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ABSTRACT **The worldwide epidemic of obesity and type 2 diabetes has greatly increased interest in the biology and physiology of adipose tissues. Adipose (fat) cells are specialized for the storage of energy in the form of triglycerides, but research in the last few decades has shown that fat cells also play a critical role in sensing and responding to changes in systemic energy balance. White fat cells secrete important hormone-like molecules such as leptin, adiponectin, and adipsin to influence processes such as food intake, insulin sensitivity, and insulin secretion. Brown fat, on the other hand, dissipates chemical energy in the form of heat, thereby defending against hypothermia, obesity, and diabetes. It is now appreciated that there are two distinct types of thermogenic fat cells, termed brown and beige adipocytes. In addition to these distinct properties of fat cells, adipocytes exist within adipose tissue, where they are in dynamic communication with immune cells and closely influenced by innervation and blood supply. This review is intended to serve as an introduction to adipose cell biology and to familiarize the reader with how these cell types play a role in metabolic disease and, perhaps, as targets for therapeutic development.**

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INTRODUCTION

The global epidemic in obesity and related disorders such as type 2 diabetes has fueled an explosion of interest in adipose (fat) cells. Adipose cells play several critical roles in systemic metabolism and physiology. There are at least two classes of fat cells—white and brown. White fat is specialized to store energy in the form of triglycerides, an especially efficient method because this class of molecules is highly energetic and stored anhydrously. On fasting, the release of fatty acids and glycerol to provide fuel for the rest of the body occurs via enzymatic hydrolysis called lipolysis. These crucial functions of fat, storage, and release of fatty acids are tightly controlled by the key hormones of the fed and fasted states—insulin and catecholamines. In addition to these classic functions, the importance of white fat tissue as a central signaling node in systemic metabolism was first identified by the cloning of adipsin and leptin, two important "adipokines" (Cook *et al.*, 1987; Zhang *et al.*, 1994). In fact, fat cells and fat tissues secrete many molecules with crucial roles in metabolism, including tumor necrosis factor α (TNF-α), adiponectin, resistin, and RBP4, among others (Rosen and Spiegelman, 2014). Healthy and robust adipose development is absolutely required for proper metabolic control. Of importance, defects in adipose differentiation do *not* lead to healthy, lean animals but instead to lipodystrophy, a serious disease by which other tissues, especially the liver, subsume the function of fat storage, with deleterious effects, including insulin resistance, diabetes, hepatomegaly, and hypertriglyceridemia (Garg, 2011).

TYPES OF FAT

In contrast to white fat, brown fat is specialized to dissipate chemical energy in the form of heat, defending mammals against hypothermia. It does so by running futile metabolic cycles, most notably the futile cycle of proton exclusion from and leak back into the mitochondrial matrix via the electron transport chain and uncoupling protein 1 (UCP1; reviewed in Cohen and Spiegelman, 2015). UCP1 expression is strictly limited to brown and beige fat cells. Although UCP1 was typically believed to be regulated transcriptionally, a recent study showed that UCP1 can also be regulated posttranslationally, by reactive oxygen species–driven sulfenylation of a key cysteine residue (Chouchani, Kazak, *et al.*, 2016). Recently a separate futile cycle involving creatine phosphorylation/dephosphorylation

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Abbreviations used: BMP7, bone morphogenetic protein 7; FGF21, fibroblast growth factor 21; HIF-1α, hypoxia-inducible factor 1α; IKKε, I-kappa-B kinase epsilon; ILC2s, type 2 innate lymphoid cells; NAFLD, nonalcoholic fatty liver disease; RBP4, retinol-binding protein 4; TBK1, TANK binding kinase 1; TNF-α, tumor necrosis factor α; Tregs, regulatory T-cells; UCP1, uncoupling protein 1.

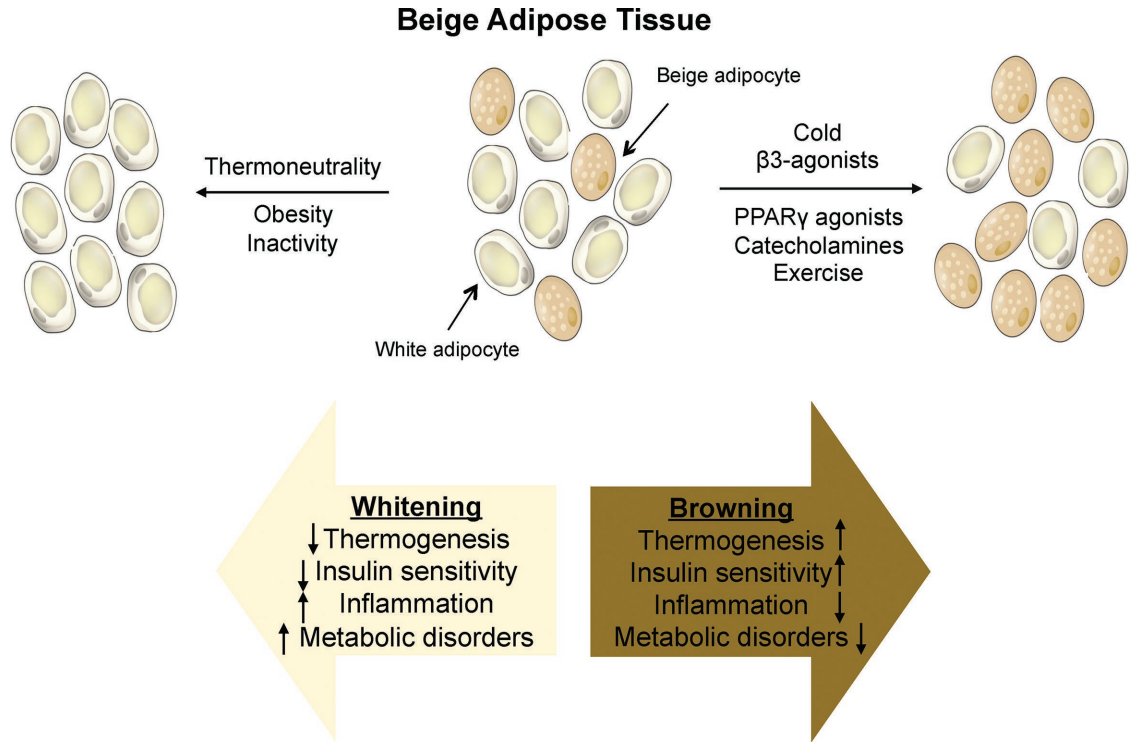

FIGURE 1: Depiction of beige adipose tissue, which consists of a mixture of white and beige adipocytes. A schematic of stimuli that lead to increased ("browning") or decreased ("whitening") beige fat activity, together with the physiological consequences.

was identified in mitochondria of beige fat cells, a type of brown-like adipocyte (Kazak *et al.*, 2015). Of importance, brown fat, in all of its dimensions, plays a role in defending animals against metabolic diseases such as obesity, type 2 diabetes, and hepatic steatosis (the earliest manifestation of nonalcoholic fatty liver disease [NAFLD]). The first evidence in this regard was the observation that mice with genetically ablated UCP1$^+$ cells are prone to obesity and diabetes (Lowell *et al.*, 1993), whereas those with genetically elevated brown fat function are markedly protected from the same disorders (Cederberg *et al.*, 2001).

Until recently, the term "brown fat" was used to refer to UCP1$^+$ cells in two distinct anatomical locations: 1) developmentally formed depots in the interscapular and perirenal regions, composed mainly of UCP1$^+$ adipocytes, which have many small lipid droplets (termed multilocular) and dense mitochondria, giving the tissue its characteristic brown color; and 2) UCP1$^+$ cells, which are interspersed in many white fat depots, particularly in the subcutaneous regions of rodents and humans. These two types of "brown fat" are not only distinct cell types (Wu *et al.*, 2012), but they are also from completely different cell lineages (Seale *et al.*, 2008). The developmentally formed brown fat cells, now termed "classical brown fat cells," are derived from a skeletal muscle–like lineage, as marked by Myf5 or Pax7 (Seale *et al.*, 2008; Lepper and Fan, 2010). The beige cells are derived, at least in part, from a vascular smooth muscle–like lineage, as marked by the Myh11 promoter (Long *et al.*, 2014; Berry *et al.*, 2016).

Most studies have not distinguished between the functional roles of these two types of UCP1$^+$ fat cells, as cold exposure or β-adrenergic stimulation activates both cell types. Recently a murine model has been developed that lacks beige fat cells but has fully functional brown fat (Cohen *et al.*, 2014). These mice develop mild obesity on a high-fat diet compared with controls. Moreover, this obesity occurs exclusively via an excess of subcutaneous fat, a rather unusual finding. These animals have severe hepatic insulin resistance and hepatic steatosis, suggesting that beige fat protects the liver; whether this occurs through oxidation of circulating lipids by beige cells or through production of a secreted hormone that protects the liver from fat accumulation is not known. An increasing number of factors have been identified that lead to increased ("browning") or decreased ("whitening") beige fat activity (Figure 1).

CELL BIOLOGY OF ADIPOSE TISSUE

Adipose tissue was once viewed as a passive repository for triglyceride accumulation within adipocytes but is now appreciated to be a complex tissue containing a host of interacting cell types, including fat cells, immune cells, endothelium, fibroblasts, neurons, and stem cells. Although adipocytes account for >90% of fat pad volume, these other cells types (collectively referred to as the stromal vascular fraction), predominate by overall number (Kanneganti and Dixit, 2012). Several immune cell subsets are now known to accumulate in adipose tissue and serve important functions. This can be traced back to the observation that adipose tissue produces TNF-α and other proinflammatory cytokines, with levels increased in the setting of obesity; these mediate local and systemic insulin resistance (Hotamisligil *et al.*, 1993). These cytokines are largely produced by macrophages within the adipose tissue (Weisberg *et al.*, 2003; Xu *et al.*, 2003). Histologically, macrophages can be seen surrounding adipocytes in what have been termed "crown-like structures" (Cinti *et al.*, 2005)

In recent years, the role of immune cell subsets in adipose tissue has become increasingly well understood. In addition to proinflammatory or M1 macrophages, fat also contains alternatively activated

or M2 macrophages, with the M1/M2 ratio increasing in obesity (Lumeng *et al.*, 2007). These cell types serve an important role in tissue remodeling. Moreover, M2 macrophages can promote beige fat activation. Cold exposure leads to a polarization toward the M2 phenotype, and these M2 cells can produce and secrete catecholamines that stimulate beige fat cells (Nguyen *et al.*, 2011). Eosinophils and type 2 innate lymphoid cells (ILC2s) within adipose tissue are also central to beige fat biogenesis. Eosinophils produce interleukin (IL)-4 and IL-13, which activate M2 macrophages, and eosinophils themselves can be activated by muscle-derived meteorin-like protein (Qiu *et al.*, 2014; Rao *et al.*, 2014). ILC2s stimulate beige fat via production of IL-33 and enkephalin (Brestoff *et al.*, 2015; Lee *et al.*, 2015). Regulatory T-cells (Tregs) are present in visceral adipose tissue but decrease in number with the development of obesity, promoting the development of insulin resistance (Feuerer *et al.*, 2009). Of interest, the properties of visceral fat Tregs depend on the expression of peroxisome proliferator-activated receptor γ (Cipolletta *et al.*, 2012). In addition to these immune cell types, roles have also been defined for other T-cell subsets, B-cells, neutrophils, mast cells, and natural killer T-cells (Brestoff and Artis, 2015).

Adipose tissue phenotypes also depend on blood supply and innervation, although the regulation of these processes has been comparatively less studied. As fat mass expands in the setting of overnutrition, local hypoxia can develop, and the oxygen-sensitive transcription factor hypoxia-inducible factor 1α (HIF1α) can become activated (Krishnan *et al.*, 2012). Genetic and pharmacologic studies show that adipose-specific deletion or inhibition of HIF-1α can protect against obesity-related metabolic dysfunction (Jiang *et al.*, 2011; Sun *et al.*, 2013). Data also indicate that white and brown adipose tissue can make vascular endothelial growth factor A and other factors to enhance its blood supply (Fredriksson *et al.*, 2000; Mick *et al.*, 2002). Adipose tissue, particularly brown fat, is also extensively innervated with sympathetic fibers that stimulate lipolysis in the setting of fasting, leptin administration, and cold exposure (Bartness *et al.*, 2010a,b; Zeng *et al.*, 2015). In contrast, parasympathetic fibers may stimulate lipid accumulation (Kreier *et al.*, 2002). Brown and beige adipocytes both express high levels of the β3-adrenergic receptor, and pharmacologic activation by CL 316,243 promotes thermogenesis (Himms-Hagen *et al.*, 1994). The factors that regulate the innervation of fat cells remain an area of active investigation.

UNANSWERED QUESTIONS AND PROSPECTS FOR HUMAN THERAPEUTICS

Successful targeting of adipose tissue for therapeutic benefit will depend on further clarification of several key unanswered questions. First, what is the full complement of transcriptional regulators that govern the development and maintenance of white, brown, and beige fat? Second, what is the complete spectrum of phenotypes of each type of adipocyte? For example, it is becoming increasingly clear that brown and beige fat do much more than generate heat and may be important endocrine organs (Kajimura *et al.*, 2015). Third, how do different types of fat cells signal to other cell types and tissues, and how do these signals affect systemic metabolism and susceptibility to diabetes, hypertension, cardiovascular disease, and cancer? Finally, can key molecular regulators of adipose tissue be modulated to engineer healthier adipose tissue? Achieving this goal will require a basic understanding of how important factors like PRDM16 are physiologically regulated (e.g., transcriptionally, translationally, posttranslationally).

Ultimately, any discussion of fat tissues as a target for human therapeutics has to go back to the notion of adipose tissues as the healthiest site for deposition of excess caloric energy (Unger *et al.*, 2013). We know from human genetics that any inhibition of fat development will cause ectopic lipid deposition and serious disease (Savage *et al.*, 2003). With that in mind, what are potential targets relating to fat tissues? First, with respect to white fat, we might target abnormalities that link the adipose tissues to the consequences of obesity, including diabetes, cardiovascular disorders, and fatty liver disease. As mentioned earlier, adipose tissues in obesity demonstrates aspects of inflammation, including secretion of inflammatory cytokines; neutralization of cytokines such as TNFα improves insulin resistance in rodents (Hotamisligil *et al.*, 1994). Similarly, antagonism of the inflammatory protein kinases I-kappa-B kinase epsilon (IKKε) and TANK binding kinase 1 (TBK1) has been shown to improve diabetes in mice (Reilly *et al.*, 2013). The challenge going forward will be to obtain therapeutic benefit in diabetes or cardiovascular diseases without causing the toxicity associated with generalized suppression of inflammation.

For brown and beige fat, the challenge will be to increase their amounts and activities in humans in a safe and effective way. That increased adaptive thermogenesis through brown and beige fat in rodents protects from obesity and diabetes is completely settled science (Cederberg *et al.*, 2001; Seale *et al.*, 2011). It is also clear that adult humans have substantial stores of beige fat and perhaps some classical brown fat as well (Sharp *et al.*, 2012; Wu *et al.*, 2012; Cypess *et al.*, 2013; Jespersen *et al.*, 2013; Lidell *et al.*, 2013). Cold exposure or administration of a β-3-adrenergic compound have been shown to increase activity of these thermogenic fat depots, as ascertained by fluorodeoxyglucose positron-emission tomographic imaging (Cypess *et al.*, 2009, 2015; van Marken Lichtenbelt *et al.*, 2009; Virtanen *et al.*, 2009). Of course, whether human thermogenic fat can be activated and/or increased in amount to play a strong therapeutic role in diabetes and obesity remains to be seen. Several polypeptides, such as fibroblast growth factor 21 (FGF21) and bone morphogenetic protein 7 (BMP7), can do this in rodents (Tseng *et al.*, 2008; Fisher, Kleiner, *et al.*, 2012), but whether the same will be seen in humans with a favorable toxicity profile remains to be seen. Additional secreted proteins with thermogenic actions on adipose tissues continue to be discovered (atrial and ventricular natriuretic peptides and Slit2; Bordicchia *et al.*, 2012; Svensson *et al.*, 2016). It is also worth noting that rodent data cited earlier suggest a hepatoprotective role for beige fat, and so diseases such as NAFLD may well be the first therapeutic targets for agents that increase beige fat function. The extent to which the diverse metabolic benefits of brown and beige fat are due to enhanced thermogenesis per se or to an endocrine role of these tissues remains an important point to be clarified.

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Owing to space constraints, we regret that we could not reference all of the important contributions that have been made to this field.

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