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Review

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.

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 human 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 overnutrition 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 "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 correlates with a higher incidence of metabolic disease (Global BMI Mortality Collaboration et al., 2016; Padwal et al., 2016). However, there is substantial interindividual 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 prediabetic 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 timescales, 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-α, IL-6, IL-1β, IL-8, IL-18, and 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, thermogenic adipose tissue 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 regions, 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 adult humans have distinct deposits of 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 thermogenic 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 by clearing triglyceride-rich lipoproteins, acylcarnitines, glucose and other potentially toxic metabolites such as branched-chain amino acids (BCAAs), which 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

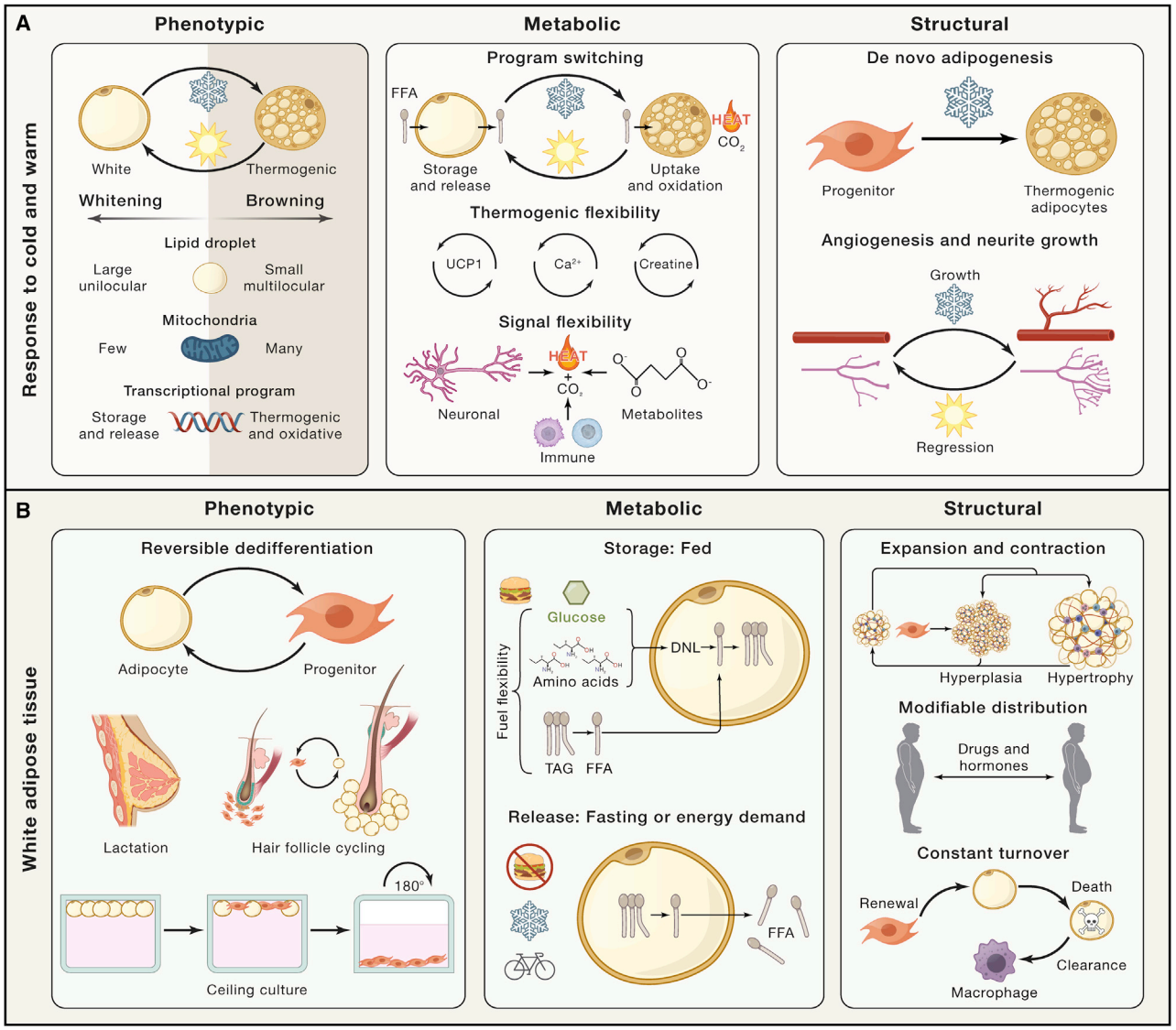

Figure 1. Adipose-tissue plasticity

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 and warm environments. Phenotypic: in response to cold, individual adipocytes remodel their internal architecture to facilitate thermogenesis in a process called beiging or browning. Beiging involves alterations to the structure of lipid droplets, robust mitochondrial biogenesis, and upregulation of a transcriptional program that supports high levels of local fuel oxidation. These changes are reversible and regress with the removal of cold 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 Uncoupling Protein 1 (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 the 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 (e.g., exercise, cold exposure), 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 (or a more metabolically maladaptive central distribution 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.

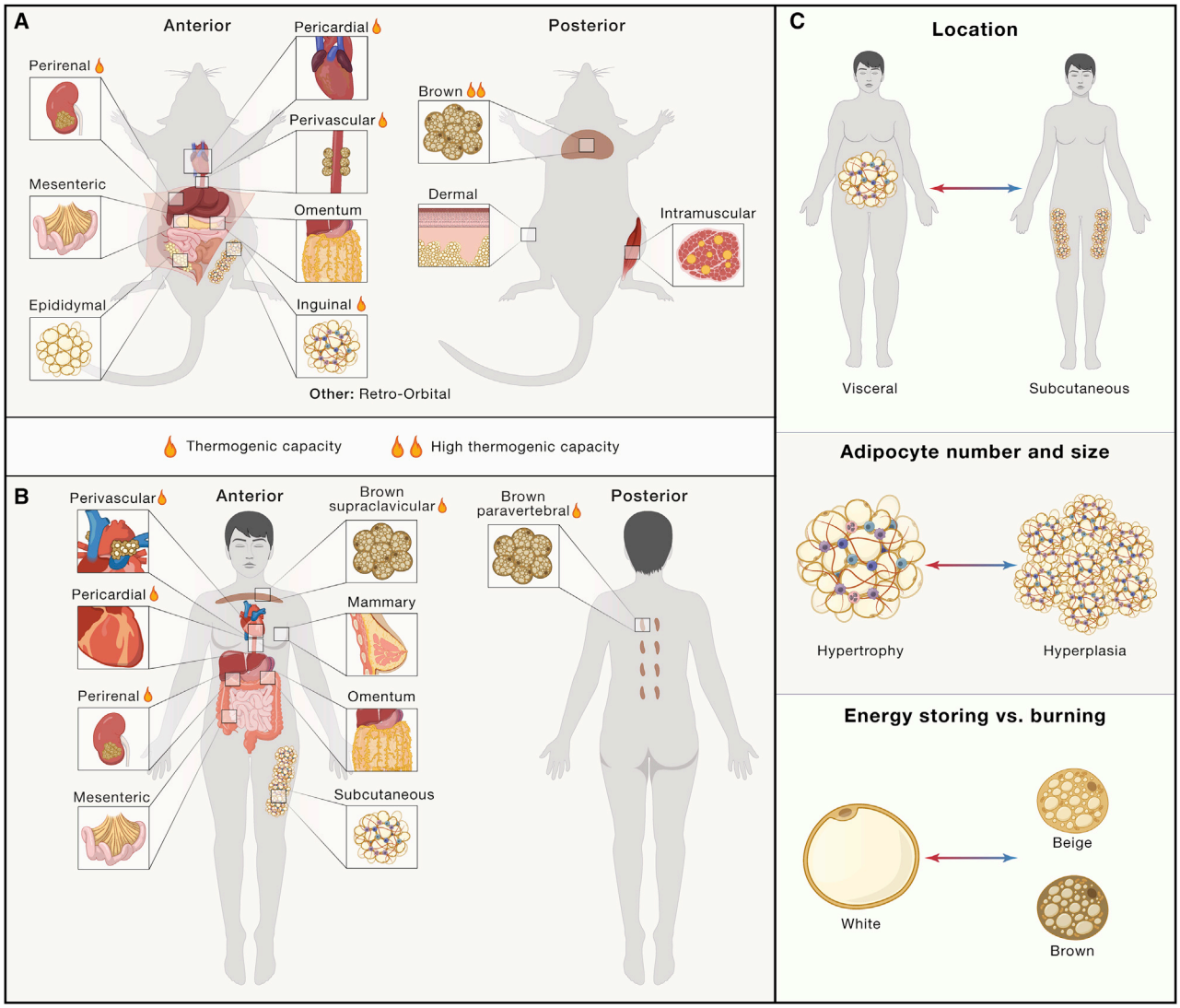

Figure 2. Location of major adipose-tissue depots in mice and humans

(A and B) Both mice (A) and humans (B) have thermogenic brown adipose tissue (interscapular, cervical, paravertebral). Mouse epididymal (gonadal) white adipose tissue (eWAT) is comparable to human visceral white adipose tissue (e.g., omental, mesenteric), while murine inguinal white adipose tissue (iWAT) is comparable to human subcutaneous adipose tissue. Fat depots differ in their propensity for thermogenesis.

(C) Three axes of adipose tissue variance relevant to metabolic health: location (visceral versus subcutaneous); expansion mechanism (hypertrophy versus hyperplasia); and metabolic phenotype (energy-storing versus energy-burning). Left: metabolically unfavorable. Right: metabolically favorable.

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 (DNL). 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 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 free fatty acids (FFA) from circulating chylomicrons and very-low-density lipoproteins (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

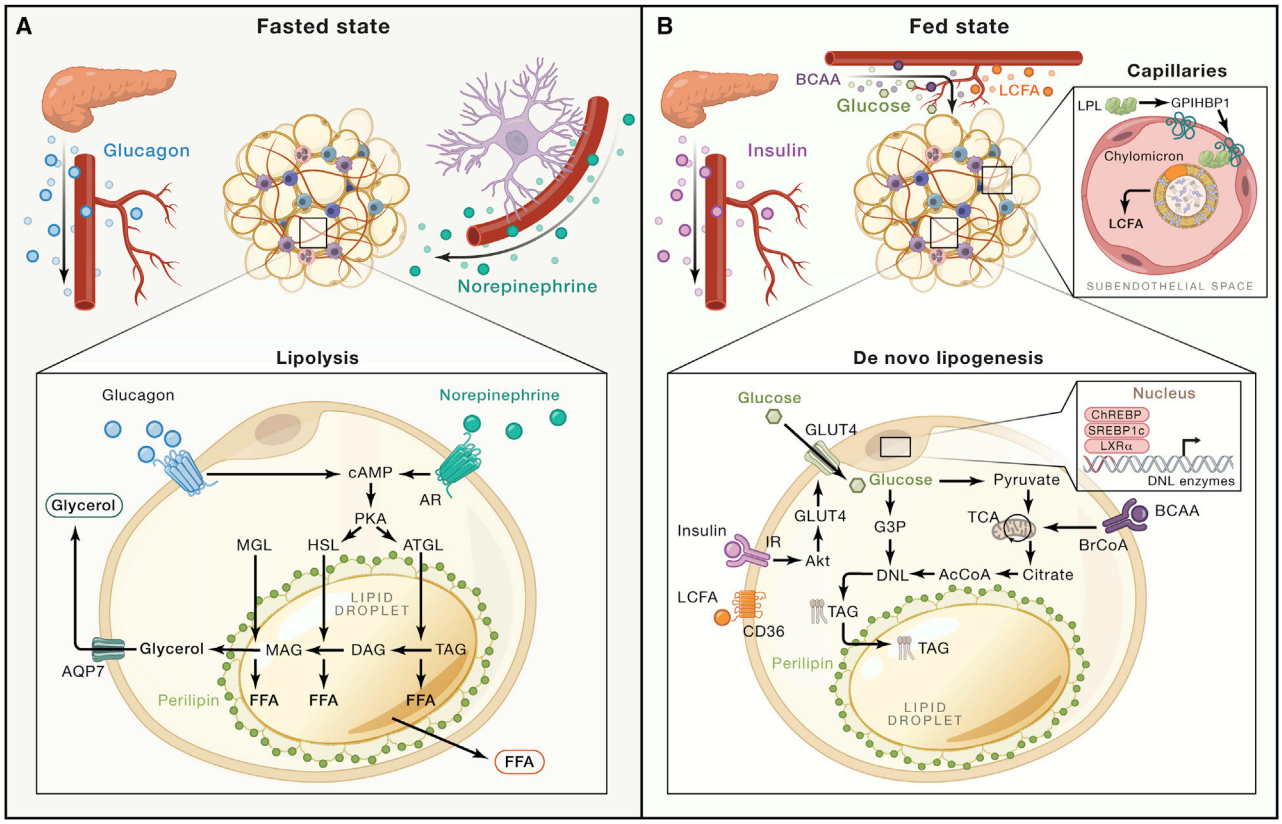

Figure 3. Metabolic plasticity of white adipose tissue

(A) Fasted state: adipocytes release FFAs and glycerol via lipolysis in response to external stimulation (i.e., norepinephrine, glucagon). Binding of norepinephrine to adrenergic receptors (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, HSL, and MGL. 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 (e.g., insulin) and by transcription factors such as carbohydrate response element-binding protein (ChREBP), liver X receptor alpha (LXRα), and sterol response element-binding protein 1c (SREBP1c). TAGs stored in lipid droplets are released by lipolysis during periods of energy demand.AQP7: Aquaporin 7. ATGL: Adipose Triglyceride Lipase. BrCoA: BCAA catabolism products (e.g. Acetyl-CoA, Propionyl-CoA, Succinyl-CoA). GPIHBP1: Glycosylphosphatidylinositol Anchored High Density Lipoprotein Binding Protein 1. HSL: Hormone Sensitive Lipase. LPL: Lipoprotein Lipase. TCA: Tricarboxylic Acid Cycle. G3P: Glyceraldehyde 3 Phosphate.

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 fatty acid (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, FAs 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 the action of diacylglycerol acyltransferase enzymes (DGAT1 and DGAT2) (Carpentier, 2021).

Adipocytes also synthesize acyl chains through DNL. Adipose tissue and the 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 the export of citrate to the cytoplasm, which is converted through a series of steps into acetyl-CoA, malonyl-CoA, and finally into FAs. 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), liver X receptor alpha (LXRα), and sterol response element-binding protein 1c (SREBP1c) stimulate the expression of the 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 DNL to growth-factor responses (Tang et al., 2016) (Figure 3).

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, 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 FFAs (Figure 3). Sympathetic nerve-derived catecholamines stimulate lipolysis, and this process is repressed by insulin (Frühbeck et al., 2014). In particular, epinephrine and norepinephrine release are induced by fasting or exercise and signal through the adrenergic receptor-protein kinase A (PKA) pathway in adipocytes to increase lipolysis. Lipolysis depends on the inhibitory phosphorylation of the lipid-droplet surface protein perilipin 1 (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 CG1-58 and subsequent activation of adipose triglyceride lipase (ATGL). Activated ATGL then moves to the lipid-droplet surface to hydrolyze triglycerides. PKA also phosphorylates HSL, which binds to PLIN1 to favor the hydrolysis of diacylglycerol to monoacylglycerol. After hydrolysis of monoacylglycerol by monoacylglycerol lipase (MGL), 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, parathyroid hormone , and glucagon also provide regulatory roles in lipolysis (Frühbeck 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 IN 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, in a process termed browning or beiging. 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 remarkable flexibility of these tissues.

Beige adipocytes can be generated within WAT depots via three mechanisms: (1) the differentiation of progenitor cells into new beige adipocytes (i.e., *de novo* beige adipogenesis), (2) phenotypic conversion of mature white adipocytes into beige adipocytes through the activation (or reactivation) of the thermogenic program, and (3) the proliferation of mature beige adipocytes (Park et al., 2021; Shao et al., 2019; Wang et al., 2013). Activation of the thermogenic 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 (nonstimulated) 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 and thermogenesis and stimulates transcription of genes driving the thermogenic program in brown and beige adipocytes. UCP1 function and thus thermogenic respiration is acutely activated by long chain fatty acids (LCFA) and inhibited by purine nucleotides (Bertholet and Kirichok, 2017; Fedorenko et al., 2012).

A key hub of the thermogenic transcriptional response is the co-activator protein PPARγ co-activator-1α (PGC1-α), which is upregulated by cold exposure (Puigserver et al., 1998) and is a master regulator of mitochondrial biogenesis. 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 function 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 (Labbé 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, which is 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 type 2 innate lymphoid cells (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 mechanisms involved remain uncertain (Fischer et al., 2017; Henriques et al., 2020; Qiu et al., 2014). ILC2s , activated by IL-33, promote beiging through two proposed pathways: (1) the production of methionine-enkephalin peptides, which 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 cross-talk between mesenchymal cells and immune cells in regulating adipose-tissue phenotypes (Mahlakõiv 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 γδ T cells in regulating innervation, especially in BAT. Specifically, IL-17 secreted from γδ T cells acts on brown adipocytes, leading to transforming growth factor β (TGFβ) production and increased sympathetic innervation. Deletion of the γδ T cells or IL-17 receptor 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 rewarming (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 approximately 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 the 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 serine/threonine 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 with white adipocytes, "previously beige" adipocytes display increased levels of H3K4me1, a chromatin mark associated with active or primed enhancers, on certain thermogenic program 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 reactivation of previously beige adipocytes during beiging 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 naive animals *de novo* beige-adipocyte differentiation from progenitor cells is favored (Shao et al., 2019). Interestingly, a subset of 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 fatty acids via oxidative metabolism (Gonzalez-

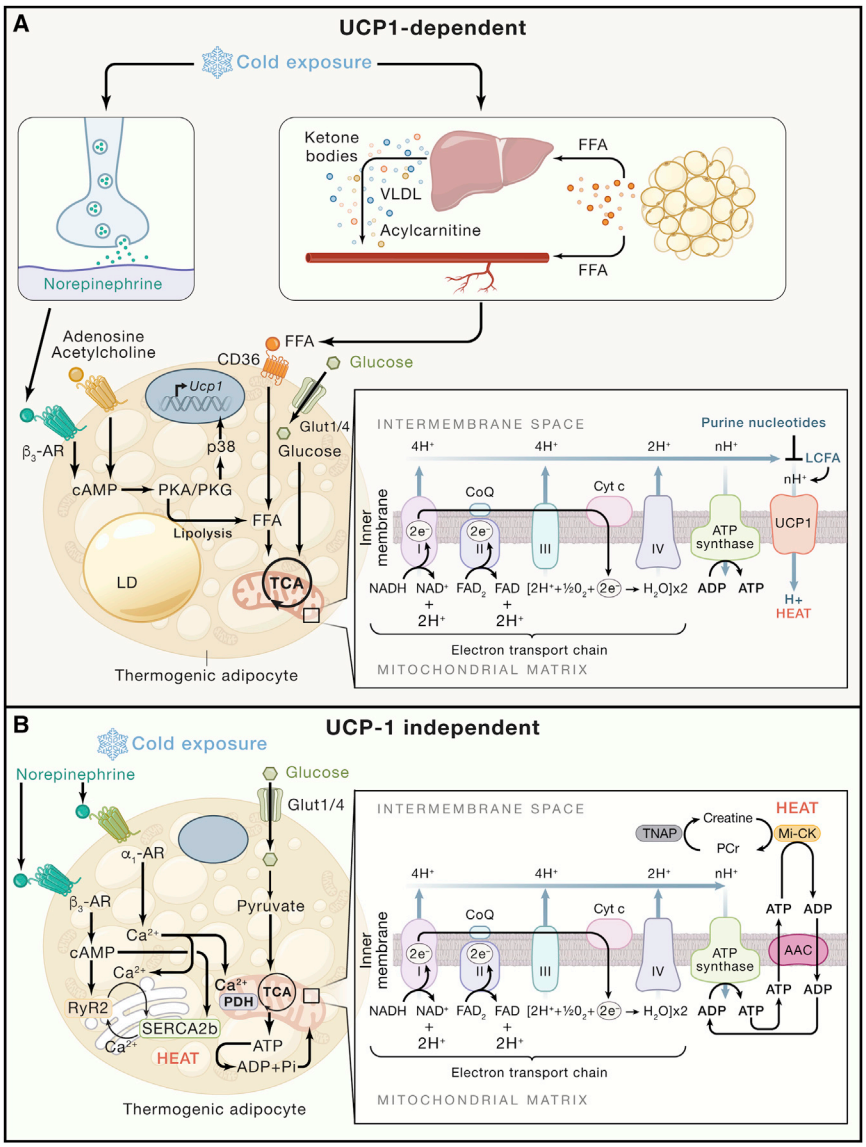

Figure 4. Metabolic plasticity of thermogenesis

(A) UCP1 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 FFAs). FFAs secreted by WAT also drive 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 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. AR: Adrenergic Receptor. AAC: ATP/ADP carrier. LD: lipid droplet. Mi-CK: Mitochondrial Creatine Kinase. PCr: Phosphocreatine. PDH: Pyruvate Dehydrogenase. RyR2: Ryanodine Receptor 2. SERCA2b: Sarco/endoplasmic Reticulum Ca^{2+} ATPase 2b. TNAP: Tissue-nonspecific Alkaline Phosphatase. UCP1: Uncoupling Protein 1.

Hurtado et al., 2018). Classically, adipose-tissue thermogenesis is driven by sympathetic nerve-mediated adrenergic signaling, which stimulates lipolysis (Figure 4). 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 the core lipogenesis enzymes DGAT1 and DGAT2 in UCP1+ adipocytes produces lipid-droplet-less adipocytes, which nevertheless remain competent for thermogenesis (Chitraju et al., 2020).

Brown fat in mice and humans also oxidizes 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 was 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. In general, BAT is more densely innervated than WAT depots; however, a recent study showed that >90% of adipocytes in inguinal WAT are closely apposed to sympathetic

fibers, which likely relates to the high beiging potential of this depot specifically (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 the 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 key thermogenic 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), TGFβ, 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 regresses 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 dedifferentiate and redifferentiate, cycling between a progenitor-cell and an adipocyte state.

The capacity for adipocytes to dedifferentiate *ex vivo* was noted in the 1980s 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 dedifferentiate into a fibroblastic pre-adipocyte state (Côté et al., 2019). However, whether dedifferentiation of adipocytes occurs *in vivo* had been unclear until recently. Bi et al. demonstrated that activation of Notch signaling induces the dedifferentiation 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 dedifferentiate 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 dedifferentiation during hair-follicle cycling and wound healing (Shook et al., 2020; Zhang et al., 2019). In the dermis, mature adipocytes dedifferentiate and give rise to myofibroblasts, specialized contractile fibroblasts which secrete extracellular matrix (ECM) for wound repair. Adipocytes undergoing dedifferentiation 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 dedifferentiation 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 with 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 adipose tissue (SAT) into a superficial and a deep compartment (sSAT and dSAT, respectively). 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, 500–1,000-μm lobules of adipocyte-rich stroma are encapsulated by fibrous septa, representing the smallest structural unit of SAT (Estève 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 with abdominal sSAT, the dSAT is more prone to inflammation and contains more saturated lipids; furthermore, adipocyte progenitor cells from this layer appear 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 has been 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 that separates the subcutaneous

structures from the overlying dermis, and which some have speculated to be an evolutionarily analogous structure to the fascia superficialis (Fodor, 1993). Both depots are encased on all sides by a thin fibrous membrane containing mostly dipeptidyl peptidase-4 (DPP4) expressing 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 nonlobular 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 with 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 and 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 (Estève 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 thermogenic fat for heat generation promotes 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) versus "gynoid" (subcutaneous/peripheral) obesity by the French physician Jean Vague in the 1950s, 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 SAT, 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-adrenergic receptor (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 the total amount) of the visceral fat is lost compared with 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 represent only 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 SAT, 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-1β, and retinol-binding protein-4 (RBP4). For example, IL-6 levels are 50% higher and leptin levels are 20%

lower in the portal compared with 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 impaired insulin sensitivity whereas systemic-draining visceral-fat transplants improved 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 intraperitoneal 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 off 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 well-known 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 *trans* women and an android distribution in *trans* men, 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, thiazolidinediones (TZDs), which promote insulin sensitivity, induce the preferential expansion of SAT (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 interindividual 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 (Björntorp, 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 0–2 and 12–18 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 ages 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; Björntorp 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 nonobese 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 finding, 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; Klöting 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 the 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 with 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 ECM, allowing for unrestricted expansion. Importantly, other genetic models which increase adipose ECM flexibility during the progression to obesity (e.g., matrix metalloproteinase 14 (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 peroxisome proliferator-activated receptor gamma (PPARγ), the master regulator of adipogenesis (Tontonoz et al., 1994). Activation of PPARγ leads to enhanced adipocyte differentiation (hyperplasia) and, in some depots, to enhanced expansion capacity (hypertrophy) (Tang et al., 2011). Although PPARγ is expressed in other cell types, notably macrophages, endothelium, muscle, and liver, the utility of TZDs as antidiabetic drugs is believed to come, in large part, from their ability to promote healthy adipose-tissue expansion (Yki-Järvinen, 2004).

Elegant mouse genetic studies further show that enhancing *de novo* adipocyte differentiation by overexpression of PPARγ 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 PPARγ 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 homolog (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). 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; Lotta 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 1960s (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 $^{2}H_{2}O$ 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, ^{15}N-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 that are more primed for differentiation (see below) (Merrick et al., 2019). Therefore, assessments of turnover which rely on assessing proliferation may understate 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, beiging, 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 versus 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 profibrogenic 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 nonadipocyte cells, which pellet after tissue digestion, and therefore the identity of the adipogenic cells was unclear. A ground-breaking 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 (i.e., CD45- and CD31-, hereafter abbreviated "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 showed that *Pdgfrb*-expressing cells develop into *Pparg*+ mural cells and white adipocytes. Further lineage-tracing experiments showed that *Pdgfrb*-expressing cells generate new adipocytes in the epididymal WAT upon HFD 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 perivascular niche and are identifiable as a population of PDGFRβ+ 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 PDGFRβ to identify APCs results in the inclusion of numerous, nonperivascular cell types. Indeed, PDGFRβ is also expressed by adventitial fibroblasts, which co-express PDGFRα and potentially represent a major source of APCs (Cattaneo et al., 2020; Hong et al., 2015; Vishvanath et al., 2016).

PDGFRα 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 PDGFRα is a common marker of APCs in WAT and BAT depots (Berry and Rodeheffer, 2013; Lee and Granneman, 2012; Lee et al., 2012). These PDGFRα+ 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).

A recent, elegant 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+* (±*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 Berry and Rodeheffer, who demonstrated that Lin−/CD29+/CD34+ cells could be subdivided into more

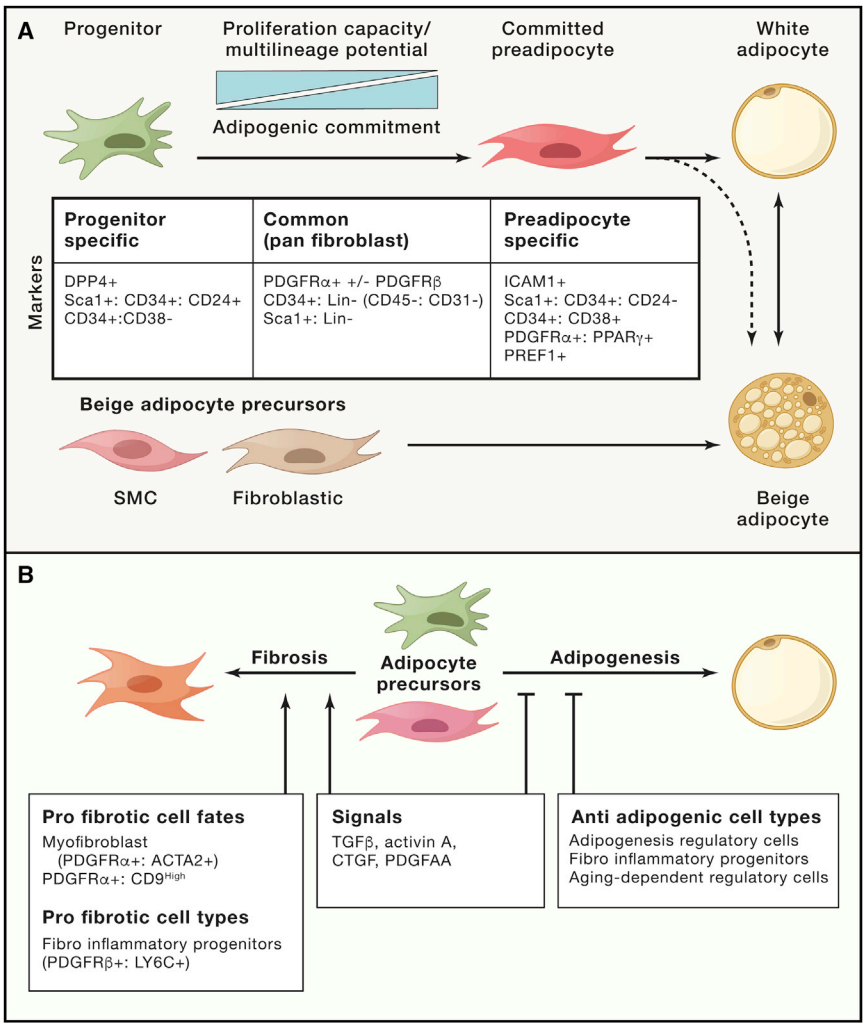

Figure 5. Adipocyte progenitors and their contribution to adipose-tissue homeostasis

(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*, and lacking hematopoietic or endothelial markers (i.e., CD45-:CD31-, abbreviated "Lin-"). These fibroblastic progenitors 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. ACTA2: Actin Alpha 2. CTGF: Connective Tissue Growth Factor. DPP4: Dipeptidyl Peptidase-4. ICAM1: Intercellular Adhesion Molecule 1. Ly6C: Lymphocyte Antigen 6C. PDGFAA: Platelet Derived Growth Factor AA. PDGFRα/β: Platelet Derived Growth Factor Receptor Alpha/Beta. PREF: Preadipocyte Factor 1. Sca-1: Stem Cell antigen-1.

and less committed cell populations based on CD24 expression. Compared with CD24+ cells, CD24− cells are less proliferative and show higher expression of adipocyte identity genes such as *Pparg*, *Lpl*, adiponectin (*AdipoQ*), and fatty acid binding protein 4 (*Fabp4*) (Berry and Rodeheffer, 2013). Moreover, transplantation studies indicate that CD24+ cells produce CD24− cells during adipogenesis (Jeffery et al., 2015, 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 [ASCs] 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; Sárvári et al., 2021; Schwalie et al., 2018; Shao et al., 2021a). Likewise, studies of human SAT 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; Sárvári et al., 2021).

Computational lineage predictions suggest that 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 the least to the 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 envelops adipose-tissue depots and subdivides it into lobes, produce preadipocytes and adipocytes *in vivo* (Merrick et al., 2019; Stefkovich et al., 2021) (Figure 5).

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} adipogenesis-regulatory cells (AREGs) 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 antiadipogenic effects are presumed to come from secretion of inflammatory mediators (ARCs and FIPs) or other secreted factors (AREGs). Of note, the antiadipogenic 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 *Pdgfra*+/*Wt1*+ cells 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 that develops into mouse 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 aging. 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 versus 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, PDGFRα+ 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 (Han et al., 2021; Oguri et al., 2020). Additionally, smooth-muscle-related cells expressing certain SMC marker genes (i.e., *Myh11*, *Acta2*, and *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/EBPβ and C/EBPδ 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 signal transducer and activator of transcription 5A (STAT5a). Among the second wave of factors, the master adipogenic factor PPARγ plays a dominant role in activating the expression of adipocyte-selective genes to confer the mature fat-cell phenotype (Siersbæk 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 versus the energy-burning thermogenic beige/brown phenotype. Notably, similar transcription factors and gene expression programs are active in both brown and beige adipose tissue, reflecting their similar thermogenic function despite their distinct developmental origins. The transcriptional co-activator PGC-1α along with interferon regulatory factor 4 (IRF4), estrogen related receptor (ERR) factors, c/EBPβ, 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 early B cell factor 2 (EBF2), nuclear factor I A (NFIA), zinc finger CCCH-type containing 10 (ZC3H10), and PR domain zinc finger protein 16 (PRDM16) (Shapira and Seale, 2019). Interestingly, sustained use of synthetic PPARγ agonists, like TDZs, 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, 2021b; Stine et al., 2016). Transducin-like enhancer protein 3 (TLE3) also represses the thermogenic program of adipocytes both by impeding the function of the prothermogenic 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 antiadipogenic growth-factor signals. Antiadipogenic signals include canonical and noncanonical WNT pathways (especially WNT5, WNT6, WNT10a, and WNT10b); TGFβ; platelet-derived growth factor (PDGF); and hedgehog signaling (Ghaben and Scherer, 2019). Proadipogenic signals include: insulin; bone-morphogenic-protein (BMP) signaling (especially BMP2 and BMP4); and ECM composition (Ghaben and Scherer, 2019). For example, BMP2 and BMP4 induce activation of SMAD4 and its heterodimeric partners, which subsequently stimulates the transcription of *Pparg* and drives adipogenic commitment (Huang et al., 2009). Of note, ZFP423, a regulator of adipogenic commitment and *Pparg* expression, sensitizes cells to the proadipogenic 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 PPARγ has yet to be identified. A notable recent study showed that omega-3 fatty acids stimulate preadipocytes to undergo differentiation via the free fatty acid receptor 4 (FFAR4) G-protein coupled receptor, located specifically in cilia (Hilgendorf et al., 2019).

A number of pathways have been proposed to inhibit adipocyte differentiation, although in many cases *in vivo* evidence is lacking. The presence of committed preadipocyte cells expressing detectable levels of PPARγ 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 a metabolically unhealthy phenotype, due to lipodystrophy, which results 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, to die via necrosis, and to 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 proadipogenic 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 Consortium, 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 senescence-associated secretory phenotype (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 antiadipogenic 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, which 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 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

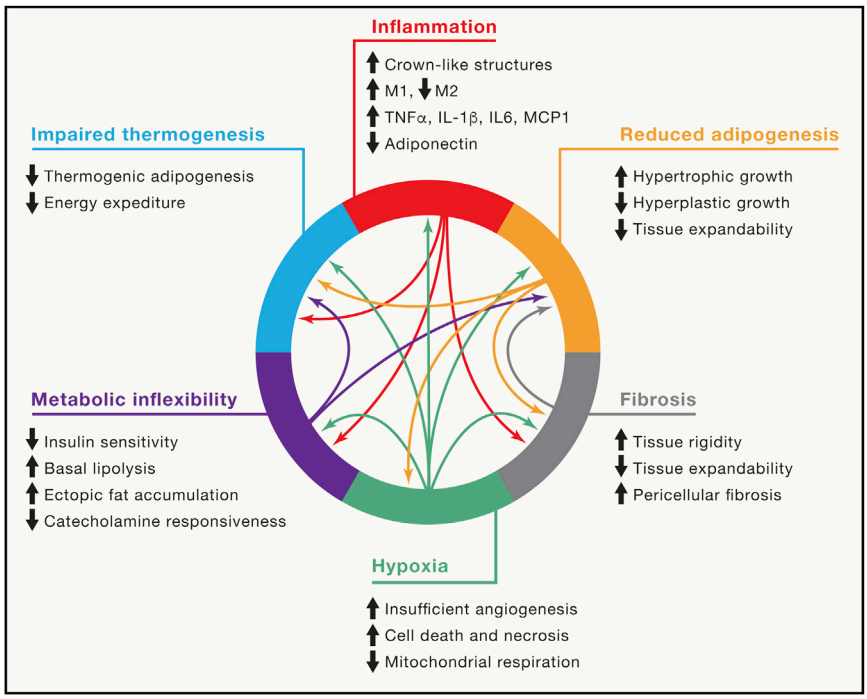

Figure 6. The hallmarks of adipose-tissue dysfunction

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 drive 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. IL: Interleukin. M1 and M2: Macrophage Polarization Type 1(M1) and Type 2 (M2). MCP-1:Monocyte Chemoattractant Protein-1. TNFα : Tumor Necrosis Factor Alpha.

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 (TGFβ) especially, as well as many other factors, including activin A, connective-tissue growth factor (CTGF), platelet-derived growth factors (PDGF), 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 HIF1α exerts potent profibrotic, 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 profibrotic 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 profibrogenic 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 profibrotic and adipogenic activities of APCs are opposed. As an example, expression of constitutively active PDGFRα in APCs results in lipodystrophy and profoundly fibrotic tissue, while deletion of PDGFRα has opposite effects (Marcelin et al., 2017; Sun et al., 2017, 2020a). Additionally, HIF1α inhibits APC differentiation through inhibitory phosphorylation of PPARγ, 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, $CD9^{High}$/PDGFRα+ cells, which increase in visceral fat during obesity, exhibit a profibrotic phenotype and are less adipogenic (Marcelin et al., 2017). Hepler et al. identified a related population of PDGFRβ+/LY6a+/CD9+ cells in visceral WAT, which they termed fibroinflammatory progenitors (FIPs) (Hepler et al., 2018). FIPs are transcriptionally similar to DPP4+ progenitor cells in subcutaneous WAT, suggesting that the division between profibrotic and proadipogenic 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 general transcription factor II-I repeat domain containing protein 1 (GTF2IRD1) recruits the transcription factors PRDM16 and euchromatic histone lysine methyltransferase 1 (EHMT1) to the promoters and enhancers of TGFβ responsive profibrosis genes, repressing their expression and suppressing fibrosis. Importantly, this mechanism occurs independently 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-versus-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, PDGFRα+ fibroblastic cells also give rise to both adipocytes and profibrogenic cells (Uezumi et al., 2011). Furthermore, in models of idiopathic pulmonary fibrosis (IPF), treatment with PPARγ 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., which show increased concentrations of the inflammatory cytokine TNFα in the adipose tissue of obese rats (Hotamisligil et al., 1993). Neutralization of TNFα signaling improveed 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 proinflammatory "type 1" state (Lumeng et al., 2007a, 2008; Nguyen et al., 2007). These "type 1" macrophages represent a major source of proinflammatory cytokines and can be found surrounding dead or dying adipocytes in adipose tissue, forming characteristic crown-like structures. Ablation of proinflammatory 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 in adipose tisue 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 proinflammatory 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 the inflammatory response and improves metabolic parameters.

Another important immune cell type in adipose tissue is innate lymphoid type 2 cells (ILC2s). 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 beige adipocytes (Hams et al., 2013; Molofsky et al., 2013). Like Treg cells, adipose-tissue ILC2s 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 lipopolysaccharide (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 proinflammatory 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 the 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 triggering receptor expressed on myeloid cells 2 (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 proinflammatory 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 not been successful thus far. 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 postprandial 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-AR expression, increased antilipolytic 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 TNFα and monocyte chemoattractant protein-1 (MCP-1) by adipocytes and by infiltrating macrophages activates c-Jun-N-terminal kinase (JNK) signaling, which phosphorylates the insulin receptor and reduces its activity (Hirosumi et al., 2002). Similarly, chronic inflammation diminishes catecholamine responsiveness. For example, TNFα-induced expression of the kinases IKKe and TBK1 activates the phosphodiesterase PDE3B, which directly reduces the levels of cAMP. This reduction in cAMP signaling reduces hormone-sensitive lipase (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 postprandial blood flow and vascular density (Trayhurn, 2013). In obesity, rather than stimulating angiogenesis, HIF1α, the master regulator of the hypoxia response, promotes the expression of proinflammatory and profibrotic genes (Halberg et al., 2009; Lee et al., 2014b). Interestingly, expression levels of an antiangiogenic form of VEGF ($VEGFA_{165}b$) 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 promoting 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 proinflammatory aging-dependent regulatory cells (ARC) in SAT, which impede adipocyte differentiation and thus presumably impair *de novo* beige adipogenesis with age (Nguyen et al., 2021). Targeting thermogenic adipose tissue 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; and (4) capacity for dedifferentiation 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 TZDs, 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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DECLARATION OF INTERESTS

The authors declare no competing interests.

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