oxygen-sensing prolyl hydroxylase enzymes (PHD1, -2, and -3), resulting in ubiquitylation and proteosomal degradation of HIF α . In contrast, reduced oxygen tension inhibits PHDs, resulting in subsequent accumulation of HIF α . There are two major subunits of HIF α , HIF-1 α and HIF-2 α , which have opposing roles. HIF-1 α activation is classically described as pro-angiogenic, but recent studies have shown it to also be pro-fibrotic and proinflammatory (4). In contrast, HIF-2 α attenuates HFD-induced inflammation (3). Although HIF-1 α and HIF-2 α pathways can interact, HIF-1 α signaling dominates in obesity, playing a causative role in early AT dysfunction (3). An important determinant of HIF-1 α activity relative to HIF-2 α activity is the abundance of specific PHD enzymes, which have differential specificities for prolyl hydroxylation sites between the HIF α isoforms (5). The importance and intricacy of PHD-specific regulation of HIF α can be illustrated by the phenotypes of mice deficient in either PHD1 or PHD2. PHD1 KO mice exhibit a greater fat pad mass and impaired glucose tolerance but do not show exacerbated diet-induced metabolic changes compared with WT mice (6). In contrast, adipocyte-specific deletion of PHD2 produces mice that are resistant to HFD-induced obesity and, despite elevated levels of both HIF-1 α and HIF-2 α , the mice display improved glucose tolerance and insulin sensitivity (7). Therefore, although further studies are required to understand the exact role for HIF-1 α and HIF-2 α in the regulation of AT expansion, it is clear that hypoxia signaling is paramount to the early response of AT to nutrient excess.

Conflict of interest: The authors have declared that no conflict of interest exists.

Reference information: *J Clin Invest.* 2017;127(1):74–82. doi:10.1172/JCI88883.

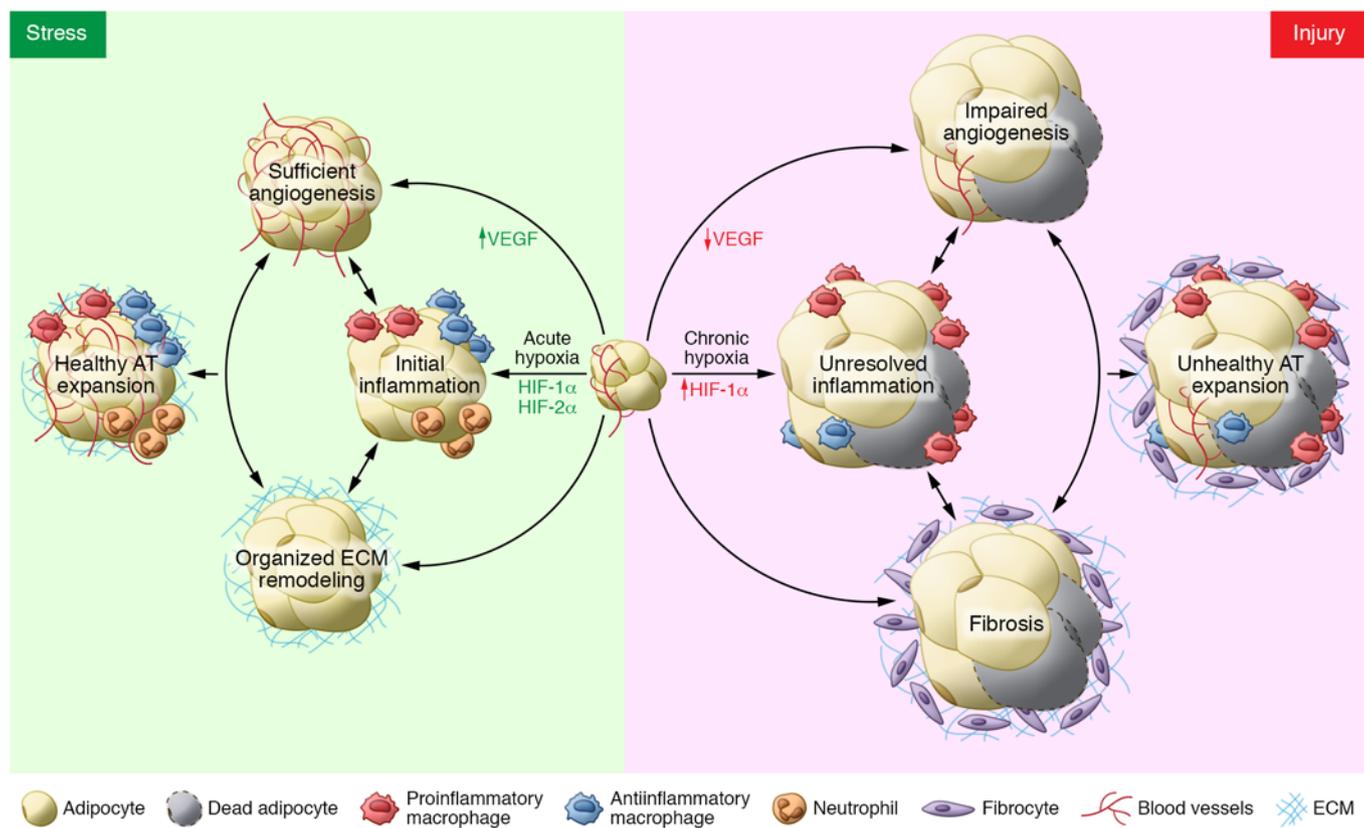


Figure 1. The complex contributions of angiogenesis, inflammation, and fibrosis in healthy and unhealthy AT expansion. Hypoxia during early AT expansion induces stress signaling that facilitates angiogenesis via upregulation of an array of genes including VEGF, initiates inflammatory cell infiltration, and induces organized ECM remodeling to encourage appropriate AT expansion. However, persistent stress signaling translates into injury signaling, caused by long-term and severe hypoxia. This hypoxic condition fails to induce VEGF, resulting in impaired angiogenesis. Limited oxygen supply also triggers chronic low-grade inflammation predominantly characterized by proinflammatory macrophage infiltration. Upregulation of HIF-1 α also increases the fibrotic program in AT. The integrated responses among those three factors promote significant hypertrophy in adipose tissue, and the consequence is an unhealthy AT expansion as well as obesity-associated metabolic consequences.

Acute inflammation. In lean individuals, resident AT immune cells are indispensable for adipocyte function; however, AT inflammation is one of the most widely recognized contributors to metabolic dysfunction in obesity. Cells of both the innate and adaptive immune systems have been identified in AT (8, 9). Macrophages comprise the largest population of resident immune cells in visceral AT, constituting up to 10% of AT cells in mice (10). Macrophages are responsible for many housekeeping processes, such as removal of apoptotic and necrotic cells, modulation of angiogenesis, ECM remodeling, and differentiation of adipocyte precursors (8). Macrophages are often characterized by their “polarized” state, displaying more of a proinflammatory or antiinflammatory phenotype. Pathologic activation of both innate and adaptive immunity has been observed during obesity, even though the degree to which each component participates differs temporally. For instance, ApoE3-Leiden mice, which exhibit a more humanized lipid metabolism compared with WT mice, display increased acute-phase response genes within one week of HFF, whereas chronic inflammatory responses are increased after six weeks of HFF (2). This is not seen under all circumstances, as CD8⁺ T cell infiltration has been observed to precede and induce AT macrophage accumulation in other settings (11).

As an early event in AT expansion, hypoxia likely plays a fundamental role in the initiation of inflammation. Adipocytes release proinflammatory factors such as IL-6 and macrophage migration inhibitory factor (12). Immune cells, particularly monocytes, migrate to areas of hypoxia, where they differentiate into proinflammatory macrophages (13, 14). Macrophage infiltration into AT occurs within one day of HFF (15). Although few studies have directly addressed the effect of mild hypoxia during early AT expansion on AT inflammatory processes, there have been reports of both pro- and antiinflammatory responses during the first four days of HFF. Lee et al. demonstrated a mild proinflammatory response in the epididymal adipose depot of mice fed a HFD for three days (16), and the proinflammatory polarization of macrophages was of particular importance in this response. In contrast, others have reported that acute HFD stress leads to activation of antiinflammatory macrophages (17, 18). This effect is mediated through NKTs, which are activated by lipid antigens presented on CD1d molecules of antigen-presenting cells. The actions of NKT cells are likely beneficial for the adaptation of AT to acute HFF because mice deficient in CD1d do not activate AT NKT cells and display exacerbated insulin resistance (17). Other studies have corroborated these findings by demonstrating

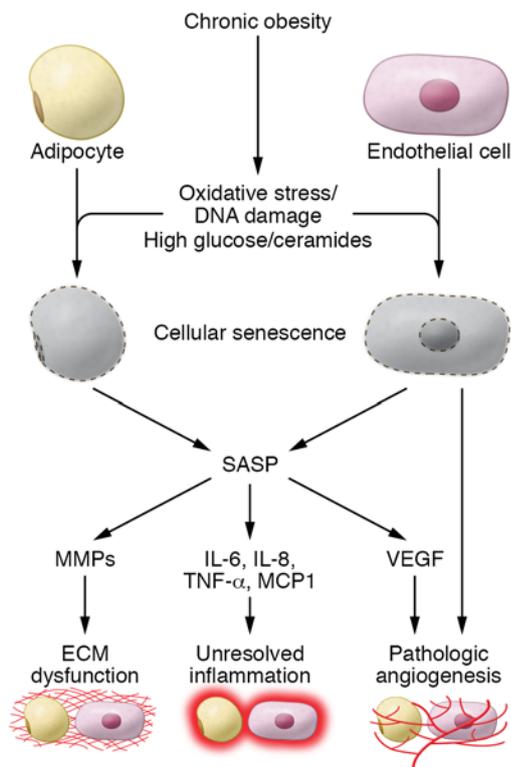


Figure 2. The role of senescence in obesity-associated AT dysfunction.

Chronic obesity can cause AT oxidative stress, DNA damage, and increased exposure to high glucose and ceramide concentrations. These deleterious factors can drive cellular senescence in many cell types. Adipocyte and endothelial cell senescence have been specifically studied in the context of obesity. Mature adipocytes and endothelial cells as well as their precursors can undergo senescence and take on the SASP. SASP factors can promote AT dysfunction through dysregulation of AT ECM remodeling, inflammation, and angiogenesis.

effect on AT vascularity and insulin sensitivity (35). In addition to authentic angiogenic factors, AT secretes several important adipokines and endogenous angiogenesis inhibitors to modulate the balance of new blood vessel formation, including adiponectin (36, 37), leptin (34), resistin (38), visfatin/thrombospondin 1 (39), and plasminogen activator inhibitor (40). Moreover, adipocytes can secrete pro-angiogenic lipid species such as monobutyrin (41).

Angiogenesis can be divided into two major subtypes: (a) sprouting angiogenesis, which is slow and the major developmental form of vessel growth and (b) looping/splitting angiogenesis, which is fast and occurs predominantly during adult wound healing. VEGF is a major regulator of sprouting angiogenesis (42). During sprouting, new vessels are headed by specialized endothelial cells (tip cells), which migrate in response to chemotactic and pro-angiogenic factors such as VEGF. In looping/splitting angiogenesis, existing vessels are split into two by the force of contracting myofibroblasts associated with a wound. There is no direct evidence for the existence of these types of angiogenesis in obese adult AT, but we could speculate that distinct angiogenic mechanisms participate during AT expansion and are dependent on the extent of hypoxia and the complex regulation in early or late stages of expansion. For example, VEGF is upregulated in AT of mice fed a HFD for three days, but there are reports of both enhanced and reduced VEGF expression in chronic obesity (3, 13, 43, 44), suggesting that sprouting angiogenesis is activated early and may or may not persist. In contrast, the combination of low VEGF signaling and increased myofibroblast activation suggests that splitting angiogenesis occurs more frequently during chronic obesity. Further studies are required to understand how these different types of angiogenesis contribute to obesity-associated vascular dysfunction. However, given the circumstantial evidence, it seems clear that, compared with any other tissue, a disproportionate level of vascular remodeling persists in AT, even at steady state.

Appropriate ECM remodeling. The ECM of AT has a multi-faceted function in tissue homeostasis. It consists of various components including collagens, fibrillins, proteoglycans and non-proteoglycan polysaccharides. These elements act as a scaffold to maintain tissue structure, contribute to inflammatory signaling and sequester various growth factors for time- and context-dependent release. The ECM is maintained through secretion of ECM components by both resident adipocytes and stromal cells, particularly fibroblasts. Many ECM molecules, such as hyaluronic acid (HA), signal through direct binding of cell receptors (45). HA is a non-sulfated glycosaminoglycan that exists as a heterogeneous mixture of greatly varying molecular weights, allowing for modulation of multiple signaling pathways involved in angiogenesis,

that acute HFF induces antiinflammatory immune cell actions, cell repair signaling, and angiogenesis (1, 17, 18). Thus, the early response to AT expansion is likely the result of a balanced action between pro- and antiinflammatory signals.

Healthy angiogenesis. The basic function of blood vessels in regulating AT biology has been well studied. The vasculature supplies adipocytes with the necessary oxygen, nutrients, hormones, and growth factors for AT viability and growth and removes metabolic waste products. The vasculature may also make a direct contribution to the maintenance of AT. For example, circulating stem cells can contribute to adipogenesis (19). Interestingly, populations of white adipocyte progenitors have been identified in the mural compartment of vascular structures. Tang et al. demonstrated that these mural cells with high adipogenic potential can be identified by expression of PDGFR- β (20). Additionally, Gupta et al. used a genetic reporter mouse driven by the promoter of a transcription factor that is important for pre-adipocyte determination, ZFP423, to demonstrate a perivascular origin of pre-adipocytes for both white and brown ATs (21–23).

Local hypoxia is a potent stimulus for new blood vessel formation through the pro-angiogenic actions of HIF-1 α and HIF-2 α (24, 25). AT-derived angiogenic factors have been intensively studied, including VEGF-A (26, 27), VEGF-C (28), placental growth factor (29), HGF (30), IGF-1 (31), angiopoietin-1 and -2 (ANG-1 and ANG-2) (32, 33), and FGF2 (34). Among these factors, the VEGF-A/VEGFR2 signaling pathway is considered to play a particularly important role in angiogenesis and is one of the most potent activators of being in the subcutaneous depots of rodents. Additionally, new angiogenic modulators have recently been identified as part of the secretory repertoire of adipocytes. For example, the VEGF-B/VEGFR1 pathway also has a positive

cell migration, inflammation and ECM remodeling. Other ECM molecules rely on the proteolytic release of biologically active peptides. For example collagen VI is cleaved at the C5 domain of the $\alpha 3$ subunit and the resulting peptide induces multiple processes including increasing collagen deposition, endothelial cell migration and macrophage infiltration (46, 47).

Bona fide fibrosis, defined as excessive ECM protein deposition, is a pathological consequence of long-term obesity, but acute ECM remodeling is essential for early healthy AT expansion. Genes involved in collagen breakdown, particularly MMP14, are significantly upregulated in mice fed a HFD for one week (48). Mice with heterozygous deletion of MMP-14 are not able to appropriately re-model the ECM, resulting in reduced capacity for adipose expansion (48). Conversely, mice deficient in collagen VI exhibit unimpeded tissue expansion (49). Thus, in early AT expansion, there is a strong ECM degradation signal to promote AT growth, which is important for maintaining whole body metabolic homeostasis (49).

The integrated response. Hypoxia is a powerful trigger for ECM remodeling and inflammation as a means to facilitate angiogenesis. A potential mechanism for hypoxia-induced angiogenesis during early AT expansion is the production of pro-angiogenic, low-molecular-weight HA (LMW-HA). Gao et al. have demonstrated that LMW-HA production likely occurs in cells cultured in low oxygen through enhanced activity of the HA-degrading enzyme hyaluronidase (50). HA is also a potent inducer of the innate immune response through binding and activation of immune cell TLR2 and -4 (51). Finally, HA can participate in TGF- β 1-dependent proliferation of fibroblasts to promote fibrosis. Immune cells can also promote angiogenesis in hypoxia. The hypoxic regions at the tip of the mouse epididymal fat pad have been shown to promote infiltration of a specific species of macrophage expressing the lymphatic endothelial marker LYVE-1. These LYVE-1⁺ macrophages promote angiogenesis through secretion of MMP-9, -12, and -7 to remodel the ECM and angiogenic factors like VEGF to stimulate the growth of a dense vascular network. This new vasculature provides the framework for adipogenesis and growth of the fat pad (52). Furthermore, the actions of MMP-9 both break down the fibrous collagen matrix to make space for new blood vessels and release matrix-bound VEGF (53).

There is a reciprocal interaction between immune cell function and angiogenesis. Blood vessels facilitate the physical infiltration of macrophages and leukocytes into tissues. These cells in turn promote endothelial cell proliferation and new blood vessel formation by secreting pro-angiogenic factors (54–56). Additionally, several cytokines produced by inflammatory cells have angiogenic effects, including TNF, IL-6, and IL-8 (57, 58). Adipocyte- and endothelial cell-derived ANG-2 exerts effects on both endothelial cells and immune cells. ANG-2 binds the endothelial cell RTK Tie2, activating endothelial cell migration and proliferation, but also initiating an NF- κ B-dependent inflammatory signaling cascade (59). Thus, endothelial cells recruit more inflammatory cells. Interestingly, both ANG-2 and VEGF-A promote an antiinflammatory macrophage polarization (60, 61). The relationship between angiogenesis and immune cell signaling is complex, but at least in the context of a non-pathologic hypoxic stress signal, well-controlled inflammation and angiogenesis promote the healthy expansion of AT with a net beneficial effect on metabolic homeostasis.

The adipocyte injury signal: wound healing

There is a physiologic limit to adipocyte cell size. Adipocytes in the mouse inguinal and epididymal fat pads can increase in size three- and seven-fold, respectively, during 12 weeks of HFF (62). Cell size correlates strongly with the frequency of adipocyte death. The percentage of dead epididymal adipocytes increases progressively from 0.1% at one week to as much as 16% at 12 weeks of HFF (62). Independent of how adipocytes die — from necrosis or apoptosis — the reaction of AT to adipocyte death can be likened to the initiation of a wound healing response, triggering a considerable increase in immune cell infiltration. Monocyte recruitment and differentiation to proinflammatory macrophages are of particular importance. These macrophages surround the dead adipocytes, forming what is described histologically as “crown-like structures” (62–64). Concomitantly, activated myofibroblasts in the area secrete collagen to maintain the integrity of the damaged tissue (65). As macrophages and neutrophils clean the damaged area, they produce toxic products such as ROS and reactive nitrogen species (RNS) that further injure surrounding cells and promote fibrosis. Dermal white AT plays an important role in topical wound healing, with the adipocytes as critical mediators (66). Inflammation during wound healing is self-limiting, provided the force producing the injury signal is removed. In fact, early removal of macrophages is essential to proper wound healing and prevention of scarring (67). However, in obesity the injury signal persists, causing chronic activation of myofibroblasts and immune cells, resulting in further tissue damage, fibrosis, and ultimately AT dysfunction.

Unresolved inflammation. The regulation of immune cell function and cytokine production in obese AT has been well studied (68), and it is now evident that the balance of pro- and antiinflammatory signals is critical for disease progression. Although a proinflammatory program is activated during early AT expansion, the immune response is dominated by antiinflammatory signals. During chronic obesity, both innate and adaptive immunity are activated and skewed to a proinflammatory response triggered by adipocyte death, hypoxia, and reduced fatty acid storage capacity in dysfunctional adipocytes.

One of the best studied consequences of proinflammatory signaling in AT is insulin resistance. TNF- α , produced by immune cells, was the first cytokine demonstrated to directly impede insulin action in the adipocyte (69). It downregulates the major insulin-responsive glucose transporter GLUT4 and inhibits insulin-dependent tyrosine phosphorylation of the insulin receptor and IRS-1 through ceramide production (70, 71). Other factors produced by inflammatory cells have since been shown to inhibit insulin signaling in the adipocyte including IL-6, IFN- γ and CCL2 (72, 73). Adaptive immune cells also produce factors that influence insulin signaling. B cells are divided into two main subfamilies, B1 and B2. B1 cells produce germline-encoded natural IgM and IgA antibodies. B2 cells are responsive to T cells upon antigen stimulation and produce a host of different adaptive antibodies as well as cytokines. In addition to cytokines, B2 cells also produce over a hundred different antibodies that have been associated with insulin resistance in human AT (8, 9).

It has been suggested that the production of ROS and RNS by immune cells contribute to the oxidative damage observed in obese AT (74, 75). Although this theory has yet to be directly tested, the degree of macrophage infiltration into obese AT and what we know about macrophage function in wound healing make this a plausible scenario. During the inflammatory phase of wound healing, activation of monocytes and macrophages induces a “respiratory burst,” in which large quantities of superoxide are generated by a membrane-bound NADPH oxidase. This respiratory burst precedes the production of multiple ROS including hydroxyl radicals and the longer-lived pro-oxidant hydrogen peroxide. This response makes macrophages lethal to pathogens, but it does not come without collateral damage. Macrophages need to be removed before surrounding tissues can be repaired (76). Increased oxidative stress and reduced antioxidant defense have been demonstrated in obese AT (74). Further work will be needed to determine whether this is a direct consequence of AT macrophage activation.

Interestingly, in humans, oxidative stress may precede AT inflammatory cell infiltration. Men fed a high-calorie diet for one week gained significant weight accompanied with insulin resistance (77). Oxidative damage was detected in AT biopsies without an increase in inflammatory cell markers. In mice, the chronology of oxidative stress and inflammation onset is not known; however, treatment of mice with antioxidants can attenuate diet-induced inflammation and insulin resistance, suggesting a strong influence of oxidative stress on obesity-associated disease (78–80). In addition, oxidative modification of cellular components such as glutathionylated 4-hydroxy-2-nonenal can directly stimulate a macrophage-mediated proinflammatory response (81).

Although the detrimental consequences of proinflammatory signaling in obese AT are evident, our group has shown that it is also required for appropriate expansion of AT and safe storage of potentially toxic lipid species (82). In fact, TNF- α plays an essential role in the adaptation of AT to HFF. Adipose-specific overexpression of a dominant-negative form of TNF- α causes severe glucose intolerance and a reduction in the insulin-sensitizing adipokine adiponectin in mice fed a HFD (82). Thus, AT inflammation in obese individuals cannot be assigned a strictly pathologic role.

Impaired angiogenesis. Under the persistent metabolic challenge of chronic HFF, the demand for oxygen is great but the capacity to form new blood vessels is poor. The consequence is disorganized and pathologic angiogenesis; however, the underlying mechanisms are not fully understood. Long-term HFF results in abnormal regulation of VEGF-A expression; not only do the VEGF-A levels decrease in the AT of obese mice and humans (13), but there is also evidence that the presence of VEGF in obese mice can block the regulation of neovascularization and vessel normalization (83). Interestingly, unlike acute AT expansion, the hypoxia response of obese *ob/ob* mice fails to induce VEGF expression, and instead a decrease in vascular density is observed (4). Although the majority of studies have shown that obese AT is hypoxic, we do not know if the degree of hypoxia is sufficient to enforce persistent angiogenesis, particularly in human AT. Indeed, it has been argued that the oxygen tension in AT is not low enough to be defined as hypoxic (84). Regardless of the intensity of the hypoxia, our studies have shown that acti-

vating the classic hypoxia response is not sufficient to ameliorate angiogenic deficiencies in obese mice. Genetic introduction of dominant-active HIF-1 α in mice was unable to induce an angiogenic response but instead increased the expression of several fibrotic genes (4). Conversely, either overexpression of a dominant-negative HIF-1 α or pharmacologic inhibition of HIF-1 α significantly reduced AT fibrosis and improved AT function in mice fed a HFD (85). Further efforts are required to understand how AT angiogenesis is disrupted in obesity and how this can be therapeutically overcome.

One potential determinant of vessel density and integrity is the type of angiogenesis that occurs during long-term obesity. We can speculate that, as a result of myofibroblast activation in obese AT, new vascular networks are formed frequently through intussusception, or splitting of existing vessels. This rapid form of angiogenesis can be beneficial in the special conditions of cutaneous wound healing but may contribute to vascular dysfunction in obese fibrotic AT. Mechanical forces that exist during the rapid growth of a tumor induce this kind of angiogenesis (86). As in obese AT, tumor vasculature is significantly more permeable than that of normal tissue (87), a characteristic that promotes immune cell extravasation and fibrosis. Thus, future therapeutic strategies will benefit from an understanding of the mechanisms behind the pathologic angiogenesis observed in obesity.

Given the importance of appropriate vascularization for AT expansion, angiogenesis has been proposed as a potential therapeutic target to treat obesity and its related metabolic problems. Chemical angiogenic inhibitors such as angiostatin, endostatin, and thalidomide as well as the VEGFR2-blocking antibody TNP-470, have been tested in diet-induced obese mice or genetically obese *ob/ob* mice and shown to significantly reduce body weight and fat pad mass (88, 89). Additionally, a recent study suggested treatment with docosahexaenoic acid might improve insulin resistance through attenuation of AT angiogenesis (90). Several natural products extracted from plants (91–93) have also effectively reduced body weight in *ob/ob* and HFD-induced obese mice by inhibiting angiogenesis. Despite these results, the inhibition of angiogenesis as a therapeutic strategy for obesity remains a controversial notion (94). Any metabolic improvements afforded by these systemically introduced compounds cannot be directly attributed to reduced angiogenesis in AT. Our group has reported that WAT-specific overexpression of VEGF-A resulted in differential metabolic effects in mice challenged with a HFD or mice that are genetically obese (22). In HFD-challenged mice, VEGF-A significantly increased WAT vascularization and beiging, which augmented energy expenditure and prevented unfavorable metabolic changes, while blockade of VEGF-A/VEGFR2 caused aggravated systemic insulin insensitivity. In contrast, in *ob/ob* mice with pre-existing obesity, inhibiting the VEGF-A/VEGFR2 signaling pathway resulted in improved insulin sensitivity and decreased body weight.

Several key questions about the role of angiogenesis in AT remain. First, what types of pro-angiogenic mechanisms are in effect either simultaneously or sequentially (and in which order) during the expansion of AT? Second, how do we properly define the stages of AT expansion and further identify when angiogenesis is appropriate and when it is pathologic? Third, can

modulators of angiogenesis be activated locally in different fat pads and achieve distinct effects? And finally, would an angiogenic strategy be more effective if combined with other existing anti-obesity therapies?

Fibrosis and ECM dysfunction. Fibrosis occurs during chronic obesity and has been accepted as a major contributor to obesity-associated AT dysfunction. Although deposition of fibrous collagen proteins is well known to promote metabolic dysfunction in obesity, many other ECM components are dysregulated in obese AT. Recent studies have shown an important role of microfibril-associated glycoprotein 1 (MAGP1) in obese AT. MAGP1 binds active TGF- β , sequestering it in the ECM (95). The actions of MAGP1 have been shown to protect mice from obesity and associated metabolic defects (95). Additionally, HA accumulates in insulin-resistant AT in obese mice and is thought to negatively regulate adipocyte insulin signaling (96). Fibrosis has been suggested to limit AT angiogenic capacity. Genetic knockout of the collagen-binding receptor integrin in the muscle of diet-induced obese mice increases vascularization (97).

Interestingly, the detrimental effects of fibrosis-mediated restriction of AT expansion during obesity on metabolic health may be dependent on the adipose depot affected. Mice genetically deficient in interferon regulatory factor 5 display enhanced expansion of subcutaneous AT but limited expansion of visceral AT on a HFD (98). Restricted epididymal AT expansion was associated with a large increase in antiinflammatory macrophage infiltration, collagen deposition, and improved insulin sensitivity. Thus, the ability of fibrosis to affect AT processes and signaling pathways in a depot-specific manner makes it a fertile topic for future research.

An integrated response: a role for cellular senescence in obesity. Recent studies have identified an important role for cellular senescence in many diseases including those associated with obesity. Senescent cells can originate from most cell types and are potent modulators of inflammation, angiogenesis, and fibrosis. Cells undergo so-called replicative senescence during natural aging when they have reached the genetically determined limit of division. Division-competent cells can also be pushed to senescence through damaging stresses such as oncogene induction, oxidative stress, and double-strand DNA breaks (99). Although growth arrested, senescent cells are highly metabolic and exhibit a senescence-associated secretory phenotype (SASP), producing factors that have profound effects on neighboring cells such as proinflammatory cytokines (IL-6, IL-8, TNF- α , monocyte chemoattractant protein 1 [MCP-1]), VEGF, MMPs, and PDGF-AA (100). Targeting senescent cells or their products alleviates age-related dysfunction of adipocyte progenitors and metabolism, as elegantly demonstrated in mice expressing a *p16INK4a* promoter-driven inducible caspase-8 (INK4-ATTAC mice) (101). In this model, the loss of senescent cells blunted age-related fat loss and enhanced adipogenic transcription factor expression within three weeks.

Little is known about the role of cellular senescence in obesity-associated disease. However, several studies suggest that senescence may be a contributing factor (Figure 2). Increased AT DNA damage and resulting cellular senescence have been identified in mice fed a HFD for 20 weeks (102). This study established a strong link between cell senescence and obesity by demonstrating that

inducing genomic instability through ablation of polymerase η increased the number of senescent AT cells and exacerbated AT dysfunction. The prevention of cellular senescence through inhibition of the major senescence regulator p53 attenuates metabolic abnormalities (102, 103). Obesity is thought to promote cell senescence through various means including oxidative stress, high glucose concentrations in the microenvironment, and increased IGF and ceramides (104). Surprisingly, preadipocytes and mature adipocytes as well as endothelial cells can become senescent during obesity (105–109). Reduced blood flow, as seen in obese AT, is sufficient to trigger endothelial cell senescence (105, 110). Systemically, the circulating endothelial cell precursor population undergoes premature senescence in obese individuals (107), contributing to the impairments in vascular function and repair observed in obesity. Thus, cell senescence of adipocytes, endothelial cells, or their precursors might have major effects on AT homeostasis.

SASP factors are also likely to have considerable effects on AT immunity, angiogenesis, and fibrosis. SASP has recently been shown to have a beneficial effect on wound healing by recruiting immune cells to clear dead cells, promote angiogenesis, limit fibrosis, and stimulate wound closure (100, 111, 112). In contrast, the proinflammatory cytokines released from senescent cells, including MCP-1, TNF- α , and IL-1 β , are among those considered most important for the progression to dysfunctional AT and chronic fibrotic disease (65, 113). Therefore, in chronic obesity the beneficial effects of time-limited cellular senescence in wound healing are replaced by the detrimental effects of chronic SASP.

AT inflammation, fibrosis, and angiogenesis in human obesity. Although rodent models are crucial for our continued understanding of adipocyte biology in obesity, the translation of these findings to human disease is the ultimate goal. Briefly outlined below are findings from human studies on this subject.

Inflammation. Weisberg et al. demonstrated that subcutaneous AT macrophage infiltration was increased in obese patients, similar to that observed in obese mice (10). Several detailed studies have also reported a higher macrophage count in visceral AT of insulin-resistant obese patients compared with lean or insulin-sensitive obese counterparts, an effect that was significantly mitigated following bariatric surgery (10, 114–116). However, conflicting findings concerning AT inflammation in humans have also been reported. Boden et al. showed that healthy men can become insulin insensitive during acute excessive caloric intake but do not display inflammatory changes in AT (77). Moreover, others have shown that 12 months after bariatric surgery, AT inflammation remained elevated in approximately 40% of patients (117). Therefore, whether inflammation is the trigger or result of obesity-associated metabolic defects remains an important area for human studies.

Angiogenesis. Similar to findings in mice, human studies have reported that VEGF expression is reduced in obese humans (13). Consistent with mouse models of adipocyte-specific overexpression of VEGF-A, higher VEGF-A expression correlated with improved capillary density and insulin sensitivity in non-diabetic obese individuals (118). However, contradictory reports have demonstrated elevated levels of VEGF in obese subjects as well as a positive correlation between VEGF expression and insulin resistance (43). Thus, in both rodent models and human studies, VEGF-A-mediated AT angiogenesis exerts dichotomous effects in AT expansion and function.

Fibrosis. The majority of human studies have reported fibrosis in obese AT (119–121). However, a recent human study compared obese patients with or without T2D and found significantly less fibrosis in the visceral AT of the diabetic subjects (122). Another independent study also reported less collagen content in visceral AT from metabolically unhealthy obese patients (123). Less fibrosis was associated with adipocyte hypertrophy, reduced pre-adipocyte hyperplasia, and AT dysfunction (122). Thus, the role of ECM remodeling and fibrosis in human AT dysfunction is stage dependent and likely fat depot specific. This debate is also a reflection of the fact that no consensus has emerged as to how we should define fibrosis. Is it the total tissue collagen content? Is it a reflection of how intensely the collagen bundles are cross-linked? Is it about interstitial pericellular collagen accumulation, or is it about the density of the septa that separate individual functional units within adipose depots (124)?

Concluding remarks

We are beginning to understand the importance of the complex interactions between inflammation, the ECM, and angiogenesis in the context of obesity. Therapeutic strategies are extremely

challenging at all three levels. The limited successes for pharmacologic intervention at the level of inflammation (94, 125), the established unmet needs in the area of anti-fibrotic therapies, and obvious difficulties in promoting a healthy pro-angiogenic response versus the creation of a tumor-friendly angiogenic environment pose major obstacles to attempts to promote exogenously healthy expansion of WAT and will remain an important challenge for future research endeavors.

Acknowledgments

The authors are supported by National Institute of Health grants R01-DK55758, R01-DK099110, P01-DK088761 and P01-AG051459, as well as a grant from the Cancer Prevention and Research Institute of Texas (CPRIT RP140412).

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