REVIEW

Adipose tissue fibrosis: the unwanted houseguest invited by obesity

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Abstract

The prevalence of obesity is increasing exponentially across the globe. The lack of effective treatment options for long-term weight loss has magnified the enormity of this problem. Studies continue to demonstrate that adipose tissue holds a biological memory, one of the most important determinant of long-term weight maintenance. This phenomenon is consistent with the metabolically dynamic role of adipose tissue: it adapts and expands to store for excess energy and serves as an endocrine organ capable of synthesizing a number of biologically active molecules that regulate metabolic homeostasis. An important component of the plasticity of adipose tissue is the extracellular matrix, essential for structural support, mechanical stability, cell signaling and function. Chronic obesity upends a delicate balance of extracellular matrix synthesis and degradation, and the ECM accumulates in such a way that prevents the plasticity and function of the diverse cell types in adipose tissue. A series of maladaptive responses among the cells in adipose tissue leads to inflammation and fibrosis, major mechanisms that explain the link between obesity and insulin resistance, risk of type 2 diabetes, cardiovascular disease, and nonalcoholic fatty liver disease. Adipose tissue fibrosis persists after weight loss and further enhances adipose tissue dysfunction if weight is regained. Here, we highlight the current knowledge of the cellular events governing adipose tissue ECM remodeling during the development of obesity. Our goal is to delineate the relationship more clearly between adipose tissue ECM and metabolic disease, an important step toward better defining the pathophysiology of dysfunctional adipose tissue.

Introduction

By 2030, 51% of the US population will be obese, and 10% of Americans will have a BMI over 40 kg/m² if current trends persist (Finkelstein et al. 2012). Prevention or reducing body weight is a critical factor in controlling diabetes, cardiovascular disease, stroke, hypertension, and cancer. Obesity is, by definition, an excess of fat mass. However, the pathogenesis of obesity involves processes that are more complex than the simple accumulation of fat. Adipose tissue biology is central to the regulation of energy balance, as it senses and responds to excess nutrient intake not only by storing energy and expanding in size but also by regulating the secretion of hunger and satiety factors that coordinate the responses of multiple organ systems (Funcke & Scherer 2019).
The white adipose tissue (WAT) is an organ that primarily stores energy as triacylglycerol during an energy surplus (lipogenesis), to be mobilized as fatty acids (lipolysis) when needed. The bulk of white adipose tissue is distributed in two major depots, the subcutaneous WAT and the visceral WAT, with the accumulation of the latter being most associated with the pathophysiology of obesity (Tchernof & Despres 2013). Distinct from WAT, brown adipose tissue is specialized for energy expenditure through the ability to stimulate thermogenesis. Adipocytes in white adipose tissue have a remarkable capacity to expand or shrink in response to nutrient status (Sakers et al. 2022). As the adipose tissue architecture changes, it is structurally and mechanically supported by the extracellular matrix (ECM), a three-dimensional network of molecules that undergo constant remodeling as they are synthesized, degraded, and cross-linked. An increase in the remodeling of ECM during WAT expansion is a physiologically appropriate response to temporary caloric excess. The ECM regulates the size of adipocytes, differentiation of preadipocytes, endothelial cells and angiogenesis, and the regulation and recruitment of immune cells (Pellegrinelli et al. 2016, Ruiz-Ojeda et al. 2019). Chronic obesity leads to inappropriate accumulation, location, or organization of ECM that is tightly associated with inflammation and dysfunction of adipose tissue and is a primary cause of obesity-related metabolic disorders, including insulin resistance, hepatic steatosis, and type 2 diabetes (Michaud et al. 2016). This is not surprising, since fibrosis is a pathological feature of most chronic inflammatory diseases. However, a degree of ECM accumulation is important for the normal structural organization and stabilization of adipose tissue and to prevent lysis of fragile adipocytes upon mechanical impact. A key pathological event during obesity is a reduced plasticity of adipose tissue due in part to the inability to break down or remodel the ECM (Sun et al. 2013).

The significance of how metabolically flexible, non-fibrotic adipose tissue is fundamental to metabolic health is exemplified by the existence of a subset of individuals that display metabolically healthy obesity (MHO) (Smith et al. 2019). They are resistant to the manifestations of the metabolic syndrome and the adverse cardiometabolic effects of excess adiposity and marked weight gain. Individuals with MHO expand their adipose tissue without the development of inflammation, and with no evidence of increased adipose tissue fibrosis or insulin resistance, features that are typically associated with metabolically unhealthy obesity (Smith et al. 2019). Further studies of metabolically healthy obesity in clinical studies and in preclinical models will define the mechanisms in adipose tissue that link obesity to cardiometabolic complications.

Prevention of obesity is the best way to ensure metabolic health. For many individuals though, obesity is inevitable. While weight loss would be the ideal approach to reverse the negative consequences of obesity, weight regain after weight loss is a substantial challenge (Dulloo & Montani 2015). In response to energy-restricted weight loss, peripheral organs send a vast array of signals to the central nervous system (CNS), which integrates this information to regulate feeding behavior and protect against the depletion of the body’s energy stores (Kim et al. 2018). However, like many other fibrotic diseases, adipose tissue fibrosis is not easily reversed, and it directly impairs adipose tissue plasticity and is a barrier against the ability to lose weight, resulting in long-term systemic metabolic dysfunction (Anderson et al. 2013, Anderson-Baucum et al. 2014, Liu et al. 2016, Zamarron et al. 2017, Zou et al. 2018, Zapata et al. 2022, Li & Chen 2023). Furthermore, the state of obesity permanently alters the cells in adipose tissue that upon weight regain, increase the risk for metabolic complications beyond the risk of obesity (Caslin et al. 2022, Ding et al. 2022). Novel approaches for achieving sustained weight loss over extended periods may focus on mechanisms that enhance the adaptability of adipose tissue or facilitate the resolution of fibrosis. In doing so, they could mitigate the persistent risk of metabolic disorders resulting from obesity. Here, we summarize the contemporary body of literature that describes the mechanisms through which the adipose tissue ECM can act as a constraint in weight maintenance and metabolic health, along with the existing approaches aimed at modulating adipose tissue ECM.

**Adipose tissue remodeling by the extracellular matrix**

White adipose tissue has the unique ability to expand and shrink in significant proportions. This is a coordinated process, controlled by diverse cell types that remodel and reshape the ECM by degrading and reassembling it. ECM provides structural support, mechanical stability, and elasticity but also accommodates expansion by guiding differentiation, migration, and survival of cells for maintaining normal homeostasis. Thus, the cells in adipose tissue play an active role in sculpting their surrounding environment (de Sousa Neto et al. 2022). The adipose tissue microenvironment consists of not only adipocytes, but also other cell populations including...
preadipocytes, fibroblasts, both innate and adaptive immune cells, and endothelial cells. Adipose tissue remodeling depends on a highly synchronized response to external stimuli among these different cell types. This includes the ECM components present in the tissue that serve as a reservoir of growth factors and cytokines that are essential to the reciprocal communication between cells and the environment (Ruiz-Ojeda et al. 2019, DeBari & Abbott 2020, Johnston & Abbott 2023).

**ECM components**

The mechanical properties of adipose tissue ECM are defined by how specific components are deposited, cross-linked, and organized together via covalent and noncovalent modifications. The maintenance of ECM is tightly controlled and many of the involved factors are regulated according to nutritional status. The adipose tissue ECM composition overlaps to a large degree with the ECM found in other tissues, albeit with some unique features (Mariman & Wang 2010). Each cell is embedded in ECM, which consists of two distinct entities – the basement membrane and the interstitial matrix. The pericellular interstitial ECM that shapes cell morphology mainly consists of collagens (I, IV, V, VI, VIII, and IX), elastin, fibrillin, glycoproteins (fibronectin, vitronectin) and glycosaminoglycan (hyaluronan) (Mariman & Wang 2010). The central role of the basement membrane is to provide a physical barrier between the epithelial cells and the connective tissue while still allowing the diffusion of gases and transport of molecules. The basal membrane is rich in collagen IV and VI, and contains laminin, entactin, perlecan, and heparan sulfate (Sharath et al. 2020). How the composition and relative abundance of ECM influence the fundamental aspects of the cells that comprise adipose tissue has recently been extensively reviewed (Sun et al. 2023).

Collagens represent the major substance of noncellular adipose tissue mass, with more than 20 subunits of 12 collagens detected (Mariman & Wang 2010, Datta et al. 2018). Since the ECM is necessary for the structural integrity of adipose tissue, total collagen does not necessarily correlate with increased fibrotic disease. It is the overabundance of the pericellular collagen that is generally found to be associated with higher body fat mass and cardiometabolic risk variables (Michaud et al. 2016). This is particularly relevant for collagen VI which is enriched in adipose tissue and forms the basal lamina surrounding adipocytes. Collagen VI together with its cleavage product *endotrophin*, is important for processes related to tissue expansion, fibrosis, and inflammation (Sun et al. 2014). Additionally, collagen IV as well as collagen I and III are particularly overrepresented in obese ECM (Divoux et al. 2010) (Fig. 1).

**ECM maintenance**

ECM turns over constantly in white adipose tissue and is mediated by enzymes promoting construction of the ECM or by components involved in its degradation. The degrading enzymes belong to either the fibrinolytic system or the matrix metalloproteinases (MMPs). The expression levels of many MMPs and tissue inhibitors of metalloproteinases (TIMPs) are frequently correlated with obesity (Mariman & Wang 2010) (Fig. 1). At the mechanistic level, several of these factors regulate processes relevant for fibrosis, such as adipocyte size, adipogenesis, angiogenesis, and differentiation (reviewed in Berg et al. 2019). Moreover, the manipulations at the level of a single metalloproteinase can cause major consequences on systemic metabolism. For example, the depletion of MMP14 (also occasionally referred to as MT1-MMP), the predominant pericellular collagenase in adipose tissue, halts white adipose tissue formation leaving tissues populated by mini-adipocytes that leads to severe lipodystrophy in mice (Chun et al. 2006). Mice with adipose tissue-specific overexpression of MMP14, on the other hand, have increased body weight, decreased energy expenditure, increased serum and liver triglyceride, and display insulin resistance (Li et al. 2020). Taken together, alterations in adipose tissue ECM composition, abundance, and cross-linking can cause both local effects as well as major effects on whole-body energy homeostasis. In addition to the relative abundance of each collagen species, the degree of collagen cross-linking determines ECM stiffness and mechanical properties. Obesity increases mature nonreducible collagen cross-linking in WAT, that is associated with insulin resistance (Liu et al. 2022a). The lysyl oxidase enzymes (LOX) are key contributors to this process by catalyzing the first step of the covalent cross-linking of collagens and elastin. In obese mice, the inhibition of LOX activity results in reduced stiffness and a significant improvement in metabolic parameters and reduces local adipose tissue inflammation (Halberg et al. 2009, Huang et al. 2021).

**ECM signaling**

The components of ECM are not inert molecules. ECM mediates bidirectional signal transduction between the
extracellular and intracellular space. This communication from the ECM to a cell is carried out by transmembrane cell-surface receptors to regulate adipogenesis, angiogenesis, inflammation, or even cell death (Hastings et al. 2019, Mezu-Ndubuisi & Maheshwari 2021). Integrins and other ECM receptors, such as CD44, are expressed in adipose tissue and are critical components for cell-matrix interactions in adipocytes (Fig. 2). Roles for specific integrins have been established in other tissues but have not been defined in regard to adipose tissue, but their activity has been shown to regulate insulin sensitivity (Williams et al. 2015). WAT-specific loss of integrins can result in systemic insulin resistance (Ruiz-Ojeda et al. 2021), and signaling through collagen, integrins, and the adaptor protein integrin-linked kinase (ILK) (Bugler-Lamb et al. 2021), as well as through hyaluronan–CD44 that is involved in obesity-associated insulin resistance in adipose tissue (reviewed in Musale et al. 2023). CD36 is an integral membrane protein that facilitates free fatty acid transport into the adipose tissue, but also binds additional ligands, including collagens (Lin et al. 2016). Evidence suggests that peptide products originating from elastin and collagen VI engage in signaling interactions within adipose tissue. However, the receptors or signaling pathways involved in adipose tissue specifically remain not well defined and deserve further attention.

**ECM regulates the structural plasticity of adipose tissue**

A fundamental property of healthy white adipose tissue is a high level of phenotypic, metabolic, and structural plasticity (Sakers et al. 2022). For example, in periods of chronic overfeeding, when lipid esterification outbalances lipolysis (metabolic plasticity), white adipose tissue can undergo a massive expansion (structural plasticity) (Fig. 3). At the level of the adipocyte, two different mechanisms underlie this elasticity: hypertrophy (increase in cell size) and hyperplasia (increase in cell number). Furthermore, the intrinsic phenotypic plasticity of adipose resident cells can either promote or decrease fibrosis by inducing autocrine, paracrine, or endocrine signaling events as they drift from one cellular identity to another.

**ECM regulates the size of adipocytes**

White adipocytes have an extraordinary capacity to enlarge and shrink, and adipocyte size can reflect adipocyte fitness. In both humans and mice, there is an inverse correlation between adipocyte size, insulin sensitivity, inflammation, and fibrosis (Divoux et al. 2010, Dankel et al. 2014, Stenkula & Erlanson-Albertsson 2018). Large adipocytes have impaired cellular function...
Hypoxia in adipose tissue triggers ECM remodeling

To handle excess energy, hypertrophic adipocytes can become very large with diameters of up to 200–300 μm. At this size, the diffusional rate of oxygen declines profoundly (Sotornik et al. 2012). This, in combination with reduced overall, but particularly postprandial blood flow occurs in insulin resistant adipose tissue during obesity, triggering local mild hypoxic conditions (Goossens et al. 2011). To avoid severe hypoxia, adipocytes stimulate angiogenesis by paracrine signaling of factors, such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and vascular endothelial growth factor (VEGF) (Herold & Kalucka 2020). At the mechanistic level, a key player in response to oxygen limitation in adipocytes is the oxygen sensitive transcription factor hypoxia-inducible factor 1α (HIF1α) (Halberg et al. 2009). Additionally, the lack of oxygen induces signals that drive adipogenesis and extracellular matrix remodeling to allow for further expansion. As such, a mild transient state of hypoxia can contribute to healthy adipose tissue expansion (Crewe et al. 2017). The importance of vascularization during healthy white adipose tissue expansion is exemplified in mice with local overexpression of VEGF-A that display increased angiogenesis, increased energy expenditure, and resistance to high-fat diet-induced metabolic complications (Sun et al. 2012). On the other hand, unresolved hypoxia leads to adipocyte death and concomitant recruitment of ‘M1-like’ polarized macrophages (to enable the clearance of dead adipocytes) that cause a chronic state of low-grade inflammation, triggering a cascade of undesired events that result in adipose tissue fibrosis (Oh et al. 2012, McNelis & Olefsky 2014). Activation of the HIF1α transcriptional program induces production of ECM components and affects enzymes that alter collagen cross-linking and abundance, such as lysyl oxidase LOX and MMP14 (Sun et al. 2013, Li et al. 2020). HIF1α induction could furthermore limit angiogenesis as elevated levels have been shown to cause insufficient VEGF-A induction (Halberg et al. 2009).

Persistent hypoxic signaling will contribute to further exaggerated ECM deposition, increasing tissue stiffness, contributing to adipose tissue dysfunction (Crewe et al. 2017, Wood et al. 2009). Experiments in mice suggest a
Depot-specific vascular reactivity of adipose tissue (Song et al. 2016, Lee et al. 2021). In these studies, upon high-fat diet feeding, the angiogenic potential is significantly reduced in visceral compared to subcutaneous depots. In line with this, only the visceral fat displayed increased ECM stiffness (Song et al. 2016). Yet, the occurrence of adipose tissue fibrosis is certainly not restricted to visceral fat (Tordjman et al. 2009, Beals et al. 2021). The depot-specific mechanisms and kinetics for how fibrosis accumulates are difficult to approach in humans, and largely unknown. It has been observed in humans that there is an increase in inflammation as a consequence of unresolved hypoxia, particularly in visceral fat that has been shown to have twice as many macrophages in omental vs subcutaneous WAT (Cancelllo et al. 2006, Tordjman et al. 2009, Wood et al. 2009). There are likely to be depot differences in relation to vascularization, macrophage infiltration and degree of fibrosis which should be explored in future studies. To date, variations of the depots studied, and the time frame of obesity development represent a major source of confounding reports in the literature.

**Adipocyte hyperplasia prevents fibroinflammatory processes**

The ability to recruit new adipocytes during development or adipose tissue expansion through hyperplasia (adipogenesis) is critical for maintaining healthy adipose tissue (Muir et al. 2016). In general, smaller, more numerous adipocytes are associated with metabolic healthy obesity and increased fat cell size correlates with impaired systemic insulin resistance (Vishvanath & Gupta 2019). Hyperplasia occurs through the differentiation of progenitor cells under the influence of various transcriptions factors, such as peroxisome proliferator-activated receptor-γ (PPARγ) and CCAAT/enhancer-binding protein-α. This process is guided by ECM components to allow preadipocytes to gradually morphologically change from a fibroblastic shape to a multilocular adipocyte and then finally to a spherical unilocular adipocyte (Pellegrinelli et al. 2016).

Increasing the adipogenic potential leads to healthy expansion of visceral white adipose tissue and improves glucose homeostasis (Shao et al. 2018). Animal models illustrate that adipose tissue has a nearly unlimited...
capacity to expand (Sun et al. 2011). Upon adipocyte-specific overexpression of mitoNEET (a mitochondrial protein) on an ob/ob background, transgenic mice develop severe obesity. However, although substantially heavier than their respective controls, mice with mitoNEET overexpression are more insulin sensitive and metabolically fit. At the histological level, their adipose tissue is characterized by smaller and more numerous adipocytes, reduced macrophage infiltration, and reduced fibrosis (Kusminski et al. 2012). It thus may be possible that recruitment of new adipocytes for lipid handling is an advantageous adaptation in relation to adipose tissue fibrosis.

The number of adipocytes in a healthy individual remains relatively constant throughout adulthood, by a dynamic process of cell death and replenishment (Spalding et al. 2008). Studies in mice show that visceral adipose tissue has greater adipogenic potential than subcutaneous adipose tissue. However, WAT mass increases in adult humans via both hypertrophic and hyperplastic growth in scWAT and visceral fat. The balance between these expansion modes may clarify why some individuals are more prone to metabolic issues, particularly due to the stronger association of visceral fat with obesity-related health risks (Fig. 3).

**ECM governs the phenotypic plasticity of adipose tissue**

As discussed earlier, adipose tissue fibrosis is characterized by exaggerated ECM deposition that restricts adipocyte hypertrophy. Unresolved hypoxia and the associated chronic low-grade inflammation as well as reduced hyperplasia are processes that contribute to and accelerate obesity-driven adipose tissue fibrosis. These processes are affected and driven by the interplay between several different cell types. Below, we highlight how adipocytes, mesenchymal stromal cells, and immune cells are linked to ECM production.

**The role of the adipocyte in regulating ECM**

Hypoxia, hypertrophy, or intracellular signaling in adipose tissue causes major alterations in the secretory phenotype of adipocytes. Obese individuals have higher circulating levels of several adipokines and cytokines including leptin, TNFα, and transforming growth factor beta (TGFβ), and reduced adiponectin levels (Straub & Scherer 2019). Although a role for these factors has been well described in other fibrotic tissues, the mechanistic insight of how these factors regulate adipose tissue fibrosis is just beginning to materialize.

Leptin is secreted mainly by white adipose tissue, correlated with the amount of fat mass and is elevated during obesity. The leptin receptor is expressed on immune cells and contributes to a fibrosis-stimulating environment through its immune-modulating functions, including the production of pro-inflammatory factors, such as TNF-α, IL-10, and IL-6 (Dessie et al. 2021, Ren et al. 2022). Several studies have established a pro-fibrotic role of leptin in fibrosis of the heart, lung, kidney, and liver, but the role of leptin in adipose tissue fibrosis remains understudied (Liu et al. 2022b). Adiponectin is produced in abundance in adipose tissue and serves as a critical marker of adipose tissue health and is lower in individuals with obesity. Among the many beneficial functions of adiponectin, it is an anti-fibrotic factor, and the underlying mechanisms are particularly well described in nonadipose tissues, such as the skin, liver, kidney, and heart (Park et al. 2015, Marangoni et al. 2017, Zhao et al. 2021). Numerous additional factors are secreted by adipocytes, including lipids, metabolites, noncoding RNAs, and extracellular vesicles (Funcke & Scherer 2019). Additional investigation is warranted into the specific contributions of these factors to local and systemic inflammation and fibrosis.

Mature adipocytes display heterogeneity, much like adipocyte progenitors or immune cells. In obese mice, there is an almost complete loss of lipogenic adipocyte subpopulations in epidydimal adipose tissue, but an increase in the abundance of lipid-scavenging adipocytes with an elevated expression of genes related to immune response and collagen deposition (Jones et al. 2020, Sarvari et al. 2021). There is also evidence suggesting that dedifferentiation of terminally differentiated adipocytes could contribute to adipose tissue fibrosis (Shen et al. 2022). Signaling through the Hippo pathway coupled with TGFβ signaling could induce a phenotypic shift in adipocytes, converting functions from energy storage to promoting extracellular remodeling. More specifically, Shen et al. showed that Lats1/2-knockout adipocytes could dedifferentiate to progenitor cells, and then convert to myofibroblasts upon TGFβ stimulation (Shen et al. 2022). Transcriptional single-cell analysis of mature adipocytes might elucidate the contribution of adipocytes to ECM; however more studies are needed to determine the relative impact of adipocyte dedifferentiation in obesity-induced adipose tissue fibrosis.

Emerging evidence suggests that a phenotypic switch in mature adipocytes could affect adipose tissue fibrosis.
In response to cold or β-adrenergic agonists, subcutaneous white adipose can undergo a process called beiging. Beiging cells are more adept at increased energy expenditure through thermogenesis and the presence of these adipocytes is associated with improved glucose and lipid metabolism in rodents (Sakers et al. 2022). Furthermore, stimulation of beiging in both white adipocytes and adipose precursor cells has been shown to alleviate adipose tissue fibrosis in mouse models (Hasegawa et al. 2018, Wang et al. 2019). Signaling through the thermogenic transcription factor PR domain containing 16 (PRDM16) has also been shown to reduce adipose tissue fibrosis by promoting a fibrogenic-to-adipogenic transition (Wang et al. 2019). However, whether these preclinical observations translate to therapeutically relevant interventions in humans remains to be seen. While there is supporting evidence for the phenotypic plasticity of adipocytes, it’s important to note that these cells are predominantly postmitotic. Consequently, the phenotypic plasticity of adipose tissue strongly relies on the proliferative activity of adipocyte progenitor cells.

**Mesenchymal stromal cells regulate ECM in adipose tissue**

In many fibrosis-prone tissues, activation of fibroblasts and myofibroblasts is associated with fibrotic transformation. The exact pathological role of these cell types in adipose tissue remains to be defined. Supporting an important role is the finding that adipocyte progenitor cells express the highest level of fibrosis markers, including collagens, relative to adipocytes, immune cells, and endothelial cells (Hepler et al. 2018). Furthermore, in human adipose, endotrophin has a fivefold higher expression in the stromal vascular fraction compared to adipocytes under the specific conditions examined (McCulloch et al. 2015).

Single-cell sequencing methodology has revealed a high degree of diversity among the mesenchymal stromal cells of adipose tissue. These observations highlight that the relative adipogenic potential and contribution to a fibrotic environment differs among these populations (Hepler et al. 2018, Maniyadath et al. 2023). This is exemplified by the fact that subcutaneous WAT and visceral WAT have distinct subsets of phenotypically unique adipocyte progenitors (Maniyadath et al. 2023). There is a population of highly adipogenic progenitors (preadipocytes, also known as adipose progenitor cells (APCs)) that are committed to differentiate into adipocytes. In addition, there are progenitor populations that have reduced adipogenic potential and exert a pro-inflammatory and fibrogenic phenotype. The transcriptional profile of these fibroinflammatory progenitor cells (FAPs) is characterized by an enrichment in gene signatures related to ECM remodeling, inflammation, collagen deposition, and adipogenesis (Maniyadath et al. 2023). FAPs increase with obesity in mice and display induced expression of collagens and inflammatory cytokine gene expression (Sarvari et al. 2021). Progenitor subpopulations with fibrostimulatory properties have also been shown to accumulate in human fat upon obesity. In obese humans, Marcellin et al. reported that PDGFRα-positive cells with high CD9 expression correlated with fibrosis level in omental fat, insulin-resistance severity, and type 2 diabetes (Marcellin et al. 2017). The identification of the subpopulations of adipocyte progenitors is a rapidly expanding field. The next step is to determine the functional properties of the progenitor cells from different adipose tissue depots to clarify whether they play a role in adipose tissue fibrosis. Another intriguing query pertains to the regulatory mechanisms that govern the proportion of APCs and FAPs in the context of obesity. The revelation of fibro/adipogenic progenitors has provided valuable insights into the intricate interplay between adipocytes, preadipocytes, and immune cells.

**Immune cells regulate ECM dynamics in adipose tissue**

Adipose tissue is an immune organ, and the temporal alteration of the immune landscape is an integral component of adipose tissue remodeling over the course of obesity. Adipose tissue immune cells comprise both innate (including macrophages, neutrophils, and mast cells) and adaptive (invariant natural killer T cells (iNKT), T cells, and B cells) nature (Ferrante 2013). A shift in the abundance of pro- vs anti-inflammatory cells and signals translates not only into activation or reduction on the overall immune response, but also contributes to adipose fibrosis by regulating ECM dynamics. As such, obesity-stimulated polarization of immune populations can unquestionably promote or repress adipose tissue fibrosis, inflammation, and insulin resistance.

Adipose tissue contains resident macrophages that regulate ECM components and secrete fibrogenic cytokines. They polarize and change their functions in response to the changing environment (Chakarov et al. 2022). The types and classification of macrophages in adipose tissue is complex. However, the most basic classifications refer to ‘M1-like’ cells expressing proinflammatory factors, and ‘M2-like’ cells express...
anti-inflammatory proteins (Lumeng et al. 2007). In the obese state, adipose tissue macrophages polarize into M1-like populations and new macrophages are recruited into adipose tissue, predominantly into visceral fat (Cancello et al. 2006). Obesity-associated accumulation of M1-like macrophages increases circulating levels of the pro-inflammatory factors tumor necrosis factor α (TNFα) and interleukin-6 (IL-6) that impair adipogenesis and cause insulin resistance (Weisberg et al. 2003, Al-Mansoori et al. 2022). Furthermore, evidence suggests that IL-6 could induce fibroblast-to-mesenchymal transition in several fibrotic organ systems (Li et al. 2022).

The development of obesity and insulin resistance is also associated with several alterations in T cell subpopulations. These changes include increased activation of pro-inflammatory CD8⁺ T cells and reduction in anti-inflammatory CD4⁺ T regulatory cells. Furthermore, there is a shift in the T helper (Th) cell pool, with a higher abundance of Th1 and Th17 cells and a reduction in the frequency of Th2 cells (reviewed in Liu & Nikolajczyk 2019). In relation to fibrosis, evidence points to that Th1 cells are anti-fibrotic as they suppress fibroblast collagen synthesis through interferon-γ (IFN-γ) signaling, whereas Th2 cells promote collagen deposition through IL-4 and IL-13 (Barron & Wynn 2011).

A shift in immune cell polarization will alter adipose tissue’s secretory profile, subsequently influencing local fibrosis and fibrosis in other organs. TGFβ is a pleiotropic cytokine recognized as a major fibrosis regulator in multiple tissues and is associated with excessive ECM deposition. Obesity strongly enhances TGFβ secretion from nonadipocyte cells within adipose tissue and induces its gene expression in adipocytes (Fain et al. 2005, Jones et al. 2020, Musale et al. 2023). TGF-β/SMAD signaling has a crucial role in regulating the adipocyte commitment of mesenchymal stromal cells and extracellular matrix remodeling in adipose tissue (Petrus et al. 2018, Li & Wu 2020). Furthermore, mechanical stress, matrix metalloproteinases and ECM components regulate the processing of the TGFβ precursor and the release of the active TGFβ dimers (Shi et al. 2011).

Many unanswered questions remain about the complex biology in adipose tissue during obesity. Advancements in single-cell technologies have revolutionized our grasp of cellular diversity in adipose tissue and its influence on ECM remodeling. The exact progression for how insulin resistance and fibrosis are generated in adipose tissue remains unclear. Still, it is recognized that it involves the coordination of the diverse cell types discussed earlier.

### Enduring effects of adipose tissue fibrosis

After weight loss, some of the consequences of obesity in adipose tissue are reversible (insulin sensitivity, adipocyte size), while others are irreversible (adipocyte number, immune cell changes, fibrosis) and have a lasting impact on weight loss, weight regain, and long-term metabolic health. Here, we will discuss the molecular and cellular mechanisms in adipose tissue that prevent full recovery after weight loss that may provide valuable insights for improved therapeutic strategies and facilitate complete post-weight loss recovery.

### Childhood is a critical phase for ECM remodeling in adipose tissue

Dynamic adipose tissue remodeling and adipogenesis occur throughout childhood and adolescence. This is a critical time during development when the total number of adipocytes an individual will carry will throughout adulthood is established. The onset of obesity during childhood can increase the number of adipocytes, a process that can have long-term and cumulative effects on obesity. In children, similar to adults, dysfunctional adipose tissue presents as adipocyte hypertrophy, lymphocyte accumulation, and ECM accumulation (Tam et al. 2012, Walker et al. 2014). Older adults who experienced childhood-onset obesity are particularly vulnerable to the cardiometabolic effects associated with perturbations in adipose tissue characteristics associated with obesity (Turner et al. 2022). This is an increasing public health issue since the prevalence of pediatric obesity continues to rise internationally. According to the WHO, 39 million children under five years of age were overweight or obese in 2020.

ECM remodels differently during childhood vs adult obesity. It is not clear if the level of collagen content correlates with the health risks of obesity (Mujkic et al. 2021). Children have fewer crown-like structures than adults. Nevertheless, overweight children do have more crown-like structures than children of a normal weight (Mujkic et al. 2021). Interestingly, in overweight children, the collagen component in scWAT does not contain collagen VI and shows little evidence of interstitial collagen surrounding adipocytes normally displayed in adults (Tam et al. 2012). Nevertheless, plasma levels of the cleaved C-terminal portion of the α3 subunit of collagen VI (endotrophin) are significantly higher in obese children and adolescents than in control subjects (Ezzati-Mobaser et al. 2020). It remains unclear if the changes in
adipose tissue ECM in obese children is related to an actual pathology or whether it reflects normal physiological growth and development of adipose tissue. To better treat childhood obesity and prevent disease risk later in life, further research is needed to understand how fibrosis contributes to adipose tissue dysfunction in children (Landgraf et al. 2015).

**Weight loss is mediated by adipose tissue fibrosis**

A 5–10% weight loss is an effective countermeasure against the complications of obesity. Unfortunately, weight relapse occurs in 95% of adults who lose weight (Anderson et al. 2001). Although still controversial (Stevens et al. 2012) prospective studies suggest that diet and repeated bouts of weight loss and gain (weight cycling) are associated with a higher risk for later weight gain, obesity, and elevated risk of metabolic disorders such as insulin resistance, type 2 diabetes, cardiovascular disease, and nonalcoholic fatty liver disease (Blair et al. 1993, French et al. 1997, Zou et al. 2021, Tan et al. 2023). In animal studies, weight cycling can be studied in a highly controlled manner, thereby overcoming the limitations of clinical studies. Thillainadesan et al. recently performed a systematic review and meta-analysis of weight cycling in rodent studies. Mice that underwent weight cycling had greater body weight, larger adipocyte size, higher fasting glucose, and impaired glucose tolerance compared with animals with late-onset obesity (Thillainadesan et al. 2022).

The physiological basis for what causes weight regain is ultimately controlled by the CNS, which has a strong drive to defend against a loss of energy stores. Therefore, any body weight loss or gain will be opposed by homeostatic mechanisms that will adjust energy intake or expenditure in an attempt to return to the original weight (Vergnaud et al. 2008, Maclean et al. 2011). At the center of this adaptive process are mechanisms that depend on signals from adipose tissue and lean mass. Beyond a certain threshold, obesity-induced alterations of cell function or fate cause irreversible changes in ECM dynamics in adipose tissue that are linked to decreased insulin sensitivity and difficulty in weight loss (Sun et al. 2013). For example, gastric bypass surgery, which generally causes rapid fat loss and increases in insulin sensitivity, is less effective for patients with high degrees of adipose tissue fibrosis (Buchwald et al. 2004, Divoux et al. 2010). Why fibrosis is slow to turnover or resolve in adipose tissue is not well understood. One proposed mechanism is that the stiff ECM seen in advanced adipose tissue fibrosis cannot be dynamically remodeled and contributes to weight regain (Lawler et al. 2016). As adipocytes shrink, stress between the ECM and cells may increase, eventually inhibiting lipolysis, leading to enlarged adipocytes without a mechanism for depleting their lipid stores (Mariman & Wang 2010, Roumans et al. 2016, 2018). Since the leukocytes and macrophages that have infiltrated during obesity remain after weight loss, they sustain inflammation and prevent the resolution of fibrosis (Jang et al. 2016). The fibrosis in adipose tissue may persist during weight loss, since the prolonged inflammatory response alters cellular function and stimulates the fibroblast-to-myofibroblast transition, which underpins many fibrotic disorders (Henderson et al. 2020). Furthermore, obesity increases the number of collagen-producing fibroblasts in adipose tissue and causes adipocytes to adopt a fibroblast-like phenotype. It is unknown if this reversible during weight loss (Jones et al. 2020).

**Fibrosis affects adipokines that regulate energy balance**

Obesity and fibrosis contribute to an unfavorable adipokine profile. Weight cycling has also been shown to imbalance adipokine production and release, promoting a vicious cycle of adipose tissue inflammation and fibrosis. Adiponectin and leptin have been identified as key adipokines affected by weight cycling and directly regulate many biological responses closely associated with weight regain (Bluher et al. 2012).

Under the conditions of normal food intake and stable weight, leptin acts as an indicator of body fat stores, and is the driving force to reduce food intake and increase energy expenditure at the level of the hypothalamus. Clinical and rodent studies have determined that plasma leptin is reduced during dynamic weight loss compared with static weight maintenance (Rosenbaum et al. 1997, Benini et al. 2001, Kochan et al. 2006). In males and females, elevated leptin levels predicted subsequent long-term weight gain (Lissner et al. 1999, Chu et al. 2001a,b). Interestingly, these studies demonstrate that during weight loss or gain, plasma leptin no longer correlates with fat mass in a predictable way. This is in part because as obesity progresses, central leptin insensitivity develops (Fam et al. 2007). Leptin resistance is reversible in humans and has been shown to be a promising therapeutic area for weight loss and improving insulin sensitivity (Wing et al. 1996, Torgerson et al. 1999, Woods et al. 2004, Enriori et al. 2007, Zhao et al. 2019). Leptin is a critical mediator of the immune response, resulting in a largely pro-inflammatory phenotype. Leptin aids in the transmigration of blood...
monocytes into adipose tissue and stimulates preadipocyte stem cells to mature into adipocytes or macrophages (Curat et al. 2004). The direct effect of leptin on adipose tissue fibrosis is not well studied, but it has been implicated in promoting fibrosis in other organs (Liu et al. 2022b). In contrast, leptin deficiency has been shown to promote anti-inflammatory effects in mast cells (Zhou et al. 2015). According to the existing literature, it is conceivable that leptin reduction in humans may be beneficial to mediate immune responses in adipose tissue, which in turn play a role in weight loss, preventing fibrosis and weight regain.

Adiponectin is an important circulating adipokine and possesses notable anti-inflammatory and anti-fibrotic effects (Park et al. 2015, Marangoni et al. 2017). In general, as obesity advances, the concentrations of circulating adiponectin tend to decrease, a trend also associated with patterns of weight cycling (Strychar et al. 2009, Barbosa-da-Silva et al. 2012). There is inconsistent evidence as to whether adiponectin levels can be restored following weight loss/regain (Barbosa-da-Silva et al. 2012, Ambeba et al. 2013). Surgically induced weight loss is accompanied by a marked and progressive rise in serum adiponectin levels (Askarpour et al. 2020, Felipe et al. 2023). However, studies involving both humans and rodents have produced mixed results as to whether caloric restriction with weight reduction impacts plasma adiponectin levels (Abbasi et al. 2006, Silha et al. 2006, Erez et al. 2011). The variations in these studies could be attributed to notable differences in sex and age differences, which are known to influence adipokine levels during weight fluctuations (Suchacki et al. 2023). It is unquestionably worthwhile to explore interventions that raise adiponectin levels, since there is a large body of evidence suggesting that adiponectin can exert important systemic benefits and inhibit hepatic stellate cell activation to counteract liver fibrosis (reviewed in Gamberti et al. 2018).

**Adipose tissue retains an obesogenic memory**

Many studies link weight cycling to an altered adipose tissue immune microenvironment as a result of obesity (Anderson et al. 2013, Anderson-Baucum et al. 2014, Zou et al. 2018, Li & Chen 2023). Zapata et al. reported a mechanism by which a ‘metabolic memory’ of obesity is localized in adipocytes (Zapata et al. 2022). In an attempt to understand the causes of weight cycling, the majority of studies have focused on obesity-induced imprinting of adipose tissue innate and adaptive immune cells after weight loss (Li & Chen 2023). The stages of immune cell recruitment in adipose tissue during weight gain have been well-described. B-cells are among the first responders, and subtypes of T-cells and macrophages gradually increase when insulin resistance occurs (Duffaut et al. 2009). To identify which specific immune cells may be involved in weight cycling, the Hasty group used CITE-seq multiomics methods to study obesity-induced imprinting of adipose tissue immune cells that persisted through weight loss and progressively worsened with weight regain. Weight cycling impaired recovery of type 2 regulatory cells, activation of antigen presenting cells, T cell exhaustion, and enhanced lipid handling in macrophages in weight cycled mice (Surendar et al. 2020, Cottam et al. 2022). It has also been demonstrated that upon weight loss, there was ongoing CD8+ T cell-driven inflammation in the liver and adipose tissue, mediated by macrophages (Surendar et al. 2020). Interestingly, adipose tissue expression of the classical M1 and M2 macrophages markers were increased during obesity but not altered by weight cycling (Anderson et al. 2013, Cottam et al. 2022). Therefore, weight loss results in metabolic improvements, but the adipose tissue remains primed for a pro-inflammatory response if weight is regained.

**Can weight loss resolve adipose tissue fibrosis?**

There is promising evidence that the comorbidities associated with obesity can be mitigated with a combination of lifestyle changes, surgery, or pharmaceutical interventions. Given the diversity of mechanisms underlying weight loss strategies, an individual’s approach toward weight loss will impact adipose tissue inflammation and fibrosis and long-term metabolic improvements.

Here, we summarize the existing literature concerning the impact of various common weight loss methods (bariatric surgery, calorie restriction, pharmacological interventions, and exercise) on the resolution of adipose tissue fibrosis.

**Bariatric surgery**

Over the past few decades, the potential of bariatric surgery to bring about long-term improvements in metabolic disease has garnered increasing attention. The health benefits of successful bariatric surgery are often superior to lifestyle interventions for weight loss (Cheng et al. 2016). Surgical weight loss can be achieved by multiple mechanisms: restricting food intake, decreasing nutrient
absorption, increasing secretion of intestinally derived hormones, and remodeling of the gut–brain axis (Abdeen & Le Roux 2016). It also offers significant lasting benefits on the function of the heart, liver, kidneys, and other organs through mechanisms associated with a partial reversal of tissue fibrosis (reviewed in Ding et al. 2022).

The research design of bariatric surgery studies offers the advantage of being able to robustly test the cause–effect value of the histological/metabolic relationships between adipose tissue fibrosis and health outcomes (Table 1). Research conducted on both animals and humans has shown that bariatric surgery can improve adipose tissue function and reduce liver fibrosis (Billeter et al. 2022). However, bariatric surgery does not improve adipose tissue fibrosis and inflammation in all individuals (Table 1). This variability may reflect study design, particularly regarding at which stage of obesity the surgery was performed. The level of adipose tissue fibrosis is found to be negatively associated with surgery-induced weight loss (Divoux et al. 2010, Abdennour et al. 2014). This is in line with the fact that durable, long-term diabetes remission following bariatric surgery is more likely when performed in patients that are in the early stages of diabetes (Purnell et al. 2021).

The level of ECM deposition in adipose tissue, particularly as reflected in collagen VI, is an impediment to the success of surgical weight loss interventions (Osorio-Conles et al. 2023). These studies suggest that appropriate ECM remodeling, primarily the reduction of ECM deposition and cross-linking and the increase of ECM degradation, may be a key molecular signature for the success of weight-loss surgeries. It will be interesting to see whether bariatric surgery will be the procedure of choice in light of the recent successes in dual agonist pharmacological interventions for weight loss (see below).

Calorie restriction

Hundreds of preclinical and clinical studies show that restricting calories anywhere from 25-75% significantly increases longevity and reduces body weight, fat mass, and markers of insulin resistance and inflammation (Balasubramanian et al. 2017, Most et al. 2017). It is by far the most effective way to prevent obesity, but caloric restriction (CR) for the treatment of obesity is temporary and reverts as soon as normal caloric intake is reinitiated (Banasik et al. 2013). One challenge is that weight regained after CR accumulates preferentially in the visceral vs subcutaneous depot. In addition, CR does not benefit everyone. The sex-specific effects of CR are often ignored, but an important finding is that pre-menopausal females resist CR-induced weight and fat loss (Suchacki et al. 2023). If CR can be prolonged long-term, mouse studies suggest that it does have distinct benefits on adipose tissue plasticity and ECM remodeling (Kurki et al. 2012, Roumans et al. 2015). However, appropriate clinical studies are lacking. Most CR studies conducted in humans have been short-term in duration and the metabolic improvements seen with CR occur after a small degree of weight loss.

The effects of caloric restriction alone do not result in the same adaptations or improvements in adipose tissue plasticity seen after surgery (Cheng et al. 2016). Studies suggest that CR does not resolve adipose tissue dysfunction caused by obesity, and contributes to long-term consequences of weight cycling (Wolfe & Sabiston 1986). Thus, the perceived short-term benefits of calorie-restricted diets, especially in young women, are likely do not outweigh the potential long-term detrimental effects. In this context, it is informative to compare CR and bariatric surgery. Bariatric surgery not only decreases the gastric pouch size and food intake but also involves duodenal bypass, leading to heightened secretion of intestinally derived hormones like glucagon-like peptide 1 (GLP-1), gastric inhibitory polypeptide (GIP), ghrelin, among others. These factors play a significant role in yielding lasting anti-diabetic effects and metabolic benefits.

As an alternative to daily CR, intermittent fasting has been increasingly popular. The common methods are fasting on alternate days, for whole days with a specific frequency per week, or during a specific daily time frame. The benefits of intermittent fasting have been demonstrated extensively in animal studies, but similar benefits for humans have not been consistently observed. However, a systematic review of 40 clinical studies found that intermittent fasting was effective for long term weight loss (Seimon et al. 2015). Our understanding of whether intermittent fasting has advantages over CR in terms of weight loss or fibrosis are not clear but is of significant interest.

Weight loss medications

Pharmacological approaches for weight loss mostly rely on approaches limiting caloric intake to promote weight loss. The ultimate goal for an anti-obesity intervention is to reset a person’s ‘set point’, a term that describes a weight range the body tries to maintain long-term. Current anti-obesity medications effectively reduce weight-related medical issues, such as improving blood pressure, serum
Table 1  Summary of studies that have determined whether bariatric surgery (BS) weight loss effects adipose tissue ECM remodeling.

<table>
<thead>
<tr>
<th>Type of plasticity</th>
<th>ECM effect</th>
<th>Factor</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural</td>
<td>Adipocyte size</td>
<td>Adipocyte hypertrophy improved after weight loss despite a persistence of fibrosis.</td>
<td>Ex-obese patients who had undergone bariatric surgery (BS) compared to lean, overweight, and obese patients.</td>
<td>(Cancello et al. 2013)</td>
</tr>
<tr>
<td>Structural</td>
<td>Angiogenesis</td>
<td>Increased angiogenesis protects against adipose hypoxia and fibrosis.</td>
<td>Metabolic disease-resistant 11beta-hydroxysteroid dehydrogenase type 1 (HSD1)-deficient mice.</td>
<td>(Michailidou et al. 2012)</td>
</tr>
<tr>
<td>Structural</td>
<td>Fibrosis</td>
<td>SPARC expression in human adipose tissue correlated with fat mass and was higher in subcutaneous WAT (scWAT).</td>
<td>Obese subjects underwent a very-low-calorie diet for 16 weeks.</td>
<td>(Kos et al. 2009)</td>
</tr>
<tr>
<td>Structural</td>
<td>Fibrosis</td>
<td>Collagen expression in WAT persists after gastric bypass surgery induced weight loss.</td>
<td>Obese bariatric surgery candidates characterized before, 3, 6, and 12 months after surgery.</td>
<td>(Abdennour et al. 2014)</td>
</tr>
<tr>
<td>Structural</td>
<td>Fibrosis</td>
<td>Diet and surgery-induced weight loss increased collagen 6a3 expression in subcutaneous adipose tissue of obese patients.</td>
<td>Obese patients undergoing bariatric surgery and on average 9.5 ± 1 months subsequently.</td>
<td>(McCulloch et al. 2015)</td>
</tr>
<tr>
<td>Structural</td>
<td>Fibrosis</td>
<td>Adipose tissue fibrosis was found negatively associated with surgery-induced weight loss.</td>
<td>Obese bariatric surgery patients were clinically characterized before, 3, 6, and 12 months after surgery.</td>
<td>(Divoux et al. 2010)</td>
</tr>
<tr>
<td>Structural</td>
<td>Fibrosis</td>
<td>Adipocyte hypertrophy and inflammatory infiltration improved after weight loss in ex-obese patient scWAT, despite a persistence of fibrosis. WAT gene expression was not fully restored, even after an extensive and stable weight loss.</td>
<td>Tissue from morbidly obese patients per-operative to bariatric surgery compared to 10 ex-obese patients at least 2 years after surgery.</td>
<td>(Cancello et al. 2013)</td>
</tr>
<tr>
<td>Structural</td>
<td>Fibrosis</td>
<td>Increased picrosirius red-stained collagen accumulation in scWAT after BS was observed along with fat mass loss. Collagen I and VI staining decreased 1 year after BS, we found increased degraded collagen I and III in scWAT, suggesting increased degradation.</td>
<td>Morbidly obese candidates underwent bariatric surgery and followed up 1 year afterwards.</td>
<td>(Liu et al. 2016)</td>
</tr>
<tr>
<td>Structural</td>
<td>Fibrosis</td>
<td>Endotrophin being correlated to the degree of fibrosis, and, notably, in an inverse manner. Collagen V α1 mRNA levels were identified as an independent predictor.</td>
<td>Subjects with severe obesity that had undergone BS 12 months after BS.</td>
<td>(Osorio-Conles et al. 2023)</td>
</tr>
<tr>
<td>Structural</td>
<td>Fibrosis</td>
<td>Before surgery scWAT fibrosis was significantly higher in diabetic vs insulin-sensitive patients, Six months after surgery and significant weight loss, fibrosis levels remained unchanged in scWAT.</td>
<td>Obese patients before and 6 months after bariatric surgery.</td>
<td>(Chabot et al. 2017)</td>
</tr>
</tbody>
</table>

(continued)
lipids, serum glucose levels, or liver steatosis (reviewed in Ahmad et al. 2021). Unfortunately, the data suggest that for all weight loss drugs to date, the set point is not altered (Wilding et al. 2022, Kosiborod et al. 2023). Since patients need to continue treatment with anti-obesity medications in order to maintain weight loss, medication will need to be taken for life to avoid weight regain. Some individuals who begin weight loss treatments may find themselves in situations where they will have to stop pharmacological weight interventions due to cost or health conditions. This could lead to weight regain during a vulnerable period that may be worse for their health than remaining obese to start out with. Therefore, the serious health risks of weight cycling should be considered if the drug is discontinued. It may be of great benefit to combine weight-loss medication with strategies that improve the plasticity of adipose tissue, angiogenesis, inflammation, or fibrosis to provide long-term health benefits.

<table>
<thead>
<tr>
<th>Type of plasticity</th>
<th>ECM effect</th>
<th>Factor</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural</td>
<td>Adipogenesis</td>
<td>High numbers of scWAT adipogenic progenitors are associated with impaired metabolic flexibility post-BS while the ones of scWAT NKT and helper T lymphocytes are associated at three years post-BS.</td>
<td>Severe obese patients that underwent BS and followed up at 1 and 3 years.</td>
<td>(Ledoux et al. 2022)</td>
</tr>
<tr>
<td>Structural</td>
<td>Adipogenesis</td>
<td>Increase of the CD45−/CD34+ /CD31− adipocyte progenitor reservoir after surgical weight loss.</td>
<td>Morbid obese participant tissue was analyzed before and at least 1 year after bariatric surgery-induced weight loss.</td>
<td>(Garcia-Rubio et al. 2018)</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Leptin</td>
<td>Primary human scWAT explants treated with leptin displayed a dose-dependent reduction in endotrophin expression with increasing leptin concentration.</td>
<td>Primary human scWAT explants.</td>
<td>(McCulloch et al. 2015)</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Cytokines/chemokines</td>
<td>Circulating concentrations of monocyte chemoattractant protein-1 (MCP-1) and IL-6 (but not TNFα) decreased.</td>
<td>ND and T2D patient at time of BS and 1 year follow-up.</td>
<td>(Camastra et al. 2017)</td>
</tr>
<tr>
<td>Phenotypic</td>
<td>Insulin resistance</td>
<td>SAT fibrosis was significantly higher in diabetic vs insulin-sensitive patients (P &lt; 0.05), and associated with IR significantly reduced in all groups (P &lt; 0.0001). No correlation was found between scWAT fibrosis and IR after surgery.</td>
<td>Diabetic vs insulin-sensitive patients scWAT collected during and 6 months after bariatric surgery.</td>
<td>(Chabot et al. 2017)</td>
</tr>
<tr>
<td>Phenotypic</td>
<td>Crown like structures</td>
<td>Before surgery: crown-like structure (CLS) density was higher in T2D than ND patients. After surgery, adipocyte size was reduced by ~50%, and CLS became rare. Accumulation of collagen fibrils, crystals, alterations of basal membrane, and signs of degeneration in adipocytes, and sparse macrophages were still present in scWAT.</td>
<td>Nondiabetic and T2D patient scWAT at time of BS and 1 year follow-up.</td>
<td>(Camastra et al. 2017)</td>
</tr>
<tr>
<td>Phenotypic</td>
<td>Liver steatosis</td>
<td>Association of adipose tissue and liver fibrosis with tissue stiffness in morbid obesity: Links with diabetes and BMI loss after gastric bypass.</td>
<td>Obese bariatric surgery candidates were clinically characterized before surgery and 3, 6, and 12 months after surgery.</td>
<td>(Abdennour et al. 2014)</td>
</tr>
</tbody>
</table>
### Table 2: Potential therapeutics for reversing or remodeling adipose tissue ECM.

<table>
<thead>
<tr>
<th>Potential therapeutic</th>
<th>Proposed mechanism</th>
<th>Effects</th>
<th>Study details</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-terminal portion of the α3 subunit of COL6 (endotrophin) antibody</td>
<td>Decreases pro-fibrotic and pro-inflammatory genes in fat</td>
<td>Suppression of endotrophin improves insulin sensitivity, attenuates adipose tissue inflammation and crown-like structures in epididymal WAT (eWAT)</td>
<td>Isotype IgG- or endotrophin antibody-treated mice after 8 weeks of HFD pretreatment</td>
<td>(Sun et al. 2014)</td>
</tr>
<tr>
<td>GM6001 (ilomastat)</td>
<td>MMP inhibition</td>
<td>GM6001 treatment reduced inflammation and collagen content in adipose tissue, and modified response to weight gain during weight loss</td>
<td>Swiss Webster Mice, diet-induced obesity model, 8 weeks treatment</td>
<td>(Caria et al. 2017)</td>
</tr>
<tr>
<td>Lysophosphatidic acid (LPA) receptor antagonist (Ki6425)</td>
<td>Ki6425 selectively inhibits LPA receptor-mediated actions, especially through LPA1 and LPA3</td>
<td>Ki6425 reduced collagen protein staining and collagen I and IV mRNAs in both inguinal and perigonadal fat; Ki6425 reduced collagen III mRNA inguinal fat from mice</td>
<td>db/db mice, 7 weeks treatment</td>
<td>(Ohta et al. 2003, Rancoule et al. 2014)</td>
</tr>
<tr>
<td>Mast cell inhibitors</td>
<td>Decreased TGFβ1 and expression of various fibrosis-related genes such as collagen 1α1, 3α1, and 6α3, Mmp9, and Timp2 in eWAT</td>
<td>Mast cell stabilization attenuated adipose tissue fibrosis, reduced obesity, and improved insulin sensitivity in mice</td>
<td>Mice, diet-induced obesity</td>
<td>(Kumar et al. 2019)</td>
</tr>
<tr>
<td>Nonthiazolidinedione PPAR gamma agonist [2-(2-[4-phenox y-2-propylphenoxy]ethyl) indole-5-acetic acid] (COOH)</td>
<td>Reduces the expression levels of a vast majority of collagens in adipose tissue</td>
<td>PPARγ agonist treatment decreased TGF-β and reduced adipose tissue collagen in mice.</td>
<td>Wild-type mice were treated with 30 mg/kg COOH or vehicle for 14 days by oral gavage</td>
<td>(Khan et al. 2009)</td>
</tr>
<tr>
<td>Adiponectin receptor agonists (ADP355)</td>
<td>Anti-fibrotic effects in skin were associated with AMPK activation and blockade of FAK activation</td>
<td>Adiponectin receptor agonist peptide inhibited ex vivo fibrotic responses and prevented and reversed skin fibrosis in mice</td>
<td>C57BL6/J mice received bleomycin or PBS daily for 14 days, and daily ADP355 or vehicle was initiated concomitantly and then continued for 21 days</td>
<td>(Marangoni et al. 2017, Yamashita et al. 2018)</td>
</tr>
<tr>
<td>Metformin</td>
<td>Represses TGF-β1-induced fibrogenesis activation of AMPK</td>
<td>Inhibits excessive ECM deposition in WAT, decreased collagen deposition surrounding adipocytes and expression of fibrotic genes including the collagen cross-linking regulator LOX</td>
<td>ob/ob mice or mice with diet-induced obesity</td>
<td>(Luo et al. 2016, Biondo et al. 2018)</td>
</tr>
<tr>
<td>Mineralocorticoid receptor (MR) agonist</td>
<td>Decreases expression of the MR and downstream molecules neutrophil gelatinase-associated lipocalin, galectin 3, and lipocalin-like prostaglandin D2 synthase</td>
<td>Reduced pericellular fibrosis, synthesis of the major subunits of collagen types I and VI, and the pro-fibrotic factor α-smooth muscle actin compared with placebo in subcutaneous adipose tissue</td>
<td>26-week, randomized, double-blind, placebo-controlled trial in patients with type 2 diabetes</td>
<td>(Johansen et al. 2021)</td>
</tr>
<tr>
<td>Isoliquiritigenin (ILG), flavonoid derived from Glycyrrhiza uralensis</td>
<td>Suppresses NLRP3 inflammasome activation</td>
<td>Improved high-fat diet-induced fibrosis in adipose tissue in vivo given ILG showed reduced fibrotic area, TNFα, COL1, and TGFβ1 expression compared to controls; no significant differences between high-fat diet plus ILG and normal diet</td>
<td>C57BL/6 mice, diet-induced obesity</td>
<td>(Watanabe et al. 2016)</td>
</tr>
</tbody>
</table>
### Integrin-targeting drugs

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study details</th>
<th>Effects</th>
<th>Proposed mechanism</th>
<th>Potential therapeutic drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ulimasov et al. 2019, Slack et al. 2022)</td>
<td>Choline-deficient, amino acid-defined, high-fat diet mouse model of NASH</td>
<td>Loss of activated hepatic stellate cells through apoptosis and suppression of hepatic pro-fibrotic signal transduction by transforming growth factor β, GSH formation, and microbial BA metabolism</td>
<td>Reversed hepatic steatosis and prevented the progression of liver inflammation and fibrosis</td>
<td>DT209, tripeptide (Gly-Gly-Leu)</td>
</tr>
<tr>
<td>(Rom et al. 2020, Qu et al. 2023)</td>
<td>Nonhuman primate NASH model, 5 months treatment</td>
<td>Fibrosis measured by analysis of liver collagen was reduced</td>
<td>Reverse hepatic steatosis, GSH formation, and microbial BA metabolism</td>
<td>Integrin-targeting drugs</td>
</tr>
</tbody>
</table>

### Exercise

In combination with a nutritionally balanced diet, exercise training can reduce fat mass, adipocyte apoptosis and inflammation, and ECM expansion (Maharjan et al. 2021, Winn et al. 2021). Evidence in animal models suggests that exercise reverses the progression of adipose tissue fibrosis (Li et al. 2021, Nigro et al. 2023). Of particular interest, many randomized controlled trials show aerobic exercise leads to higher adiponectin and lower leptin levels in diabetic or obese adults (Yu et al. 2017, Becic et al. 2018). Determining how exercise influences adipose tissue fibrosis in humans is challenging since the studies lack of precise control of dietary intake and demographic factors. However, many clinical studies clearly show that exercise improves many of the root causes of ECM accumulation in adipose and other tissues (Thompson et al. 2012, Ahn et al. 2022). Therefore, even without weight loss, exercise is an excellent way to prevent obesity-related diseases and is an effective adjuvant to surgical or pharmaceutical treatments for obesity.

### Future directions

There has been significant progress in the surgical and pharmacological treatment interventions for weight loss, however, investigation into how to prevent fibrosis, weight regain, or weight cycling is still in its infancy. To date, no anti-fibrotic therapies have been approved by the FDA, but several drug candidates have promise to target adipose tissue ECM remodeling (Table 2). Due to the clear connection between fibrosis and inflammatory response in adipose tissue, targeting inflammatory pathways is a logical strategy. However, it is important to keep in mind that fibrosis and inflammation are regulated by distinct mechanisms (McVicker & Bennett 2017). Targeting macrophages that play a role in the onset and progression of obesity-related inflammation and fibrosis has shown to have therapeutic promise (Li et al. 2023). Furthermore, several anti-inflammatory agents in preclinical studies prevent macrophage infiltration in white adipose tissue, promoting the polarization process of type M1-like to M2-like macrophages (da Cruz Nascimento et al. 2022). To develop therapeutics specific to fibrosis prevention, it is crucial that we gain a deeper understanding of the specific factors that contributing to the pathogenesis of fibrosis. Then, in order to test these drugs, it will be fundamental to generate new and improved preclinical models that more closely replicate fibrotic diseases in humans.
It is becoming clear that weight loss alone does not fully address the inflammation or fibrosis in adipose tissue caused by obesity. There are several challenges to treating established fibrosis in adipose tissue. Adipose tissue fibrosis is not commonly measured, and to determine the efficacy of an anti-fibrotic therapy, it will be necessary to begin to accurately measure and diagnose adipose tissue fibrosis in the clinic. Several noninvasive methods have been developed (Table 3), and imaging methods for biomedical purposes are becoming more advanced (Wang 2018, Welle et al. 2022). Another obstacle for the clinical application of antifibrotic therapies is that the effectiveness of the treatment may vary depending on the stage of obesity/and or fibrosis (McVicker & Bennett 2017). For example, when MMP14 is overexpressed in adipose tissue in the early-stage obesity mice showed a healthier metabolic profile, including ameliorated fibrosis (Li et al. 2020). In contrast, the overexpression of MMP14 in the adipose tissue from mice with established obesity, led to enlarged adipocytes and increased body weight. Therefore, it’s possible obese patients may benefit most from MMP inhibitors that digest/modify the dense adipose tissue extracellular matrix and allow for its healthy expansion (Caria et al. 2017). In addition, MMP14 inhibition may prevent the digestion of collagen VI α3 to produce endotrophin, a potent stimulator of fibrosis (Li et al. 2020).

With the advent of effective but expensive weight loss interventions in the form of dual and triple agonists targeting incretin receptors, it is likely that individuals will cycle on and off these medications. It will be important to monitor these trends carefully with the implications for long-term adipose tissue homeostasis. Since obesity is a multifaceted disease, and adipose tissue is a complex endocrine and immune organ a holistic approach involving multiple types of interventions may be necessary for successful treatment and recovery. Thus, it would be practical to design and test antifibrotic therapies in combination with other drugs for the treatment of obesity or type 2 diabetes.

### Table 3  Methods for quantifying adipose tissue fibrosis.

<table>
<thead>
<tr>
<th>Method</th>
<th>Measurement</th>
<th>Sample</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound based assess shear wave speed (Adiposcan, developed from Fibroscan for liver)</td>
<td>Transient elastography</td>
<td>Noninvasive, in vivo</td>
<td>(Abdennour et al. 2014, Sasso et al. 2016)</td>
</tr>
<tr>
<td>Three-dimensional (3D) chemical Shift-Encoded MRI</td>
<td>Adipose tissue volume</td>
<td>Noninvasive, in vivo</td>
<td>(Nemeth et al. 2019)</td>
</tr>
<tr>
<td>Magnetic resonance elastography (MRE)</td>
<td>Shear wave elastography Tissue stiffness</td>
<td>Noninvasive, in vivo</td>
<td>(Jensen et al. 2023)</td>
</tr>
<tr>
<td>Acoustic radiation force impulse (ARFI) elastography</td>
<td>Point shear wave elastography Tissue stiffness</td>
<td>Noninvasive, in vivo</td>
<td>(Tozaki et al. 2011, Wojcinski et al. 2013)</td>
</tr>
<tr>
<td>Fibrosis score of adipose tissue (FAT score)</td>
<td>Perilobular and pericellular fibrosis</td>
<td>Breast adipose tissue Biopsy</td>
<td>(Bel Lassen et al. 2017)</td>
</tr>
<tr>
<td>High-resolution ex vivo MRI</td>
<td>3D fibrosis volume</td>
<td>Biopsy</td>
<td>(Bouazizi et al. 2021)</td>
</tr>
<tr>
<td>Hematoxylin and eosin</td>
<td>Adipocyte morphology Fibrillar collagen</td>
<td>Biopsy staining, histology samples</td>
<td>(Woessner 1961, Krishna et al. 2013, Lattouf et al. 2014)</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>Collagen, keratin</td>
<td>Biopsy, cultured cells</td>
<td>(Amer et al. 2018, Kandhi et al. 2023)</td>
</tr>
<tr>
<td>Picrosirius red</td>
<td>Cell type frequency or intensity of fibrosis markers</td>
<td>Histology samples</td>
<td>(Di Caprio &amp; Bellas 2020)</td>
</tr>
<tr>
<td>Masson's trichrome</td>
<td>3D imaging of collagen volume and architecture</td>
<td>Biopsy</td>
<td>(Huang et al. 2008)</td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>Label-free 3D imaging to evaluate collagen dispersion and structure</td>
<td>Biopsy, cultured cells</td>
<td>(Khan et al. 2009)</td>
</tr>
<tr>
<td>Confocal microscopy</td>
<td>Adipocytes and ECM fibers Ultrastructure of cells: interstitial space, caveolae, vasculature Fresh tissue</td>
<td>Biopsy</td>
<td>(Giordano et al. 2013)</td>
</tr>
<tr>
<td>Second harmonic generation microscopy</td>
<td>Cell type frequency or intensity of fibrosis markers</td>
<td>Biopsy, cultured cells</td>
<td>(Palmeri &amp; Nightingale 2011, Lackey et al. 2014)</td>
</tr>
<tr>
<td>Scanning electron microscopy</td>
<td>3D imaging of collagen volume and architecture</td>
<td>Histology samples</td>
<td>(Di Caprio &amp; Bellas 2020)</td>
</tr>
<tr>
<td>Transmission electron microscopy</td>
<td>Label-free 3D imaging to evaluate collagen dispersion and structure</td>
<td>Biopsy</td>
<td>(Huang et al. 2008)</td>
</tr>
<tr>
<td>Mechanical testing</td>
<td>Adipocytes and ECM fibers</td>
<td>Biopsy</td>
<td>(Khan et al. 2009)</td>
</tr>
<tr>
<td>Gene expression</td>
<td>Ultrastructure of cells: interstitial space, caveolae, vasculature</td>
<td>Biopsy, cultured cells</td>
<td>(Giordano et al. 2013)</td>
</tr>
</tbody>
</table>

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Declaration of interest

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