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Adipose tissue plasticity in health and disease

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Summary

Adipose tissue, colloquially known as “fat”, is an extraordinarily flexible and heterogeneous organ. While historically viewed as a passive site for energy storage, we now appreciate that adipose tissue regulates many aspects of whole-body physiology, including food intake, maintenance of energy levels, insulin sensitivity, body temperature, and immune responses. A crucial property of adipose tissue is its high degree of plasticity. Physiologic stimuli induce dramatic alterations in adipose tissue metabolism, structure, and phenotype to meet the needs of the organism. Limitations to this plasticity cause diminished or aberrant responses to physiologic cues and drive the progression of cardiometabolic disease along with other pathological consequences of obesity.

Villanueva eTOC blurb

Fat is an exceptionally heterogeneous and dynamic organ with many functions beyond energy storage. Cellular, molecular, and systems level studies shed light on what fat is and does; how it is regulated; and how it dysfunctions in obesity, metabolic syndrome and aging.

Keywords

Adipose tissue; adipocyte; adipocyte progenitor; brown fat; beige fat; thermogenesis; obesity; diabetes

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Declaration of Interests

The authors declare no competing interests.

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Introduction

Adipose tissue is defined by the presence of specialized lipid handling cells called adipocytes, which function as the body's primary energy reservoir. Throughout much of our evolution, access to food was sporadic and stores of adipose tissue were advantageous for surviving extended periods of food insecurity. However, in current times, chronic over nutrition is driving an epidemic of obesity and cardiometabolic disease (e.g., type 2 diabetes, coronary artery disease and stroke) in large parts of the world. Furthermore, obesity increases the risk of developing numerous cancers and predisposes to adverse outcomes in other diseases (Donohoe et al., 2017). The increased mortality among obese patients in the COVID-19 pandemic is a notable example. This expanding health crisis is reversing recent gains in life expectancy and imposes an enormous strain on healthcare systems (Mehta et al., 2020).

The association between excess adiposity and disease has been recognized since antiquity, with notable thinkers like Hippocrates writing over 2,000 years ago "sudden death is more common in those who are naturally fat than in the lean" (Haslam and Rigby, 2010). Indeed, obesity, especially central (abdominal) obesity, is associated with several metabolic pathologies, including hyperglycemia, low HDL cholesterol, hypertriglyceridemia, and hypertension, which together are often called "the metabolic syndrome" (Lanktree and Hegele, 2017). Recent discoveries have revealed a complex and nuanced relationship between adipose tissue and health. Epidemiologic studies indicate that excess fat mass strongly correlate with a higher incidence of metabolic disease (Di Angelantonio et al., 2016; Padwal et al., 2016). However, there is substantial inter-individual variation, with some obese people remaining metabolically healthy and some thin people exhibiting metabolic disease. Furthermore, patients with lipodystrophy have low amounts of adipose tissue yet suffer many of the same ailments as those with severe obesity.

The distribution of adipose tissue into multiple heterogeneous depots and their myriad functions add to the challenges in deciphering the roles of adipose tissue in disease. Beyond its critical role in energy storage, adipose tissue produces hormones that regulate many physiological processes, serves as a hub for inflammatory responses, provides mechanical cushioning and insulation, and participates in heat production for the regulation of body temperature (Rosen and Spiegelman, 2014; Zwick et al., 2018). All these processes may change in adaptive or maladaptive ways during weight loss or gain.

How then should we consider the relationship between adipose tissue and metabolic health? Adipose tissue plays a central role in maintaining whole body insulin sensitivity and energy levels. Adipose tissue regulates insulin action via the secretion of insulin-sensitizing factors like Adiponectin and by sequestering lipids, which would otherwise accumulate in other tissues and have deleterious effects. Indeed, adipose tissue insufficiency (as in lipodystrophy) or dysfunction (as in obesity) leads to the excessive deposition of lipids in other organs like liver and muscle, which is a hallmark of and major contributor to insulin resistance (Petersen and Shulman, 2018). Insulin resistance and high insulin secretion define the pre-diabetic state, which often progresses to type 2 diabetes and contributes to the pathogenesis of other disease processes.

This review discusses the function and regulation of adipose tissue, emphasizing its ability to undergo profound metabolic, structural, and phenotypic remodeling in response to physiologic cues (Figure 1). We further consider how the maintenance of adipose tissue plasticity helps to preserve metabolic health.

Overview of Adipose Tissue

Placental mammals have three main types of adipocytes – white, beige, and brown, organized into discrete depots throughout the body (Figure 2). White adipocytes are specialized for lipid storage and release, while beige and brown adipocytes are specialized thermogenic cells able to expend nutritional energy in the form of heat.

White Adipose Tissue (WAT)—WAT is the most abundant form of adipose tissue, found in almost every area of the body (Zwick et al., 2018) (Figure 2). The major WAT depots are classified according to their anatomic location as either subcutaneous or visceral. In humans, visceral fat is located in the peritoneal cavity, corresponding to the omental and mesenteric depots (Chusyd et al., 2016). Subcutaneous fat is located beneath the skin and typically represents 80% or more of total fat mass in humans, concentrated in the abdominal and gluteofemoral depots (Karastergiou and Fried, 2017). Mice and rats have somewhat analogous visceral (mesenteric, perirenal, and gonadal) and subcutaneous (inguinal and axillary) depots (Figure 2). A notable difference is that murine gonadal fat drains into the systemic circulation while human visceral fat drains into the portal circulation (Rytka et al., 2011). In addition to the major fat depots discussed above, smaller deposits of adipocytes serve important mechanical and signaling roles in diverse locations, such as the muscle, breast, bone marrow, orbits, face, joints, feet and dermis (Zwick et al., 2018).

White adipocytes generally possess a single large lipid droplet occupying most of the cell and relatively few mitochondria. A major function of these cells is to store and release energy in response to changes in systemic energy levels. These processes occur on multiple time scales, with lipolysis (fatty acid release) versus lipogenesis (fatty acid uptake/synthesis) acting in the acute setting, the balance of which drives tissue expansion and contraction over longer periods.

WAT is an essential endocrine organ, secreting numerous hormones and other factors, collectively termed adipokines. Adipokines play major roles in regulating whole body metabolism, including promoting insulin sensitivity (e.g. Adiponectin), insulin resistance (e.g. Resistin, RBP4, Lipocalin), and inflammation (e.g. TNF, IL6, IL-1b, IL-8, IL-18, sFRP5) (Funcke and Scherer, 2019). Leptin is particularly well studied as it plays a major role in controlling energy homeostasis. High levels of Leptin signal high levels of energy storage in adipose tissue. Leptin acts in the hypothalamus and other brain regions to promote satiety and augment energy expenditure (Pan and Myers, 2018). Rare loss-of-function mutations in Leptin or the Leptin receptor cause severe forms of monogenic obesity. In common forms of obesity, the brain becomes resistant to higher levels of Leptin. An intriguing recent study shows that reducing leptin levels in obese mice alleviates Leptin resistance, decreases obesity and improves metabolic parameters (Zhao et al., 2019).

Brown and beige adipose tissue—Brown and beige adipocytes, while representing a small proportion of total adipose tissue, can exert a sizable metabolic impact due to their capacity to engage in thermogenesis. When fully active, BAT can increase whole body energy expenditure by over 100% in mice and by 40–80% in humans (Angueira et al., 2020; Ouellet et al., 2012). Both cell types are characterized by multilocular lipid droplets, high mitochondrial density, and expression of Uncoupling Protein 1 (UCP1) (Figure 2). Upon activation, UCP1 separates nutrient catabolism from ATP synthesis by dissipating the proton gradient in the inner mitochondrial membrane, releasing potential energy in the form of heat (Cannon and Nedergaard, 2004).

Brown adipocytes develop in dedicated deposits of brown adipose tissue (BAT) that are specified prior to birth whereas beige adipocytes develop in WAT depots, predominantly in response to cold exposure. The major murine BAT depot is located in the interscapular region, with additional depots found in cervical, axillary, perivascular, and perirenal regions (Zhang et al., 2018) (Figure 2). Human infants also possess an interscapular BAT depot, which later regresses and is absent in adults (Lidell et al., 2013). Adult humans possess substantial, though variable, amounts of BAT and beige fat tissue in the paravertebral junctions, cervical/axillary region, along the trachea and blood vessels, and in perirenal/adrenal locations (Ouellet et al., 2011). Several groups have isolated populations of thermogenic adipocytes from adult humans: some report more transcriptional similarity to mouse beige adipocytes, while others report more similarity to mouse brown adipocytes (Jespersen et al., 2013; Lidell et al., 2013). The results from these studies are probably influenced by the biopsy site and history of cold exposure, so it is likely that humans have both brown and beige adipocytes.

Thermogenic fat is critical for adaptation to environmental cold in mice and humans, but current interest in these tissues focuses on their ability to act as a metabolic sink for excess nutrients. Many studies have shown that mice with increased thermogenic fat activity are protected against weight gain and metabolic dysfunction (Harms and Seale, 2013). Moreover, transplantation of brown or beige fat into obese mice enhances insulin sensitivity and decreases fat mass (Liu et al., 2015; Min et al., 2016). Similarly, in humans, augmenting brown fat activity is associated with beneficial metabolic effects (Chondronikola et al., 2016). In addition to suppressing weight gain by elevating energy expenditure, thermogenic adipocytes improve systemic metabolism and insulin-action via clearing triglyceride-rich lipoproteins, acylcarnitines, glucose and other potentially toxic metabolites such as branched chain amino acids (BCAAs) that have been closely linked to metabolic dysfunction (Bartelt et al., 2011; Yoneshiro et al., 2019).

Metabolic Plasticity of White Adipocytes

WAT metabolism rapidly shifts to meet the energetic needs of the organism, which vary greatly during times of fasting, feeding, cold, and exercise. WAT switches between two opposing metabolic programs, one driving nutrient uptake and the other nutrient release, to ensure that other organs always have an adequate, but not excessive, level of energy (Figure 3). The metabolic plasticity of white adipocytes is controlled by hormonal and neuronal signals acting through a cadre of effector proteins and transcriptional regulators.

Nutrient Uptake and Lipogenesis—During periods of positive-energy balance and after feeding, WAT takes up nutrients from the bloodstream and stores them as lipids. This process is mediated by both fatty acid uptake and through the conversion of other nutrients (e.g., glucose) into lipids via *de novo* lipogenesis. The major signal for nutrient uptake into adipocytes is the hormone insulin, secreted by pancreatic β -cells in response to increased circulating levels of glucose and fatty acids (Petersen and Shulman, 2018). Insulin drives lipid storage in adipocytes by: (1) stimulating glucose uptake, (2) promoting *de novo* lipogenesis (DNL), and (3) suppressing lipolysis (Carpentier, 2021). Insulin signaling is also critical for the differentiation and maintenance of adipocytes; genetic deletion of the insulin receptor or downstream effectors in adipocytes causes varying degrees of lipodystrophy along with insulin resistance (Sakaguchi et al., 2017; Shearin et al., 2016; Vazirani et al., 2016).

Adipocytes contain specialized machinery to take up FFAs from circulating chylomicrons and very low-density lipoprotein (VLDL) (Figure 3). A major constituent of this machinery is lipoprotein lipase (LPL), an enzyme responsible for the hydrolysis of triacylglycerols (TAG) into FFAs and monoacylglycerols. LPL, produced from adipocytes is transported to the apical membrane of capillaries in adipose tissue via the action of the GPI anchored protein GpiHBP1 (Davies et al., 2010). After LPL releases FFAs, specialized FA binding and transport proteins (FATPs), such as FATP1 and CD36, facilitate the uptake of fatty acids into adipocytes. Insulin stimulates the translocation of FATP1 to the plasma membrane to promote FA uptake. Once taken up by adipocytes, FFAs are activated by acyl-CoA synthetase to generate acyl-CoAs, which are the substrate for successive acylation reactions with glycerol through the Kennedy pathway. The last step in triglyceride synthesis joins an acyl-CoA and diacylglycerol (DAG) through diacylglycerol acyltransferase enzymes (DGAT1 and DGAT2) (Carpentier, 2021).

Adipocytes also synthesize acyl chains through DNL. Adipose tissue and liver are the two major sites for DNL, with adipose tissue accounting for more whole-body lipogenesis in humans and the liver accounting for more in rodents (Song et al., 2018). DNL is essential for maintaining energy balance, since it converts excess energy from carbohydrates and protein into fatty acids and ultimately triglycerides, for storage in lipid droplets. DNL initially involves the breakdown of nutrients through the TCA cycle, followed by export of citrate to the cytoplasm which is converted through a series of steps into Acetyl-CoA, Malonyl-CoA, and finally into FFAs. DNL is regulated at multiple levels, including: (1) the buildup of malonyl coA, which signals to suppress FA oxidation, and (2) transcriptional activation of key enzymes in the DNL pathway. In particular, carbohydrate response element binding protein (ChREBP), LXRA, and sterol response element binding protein 1c (SREBP1c) stimulate the expression of key DNL enzymes fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) (Herman et al., 2012). ChREBP is a major transcriptional regulator of DNL in adipocytes, and its expression is controlled by mammalian rapamycin complex 2 (mTORC2), linking the regulation of *de novo* lipogenesis to growth factor responses (Figure 3) (Tang et al., 2016).

Adipocyte DNL maintains insulin sensitivity by converting excess nutrients into lipids for sequestration in adipocytes. Additionally, DNL in adipocytes results in the production of

several lipid species with anti-inflammatory and insulin sensitizing effects (Yilmaz et al., 2016; Yore et al., 2014). These lipids largely correspond to branched fatty acid esters of hydroxy fatty acids (FAHFA), of which there are many variants, based on the position of the branched ester (Zhou et al., 2019). Among these, palmitic acid esters of hydroxy stearic acid (PAHSA) have been singled out for their insulin sensitizing properties. PAHSAs signal through GPR120 to enhance insulin stimulated glucose uptake into adipocytes and also have direct and indirect insulin sensitizing effects in the liver (Yang et al., 2018; Zhou et al., 2019). Finally, branched chain amino acids (BCAAs) are also used as substrate for DNL, thereby limiting their buildup in circulation, which has been linked to insulin resistance (Yoon, 2016).

Energy Mobilization through Adipose Tissue Lipolysis—Lipolysis is the process of hydrolyzing triacylglycerols into glycerol and free fatty acids (FFAs) (Figure 3). Sympathetic nerve-derived catecholamines stimulate lipolysis, and this process is repressed by insulin (Fruhbeck et al., 2014). In particular, epinephrine and norepinephrine release are induced by fasting or exercise and signal through the adrenergic receptor-PKA pathway in adipocytes to increase lipolysis. Lipolysis depends on the inhibitory phosphorylation of the lipid droplet membrane protein PLIN1 (Sztalryd and Brasaemle, 2017). In a basal or anabolic state, PLIN1 is bound to comparative gene identification 58 (CGI-58) (Chouchani and Kajimura, 2019). Upon stimulation of lipolysis, PLIN1 is phosphorylated, triggering the release of CGI-58 and subsequent activation of adipose triglyceride lipase (ATGL). ATGL moves to the lipid droplet surface to hydrolyze triglycerides. PKA also phosphorylates HSL, which binds to PLIN1 to favor the hydrolysis of diacylglycerol, and subsequently monoacylglycerol. The final products, glycerol and FFAs are exported into the bloodstream (Figure 3). While lipolysis is viewed as the main pathway for lipid release, a recent study demonstrates that lipids are also exported from adipocytes in exosomes, providing an important local signal for macrophage differentiation (Flaherty et al., 2019). Lipolysis is further regulated by several endocrine factors. Leptin promotes lipolysis via stimulation of neuro-adipose junctions (Zeng et al., 2015). Growth hormone (GH), adrenocorticotropic hormone, cortisol, thyroid hormones, PTH, and glucagon also provide regulatory roles in lipolysis (Fruhbeck et al., 2014). By contrast, insulin signaling functions as the major anti-lipolytic factor by blocking production of intracellular cAMP, leading to suppression of PKA-activity and lipolysis.

Thermogenic Adaptation of White Adipose Tissue

A striking example of adipose tissue plasticity is observed during environmental cold exposure. Initially, animals shiver and activate pre-existing BAT to help defend their body temperature. Longer exposure recruits additional thermogenic capacity, mediated by increases in BAT mass and elevated expression of thermogenic genes (Cannon and Nedergaard, 2004). In WAT, especially in rodents, cold exposure induces the development of mitochondria-rich, thermogenic beige adipocytes. The rapid induction of beige adipocytes is accompanied by remarkable changes in tissue structure, including increased nerve fiber arborization and angiogenesis. Importantly, these cold-induced changes in BAT and WAT are reversible and regress in the absence of cold, highlighting the flexibility of the tissue.

Beige adipocytes can be generated via three mechanisms: (1) the differentiation of progenitor cells into new beige adipocytes (i.e. *de novo* beige adipogenesis), (2) the activation (or re-activation) of the thermogenic program in mature adipocytes and (3) the proliferation of mature beige adipocytes (Park et al., 2021; Shao et al., 2019; Wang et al., 2013). Activation of the beige program in adipocytes involves upregulation of thermogenic genes such as *Ucp1*, mitochondrial biogenesis and lipid droplet remodeling from a unilocular to multilocular morphology (Kim et al., 2019).

BAT undergoes an analogous thermogenic recruitment process during cold exposure. Histological studies show that expression of UCP1 in brown adipocytes is not homogeneous, suggesting a level of cellular heterogeneity in BAT (Cinti et al., 2002). A recent study identified two distinct populations of thermogenic cells in mouse BAT, classical brown adipocytes and ‘low-thermogenic’ brown adipocytes exhibiting fewer mitochondria, lower levels of UCP1, and larger lipid droplets (Song et al., 2020). Interestingly, cold exposure activated the ‘low-thermogenic’ cells to become highly thermogenic. Another recent study identified a new subset of ‘thermogenesis-inhibitory’ adipocytes in mouse and human BAT that restrain the thermogenic capacity of brown adipocytes via local production of acetate (Sun et al., 2020b). These inhibitory adipocytes are enriched in BAT under thermoneutral (non-stimulated) conditions, suggesting that BAT function is regulated by the coordinated activity of distinct adipocyte subpopulations.

Adrenergic signaling is the major physiologic signal controlling both the formation and thermogenic activity of brown and beige adipocytes. Adipose tissue, especially BAT, is densely innervated by sympathetic neurons (Morrison, 2016). Upon cold exposure, sympathetic neurons release the neurotransmitter norepinephrine (NE), which activates the β -adrenergic receptor-cAMP-PKA pathway in adipocytes. This signaling cascade induces lipolysis, thermogenesis and stimulates brown fat-selective gene transcription in brown and beige adipocytes. UCP1 function and thus thermogenic respiration is acutely activated by long-chain FAs and inhibited by purine nucleotides (Bertholet and Kirichok, 2017; Fedorenko et al., 2012).

A key hub of the thermogenic transcriptional response is the coactivator protein PPAR γ coactivator-1 α (PGC1- α), which is upregulated by cold exposure (Puigserver et al., 1998). PGC1- α is phosphorylated and activated by p38 mitogen-activated protein kinase (MAPK) in response to β -adrenergic signaling (Cao et al., 2004). PGC1- α co-activates several transcription factors, including PPAR and ESRR family members. Thyroid Receptor and IRF4 to increase the transcription of *Ucp1* and other mitochondrial genes involved in thermogenesis (Shapira and Seale, 2019).

Adrenergic stimulation of adipocytes also activates the nutrient-sensing mTOR pathway, a central integrator of cell and tissue metabolism that functions in two distinct complexes, mTORC1 and mTORC2 (Ye et al., 2019). PKA phosphorylates Raptor and activates the mTORC1 complex in β -adrenergic agonist-stimulated adipocytes (Liu et al., 2016). Mice with genetic loss or inhibition of Raptor display reduced WAT beiging and impaired brown fat activity (Labbe et al., 2016; Liu et al., 2016; Tran et al., 2016). The mTORC2 complex, containing the Rictor subunit, is also required for glucose uptake and glycolysis in brown

fat tissue during cold exposure (Albert et al., 2016). Interestingly, inhibition of mTORC2 in brown adipocytes reduces glucose uptake and lipid storage while also leading to enhanced lipid catabolism, associated with protection against cold and obesity (Jung et al., 2019). By contrast, loss of mTORC2 in all adipocytes leads to systemic insulin resistance, which can indirectly decrease BAT function (Tang et al., 2016).

A new study shows that cold and β -adrenergic signaling also activate expression of the ligand-independent G-protein coupled receptor, GPR3 in brown adipocytes (Sveidahl Johansen et al., 2021). GPR3 amplifies the β -adrenergic response to enable high levels of thermogenesis. Forced expression of GPR3 in adipose tissues dramatically augments energy expenditure and can reduce obesity in mice. Finally, numerous other extracellular signals, hormones, and metabolites (e.g. FGF21, natriuretic peptides, acetylcholine, and Irisin) promote WAT beiging and add an additional layer of regulation to the control of thermogenesis (Cohen and Kajimura, 2021).

Immune cells and beiging—Immune cells, including M2 macrophages, mast cells, eosinophils and ILC2s regulate adipose tissue remodeling and thermogenesis during cold exposure. Type 2 cytokines, especially IL-4, promote beige fat biogenesis and ameliorate obesity, although the involved mechanisms remain uncertain (Fischer et al., 2017; Henriques et al., 2020; Qiu et al., 2014). Innate lymphoid ILC2 cells, activated by IL-33, promote beiging through two proposed pathways: (1) the production of methionine-enkephalin peptides, that act on adipocytes to stimulate UCP1 expression (Brestoff et al., 2015); and (2) the induction of IL-4 and IL-13, which act on adipocyte progenitor cells to promote beige adipocyte differentiation (Lee et al., 2015). Recent work has identified stromal cells as a critical source of IL-33 in adipose tissue, illustrating the crosstalk between mesenchymal cells and immune cells in regulating adipose tissue phenotypes (Mahlakoiv et al., 2019; Shan et al., 2021; Spallanzani et al., 2019). The anti-inflammatory cytokine IL-10 suppresses thermogenic genes in adipocytes. Deletion of the IL-10 receptor in adipocytes augments thermogenesis and reduces obesity (Rajbhandari et al., 2019). Additionally, recent studies demonstrate an important role for $\gamma\delta$ T cells in regulating innervation, especially in BAT. Specifically, IL-17 secreted from $\gamma\delta$ T cells acts on brown adipocytes, leading to TGF β production and increased sympathetic innervation. Deletion of the $\gamma\delta$ T cells or IL-17R on brown adipocytes reduces energy expenditure in mice and exacerbates obesity (Hu et al., 2020).

Adipose tissue whitening—The thermogenic phenotype of fat cells, especially beige fat cells, is unstable, requiring persistent stimulation. Elegant cell tracking studies revealed that UCP1+ beige fat cells become unilocular white-appearing adipocytes following re-warming (Roh et al., 2018; Rosenwald et al., 2013). During this “whitening process”, fat cells lose UCP1 expression and mitochondrial density, and remodel their lipid droplets from a multilocular to a unilocular architecture over the course of ~4 weeks (Roh et al., 2018). This process involves direct conversion of beige adipocytes rather than proceeding through a progenitor cell state and depends on mitochondrial clearance (Altshuler-Keylin et al., 2016). Decreased adrenergic signaling in beige fat cells induces the recruitment of the E3 ubiquitin ligase complex, Parkin, to mitochondria, triggering mitophagy. Impairing this process by

deletion of autophagy components, *Atg5*, *Atg12*, or *Parkin* prevents the “beige-to-white” phenotype transition (Lu et al., 2018). Mitophagy in adipocytes is also driven by the kinases STK3 and STK4. STK3 and STK4 are highly expressed in ‘white- appearing’ (unstimulated) adipocytes and downregulated during cold exposure. Genetic loss or inhibition of STK3/4 activity increases mitochondrial content and uncoupled respiratory activity in beige and brown adipocytes via reducing mitophagy (Cho et al., 2021). Remarkably, inhibiting mitochondrial clearance in the above mouse models ameliorates obesity and improves systemic metabolism, though this may be expected to cause aberrant mitochondrial function over the long term. A similar whitening process occurs in BAT with exposure to warmer temperatures and during aging.

The gene expression profile of ‘previously beige adipocytes’ is nearly indistinguishable from ‘white’ adipocytes (never beige) after rewarming (Roh et al., 2018). However, ‘previously beige’ cells rapidly reactivate the thermogenic program upon a second exposure to cold (Rosenwald et al., 2013). Compared to white adipocytes, ‘previously beige’ adipocytes display increased levels of H3K4me1, a chromatin mark associated with active or primed enhancers, at certain thermogenic genes, indicating an epigenetic memory of cold exposure (Roh et al., 2018). Because of the plasticity of mature adipocytes, the balance between new beige adipocyte differentiation and re-activation of ‘previously beige’ adipocytes during being depends on the environmental exposure history of the animal. For example, in mice that have recently undergone cold exposure, reactivation of dormant beige cells predominates, whereas in cold naïve animals, *de novo* beige adipocyte differentiation from progenitor cells is favored (Shao et al., 2019). Interestingly, UCP1+ cells, specifically in the central region of iWAT (near the lymph nodes) exhibit proliferative capacity and generate new beige adipocytes in response to β -adrenergic stimulation (Park et al., 2021).

Metabolic Programming for Thermogenesis—The thermogenic capacity of brown and beige adipocytes relies on burning FAs via oxidative metabolism (Gonzalez-Hurtado et al., 2018). Classically, adipose tissue thermogenesis is driven by sympathetic nerve-mediated adrenergic signaling, which stimulates lipolysis (Figure 4). Free fatty acids (FFAs) serve as both a fuel for thermogenesis and as an allosteric activator of UCP1 function. Surprisingly, lipolysis in UCP1+ adipocytes is dispensable for thermogenesis. However, disrupting lipolysis in all adipocytes compromises thermogenesis in the absence of food, demonstrating that white fat cells can supply the FFAs necessary to support adipose tissue thermogenesis (Schreiber et al., 2017; Shin et al., 2017). Furthermore, even lipid droplets in BAT are dispensable for thermogenesis. Deletion of core lipogenesis components DGAT1 and DGAT2 in UCP1+ adipocytes produces lipid droplet-less adipocytes, that remain competent for thermogenesis (Chitraju et al., 2020).

Brown fat in mice and humans also oxidize branched-chain amino acids (BCAAs) during cold exposure (Yoneshiro et al., 2019). This pathway likely contributes to the metabolic benefits of BAT given the well-established link between elevated circulating BCAAs and insulin resistance. Interestingly, recent studies show that brown fat cells take up and concentrate large amounts of the TCA intermediate succinate, which promotes thermogenic respiration (Mills et al., 2018). Mechanistically, the oxidation of succinate generates reactive oxygen species that promote UCP1-activity (Chouchani et al., 2016).

Adipocytes can also carry out thermogenesis through an expanding array of UCP1-independent mechanisms. The existence of such mechanisms were initially invoked when it was observed that UCP1-null mice can adapt to the cold if ambient temperature is gradually decreased (Ukropec et al., 2006). These alternative mechanisms have been extensively reviewed elsewhere and include Ca^{2+} futile cycling, creatine-dependent substrate cycling, and triacylglycerol futile cycling (Chouchani and Kajimura, 2019; Ikeda et al., 2017; Kazak et al., 2015). Of note, the futile creatine cycle is required for high fat diet-induced energy expenditure in adipocytes. Ablation of this pathway in mice sensitizes them to obesity and metabolic complications (Kazak et al., 2017) (Figure 4).

Structural Remodeling to Optimize Thermogenesis—Sympathetic innervation is critical for brown and beige fat thermogenesis. BAT is more densely innervated than WAT depots; however, a recent study shows that >90% of adipocytes in inguinal WAT are closely opposed to sympathetic fibers, which likely relates to the high beiging potential of this depot (Jiang et al., 2017; Murano et al., 2009). Sympathetic arborization increases (by over 3 fold) during cold exposure and is likely essential for sustaining high levels of thermogenic activation (Cao et al., 2018b). Indeed, ablation of nerve fiber arborizations blunts the development of beige adipocytes in response to cold exposure (Cao et al., 2019; Jiang et al., 2017). The expansion of neurites is reversible and neurite density normalizes to baseline within approximately 4 weeks after removal of cold stimulus (Cao et al., 2018b). The growth and branching of sympathetic neurites during cold exposure is regulated by adipocytes. For example, adipocyte-specific deletion of the brown fat transcription factor PRDM16 impairs nerve fiber growth and branching in WAT following cold exposure (Chi et al., 2018). Adipose cells have been shown to produce a variety of neurotrophic factors, including Nerve Growth Factor (NGF), Neuregulin-4 (NRG4), $\text{TGF}\beta$ and S100b (Hu et al., 2020; Rosell et al., 2014; Zeng et al., 2019).

Vascular density also increases in adipose tissue during cold exposure to support the increase in metabolic activity (Xue et al., 2009). Vascular density doubles within just 5 days and is reversible upon warming (Cao et al., 2018a). As with angiogenesis in other organs, vascular endothelial growth factor (VEGF) is a critical regulator of this process in adipose tissue. Knockout of VEGF in adipose cells leads to whitening of both brown and beige fat (During et al., 2015; Shimizu et al., 2014). Interestingly, overexpression of VEGF stimulates browning of WAT and BAT, suggesting that VEGF and/or angiogenesis plays an instructive role in beiging, in addition to supporting the increase in tissue metabolism (During et al., 2015; Sun et al., 2014).

Phenotypic Plasticity of Adipose Tissue

Intriguing work over the past few years shows that adipocytes are not necessarily terminally differentiated. Under certain conditions adipocytes reversibly de-differentiate and re-differentiate, cycling between a progenitor cell and adipocyte state.

The capacity for adipocytes to dedifferentiate *ex vivo* was noted in the 1980's with the development of the ceiling culture method to isolate adipogenic primary cell lines. In this method, mature adipocytes are induced to adhere to the top surface of a flask, where

they de-differentiate into a fibroblastic pre-adipocyte state (Cote et al., 2019). However, whether de-differentiation of adipocytes occurs *in vivo* had been unclear until recently. Bi et al. demonstrated that activation of Notch signaling induces the de-differentiation of adipocytes, leading to the development of liposarcomas (Bi et al., 2016). In lactation, the posterior mammary fat pads (inguinal fat) in mice remodel, with proliferation of mammary alveolar structures and a relative loss of adipocytes in the areas of high ductal density. During this process, mature adipocytes de-differentiate into proliferative fibroblasts that retain their adipocyte differentiation capacity *in vitro* and *in vivo* (Wang et al., 2018). Similarly, adipocytes within the dermis undergo reversible de-differentiation during hair follicle cycling and wound healing (Shook et al., 2020; Zhang et al., 2019). In the dermis, mature adipocytes de-differentiate and give rise to myofibroblasts, specialized contractile fibroblasts which secrete extracellular matrix for wound repair. Adipocytes undergoing de-differentiation stimulate lipolysis and release of FFA, which also plays a critical role in regulating the wound inflammatory response (Shook et al., 2020). It remains unknown if adipocyte de-differentiation occurs in the major WAT and BAT depots under other physiological conditions, such as during fasting, weight loss, or wound healing outside of the skin.

Adipose Tissue Expandability

Adipose tissue has an unparalleled ability to expand, and contract compared to other organs. In humans, the proportion of body fat varies widely, ranging from normal levels of 10–20% in men and 15–30% in women, to below 5% in bodybuilders and anorexic patients, and above 70% in severe obesity. These differences in fat mass are driven by long term calorie surplus or deficit, and the structural changes are enabled by the coordinated action of several cell types, including adipocytes, adipocyte progenitor cells, and immune cells.

The Structure of Adipose Tissue—Human subcutaneous WAT is organized by fibrous septa that define progressively smaller tissue compartments at each scale. The highest-level division is formed by a collagen and elastin rich sheet called the fascia superficialis, often referred to as Scarpa's fascia in the abdominal wall and a "membranous layer" in other body regions (Markman and Barton, 1987). The fascia superficialis runs parallel to the plane of the skin and separates subcutaneous fat into a superficial (sSAT) and a deep compartment (dSAT). At the next level, thinner fibrous septa, sometimes referred to as retinacula cutis superficialis (in sSAT) and profundus (in dSAT), define centimeter scale compartments, and anchor the fascia superficialis to the dermis above and to the deep fascia below. Together, the fascia superficialis, retinacula cutis, and compartments of sSAT and dSAT, are referred to as the superficial fascial system, and are identifiable in nearly all areas of the body (Lockwood, 1991). Finally, within the compartments of the superficial fascial system, 500 μm -1000 μm lobules of adipocyte rich stroma are encapsulated by fibrous septa, representing the smallest structural unit of subcutaneous adipose tissue (Esteve et al., 2019).

The distinctions between sSAT and dSAT are not purely anatomic. During obesity, both the abdominal sSAT and dSAT expand, with males exhibiting a tendency to expand the abdominal dSAT preferentially (Kim et al., 2016). Compared to abdominal sSAT, the dSAT is more prone to inflammation, contains more saturated lipids, and adipocyte progenitor cells

from this layer are more resistant to differentiation (Cancello et al., 2013). Accordingly, expansion of the dSAT, especially in men, is associated with adverse metabolic outcomes (Kelley et al., 2000).

Mice have two main subcutaneous WAT depots, the posterior inguinal WAT (iWAT) and anterior axillary WAT (axWAT). The inguinal WAT is heavily studied, due to its larger size, high propensity for beiging, and ease of dissection. Both the iWAT and axWAT lie directly beneath the panniculus carnosus, a layer of striated muscle which separates the subcutaneous structures from the overlying dermis, that some have speculated is an evolutionarily analogous structure to the fascia superficialis (Fodor, 1993). Both depots are encased on all sides by a thin fibrous membrane containing mostly DPP4+ fibroblasts that can also serve as adipocyte progenitors (Merrick et al., 2019; Stefkovich et al., 2021). At the next scale, the tissue can be subdivided into lobular areas (the central areas within the tissue) and non-lobular areas (surrounding, at the periphery) (Barreau et al., 2016; Peurichard et al., 2017). The lobular areas are delineated by fibrous septations, analogous to those found in humans that create discrete compartments of adipocytes on the order of 300 μm (Chi et al., 2018; Dichamp et al., 2019). Several studies have noted clear anatomic regionality within the iWAT, with the more ventral regions and central lobular areas being more prone to cold-induced beiging, as compared to the peripheral and posterior regions (Barreau et al., 2016; Chi et al., 2018).

The structure of visceral adipose tissue has been less well studied. A defining feature of visceral fat is that, like other intraperitoneal organs, it is surrounded by a layer of mesothelium (Chau et al., 2014). Thus, both visceral and subcutaneous fat are encased by a lining of specialized cells (mesothelial cells for visceral fat, DPP4+ fibroblasts for subcutaneous fat), although in contrast to subcutaneous fat, this lining does not appear to contribute to adipocyte generation in visceral adipose tissue (Westcott et al., 2021). Furthermore, a recent report demonstrates the presence of lobules in human visceral adipose tissue, analogous to those present at the smallest scale in subcutaneous fat (Esteve et al., 2019). By contrast, mouse visceral fat depots do not have a readily apparent lobular structure.

Adipose Tissue Expansion—Adipose tissue expansion is intricately linked to metabolic health. While high fat mass generally correlates with poor metabolic health, a high capacity for expansion protects against metabolic disease. The apparent contradiction in this relationship can be understood by considering the fate of excess nutrients. Once ingested and absorbed, excess nutrients must be either burned or stored. WAT is uniquely capable of safely storing large quantities of excess nutrients as lipids. In contrast, accumulation of excess lipids in other tissues drives insulin resistance (Petersen and Shulman, 2018). Therefore, the proper partitioning of excess nutrients into WAT for storage or into BAT for heat generation promote metabolic health. Notably, the site of adipose tissue expansion (into visceral or subcutaneous depots) and the mechanism of expansion, via increases in adipocyte number (hyperplasia) or size (hypertrophy), have profound impacts on metabolic health.

Adipose Tissue Distribution: Metabolic Consequences—Fat tissue distribution is highly variable, driven by differences between sexes, genetics, development, aging, and in

response to hormones or drugs. The most common distinction between types of adipose tissue distribution is whether fat is stored viscerally or subcutaneously, and countless studies have examined the relative effects of visceral versus subcutaneous adiposity on overall health. Almost universally, since the first descriptions of “android” (central) vs “gynoid” (subcutaneous/peripheral) obesity by the French physician Jean Vague in the 1950’s, studies have shown that increased visceral/central adiposity correlates with worse insulin resistance and an increased risk of cardiometabolic disease, even in normal weight subjects (Chait and den Hartigh, 2020). By contrast, the preferential expansion of subcutaneous adipose tissue, especially in the superficial region, is associated with a more favorable metabolic profile (Kelley et al., 2000). It should be noted that, despite its metabolic importance, visceral fat represents only a small portion (~6–20%) of total fat mass, with this proportion generally higher in males (Karastergiou and Fried, 2017).

Differences in body fat distribution may also explain the existence of “metabolically healthy obese” and “metabolically unhealthy normal-weight” individuals (Smith et al., 2019). Estimates from the United States suggest that 23.5% of normal weight adults are metabolically unhealthy while 31.7% of obese are metabolically healthy (Wildman et al., 2008). Metabolically healthy obese people have unexpectedly low levels of visceral adiposity for their body weight while the situation is exactly reversed in those who are metabolically unhealthy but normal weight.

What makes visceral fat unhealthy and why do we have it? Visceral adipocytes are more metabolically and lipolytically active, exhibiting higher levels of both basal and catecholamine-induced lipolysis. Mechanistically, these differences may be due to increased expression of the stimulatory β -AR, lower expression of the inhibitory α -AR and reduced insulin-mediated lipolysis suppression in visceral adipocytes (Item and Konrad, 2012). Consistent with these observations, fasting and weight loss in mice induce the preferential mobilization of visceral fat stores, with visceral depots losing mass earlier and losing a greater proportion of their mass overall (Ding et al., 2016; Tang et al., 2017). Similarly, studies of weight loss in humans consistently show that a greater proportion (but not total amount) of the visceral fat is lost compared to subcutaneous fat (Merlotti et al., 2017). It is reasonable to speculate that a rapidly mobilized source of energy for internal organs may be advantageous under certain conditions.

The high lipolytic activity of visceral fat also underlies the basis for the “portal hypothesis”, which posits that visceral depots, since they drain into the portal circulation, expose the liver to high levels of FFAs, which impair hepatic insulin action. However, this version of the portal hypothesis has fallen out of favor because studies in humans show that, while the proportion of portal vein and circulating FFAs from visceral fat increase in obesity (from 5 to 20% and from 6% to 14% respectively), the visceral fat-derived FFAs still only represent a small proportion of the total circulating pool (Nielsen et al., 2004).

Alternative versions of the portal hypothesis highlighting a central role for inflammation are more compelling. Visceral adipose tissue is more prone to immune cell infiltration and inflammatory cytokine production than subcutaneous adipose tissue, especially in obesity (Item and Konrad, 2012). Several factors that are preferentially produced by visceral fat

and secreted into the portal circulation have been linked to the development of insulin resistance, including IL-6, IL-1b, and Retinol Binding Protein-4. For example, IL-6 levels are 50% higher and leptin levels are 20% lower in the portal compared to systemic circulation of severely obese subjects (Fontana et al., 2007). Transplantation studies further support the idea that increased intraperitoneal adipose tissue, whether from a visceral or subcutaneous source, is not harmful *per se* and may even be protective. Instead, the portal delivery of inflammatory cytokines appears to drive the detrimental effects of visceral fat. (Item and Konrad, 2012). In transplant experiments, portal-draining visceral fat transplants impair insulin sensitivity whereas systemic-draining visceral fat transplants improve insulin sensitivity. Furthermore, portal-draining transplants from IL-6-deficient mice did not reduce host insulin sensitivity (Rytka et al., 2011).

The inflammatory properties of visceral adipose tissue may have been selected for during evolution, by providing a defense against intra-peritoneal pathogens and helping to heal abdominal injuries (West-Eberhard, 2019). Consistent with this notion, the omentum has important immunological functions and contains lymphoid cells organized into structures called milky spots, which are key mediators of peritoneal immunity (Meza- Perez and Randall, 2017). Moreover, the omentum and mesenteric fat commonly adhere to sites of injury, including ruptured bowels, ovaries, or surgical trauma (West-Eberhard, 2019). These fat depots can even wall off foreign bodies within the abdomen. A dramatic example of these properties is the phenomenon of creeping fat in Crohn's disease, in which mesenteric adipose tissue adheres to sites of gut barrier dysfunction, walling of the diseased areas and preventing dissemination of bacteria (Ha et al., 2020). Overall, the metabolic and immunological properties of visceral fat, which serve important protective roles, also trigger metabolic dysfunction in the setting of obesity.

Distributional Plasticity—Body fat distribution is not fixed and can be modified by hormones. Redistribution of adipose tissue is accomplished by varying the rates of nutrient uptake and lipolysis until a new steady state distribution is achieved. A famous example occurs during Cushing's syndrome, which results from excess secretion or administration of glucocorticoids. In addition to promoting weight gain via effects on the CNS, glucocorticoids induce a redistribution of lipids to visceral adipose tissue, while causing wasting of adipose tissue from the extremities (Lee et al., 2014a).

Androgens and estrogens also produce characteristic effects on adipose tissue leading to the "android" and "gynoid" adipose tissue distributions in men and women, respectively (Karastergiou and Fried, 2017). The plasticity of this distribution is most apparent in studies of gender transition, in which estrogen or androgen treatment produce characteristic shifts toward a gynoid distribution in transwomen and an android distribution in transmen, respectively (Klaver et al., 2018). Prior to puberty there are discernable but small differences in the fat distribution of male and female children which become much more pronounced as sex hormone levels rise (Shen et al., 2009). Likewise, during the transition to menopause, as estrogen levels fall, women begin to accumulate adipose tissue in a more android pattern, with an increase in the amount of centrally stored adipose tissue; these effects are reversed by estrogen replacement therapy (Lovejoy et al., 2008; Reubinoff et al., 1995). Reciprocally,

androgens tend to promote preferential visceral fat accumulation in women, as observed in polycystic ovarian syndrome (Dumesic et al., 2016).

Finally, several drugs produce stereotyped effects on adipose tissue distribution. For example, certain HIV medications promote peripheral subcutaneous fat wasting (lipoatrophy) and central fat accumulation (Koethe et al., 2020). Conversely, TZDs, which promote insulin sensitivity, induce the preferential expansion of subcutaneous adipose tissue (Miyazaki et al., 2002).

Adipocyte hypertrophy and hyperplasia—Adipose tissue expands through adipocyte hypertrophy (increases in fat cell size) and/or hyperplasia (increases in fat cell number). Hypertrophic growth is linked with higher levels of adipose tissue inflammation, fibrosis, and hypoxia, along with poor metabolic health (Vishvanath and Gupta, 2019). In contrast, hyperplastic growth does not provoke these pathologic changes and is generally more metabolically favorable.

Association studies in humans provide evidence for the divergent consequences of hypertrophic versus hyperplastic expansion. First, obese subjects, have both more adipocytes and larger, more hypertrophic, adipocytes than normal weight controls (Salans et al., 1973). Adipocyte size increases up to the point of moderate obesity, after which subsequent increases in fat mass are characterized by increases in adipocyte number (Hirsch and Batchelor, 1976). Notably, there is substantial inter-individual variation; at any given fat mass, people can exhibit a more hypertrophic or more hyperplastic adipose tissue phenotype. Second, these studies showed that hypertrophic adipose tissue is associated with poor metabolic health, including increased fasting insulin, decreased insulin sensitivity, and elevated blood glucose levels (Bjorntorp, 1971). Importantly, a body of recent work continues to support these conclusions (McLaughlin et al., 2016). Third, longitudinal and cross sectional studies suggest that the total number of adipocytes increases throughout childhood before stabilizing in adulthood (Spalding et al., 2008). Normal weight children experience two developmental periods (from age 0–2 and from age 13–18 years) characterized by rapid increases in adipocyte number; in contrast, obese children produce significantly more adipocytes than lean children and show ever increasing adipocyte numbers from age 0–18 (Knittle et al., 1979). By the time they reach adulthood, those who were obese as children have about twice as many fat cells as their normal weight counterparts. The apparent stabilization of adipocyte numbers in adulthood has led to considerable confusion, with many erroneously believing that people have a “fixed” number of adipocytes.

While many obese children become obese adults, most obese adults were not obese as children. When do obese adults make their extra adipocytes? Adults produce new adipocytes during the normal process of adipose tissue turnover (Spalding et al., 2008). Therefore, it seems likely that independent of the age of onset, adipocyte numbers increase during the development of obesity. To prove this, a longitudinal study quantifying adipocyte numbers in the transition from leanness to obesity during adulthood would be needed. The converse experiment, tracking adipocyte numbers during weight loss, has been performed. Weight loss induced by dietary changes or bariatric surgery leads to a reduction in subcutaneous

adipocyte size but a maintenance of adipocyte number (Andersson et al., 2014; Bjorntorp et al., 1975). These results suggest that adipocyte number might function as a one-way ratchet, expanding in obesity, but not declining after weight loss. This may have evolved to allow the quick expansion of adipose tissue to accommodate calories during cycles of feast and famine.

Hypertrophic adipose tissue is dysfunctional—Hypertrophic expansion of adipose tissue is a risk factor, independent of body mass index, for the development of the metabolic syndrome (Weyer et al., 2000). Interestingly, the WAT of non-obese patients with insulin resistance or diabetes is characterized by large hypertrophic adipocytes further indicating a link between adipocyte hypertrophy (rather than total fat mass) and metabolic dysfunction (Acosta et al., 2016). Molecular and functional analyses of large versus small adipocytes from the same individual provide some insights for why this is the case. In particular, large adipocytes undergo higher rates of lipolysis, and produce higher levels of inflammatory cytokines (Laurencikiene et al., 2011; Xiao et al., 2016). Additionally, small adipocytes may secrete higher levels of the insulin sensitizing hormone adiponectin (Meyer et al., 2013). Consistent with this, WAT from insulin resistant patients features larger adipocytes, more fibrosis, hypoxia, and inflammation (Hepler and Gupta, 2017). At a tissue level, this dysfunctional fat produces lower levels of insulin sensitizing adipokines such as adiponectin (Henninger et al., 2014; Kloting and Bluher, 2014).

Pharmacologic and Genetic Manipulation of Tissue Expandability—Genetic and pharmacological studies suggest that it is not hypertrophic adipocytes *per se* that drive systemic metabolic dysfunction, but rather a failure of adipose tissue “expandability”. In this model, hypertrophic adipocytes are a symptom more than a cause of dysfunctional adipose tissue. Once adipose tissue becomes “full” and can no longer take up excess nutrients, ectopic lipid begins to accumulate in peripheral organs leading to metabolic decline.

The first line of evidence for this concept comes from two genetic models of healthy obesity, one characterized by extreme adipose tissue hyperplasia and the other by extreme hypertrophy. Leptin deficient (*ob/ob*) mice, a model of severe obesity, exhibit glucose intolerance, hyperphagia, and adipose tissue replete with large hypertrophic adipocytes and inflammatory macrophages. Strikingly, the metabolic dysfunction of *ob/ob* mice is ameliorated by concomitant overexpression of the insulin sensitizing hormone adiponectin (AdiponectinTG) or by knockout of collagen 6 (*Col6* KO) (Khan et al., 2009; Kim et al., 2007). Both models are characterized by massively increased adipose tissue mass which normalizes insulin sensitivity, presumably by preventing ectopic lipid deposition in other tissues. The adipose tissue of *ob/ob* AdiponectinTG mice exhibits extreme hyperplasia and contains many small adipocytes. Interestingly, the adipose of *ob/ob Col6* KO mice contains enormous, highly hypertrophic adipocytes.

If hypertrophic adipocytes are truly harmful, why do *ob/ob Col6* KO mice have less severe metabolic disease than control *ob/ob* mice? Collagen 6 is selectively produced by adipocytes compared to other cell types (Divoux et al., 2010). It surrounds fat cells and is responsible for the pericellular fibrosis that restrains adipocytes from expanding past a certain size. Mice lacking collagen 6 therefore have a more permissive extracellular matrix, allowing

for unrestricted expansion. Importantly, other genetic models which increase adipose ECM flexibility (ex. MMP14 overexpression) produce similar results to *Col6* KO (Li et al., 2020). Thus, it appears that hypertrophic adipocytes are not deleterious because they are large, but rather because they are prevented by the ECM from getting even larger.

Further evidence comes from experiments with thiazolidinediones (TZDs) which demonstrate that augmenting the expansion capacity of adipose tissue is beneficial in metabolic disease. TZDs are pharmacological ligands for PPAR γ , the master regulator of adipogenesis (Tontonoz, 1994). Activation of PPAR γ leads to enhanced adipocyte differentiation (hyperplasia) and, in some depots, to enhanced expansion capacity (hypertrophy) (Tang et al., 2011). Although PPAR γ is expressed in other cell types, notably macrophages, endothelium, muscle, and liver, the utility of TZDs as anti-diabetic drugs is believed to come, in large part, from their ability to promote healthy adipose tissue expansion (Yki-Jarvinen, 2004).

Elegant mouse genetic studies further show that enhancing *de novo* adipocyte differentiation by overexpression of PPAR γ in a subset of progenitor cells in visceral adipose tissue improves insulin sensitivity in mice fed a high fat diet (HFD), without affecting body weight. Reciprocally, deletion of PPAR γ in these cells provokes adipose tissue fibrosis and inflammation, along with worsened insulin resistance (Shao et al., 2018). Similarly, loss of function mutations in humans and adipocyte specific deletion in mice of phosphate and tensin homologue (PTEN), a negative regulator of adipogenesis, increase nutrient partitioning into adipose tissue and enhance insulin sensitivity despite obesity (Morley et al., 2015; Pal et al., 2012). Other studies have described consistent results, with genetic models characterized by enhanced lipid sequestration into adipocytes and insulin sensitive obesity (Kusminski et al., 2012). Genome wide association studies in humans have also begun to link genetic variants associated with reduced subcutaneous adipocyte storage capacity to increased risk for insulin resistance (Chu et al., 2017; Gulati et al., 2017; Majithia et al., 2014).

Adipose Tissue Turnover—Adipose tissue is in a constant state of low-level turnover, with mature adipocytes dying and being replaced by new adipocytes. Several studies have attempted to estimate the rate of this turnover in mice and humans. The most widely cited study employed ^{14}C measurements of adipocytes, taking advantage of the spike in atmospheric ^{14}C that occurred due to nuclear tests in the 1960's (Spalding et al., 2008). This group found that about 10% of adipocytes turn over per year in both lean and obese subjects. They note that obese people have similar rates of turnover when normalizing to the number of adipocytes, but higher absolute levels of turnover due to their increased number of adipocytes. Follow up work using ^{14}C measurements to track long term lipid flux in adipose tissue further indicated that there is no long-term lipid pool in fat; i.e. all lipid in the tissue (and thus presumably every adipocyte) is subject to turnover (Arner et al., 2019). Other studies tracking the proliferation of cells and turnover of substrates in slow turnover tissues using $^2\text{H}_2\text{O}$ long term labeling suggested more rapid turnover, of 0.16–0.29% of adipocytes and 4.5% of stromal-vascular cells per day (Neese et al., 2002).

In mice, the rates of adipocyte turnover are higher than in humans. Several studies employing distinct methods largely agree that ~5% of cells in the stromal vascular fraction are replicating at any time and that 1–5% of adipocytes are replaced each day (Neese et al., 2002; Rigamonti et al., 2011). As in humans, obese mice exhibit higher rates of proliferation and adipocyte turnover (Rigamonti et al., 2011). Notably, ¹⁵N thymidine labeling studies in mice indicate that the renewal and differentiation of adipocyte progenitors are uncoupled (Kim et al., 2014). The biological basis of this phenomenon is likely accounted for by specialization of adipocyte progenitor cells into discrete cell types, some of which are more proliferative and others which are more primed for differentiation (see below) (Merrick et al., 2019). Therefore, assessments of turnover which rely on assessing proliferation may underestimate the true rate of *de novo* adipogenesis, as committed preadipocytes may differentiate without first dividing.

The turnover of adipose tissue requires the coordinated action of multiple cell types. Dying adipocytes must be cleared away in an orderly manner to avoid the harmful effects of releasing lipids into the tissue. This clearing process is dependent upon adipose tissue macrophages, which engulf dying adipocytes and are detected within the tissue as crown-like structures. Interestingly, there is evidence that macrophages recruit adipocyte progenitor cells to sites of dying adipocytes via a CD44-Osteopontin axis, thereby linking the process of adipocyte death to adipogenesis (Lee et al., 2013).

Adipocyte progenitor cells (APCs)

The activity of APCs is a key mechanism by which adipose tissue achieves its plasticity. The major adaptive processes in adipose tissue, including expansion, being, and maintenance of adipocyte number, all involve *de novo* adipogenesis and therefore rely on the proper functioning of APCs. Could imbalances between the rate of adipocyte loss vs. replacement lead to metabolically maladaptive adipose tissue remodeling during aging? Likewise, since APCs must differentiate regularly, could we modulate their cell fate decisions, to encourage the formation of thermogenic adipose tissue instead of white adipose? Finally, do APCs make maladaptive cell fate decisions, for example by differentiating into pro-fibrogenic cell types, and can these decisions be intervened upon?

It has been known for decades that the stromal-vascular fraction (SVF) of adipose tissue contains cells capable of differentiating into adipocytes. The SVF is a heterogeneous mixture containing all the non-adipocyte cells which pellet after tissue digestion, and therefore the identity of the adipogenic cells was unclear. A groundbreaking study in 2008 utilized candidate cell surface markers to prospectively isolate and characterize APCs in WAT (Rodeheffer et al., 2008). In this study, APCs were defined based on their lack of expression of hematopoietic and endothelial cell markers (CD45 and CD31, hereafter called Lin-) and their selective expression of CD29, CD34, LY6A/Sca1, and CD24. This refined cell population produced adipocytes *in vitro* and *in vivo* following cell transplantation (Rodeheffer et al., 2008).

Another landmark study from Graff and colleagues identified a population of *Pparg*-expressing APCs residing alongside blood vessels in WAT. In addition to *Pparg*, these cells express the mural (vessel wall cell) marker *Pdgfrb* (Tang et al., 2008). Genetic lineage

tracing studies in mice show that *Pdgfrb*-expressing cells develop into *Pparg*⁺ mural cells and white adipocytes. Further lineage tracing experiments show that *Pdgfrb*-expressing cells generate new adipocytes in the epididymal WAT upon high fat diet feeding, contributing to 10–30% of the total adipocytes in this depot after several weeks (Gao et al., 2018; Vishvanath et al., 2016). Together, these findings led to the conclusion that (at least a subset of) APCs occupy a peri-vascular niche and are identifiable as a population of PDGFR β ⁺ cells, often termed mural cells, which are distinct from smooth muscle cells. Many papers in the field have taken this view. However, a parallel body of work suggests that use of the marker PDGFR β to identify APCs results in the inclusion of numerous, non-perivascular cell types. Indeed, PDGFR β is also expressed by adventitial fibroblasts that co-express PDGFR α and potentially represent a major source of APCs (Cattaneo et al., 2020; Hong et al., 2015; Vishvanath et al., 2016).

PDGFR α was first identified as a marker of adipogenic cells in regenerating muscle (Joe et al., 2010; Uezumi et al., 2010). Lineage tracing studies indicate that PDGFR α is a common marker of APCs in WAT and BAT depots (Berry and Rodeheffer, 2013; Lee and Granneman, 2012; Lee et al., 2012). These PDGFR α ⁺ cells are characterized as adventitial fibroblasts with multiple elongated processes touching components of the ECM and vasculature. Numerous confirmatory studies have been done, by separate groups employing different *Pdgfra-Cre* lineage reporters, which consistently demonstrate tracing of *Pdgfra*⁺ cells to adipocytes in both visceral and subcutaneous fat (Berry et al., 2014; Cattaneo et al., 2020; Han et al., 2021; Sun et al., 2020a).

An elegant recent study utilized intersectional lineage tracing with Cre/Lox and Dre/Rox reporters, showing that *Pdgfra*⁺/*Pdgfrb*⁺ and *Pdgfra*⁺/*Pdgfrb*⁻ progenitors, but not *Pdgfra*⁻/*Pdgfrb*⁺ cells, generated adipocytes during basal turnover and cold-induced adipogenesis in subcutaneous WAT and during wound healing-induced adipogenesis in dermal WAT (Han et al., 2021). Consistent with this, another recent lineage tracing study revealed that *Pdgfra*⁺ cells but not *Tbx18*⁺ pericytes contribute to adipocyte formation (Cattaneo et al., 2020). Taken together, these results suggest that *Pdgfra*⁺ (\pm *Pdgfrb*) adventitial fibroblasts rather than mural cells are the primary source of new adipocytes in WAT (Figure 5).

An APC hierarchy?—Recent single cell transcriptomic-based studies have enabled an unbiased analysis and further refinement of APC populations, suggesting specialization of APCs for different functions (Ferrero et al., 2020). One source of APC specialization relates to their degree of adipocyte lineage commitment. This concept was originally introduced by Rodeheffer *et al.*, who demonstrated that Lin⁻/CD29⁺/CD34⁺ cells could be subdivided into more and less committed cell populations based on CD24 expression. Compared CD24⁺ cells, CD24⁻ cells are less proliferative and show higher expression of adipocyte identity genes such as *Pparg*, *Lpl*, *AdipoQ*, and *Fabp4* (Berry and Rodeheffer, 2013). Moreover, transplantation studies indicate that CD24⁺ cells produce CD24⁻ cells during adipogenesis (Jeffery et al., 2015; Jeffery et al., 2016).

Recent single cell transcriptomic studies have further refined this concept, identifying distinct cell types on the basis of unique gene expression signatures. Studies in mouse adipose tissue consistently identify a continuum of adipogenic cells which subdivides into

two broad categories, namely progenitor cells (also called adipocyte stem cells (ASC) or interstitial progenitors) and preadipocytes (Burl et al., 2018; Cho et al., 2019; Ferrero et al., 2020; Han et al., 2021; Hepler et al., 2018; Merrick et al., 2019; Nguyen et al., 2021; Sarvari et al., 2021; Schwalie et al., 2018; Shao et al., 2021a). Likewise, studies of human subcutaneous adipose tissue have identified a similar continuum of APCs (Hildreth et al., 2021; Raajendiran et al., 2019; Vijay et al., 2020).

Progenitor cells are the most “stem-like” cells found in the tissue and are characterized by expression of *Pdgfra*, as well as *Dpp4*, *Pi16*, *Cd55*, *Ly6a*, and numerous *Wnt* pathway genes. Interestingly, fibroblasts with this gene expression signature are present in nearly every tissue of the body (Buechler et al., 2021). Preadipocytes are characterized by the expression of adipocyte-related genes, including *Pparg*, *Fabp4*, *Lpl*, and *Cd36*, suggesting commitment to the adipocyte lineage. Interestingly, preadipocytes express similar levels of *Pdgfra* but higher levels of *Pdgfrb* than progenitors, suggesting that earlier studies using these markers were in fact isolating distinct APC subtypes (Han et al., 2021; Sarvari et al., 2021).

Computational lineage prediction suggest that the APCs are arranged into a lineage hierarchy, with progenitors producing committed preadipocytes, before finally generating adipocytes (Burl et al., 2018; Merrick et al., 2019). This work implies that APCs likely exist in a continuum, from least to most committed to the adipocyte lineage, rather than occupying discrete states. Consistent with this, transplantation and lineage tracing studies show that DPP4+ progenitors, mainly localized in the layer of fibrous tissue which envelopes adipose tissue depots and subdivides it into lobes, produce preadipocytes and adipocytes *in vivo* (Figure 5) (Merrick et al., 2019; Stefkovich et al., 2021).

Adipogenesis-inhibitory cells—Several recent studies have identified fibroblast populations that are capable of inhibiting adipogenesis, including: fibro-Inflammatory-Progenitors (FIPs) in visceral fat, CD142^{High} AREGs (adipogenesis-regulatory cells) in visceral and subcutaneous fat and aging-dependent regulatory cells (ARCs) in aged subcutaneous WAT (Hepler et al., 2018; Nguyen et al., 2021; Schwalie et al., 2018). Their anti-adipogenic effects are presumed to come from secretion of inflammatory mediators (ARCs and FIPs) or other secreted factors (AREGs). Of note, the anti-adipogenic properties of AREGs have been called into question, since other groups report robust adipogenesis from this population (Merrick et al., 2019; Nguyen et al., 2021). Overall, the concept that stromal cells modulate the adipogenic commitment and differentiation of APCs is compelling and suggests an added layer of regulation to adipogenesis.

Depot and Development Specific Progenitors—Several groups have investigated the embryonic origin and development of adipocytes and this work has been reviewed in recent articles (Sanchez-Gurmaches et al., 2016; Sebo and Rodeheffer, 2019). To summarize, selective marker genes have been identified for adipocyte lineage cells that give rise to the broad categories of adipose tissue depots. For example, Paired related homeobox 1 (*Prx1*) is a selective marker of the subcutaneous adipocyte lineage (Sanchez-Gurmaches et al., 2015). Wilms Tumor 1 (WT1), a transcription factor with key roles in heart and kidney development, is a selective marker gene for visceral (versus subcutaneous) APCs (Chau et al., 2014). Notably, since mesothelial cells express WT1, lineage tracing from WT1+ cells

into visceral adipocytes initially suggested that mesothelial cells contribute to adipogenesis. However, recent work demonstrates that bona fide mesothelial cells are not adipogenic; instead a population of fibroblastic PDGFR α +WT1+ accounts for this result (Westcott et al., 2021). Lineage tracing studies show that fat depots in the dorsal anterior aspect of the mouse, including interscapular BAT and WAT, develop from somitic mesodermal cells expressing *Myf5*, *Engrailed1* and *Pax7* which also give rise to dermal fibroblasts and skeletal muscle cells (Sebo and Rodeheffer, 2019).

Elegant studies using a *Pparg* lineage tracing system showed that distinct populations of APCs are responsible for adipose tissue development and maintenance (Jiang et al., 2014). *Pparg*+ cells are detectable in the region which develops into inguinal WAT as early as E10.5. Interestingly, deletion of *Pparg* in these embryonic *Pparg*+ cells at E10.5 does not affect adipose tissue formation but causes progressive lipodystrophy during ageing. These results indicate that the adult progenitor cells responsible for adipocyte renewal are specified early in development and do not mediate the initial development of adipose tissue (organogenesis). The specification of adipocyte progenitors in embryogenesis suggests that *in utero* exposures may modulate the future differentiation potential or fate of these cells.

White vs. Beige adipogenesis—Beige and white fat-specific progenitor cell populations can be isolated and cloned from subcutaneous WAT, suggesting that beige and white fat cells represent distinct cell types/lineages (Wu et al., 2012). In this regard, PDGFR α + cells expressing *Cd81* have been reported to possess enhanced beige adipogenic potential, though this marker gene appears to be quite broadly expressed in many/most fibroblasts (Oguri et al., 2020). Additionally, smooth muscle-related cells expressing certain SMC marker genes (i.e. *Myh11*, *Acta2*, *Trpv1*) contribute to beige adipocyte development (Berry et al., 2016; Long et al., 2014; Shamsi et al., 2021). Remarkably, in the absence of β -adrenergic receptor signaling, a completely different progenitor cell population expressing skeletal muscle genes, including *Myod*, are recruited to generate a distinct type of beige fat exhibiting high levels of glycolysis (Chen et al., 2019). These results show that there are multiple paths for beige adipocyte development, though the inter-relationships between these different cell types and their differentiation trajectories are uncertain.

APC Regulation and Adipogenesis—APCs differentiate into adipocytes via the process of adipogenesis. The molecular regulation of this process has been extensively studied using *in vitro* cell model systems. Adipogenesis is governed by two main waves of transcription factor activation (Lefterova et al., 2014). At the onset of differentiation, C/EBP β and C/EBP δ bind to “semi-closed” chromatin at adipogenic target genes. At later time points, these regions become transcription factor ‘hotspots’ that are bound and regulated by multiple transcription factors, including Glucocorticoid receptor (GR), retinoid X receptor (RXR), and STAT5a. Amongst the second wave of factors, the master adipogenic factor PPAR γ plays a dominant role in activating the expression of adipocyte-selective genes to confer the mature fat cell phenotype (Siersbaek et al., 2012; Steger et al., 2010).

An additional layer of transcriptional regulation in adipocytes is provided by numerous factors that determine the energy-storing white vs. energy-burning brown phenotype. The transcriptional co-activator PGC-1 α along with IRF4, ERR factors, c/EBP β , CREB and

ZFP516 are key regulators of the β -adrenergic-stimulated thermogenic gene program in adipocytes (Shapira and Seale, 2019). Several other transcriptional factors play pivotal roles in specifying thermogenic adipocyte identity, including EBF2, NFIA, Zc3h10 and PRDM16 (Shapira and Seale, 2019). Interestingly, sustained use of synthetic PPAR γ agonists, like thiazolidinediones, can promote thermogenic gene expression, in a PRDM16 dependent manner (Ohno et al., 2012). Conversely, ZFP423 enforces white fat cell identity, acting through suppression of EBF2 activity. Adipocyte-specific deletion of ZFP423 or activation of EBF2 in mice enhances beige fat formation and improves metabolic health (Shao et al., 2016; Shao et al., 2021b; Stine et al., 2016). TLE3 also represses the thermogenic program of adipocytes both by impeding the function of pro-thermogenic factors PRDM16 and EBF2 and by increasing the expression of white-selective genes. Activation of TLE3 in BAT impairs lipid oxidation and thermogenesis, while deletion of TLE3 in WAT promotes thermogenesis and energy expenditure (Pearson et al., 2019; Villanueva et al., 2013).

A full understanding of adipocyte lineage commitment (i.e., progenitor-to-preadipocyte transition) in adult tissues has been hampered by the lack of molecular markers that define these cell types. However, as discussed above, recent studies have identified distinct cell types/states which appear to be positioned at different stages along the adipogenic trajectory. The adipogenic commitment process likely involves the integration of several pro- and anti- adipogenic growth factor signals. Anti-adipogenic signals include: canonical and noncanonical WNT pathways (especially WNT5, WNT6, WNT10a, and WNT10b), TGF β , platelet derived growth factor (PDGF), and hedgehog signaling (Ghaben and Scherer, 2019). Pro-adipogenic signals include: Insulin, Bone Morphogenic Protein (BMP) signaling (especially BMP2 and BMP4), and extracellular matrix composition (Ghaben and Scherer, 2019). For example, BMP2 and BMP4 induce activation of SMAD4 and its heterodimeric partners, which subsequently stimulates the transcription of PPAR γ , driving adipogenic commitment (Huang et al., 2009). Of note, ZFP423, a regulator of adipogenic commitment and PPAR γ expression, sensitizes cells to the pro-adipogenic effects of BMP signaling (Gupta et al., 2010).

While many pathways and factors have been shown to regulate adipocyte differentiation in cell culture models, the physiologic mechanisms that control adipocyte differentiation *in vivo* remain poorly defined. There is substantial literature implicating a role for fatty acids in promoting adipocyte differentiation, suggesting that lipolysis or lipid accumulation in fat tissue provides a signal for adipogenesis. In this regard, certain fatty acids can serve as activating ligands for PPAR proteins, providing an attractive mechanistic link between diet, lipid levels and adipocyte differentiation. However, a high affinity natural ligand for PPAR γ has yet to be identified. A notable recent study showed that omega-3 fatty acids stimulate preadipocytes to undergo differentiation via the FFAR4 G-protein coupled receptor, located specifically in cilia (Hilgendorf et al., 2019).

A number of pathways have been proposed to inhibit adipocyte differentiation, though in many cases *in vivo* evidence is lacking. The presence of committed preadipocyte cells expressing detectable levels of PPAR γ suggest that the adipocyte differentiation program is actively inhibited in these cells under basal conditions. A widespread problem in the field relates to the misinterpretation of mouse models exhibiting changes in adipose

tissue mass. Such effects are often attributed to primary changes in APC activity and adipogenesis. However, adipose tissue size is highly sensitive and responsive to changes in systemic energy levels. Many papers presenting an obesity-resistant mouse model with a metabolically healthy phenotype will attribute the phenotype to a defect in adipogenesis. However, impaired adipogenesis is expected to cause lipodystrophy, resulting in ectopic lipid accumulation in muscle and liver along with insulin resistance.

Limitations to Plasticity

The functional decline of adipose tissue during obesity and aging is associated with a loss of plasticity. A prevailing model posits that maladaptive adipose tissue remodeling, characterized by fibrosis and inflammation, is triggered by a failure of angiogenesis which leads to tissue hypoxia as well as the accumulation of senescent cells (Crewe et al., 2017; Hepler and Gupta, 2017). In this model, adipose tissue expansion outstrips vascular supply, causing local hypoxia, which inhibits adipogenesis and induces hypertrophic adipocytes to secrete inflammatory cytokines, die via necrosis and spill lipid in an uncontrolled manner. Consequently, adipose tissue becomes insulin resistant, inflamed, and fibrotic, further compromising its function. All of these processes are continuous and mutually reinforcing, making it difficult to disentangle cause and effect (Figure 6).

Reduced APC function in aging?—The capacity for hyperplastic adipose tissue expansion declines during aging (Caso et al., 2013; Kim et al., 2014). Aging-induced defects in APCs include decreased expression of sirtuins, reduced expression of pro-adipogenic transcription factors, and impaired proliferative capacity (Caso et al., 2013; Khanh et al., 2018). Moreover, the adipose tissue of aged mice and humans accumulate senescent APCs (Baker et al., 2016; Tabula Muris, 2020). Clearance of senescent cells from the adipose tissue of old mice improves adipogenesis and systemic metabolism (Xu et al., 2015). Similarly, suppression of the SASP in human preadipocytes enhances adipogenic differentiation (Gustafson et al., 2019). Interestingly, aging in mice also leads to the accumulation of a distinct population of anti-adipogenic cells, specifically in subcutaneous fat, called “aging- dependent regulatory cells” (ARCs). ARCs, which express inflammatory markers, inhibit both the proliferation and adipogenic capacity of APCs (Nguyen et al., 2021).

Adipose tissue fibrosis—In healthy adipose tissue, adipocytes are embedded in a loose mesh of ECM, composed of multiple collagens (especially I, III, and VI), fibrillins, and proteoglycans, that provides structural support and modulates the activity of growth factors and signaling molecules (Marcelin et al., 2019). In contrast, fibrosis is a hallmark of dysfunctional fat, characterized by the excessive accumulation of extracellular matrix (ECM) and tissue stiffening. As with fibrosis in other organs, adipose tissue fibrosis is both a symptom of and contributor to the functional decline of the tissue.

Obesity in mice and humans is generally associated with increased adipose tissue fibrosis, especially in visceral depots, with higher levels of fibrosis correlating with more metabolic complications (Sun et al., 2013b). Fibrosis appears to cause tissue dysfunction through several mechanisms. First, adipocytes themselves are mechanosensitive and thus

dysregulated ECM can alter mechanical cues and impair adipocyte function. Indeed, mechanical compression of adipocytes impairs lipolysis, decreases the expression of adipokines like leptin and adiponectin, and increases the expression of ECM genes and proinflammatory cytokines (Pellegrinelli et al., 2014). Second, the ECM serves as a reservoir of growth factors and fibrotic ECM can alter tissue function by disrupting the signaling milieu (Marcelin et al., 2019). Third, fibrotic ECM increases the rigidity of the tissue, physically impeding adipose tissue expansion by adipocyte hypertrophy (Khan et al., 2009). Fourth, dysregulated ECM impairs the function of APCs, which must remodel the local ECM to undergo adipocyte differentiation (Chun et al., 2006). Finally, APCs are mechanosensitive and exhibit decreased adipogenic capacity on stiffer substrates (Young et al., 2013).

The signaling pathways, gene regulatory networks, and cellular mediators responsible for adipose tissue fibrosis have been extensively reviewed elsewhere (Crewe et al., 2017). Transforming growth factor beta (TGF β) especially, as well as many other factors, including Activin A, Connective Tissue Growth Factor (CTGF), Platelet Derived Growth Factor (PDGF α), and inflammatory cytokines have been implicated in the development of adipose tissue fibrosis (Iwayama et al., 2015; Yoshino et al., 2019; Zaragosi et al., 2010). Additionally, during obesity, hypoxia-induced signaling through HIF1 α exerts potent pro-fibrotic rather than angiogenic effects in adipose tissue, further driving adipose tissue dysfunction (Halberg et al., 2009; Sun et al., 2013a).

The role of APCs in adipose tissue fibrosis has received significant attention. While other cell types in adipose tissue, such as macrophages and adipocytes, produce collagens and secrete pro-fibrotic factors, fibroblasts express the highest levels of collagens and fibrosis genes (Marcelin et al., 2017). Several studies suggest that APCs may have the capacity to adopt either an adipogenic or pro-fibrogenic fate, depending on the signaling context. In this regard, fibrosis would be pathogenic not only because of direct effects on the ECM but also because of aberrant cell fate choices by APCs, compromising their capacity for adipogenesis (Figure 6). Genetic mouse models provide additional evidence that the pro-fibrotic and adipogenic activities of APCs are opposed. As an example, expression of constitutively active PDGFR α in APCs results in lipodystrophy and profoundly fibrotic tissue, while deletion of PDGFR α has opposite effects (Marcelin et al., 2017; Sun et al., 2017; Sun et al., 2020a). Additionally, HIF1 α inhibits APC differentiation through inhibitory phosphorylation of PPAR γ and targeting this pathway augments adipogenesis and ameliorates metabolic dysfunction (Shao et al., 2021a).

Several studies have defined subsets of APCs in WAT that exhibit high fibrotic and low adipogenic potential. For example, CD9High/PDGFR α + cells, which increase during obesity in visceral fat, exhibit a pro-fibrotic phenotype and are less adipogenic (Marcelin et al., 2017). Hepler *et al.* identified a related population of PDGFR β +LY6a+/CD9+ cells in visceral WAT, which they termed fibro inflammatory progenitors (FIPs) (Hepler et al., 2018). FIPs are transcriptionally similar to (DPP4+) progenitor cells in subcutaneous WAT, suggesting that the division between pro-fibrotic and pro-adipogenic adipocyte progenitor cell subtypes is conserved across depots (Burl et al., 2018; Ferrero et al., 2020; Merrick et al., 2019).

There is evidence that beige fat biogenesis and the activity of the thermogenic transcription factor PRDM16 is protective against adipose tissue fibrosis. For example, the cold inducible transcription factor GTF2IRD1 recruits the transcription factors PRDM16 and EHMT1 to the promoters and enhancers of TGF β responsive pro-fibrosis genes, repressing their expression and suppressing fibrosis. Importantly, this mechanism occurs independent of UCP1 (Hasegawa et al., 2018). Additionally, PRDM16 expression in mature beige adipocytes promotes secretion of beta-hydroxybutyrate, which promotes APC beige adipogenesis and blocks fibrosis (Wang et al., 2019).

Finally, a fibrosis vs. adipogenesis (or lipid storage) fibroblast fate axis exists in other tissues. A well-studied example occurs in the skin, in which conversion of myofibroblasts into adipocytes and vice-versa occurs during wound healing (Marangoni et al., 2015; Plikus et al., 2017; Shook et al., 2020). In skeletal muscle, PDGFR α + fibroblastic cells also give rise to both adipocytes and pro-fibrogenic cells (Uezumi et al., 2011). Furthermore, in models of idiopathic pulmonary fibrosis (IPF), treatment with PPAR γ agonists alleviates fibrosis by promoting differentiation of lung fibroblasts into lipid storing and less fibrogenic lipofibroblasts (El Agha et al., 2017).

Adipose tissue inflammation—Immune cells play many critical roles in regulating adipose tissue phenotypes in response to physiological and pathological stimuli (Lu et al., 2019). Evidence that obesity results in chronic inflammation emerged in the 1990s, through the study of Hotamisligil *et al.*, showing increased concentrations of the inflammatory cytokine TNF α in the adipose tissue of obese rats (Hotamisligil et al., 1993). Neutralization of TNF α signaling improves insulin sensitivity, establishing a link between immune responses and metabolism. Following these early studies, an extensive amount of research has demonstrated that chronic inflammation is a hallmark of adipose tissue dysfunction and systemic metabolic dysregulation.

Obesity in mice and humans dramatically increases the number of adipose tissue macrophages, linked to the activation of several inflammatory pathways (Amano et al., 2014; Patsouris et al., 2008; Weisberg et al., 2003). Seminal work showed that obesity induces a phenotypic switch in adipose tissue macrophages from an anti-inflammatory “type 2” profile to a pro-inflammatory “type 1” state (Lumeng et al., 2007a; Lumeng et al., 2008; Nguyen et al., 2007). These “type 1” macrophages represent a major source of pro-inflammatory cytokines and can be found surrounding dead or dying adipocytes in adipose tissue, forming characteristic crown-like structures. Ablation of pro-inflammatory macrophages in obese mice decreases adipose tissue inflammation and enhances insulin sensitivity (Patsouris et al., 2008). Similarly, reducing macrophage recruitment into adipose tissue ameliorates metabolic complications in high fat fed mice (Kanda et al., 2006; Weisberg et al., 2006).

T cells also increase during obesity and play prominent roles in adipose tissue inflammation (Nishimura et al., 2009; Wu et al., 2007). CD8+ effector T cells infiltrate adipose tissue at early stages of obesity development, stimulating macrophage recruitment and inflammation (Nishimura et al., 2009; Rausch et al., 2008). Of note, high fat feeding in mice led to an accumulation of a particular subset of T cells exhibiting a senescent phenotype and

expressing high levels of the pro-inflammatory factor Osteopontin (*Spp1*) in visceral adipose tissue (Shirakawa et al., 2016). Conversely, regulatory T (Treg) cells play a critical role in suppressing adipose tissue inflammation in the visceral depot (Feuerer et al., 2009; Ilan et al., 2010). Adipose tissue Treg cells are abundant in the lean state and decrease in obesity. Ablation of these cells in fat tissue increases inflammation and insulin resistance, whereas adoptive transfer of Treg cells blunts inflammatory response and improves metabolic parameters.

Another important immune cell type in adipose tissue is innate lymphoid type 2 cells (ILC2). ILC2 cells express IL-5 and IL-13, which regulate the maintenance of alternatively activated macrophages and eosinophils to limit inflammation and promote the development of thermogenic adipocytes (Hams et al., 2013; Molofsky et al., 2013). Like Treg cells, adipose tissue ILC2 decrease in the setting of obesity. ILC2 cells also decrease in abundance and lose their identity in the visceral adipose tissue of mice during aging (Goldberg et al., 2021).

The mechanisms responsible for triggering and sustaining adipose tissue inflammation have been intensively studied over the past decade. Obesity-induced alterations in the gut microbiome, along with increased gut permeability promote the translocation of endotoxins like LPS, driving inflammation in many tissues including adipose tissue. Within adipose tissue, fatty acids released from fat cells (or insufficiently sequestered by fat cells) have been proposed to elicit inflammatory responses, though these effects have not been observed consistently across studies (Tilg et al., 2020). Additionally, the chronic uptake and increased storage of fatty acids as lipid droplets in macrophages may cause lipotoxicity, leading to pro-inflammatory changes within macrophages. In support of this idea, lipid-storing macrophages, resembling foam cells, accumulate in obese adipose tissue (Lumeng et al., 2007b). More recently, single cell transcriptomic studies defined a population of lipid-laden macrophages in adipose tissue of obese animals marked by the expression of CD9. These CD9⁺ macrophages are sufficient to induce pathologic programming of adipose tissue when transferred into lean mice (Hill et al., 2018). Interestingly, the capacity for macrophages to take up and store lipid exerts beneficial, metabolically protective effects, suggesting that lipid-storage *per se* is adaptive and that other signals are necessary to provoke inflammatory changes (Aouadi et al., 2014). Further studies show that the lipid receptor TREM2 is a key functional regulator and marker of lipid-storing macrophages in rodent and human fat tissue (Jaitin et al., 2019). Notably, a recent study shows that adipocytes, in addition to releasing fatty acids via lipolysis, transfer lipids to macrophages *via* exosomes (Flaherty et al., 2019). This exosomal lipid transfer pathway is increased in obesity and promotes macrophage differentiation.

Adipocytes modulate inflammation through the production of adipokines. In particular, Leptin, which increases during obesity, exerts pro-inflammatory effects through direct actions on many types of immune cells (Francisco et al., 2018). By contrast, adiponectin promotes an anti-inflammatory profile in macrophages (Ohashi et al., 2010). An emerging concept in the field demonstrates important functions for various types of fibroblasts in modulating adipose tissue immune responses. For example, the Gupta lab has defined a subset of fibroblasts, called fibro-inflammatory progenitors that stimulate macrophage

accrual in adipose tissue during obesity development (Shan et al., 2020). Additionally, certain subpopulations of mesenchymal cells in adipose tissue are a major source of IL-33, which regulates the activity of Treg and ILC2 cells (Spallanzani et al., 2019). Unfortunately, despite a huge body of literature implicating inflammation as a driver of obesity-related metabolic disease, anti-inflammatory therapies have, thus far, not been successful. Given the pleiotropic effects of immune cells in adipose tissue, it will likely be necessary to identify approaches that selectively block the maladaptive effects of inflammation, without compromising the critical functions of immune cells that support adipose tissue health and plasticity.

Limitations to Metabolic Plasticity—Healthy WAT exhibits extensive metabolic flexibility, responding to anabolic and catabolic signals (via lipogenesis and lipolysis respectively) to preserve whole organism energy homeostasis. However, in the setting of chronic positive energy balance, WAT develops metabolic inflexibility, characterized by a decreased amplitude of response to signals regulating both the storage and mobilization of nutrients.

In the fed-state, adipose tissue metabolic inflexibility manifests as insulin resistance, resulting in decreased post-prandial glucose and lipid sequestration, unrestrained lipolysis, and elevated circulating FFA levels (Gastaldelli et al., 2017; Petersen and Shulman, 2018). The molecular pathogenesis of adipose tissue insulin resistance is complex and incompletely understood, but inflammation, hypoxia, fibrosis, and impaired expandability appear to be key contributors; comprehensive reviews offering integrated perspectives on whole body and adipose tissue specific insulin resistance have been recently published elsewhere (Czech, 2020).

In the fasted-state, adipose tissue metabolic inflexibility manifests as diminished catecholamine-stimulated lipolysis in subcutaneous WAT, despite elevations in basal lipolysis in all fat depots (Arner, 2005). This lipolysis impairment is due to alterations in the catecholamine-stimulated signaling cascade, including reduced β_2 -adrenergic receptor expression, increased anti-lipolytic α_2 -receptor activity, and decreased HSL stimulation by cAMP; these phenomena have been extensively reviewed elsewhere (Morigny et al., 2016). These changes are likely exacerbated by obesity-induced decreases in sympathetic nerve fiber density (Cao et al., 2018a; Jiang et al., 2017).

Chronic inflammation is believed to be a major contributor to the impairment in the metabolic plasticity of fat (Zatterale et al., 2019). As an example, secretion of the proinflammatory cytokines TNF α and MCP1 by adipocytes and infiltrating macrophages activates JNK signaling, which phosphorylates the insulin receptor and reduces its activity (Hirosumi et al., 2002). Similarly, chronic inflammation diminishes catecholamine responsiveness. For example, TNF α -induced expression of the kinases IKK ϵ and TBK1 activates the phosphodiesterase PDE3B, which directly reduces the levels of cAMP. This reduction in cAMP signaling reduces HSL phosphorylation and UCP1 expression thereby diminishing both lipolysis and thermogenesis (Li et al., 2019; Mowers et al., 2013).

Hypoxia is another key driver of adipose tissue dysfunction and metabolic inflexibility during aging and obesity. Hypoxia is believed to develop due to (1) the presence of hypertrophic adipocytes (reaching 200+ μm in diameter), which exceed the diffusion limit of O_2 (typically 100–200 μm in tissues) and (2) defects in post-prandial blood flow and vascular density (Trayhurn, 2013). In obesity, rather than stimulating angiogenesis, HIF1 α , the master regulator of the hypoxia response, promotes the expression of pro-inflammatory and pro-fibrotic genes (Halberg et al., 2009; Lee et al., 2014b). Interestingly, expression levels of an anti-angiogenic form of VEGF (VEGFA_{165b}) are elevated in obesity and likely contribute to impaired angiogenesis in adipose tissue (Ngo et al., 2014). Obese humans exhibit diminished adipose tissue blood flow and a notable failure to augment blood flow in response to feeding, prolonged fasting, and exercise (Frayn and Karpe, 2014). Consistent with this finding, obese mice show precipitous declines in adipose tissue capillary density (Cao et al., 2018a). Together these results suggest that targeting adipose tissue angiogenesis to promote healthy vascular growth and avoid hypoxia may be a promising future therapeutic avenue.

Thermogenesis—Obesity and aging are associated with reductions in the abundance and activity of thermogenic adipose tissue in both mice and humans (Becher et al., 2021; Wang et al., 2019). Interestingly, distinct mechanisms may underlie age- and obesity-linked declines in thermogenic fat activity. For example, Song et al. observed that the conversion of low-thermogenic cells to high-thermogenic cells in BAT is impaired in aging but not in diet-induced obesity (Song et al., 2020). Likewise, Nguyen et al. showed that aging but not obesity causes the emergence of pro-inflammatory precursor (ARC) cells in subcutaneous adipose tissue, which impede adipocyte differentiation (Nguyen et al., 2021). Targeting BAT to increase longevity has been the aim of several studies and is reviewed elsewhere (Darcy and Tseng, 2019).

Conclusions

There is an urgent need to develop new therapies to combat the expanding dual epidemics of obesity and cardiometabolic disease. Adipose tissue lies at the center of these health problems, representing a major contributor to disease pathogenesis and a promising target for therapies. As highlighted in this review, adipose tissue possesses extraordinary plasticity, including its: (1) rapid titration of metabolic programs to maintain systemic energy levels in the face of fluctuating changes in nutrient supply and demand; (2) unparalleled capacity to expand and contract to accommodate long term trends in energy balance; (3) remarkable structural and metabolic transformation during cold exposure to engage in heat production; (4) capacity for de-differentiation to regulate lactation and wound healing.

Obesity often leads to a decline in adipose tissue plasticity, which is associated with fibrosis, inflammation, progenitor cell senescence, and catecholamine resistance. Ultimately, these pathological changes impair the critical nutrient-buffering function of adipose tissue, leading to insulin resistance and metabolic disease. The central role of adipose tissue dysfunction in disease and the incredible plasticity of fat tissue supports the promise of modulating fat tissue phenotypes for therapeutic purposes. The viability of this approach has already been demonstrated with the success of thiazolidinediones, which promote healthy adipose tissue

expansion and enhance insulin sensitivity. Unfortunately, unfavorable side effects of some thiazolidinediones have caused this class of drugs to fall out of favor.

New insights into the identity and regulation of APCs, adipocytes, immune cells, and other diverse cell types in adipose tissue promise to reveal novel drug targets to promote metabolically beneficial tissue remodeling. For example, it may be possible to promote favorable APC-fate decisions, encouraging adipogenesis at the expense of adipocyte hypertrophy, fibrosis, and inflammation. Additionally, increasing the abundance and activity of thermogenic adipose tissue is a promising strategy to enhance energy expenditure to combat both metabolic disease and obesity. Many questions and opportunities for future discovery remain, which will yield new insights into adipose tissue biology and hopefully lead to improved therapies for human disease.

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Adipose Tissue Plasticity

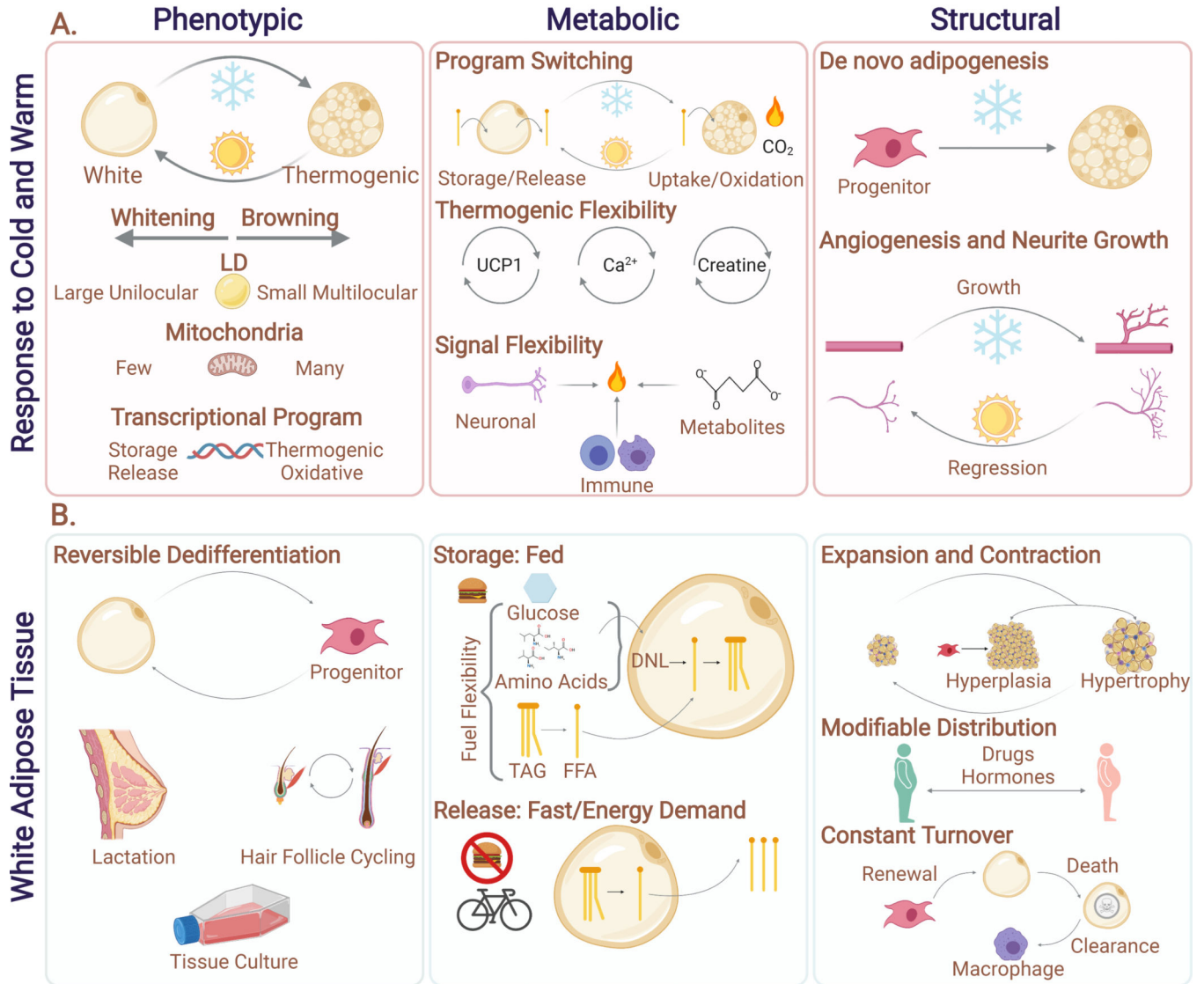


Figure 1: Adipose Tissue Plasticity.

Adipose tissue engages in multiple adaptive processes to maintain homeostasis which can be classified into distinct categories of plasticity. A) Adipose tissue changes dynamically in response to cold or warm environment. Phenotypic: In response to cold, individual adipocytes remodel their internal architecture to facilitate thermogenesis in a process called being or browning. Being involves alterations to the structure of lipid droplets, robust mitochondrial biogenesis, and upregulation of a transcriptional program which supports high levels of local fuel oxidation. These changes are reversible and regress with the removal of stimulus through the reverse process called whitening. Metabolic: Cold exposure promotes a metabolic switch from energy storage to fuel utilization and uncoupled respiration. Thermogenesis is classically achieved by a futile cycle involving UCP1, although recent work has demonstrated that adipocytes employ several mechanisms

of futile cycling that promote thermogenesis, including calcium and creatine cycling. Adipocytes respond to several cues for thermogenesis, including neuronal, immune, and metabolite derived signals, allowing tight and context specific control of heat production. Structural: During the response to cold, the structure of adipose tissue remodels to facilitate thermogenesis. Cold induces the production of new adipocytes from adipogenic progenitor cells via *de novo* differentiation. Additionally, cold induces angiogenesis and sympathetic nerve fiber branching, which regress upon removal of thermogenic stress. B) White adipose tissue plasticity. Phenotypic: In specific contexts, white adipocytes are capable of reversible dedifferentiation *in vivo* and *in vitro*, most notably during lactation (dedifferentiation) and involution (redifferentiation), hair follicle cycling, and in “ceiling culture, a specific technique for primary cell culture of isolated adipocytes. Metabolic: White adipocytes switch between two opposing metabolic programs, nutrient storage and nutrient release. Nutrient storage involves uptake of glucose, amino acids, and fatty acids (TAG: triacylglycerol. FFA: Free fatty acid). By the process of *de novo* lipogenesis (DNL) excess nutrients are converted into fatty acids allowing for efficient storage in lipid droplets. During periods of fasting or high energy demand (ex. exercise), adipocytes release nutrients into the systemic circulation by breaking down stored TAGs and releasing FFAs through lipolysis. Structural: Adipose tissue has a remarkable ability to expand and contract in response to over- and under- nutrition respectively. Expansion is mediated by a combination of one of two mechanisms: hypertrophy (increases in individual adipocyte size) and hyperplasia (increases fat cell number mediated by *de novo* differentiation of adipocyte progenitor cells). The distribution of adipose tissue is variable and can be modified toward a more metabolically favorable peripheral distribution (green) or a more metabolically maladaptive central distribution (red) by numerous factors including sex hormones, growth hormones, cortisol, and pharmaceuticals. The structure of adipose tissue is in constant flux due to persistent low-level turnover and replacement of adipocytes at a rate of ~10% per year in humans.

Location of Major Adipose Tissue Depots in Mice and Humans

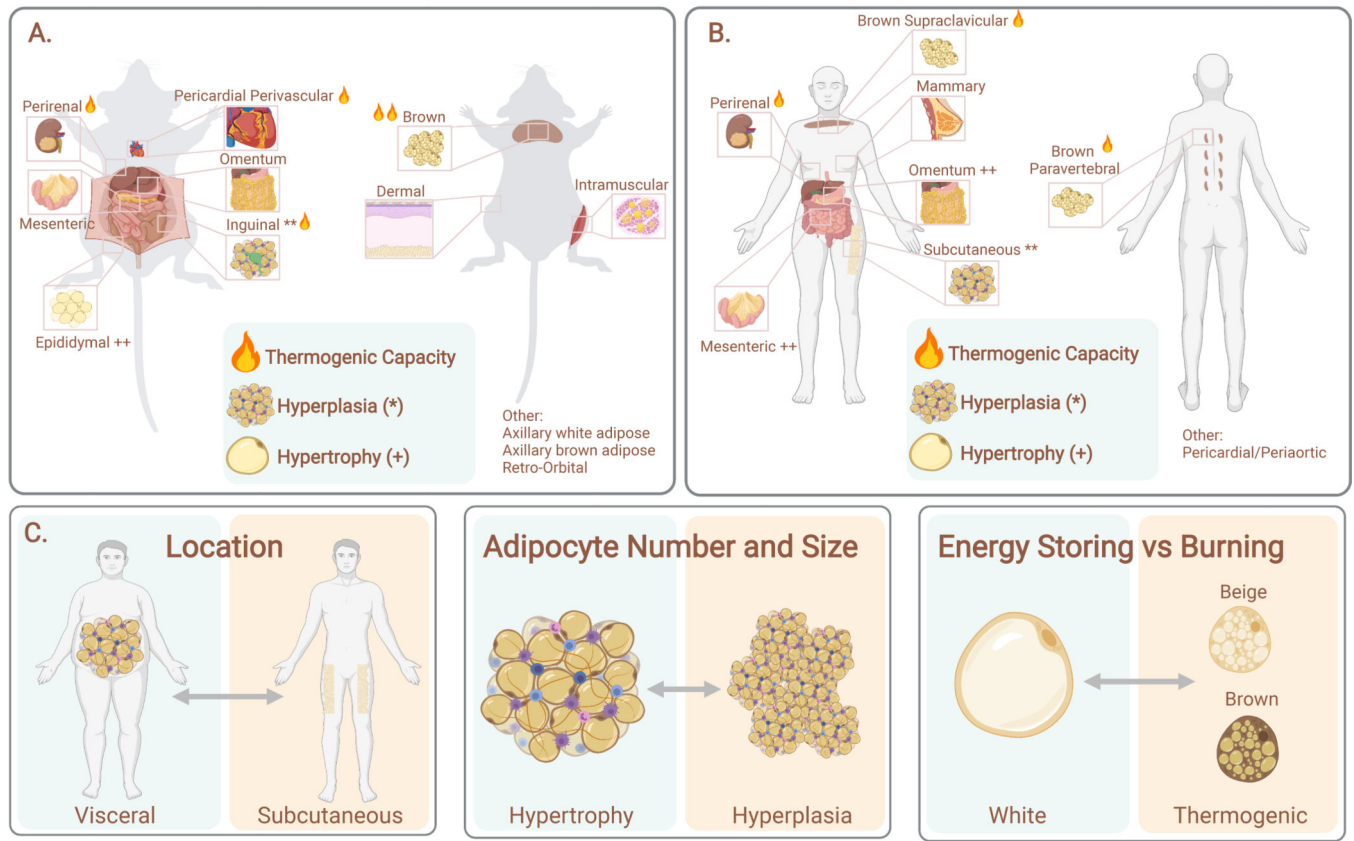


Figure 2. Location of Major Adipose Tissue Depots in Mice and Humans.

Both mice (A) and humans (B) have thermogenic brown adipose tissue (interscapular, cervical, paravertebral). Epididymal adipose tissue (eWAT) is comparable to visceral human adipose tissue (omental, mesenteric adipose tissue (MAT)), while murine inguinal adipose tissue (iWAT) is comparable to human subcutaneous adipose tissue. Fat depots differ in their propensity for thermogenesis. (C) The three axes of adipose tissue variance relevant to metabolic health: location (visceral vs subcutaneous); expansion mechanism (hypertrophy vs hyperplasia), and metabolic phenotype (energy storing vs burning).

Metabolic Plasticity of White Adipose Tissue

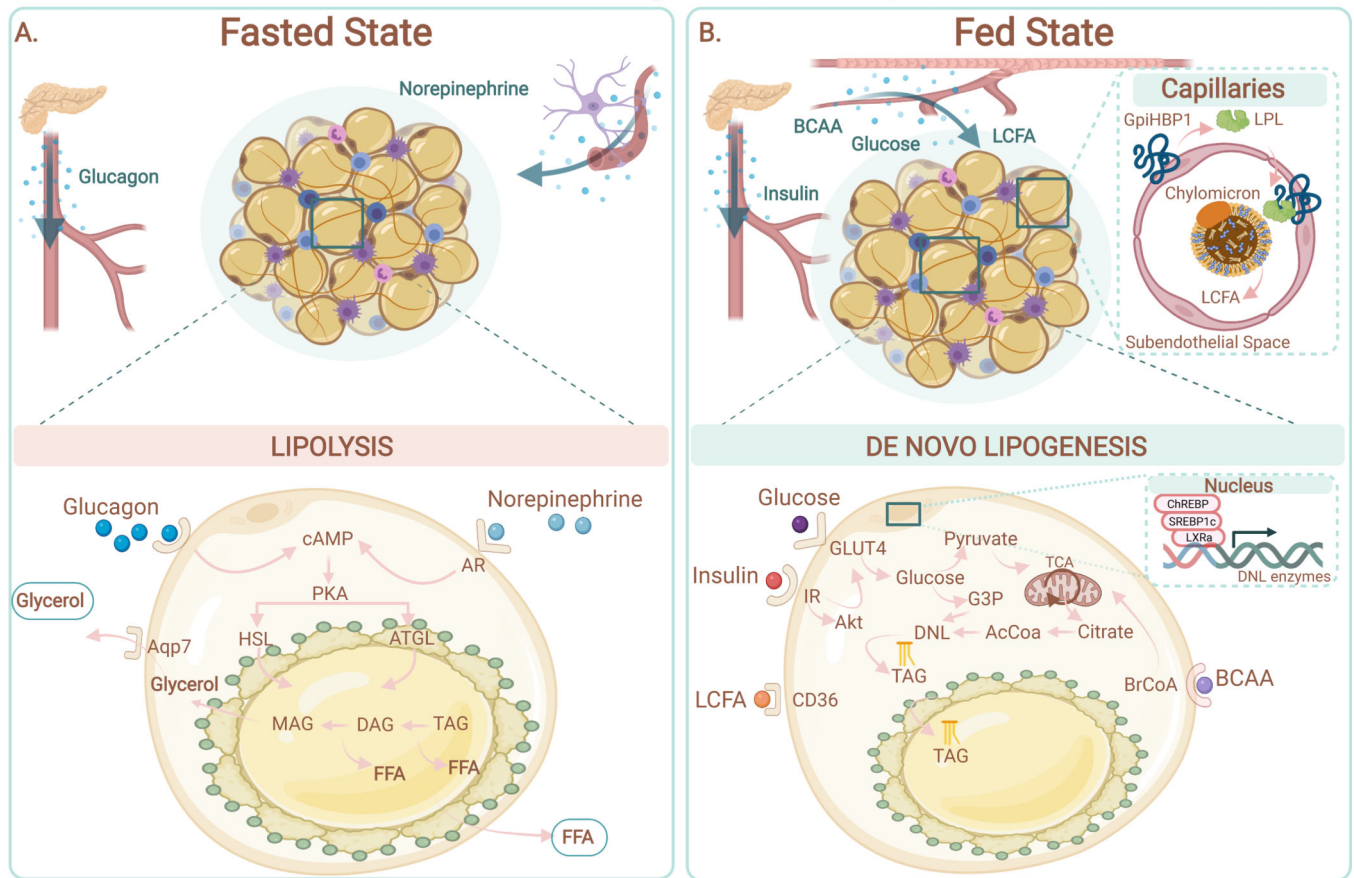


Figure 3. Metabolic Plasticity of White Adipose Tissue.

(A) Fasted state. Adipocytes release free fatty acids (FFAs) and glycerol via lipolysis in response to external stimulation (i.e., norepinephrine, glucagon). Binding of norepinephrine to the adrenergic receptor (AR) on adipocytes drives the elevation of cAMP and PKA activation. PKA stimulates the hydrolysis of triglycerides (TAG), diacylglycerol (DAG), and subsequently monoacylglycerol (MAG) through activation of the endogenous lipases ATGL and HSL. FFAs and glycerol are secreted into the systemic circulation to supply fuel to other tissues. (B) Fed State. Adipocytes have access to multiple sources of circulating nutrients, including: 1) Long Chain Fatty Acids (LCFA) from Very Low-Density Lipoprotein (VLDL) (LPL-mediated hydrolysis of triacylglycerols from VLDL in capillaries to generate FFAs); 2) glucose; 3) branched-chain amino acids (BCAA). De novo lipogenesis (DNL) uses Acetyl-CoA (AcCoA) as the primary building block for fatty acid synthesis. Synthesized fatty acids are esterified into triglycerides (TAG) and stored in lipid droplets. Expression of enzymes involved in DNL (i.e., fatty acid synthase (FAS); acetyl-CoA carboxylase (ACC)) are positively regulated by hormones (i.e., insulin) and by transcription factors such as Carbohydrate response element binding protein (ChREBP), Liver X receptor alpha (LXR α) and Sterol response element binding protein 1c (SREBP1c). TAGs stored in the lipid droplet are released by lipolysis during periods of energy demand.

Metabolic Plasticity of Thermogenesis

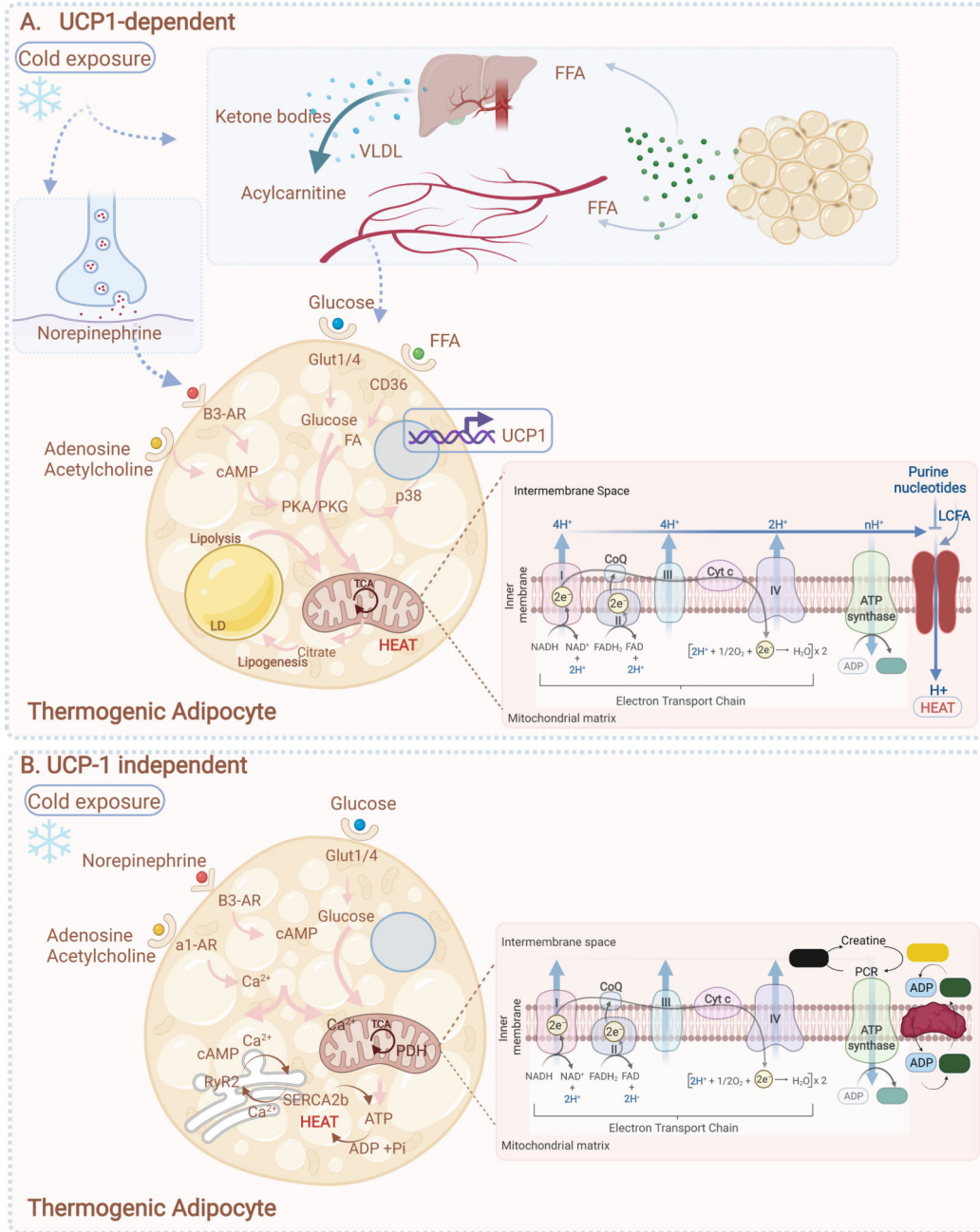


Figure 4. Metabolic Plasticity of Thermogenesis.

(A) UCP-1 dependent: Cold exposure or adrenergic stimulation increases cAMP levels, driving activation of PKA/PKG signaling and lipolysis. P38MAPK (p38) in turn promotes activation of transcriptional regulators that drive mitochondrial biogenesis and expression of thermogenic genes (*Ucp1*, *Cidea*, *Dio2*). When activated, UCP1 drives proton leak in the mitochondria, leading to uncoupling of mitochondrial respiration from ATP synthesis and driving greater consumption of fuels (e.g., glucose and free fatty acids (FFA)). FFA secreted by WAT also drives hepatic production of acylcarnitines and ketones to help

fuel thermogenesis. (B) UCP1-independent: beige thermogenic cells use Ca^{2+} futile cycling through the SERCA2b-RyR2 pathway in the endoplasmic reticulum (ER) to produce heat during cold exposure. Creatine cycling in the mitochondria is an additional mechanism to produce heat independent of UCP1. UCP-1 independent pathways require glycolysis and mitochondrial ATP-synthesis to provide fuel for futile cycling.

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Adipocyte Progenitor Cells

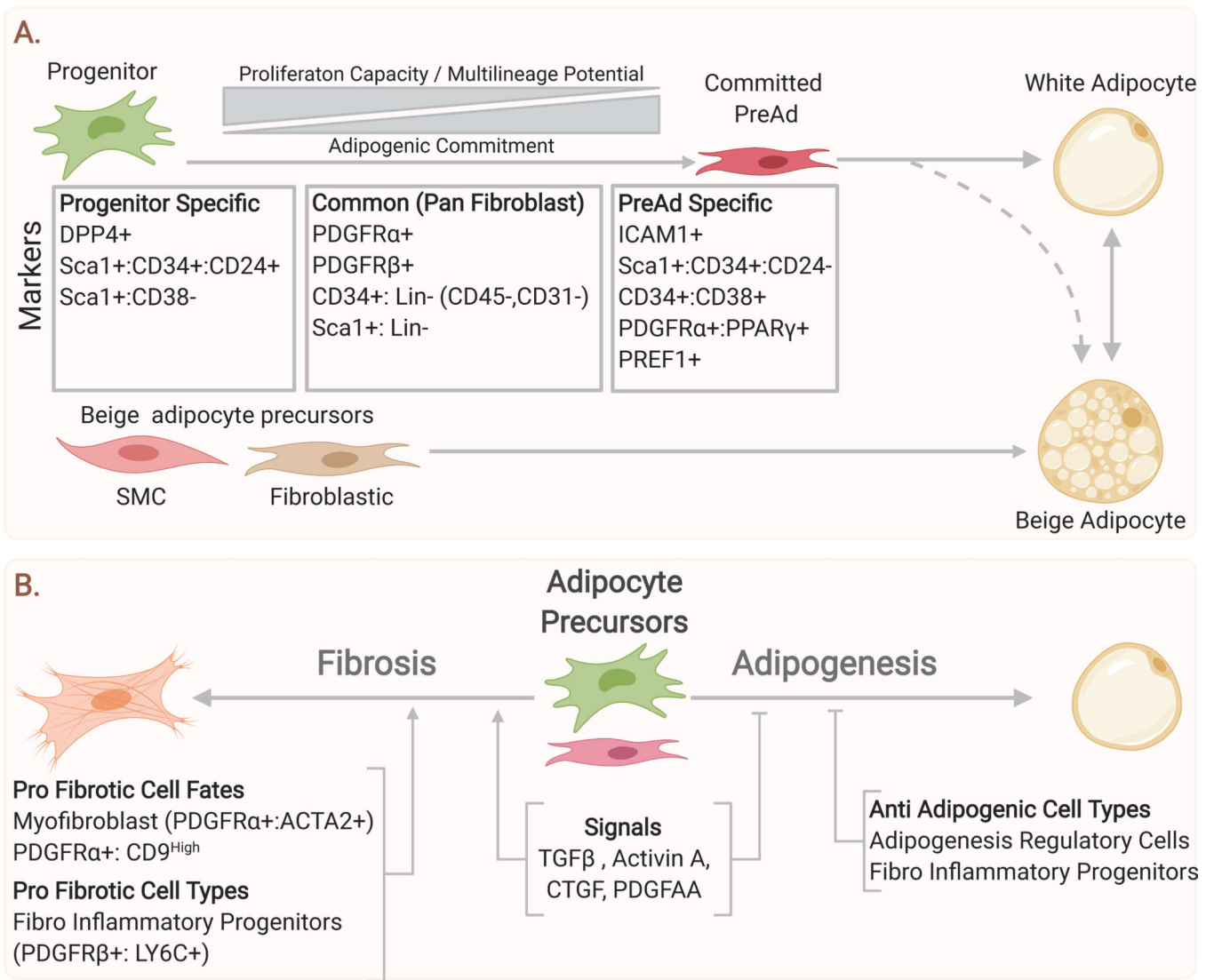


Figure 5. Adipocyte Progenitors and their Contribution to Adipose Tissue Homeostasis.

(A) Adipocyte progenitors are specialized according to their degree of commitment to the adipocyte lineage. A consensus has emerged that the major contributors to the adipocyte lineage are adventitial fibroblasts sharing a common set of fibroblastic markers including *Pdgfra* and *Cd34*. These fibroblasts can produce both white and beige adipocytes (which can interconvert in response to environmental temperature). (B) Adipocyte progenitor cells make critical cell fate decisions including whether to differentiate or adopt a more pro-fibrogenic state. Several lines of evidence suggest that, at a tissue level, there is competition between fibrosis and adipogenesis, with key mediators acting on adipocyte progenitors to alter their cell fate decisions.

The Hallmarks of Adipose Tissue Dysfunction

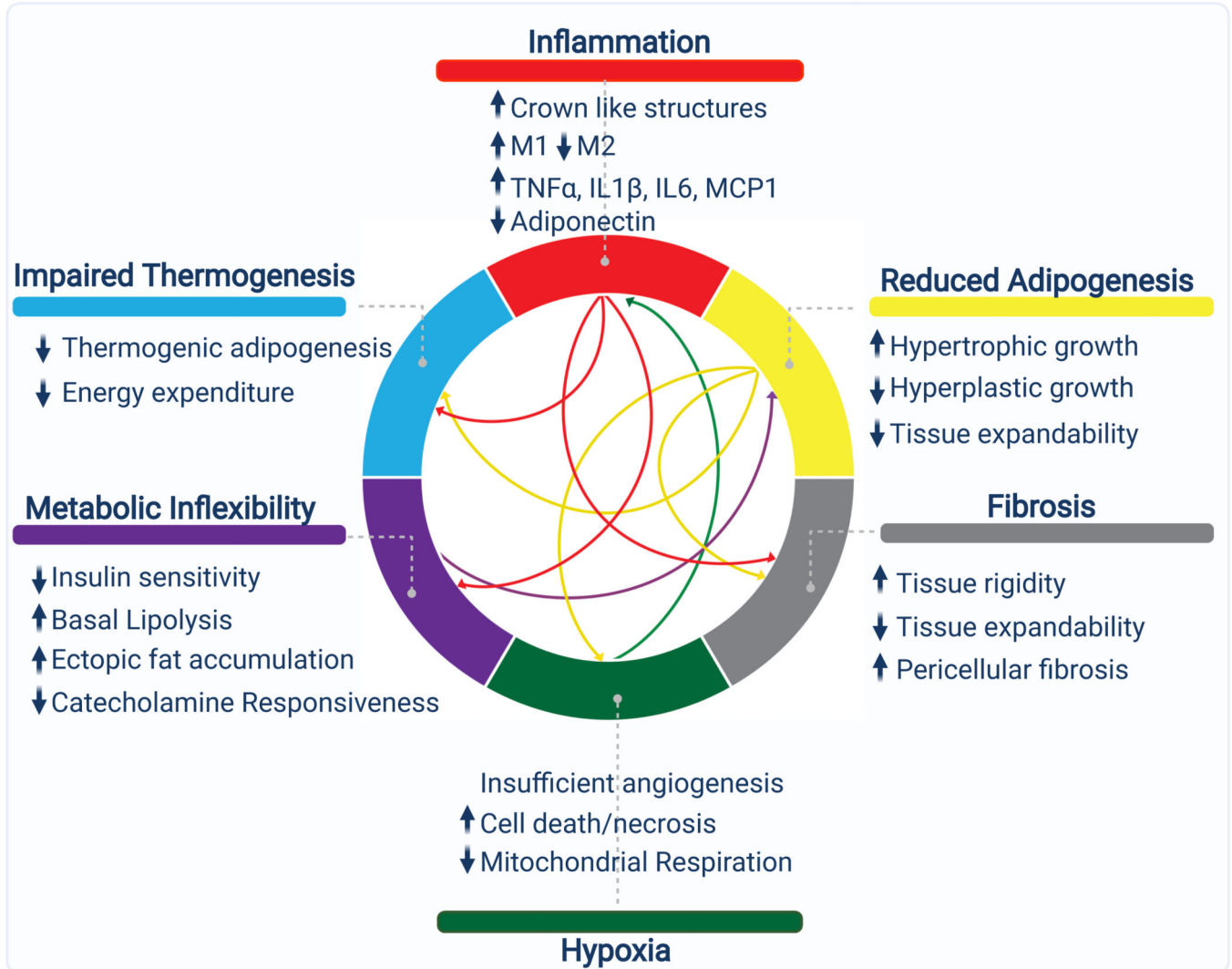


Figure 6. The Hallmarks of Adipose Tissue Dysfunction.

Several interconnected and mutually reinforcing processes contribute to adipose tissue dysfunction during the pathogenesis of metabolic disease. Excessive expansion of adipose tissue and insufficient angiogenesis drives hypoxia, which triggers an inflammatory response and promotes fibrosis. The inflammatory milieu drives the secretion of cytokines that maintain adipose inflammation, promote fibrosis, and impair metabolic flexibility by interfering with both nutrient uptake and nutrient release. Diminished progenitor cell differentiation capacity, due to progenitor autonomous defects, inflammation, or fibrotic ECM, limits expansion via hyperplasia, favoring adipocyte hypertrophy. Thermogenesis, which is highly dependent on the metabolic flexibility of adipose tissue is blunted, diminishing energy expenditure. These processes are continuous and synergistic, leading to a vicious cycle that culminates in adipose tissue dysfunction.