Review

Developmental and functional heterogeneity of thermogenic adipose tissue

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The obesity epidemic continues to rise as a global health challenge. Thermogenic brown and beige adipocytes dissipate chemical energy as heat, providing an opportunity for developing new therapeutics for obesity and related metabolic diseases. Anatomically, brown adipose tissue is distributed as discrete depots, while beige adipocytes exist within certain depots of white adipose tissue. Developmentally, brown and beige adipocytes arise from multiple embryonic progenitor populations that are distinct and overlapping. Functionally, they respond to a plethora of stimuli to engage uncoupling protein 1-dependent and independent thermogenic programs, thus improving systemic glucose homeostasis, lipid metabolism, and the clearance of branched-chain amino acids. In this review, we highlight recent advances in our understanding of the molecular and cellular mechanisms that contribute to the developmental and functional heterogeneity of thermogenic adipose tissue.

Keywords: brown adipose tissue, beige adipocyte, BAT involution, lineage tracing, browning, UCP1

Introduction

Brown adipose tissue (BAT) was first described by Conrad Gesner in hibernators as 'neither fat, nor flesh [nec pinguitudo, nec caro]' (Gessner, 1551). Hibernating mammals use BAT, also once known as the 'hibernating gland', to rewarm from hypothermic torpor (Ballinger and Andrews, 2018). Later, a little over 100 years ago, BAT was also recognized in non-hibernating mammals including humans (Hatai, 1902; Bonnot, 1908). In early 1960s, the thermogenic role of BAT during cold acclimation was experimentally established (Smith and Roberts, 1964). It was not until late 1970s that uncoupling protein 1 (UCP1) was identified as a functional marker of BAT (Heaton et al., 1978; Aquila et al., 1985) and BAT was suggested to be involved in metabolic efficiency and resistance to obesity (Rothwell and Stock, 1979). In addition, beige, brite (brown-in-white), inducible, or recruitable brown adipocyte-like cells that are multilocular and UCP1-positive were discovered in predominantly white adipose tissue (WAT) depots (Young et al., 1984; Loncar, 1991; Guerra et al., 1998; Vitali et al., 2012). Thanks to recent advances in gene targeting, lineage tracing, and single-cell sequencing, there has been an exponential growth of our knowledge in the development and function of thermogenic brown and beige adipocytes (Wang et al., 2013; Wu et al., 2013; Inagaki et al., 2016; Wang and Seale, 2016; Shao et al., 2019). In this review, I will discuss the current understanding of molecular and cellular heterogeneity of thermogenic adipose tissue between species, through life, and during metabolic stress.

The development and age-related involution of BAT

Human BAT develops during gestation and the main depots can be found in interscapular, neck, axillary, and perirenal areas, while smaller deposits are behind the sternum and along the spine (Lidell, 2019). Cellular heterogeneity already exists in fetal BAT, as interscapular, perirenal, and supraclavicular depots are predominantly multilocular adipocytes, while other depots contain mixed multilocular and unilocular fat cells (Merkl, 1974). However, molecular characterization of these BAT depots that contain unilocular white-like adipocytes is still lacking. It would be informative to determine whether they represent beige fat that has been extensively studied in mice.

BAT is maximally recruited at birth as infants do not possess sufficient muscle for shivering thermogenesis. However, as we grow, the prevalence and activity of BAT steadily decline throughout the body. The interscapular BAT (IBAT) can be found in all infants, but it rapidly atrophies in adolescents and young adults (Heaton, 1972; Sidossis and Kajimura, 2015; Figure 1A).
Perirenal BAT in newborns contains exclusively brown adipocytes, but they are gradually replaced by unilocular white adipocytes (Tanuma et al., 1975; Figure 1B).

In adult humans, metabolically active, anatomically dispersed BAT exists in cervical, supraclavicular, and axillary areas, shown by $^{18}$F-fluorodeoxyglucose-postion emission tomography/computed tomography (PET/CT) imaging and subsequent histological and molecular characterization (Cypess et al., 2009; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009; Zingaretti et al., 2009). These BAT depots around the neck appear to develop postnatally and their prevalence peaks around the age of 20 years and then reduces to <5% in middle-aged men and women (Figure 1C; Truong et al., 2004; Gelfand et al., 2005; Lee et al., 2010; Pfannenberg et al., 2010; Drubach et al., 2011; Gilsanz et al., 2011, 2012; Jacene et al., 2011; Ouellet et al., 2011; Zhang et al., 2014). Cold acclimation increases the activity of BAT and recruits brown fat even in subjects with previously undetectable metabolically active BAT (Saito et al., 2009; van Marken Lichtenbelt and Schrauwen, 2011; Yoneshiro et al., 2011; Figure 1C). However, the recruitment largely happens in young people and there is little evidence that brown fat can be recruited by cold in aged subjects with no detectable BAT (Rogers, 2015). It is worth noting that most of these studies relied only on $^{18}$F-fluorodeoxyglucose-PET/CT assessment of glucose uptake in BAT, thus possibly underestimating the prevalence and activity of BAT (Lee et al., 2013).

The mechanisms underlying age-related BAT involution remain enigmatic, but can be multi-fold: (i) neuronal and hormonal regulation as a result of reduced need for BAT to produce heat (e.g. thermal insulation, muscle shivering, lower ratio of body surface area to body volume, etc.); (ii) brown adipocyte 'whitening' or transdifferentiation to white adipocytes; and (iii) the defect in browning ability of adipose progenitors and/or the loss of their population. Although mice display human-like brown/beige fat depots in anatomically comparable regions (Cypess et al., 2013; Shinoda et al., 2015; Zhang et al., 2018), they have constitutively active interscapular BAT throughout life. In mice, aging is associated with thermogenic dysfunction, but brown adipocytes largely preserve their 'browning' ability to external cues (Sellayah and Sikder, 2014; Goncalves et al., 2017; Tajima et al., 2019). At the thermoneutral zone, brown adipocytes in mice also remain their cellular identity, despite decreased thermogenic program (Cui et al., 2016; Roh et al., 2018). Therefore, complementary animal models that possess characteristics of human BAT involution are immediately needed.

### Heterogeneity in the browning capacity of WAT depots

Cold exposure recruits BAT and induces WAT "browning". When housing mice at thermoneutral conditions, UCP1 is only well expressed in bona-fide BAT including the interscapular, cervical, axillary, and mediastinal depots. Cold-induced Ucp1 mRNA expression increases 2–3 folds in BAT depots whereas 10–200 folds in cardiac, inguinal, and retroperitoneal WAT depots that contain significant numbers of 'dormant' beige adipocytes and progenitors (Walden et al., 2012). Among beige fat, the inguinal subcutaneous WAT appears to have the highest Ucp1, while retroperitoneal WAT shows the most induction of Ucp1 by cold (Walden et al., 2012). Visceral WAT including the mesenteric and epididymal depots lacks beige adipocytes, thus expressing very little Ucp1 under thermoneutral or cold conditions (Walden et al., 2012).

On the other hand, fasting could preferentially downregulate the thermogenic program in retroperitoneal WAT, but to a much lesser extent in other WAT depots and not in BAT (Ruan et al., 2014). Although the mechanisms of differential responses to thermogenic regulators among WAT depots are largely unclear, the diversity in neuronal innervation and sympathetic activity may be one contributing factor (Ruan et al., 2014; Yang and Ruan, 2015). Retroperitoneal WAT

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**Figure 1** Progressive BAT involution with age in humans. (A) Re-plotting of the prevalence of interscapular BAT in humans, described in Heaton (1972). (B) Occupancy of brown adipocytes (BA) in human perirenal BAT with age. Data from Tanuma et al. (1975). BA occupancy: 0, absent of brown adipocytes; 0.5, only a few multilocular fat cells; 1, small accumulation of brown adipocytes; 2, medium accumulation of brown adipocytes; 3, large accumulation of brown adipocytes; 4, almost fully occupied by brown adipocytes. (C) Summary of the prevalence of supraclavicular BAT in humans at room temperature (RT) or cold acclimated, determined by $^{18}$F-PET/CT. Raw data collected from previous publications (Truong et al., 2004; Gelfand et al., 2005; Lee et al., 2010; Pfannenberg et al., 2010; Drubach et al., 2011; Gilsanz et al., 2011, 2012; Jacene et al., 2011; Ouellet et al., 2011; Zhang et al., 2014).
shows the most robust responses to diverse metabolic stimuli, such as cold (Walden et al., 2012), β3-adrenergic receptor agonism (Poher et al., 2015), fasting (Ruan et al., 2014), and calorie restriction (Narita et al., 2018), pointing to the possibility that retroperitoneal WAT could function as a first line of defense to maintain energy and lipid homeostasis when environmental temperature and food are fluctuant.

Genetic background is another determinant of the heterogeneity in WAT browning. The Kozak group elegantly demonstrated that the obesity-resistant A/J mice have significantly higher thermogenic gene expression preferentially in retroperitoneal WAT when compared with obesity-prone C57BL/6 mice (Guerra et al., 1998; Koza et al., 2000; Coulter et al., 2003). Later, they identified nine quantitative trait loci that may regulate the browning process in retroperitoneal WAT (Xue et al., 2018). A recent study found that obesity resistance in female mice derived from the En1-expressing dermomyotome (Att et al., 2006). Later, several independent fate-mapping experiments demonstrated that brown adipocytes in mice originate from myogenic precursor cells that express Myf5 (Seale et al., 2008), Pax3 (Sanchez-Gurmaches and Guertin, 2014), and Pax7 (Lepper and Fan, 2010). However, recent studies have revealed that the composition of thermogenic adipose lineage is more complex than we previously thought of (Table 1).

The Guertin group systemically evaluated the Myf5+ and Pax3+ lineages and found that most dorsal-anterior adipocytes, in both BAT and WAT depots, are derived from the largely overlapping Myf5-Cre- and Pax3-Cre-expressing precursors (Sanchez-Gurmaches et al., 2012). In contrast, Myf5-Cre and Pax3-Cre do not label any inguinal and mesenteric WAT adipocytes, though Pax3-Cre progenitors do contribute to about half of the gonadal adipocytes in male mice (Sanchez-Gurmaches and Guertin, 2014). Pax7, the expression of which is restricted to the central dermomyotome, defines progenitors of a minority of interscapular brown and white adipocytes (Sebo et al., 2018). While Mecox1-Cre, which is broadly expressed in the dermomyotome as Pax3-Cre, traces to most adipocytes in the dorsal–anterior and male gonadal depots (Sebo et al., 2018). On the other hand, HoxB6-Cre, with expression restricted to the posterior domain of lateral plate mesoderm, complements the tracing patterns of Mecox1-Cre and labels most adipocytes in the inguinal, mesenteric, and female gonadal depots (Sebo et al., 2018). Based on these data, Sebo and Rodeheffer (2019) proposed that the dorsal-anterior-located interscapular and retroperitoneal adipose depots predominately arise from a Pax3+/Mecox1+/Myf5+ lineage in the epaxial dermomyotome (Table 1), with a minor contribution from the Pax7+ central dermomyotome. Intriguingly, Mva01, a classic myogenic transcription factor that acts

### Table 1 Lineage tracing of BAT and WAT depots.

| Cre line | En1-CreER | Myf5-Cre | Pax3-Cre | Pax7-Cre | Mecox1-Cre | HoxB6-Cre | MyoD1-Cre | WT1-CreER | Ptx1-Cre | Pdgfra-Cre |
|----------|-----------|----------|----------|----------|------------|-----------|-----------|-----------|----------|-----------|
| BAT      | Interscapular | +        | All      | All      | <50%       | >85%      | –         | –         | –        | +         |
|          | Subcapsular | All      | All      | –        | –          | –         | –         | –         | –        | –         |
|          | Cervical   | 50%      | 80%      | –        | –          | –         | –         | –         | –        | –         |
|          | Periaortic | –        | 70%      | –        | –          | –         | –         | –         | –        | –         |
|          | WAT        | Interscapular | Most    | Most    | <50%       | >85%      | –         | –         | –        | +        |
|          | Anterior subQ | All      | All      | –        | –          | –         | –         | –         | –        | +        |
|          | Retroperitoneal | All1    | All      | –        | >85%       | –         | –         | +         | –        | All       |
|          | Inguinal   | –        | –        | –        | –          | All       | –         | –         | All      | All       |
|          | Mesenteric | –        | –        | –        | –          | All       | –         | +         | –        | All       |
|          | Gonadal   | 58%2    | 60%3     | –        | –          | All       | –         | +         | –        | All       |

1Approximately half of the ventral region but none of the dorsal region of the anterior subcutaneous adipocytes are positive (Sanchez-Gurmaches et al., 2015).
2Decreases with age, in short-term HFD, and after β3-adrenergic receptor activation (Sanchez-Gurmaches and Guertin, 2014).
3MyoD1+ lineage can give rise to the so-called ‘glycolytic beige fat’ during chronic cold adaptation in the absence of β-adrenergic receptor signaling (Chen et al., 2019).
4Percentage in males. No gonadal adipocytes trace to Mecox1-lineage in females (Sanchez-Gurmaches and Guertin, 2014).
5Percentage in males. Only 4% of gonadal adipocytes trace to Mecox1-lineage in females (Sebo et al., 2018).
6Percentage in females. Only 5% of gonadal adipocytes trace to HoxB6-lineage in males (Sebo et al., 2018).
downstream of Pax3 and Myf5, does not label any adipocytes at physiological conditions (Sanchez-Gurmaches and Guertin, 2014), suggesting that the adipose and muscle lineages diverge before the MyoD1 gene is expressed.

A large body of literature has documented significant differences between visceral and subcutaneous WAT depots, in terms of their morphological and molecular characteristics, thermogenic capacity, and metabolic contributions. Apparently, these two fat subtypes have different origins and developmental timing (Wang et al., 2013, 2015). The postnatally differentiated visceral fat, including perirenal, epicardial, retroperitoneal, omental, mesenteric, and gonadal depots, receives significant contributions from Wt1þ progenitors in the intermediate mesoderm and splanchnic lateral plate mesoderm (Chau et al., 2014). On the other hand, limb-associated subcutaneous fat initiates its differentiation during embryonic days 14–18 and is labelled by Prx1-Cre that traces the somatic lateral plate mesoderm (Krueger et al., 2014; Sanchez-Gurmaches et al., 2015; Table 1).

The lineage distribution of thermogenic adipose tissue is dynamic during obesity, aging, and thermal stress. WAT browning induced by cold exposure or β-adrenergic receptor activation involves the transdifferentiation of white to beige adipocytes (Barbatelli et al., 2010; Lee et al., 2015), the activation of existing ‘dormant’ beige cells (Rosenwald et al., 2013), and the de novo adipogenesis from beige adipocyte progenitors (Wang et al., 2013; Berry et al., 2016). These beige adipocyte progenitors may share the common embryonic origin with the white lineage, as they can be both tracing-labelled by constitutive Prx1-Cre (Sanchez-Gurmaches et al., 2015), Pdgfra-Cre (Lee et al., 2012, 2015; Berry and Rodeheffer, 2013; Krueger et al., 2014), and Pdgfrb-Cre (Vishvanath et al., 2016; Figure 2). However, in adult mice, the contribution of Pdgfra- and Pdgfrb-expressing cells to WAT browning is relatively limited, revealed by temporal fate-mapping experiments (see below).

Cold and β-adrenergic receptor activation induce distinct mechanisms for adaptive thermogenesis (jiang et al., 2017; Chen et al., 2019). Exposing animals to cold activates beige adipogenesis in inguinal WAT from smooth muscle-like perivascular mural cells that express Myh11 (Long et al., 2014), Tagln (Sm22), Cspg4 (Ng2), and Acta2 (Sma) (Berry et al., 2016). None of these newly differentiated beige cells can be labelled by temporally expressed Pdgfra-CreER. On the other hand, β3-adrenergic receptor activation stimulates the conversion of mature white adipocytes into beige adipocytes in both inguinal and gonadal WAT (Lee et al., 2012; Jiang et al., 2017). Similar to high-fat diet (HFD)-stimulated adipogenic differentiation in gonadal WAT (Jeffery et al., 2015), beige adipocytes induced by β-adrenergic receptor activation arise from Pdgfra-þ precursor cell (Lee et al., 2012; Cattaneo et al., 2020). Nonetheless, it is not fully resolved whether an intermediate transition is required for the fully differentiation of Pdgfra-þ precursors into mature adipocytes. Moreover, future works will be required to determine whether the Pdgfra-þ precursors are homogeneous or subpopulations of precursors exist for white and beige adipocyte lineages, respectively. Indeed, Pdgfra-þ and Pdgfrb-þ progenitors have lineage plasticity and adopt a fibrogenic phenotype in obesity (Marcelin et al., 2017).

The percentage of Myf5-derived adipocytes decreases with age, in short-term HFD feeding, and after β3-adrenergic receptor activation in retroperitoneal WAT, but not in classic BAT

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**Figure 2** Dynamic lineage contribution within WAT. During embryonic development, Pdgfra-labelled mesenchymal stem cells (MSCs) give rise to all adipocytes in WAT and some brown adipocytes in interscapular BAT (iBAT). In gonadal WAT (gWAT), HFD and β-adrenergic receptor activation stimulate the differentiation of Pdgfra-þ precursors. It is unclear whether beige adipocytes differentiate directly from Pdgfra-þ precursors or via an intermediate white phenotype (these two processes are not necessarily exclusive). In inguinal WAT (iWAT), Myh11/Acta2-þ mural cells give rise to new beige adipocytes upon cold stimulation. However, β-adrenergic receptor activation does not stimulate de novo adipogenesis, rather it promotes transdifferentiation of existing white adipocytes. Retroperitoneal WAT (rWAT) and iBAT both arise from Myf5/Pax3-þ precursor cells. While the lineage distribution within iBAT is constitutive, the percentage of Myf5-derived beige adipocytes decreases with age, in short-term HFD feeding, and after β3-adrenergic receptor activation in rWAT.
(Sanchez-Gurmaches and Guertin, 2014; Figure 2). Similarly, changes in insulin signaling by deleting PTEN or IRβ can alter the Myf5+ lineage composition within anterior subcutaneous and retroperitoneal WAT, not within BAT (Sanchez-Gurmaches and Guertin, 2014). Thermal stress in ‘β-less’ mice that lack β-adrenergic receptor signaling induces a group of non-canonical beige adipocytes that display enhanced glycolysis and originated from the MyoD1+ progenitors (Chen et al., 2019). A thorough understanding of adipose lineage plasticity will provide new insights into the pathogenesis of obesity and obesity-associated complications.

Functional heterogeneity of thermogenic adipocytes

Since its discovery, UCP1 has been wildly used as a functional marker of both brown and beige adipocytes. Indeed, UCP1 mediates most, if not all, of the non-shivering thermogenesis induced by norepinephrine (NE), and loss of UCP1 renders mice to become sensitive to acute cold exposure (Cannon and Nedergaard, 2004). In addition to NE, the sympathetic nerve system also provides purine nucleotides and nucleosides to activate the purinergic receptors on thermogenic adipocytes (Gnad et al., 2014; Ussar et al., 2014; Razzoli et al., 2016). Moreover, many hormones and cytokines derived from a whole range of tissues and cells can act independently or in parallel with the adrenergic signaling to activate and/or recruit brown/beige adipocytes (Figure 3; Wang and Yang, 2017).

When gradually acclimated to cold, UCP1-deficient mice are still able to maintain tolerance to cold (Ukropec et al., 2006). Intriguingly, or rather paradoxically, UCP1-deficient mice are resistant to diet-induced obesity when housed at ambient temperature that evokes mild cold stress in mice (Enerback et al., 1997; Liu et al., 2003). Since these early findings indicating the functional compensation for the loss of UCP1, several UCP1-independent thermogenic mechanisms have been discovered (Figure 3; Chang et al., 2019). These include the phosphocreatine/creatine futile cycle in brown and beige adipocytes (Kazak et al., 2015), Ca2+ cycling mediated by sarco/endoplasmic reticulum Ca2+-ATPase in muscle and beige adipocytes (Bal et al., 2012; Ikeda et al., 2017), mitochondrial uncoupling by N-acyl amino acids that are generated by PM2D1 in brown and beige fat cells (Long et al., 2016), the futile cycle between triglyceride breakdown and re-esterification in adipocytes (Guan et al., 2002), and the futile cycle between lipid oxidation and de novo lipogenesis in muscle and adipocytes (Solinas et al., 2004; Mottillo et al., 2014). A better understanding of these non-canonical mechanisms of thermogenic fat cells will potentially lead to therapeutic interventions for the treatment of obesity.

Even the classic BAT contains heterogeneous groups of brown adipocytes. An early study by Cinti et al. (2002) presented the non-homogeneous pattern of UCP1 expression after cold stimulus and β3-adrenoceptor agonist treatment. Very recently, Song et al. (2020) took advantage of the AdipoChaser system and SNAP-seq, identified Adipoq hi/Ucp1 hi high-thermogenic and Adipoq lo/Ucp1 low low-thermogenic brown adipocytes within the BAT. Compared to classic high-thermogenic brown adipocytes, these low-thermogenic cells...
have larger lipid droplets but lower mitochondrial content and respiration, are specialized in fatty acid uptake, and engage the creatine futile cycle. Importantly, cold exposure can convert low-thermogenic brown adipocytes into high-thermogenic cells, the efficiency of which declines with age (Song et al., 2020). However, it is unclear whether this postnatally established functional heterogeneity is due to the developmental heterogeneity of brown adipocyte precursors.

In addition to maintaining body temperature via thermogenesis, BAT and beige fat also serve as metabolic sinks for glucose, fatty acids, and branched-chain amino acids (BCAAs) to improve metabolic health (Figure 3; Harms and Seale, 2013; Wallace et al., 2018; Yoneshiro et al., 2019; Maurer et al., 2020). Cold exposure dramatically increases the uptake of glucose, fatty acids, lipoproteins, and BCAAs into the BAT, which can be reversed when animals are returned to a warm environment (Bartelt et al., 2011; Labbe et al., 2015; Yoneshiro et al., 2019). Recently, the tricarboxylic acid cycle intermediate succinate was shown to be accumulated in thermogenic adipose tissue upon cold exposure (Mills et al., 2018; Wang et al., 2019). Circulating succinate may be derived from muscle and active UCP1-dependent thermogenesis via reactive oxygen species and mitochondrial protein succinylation. Of note, fatty acids are traditionally considered as the main fuel for thermogenesis.
in BAT (Trayhurn, 1995). Glucose uptake does not necessarily reflect the thermogenic activity in BAT (Olsen et al., 2017; Skorobogatko et al., 2018), and the relative fates of glucose metabolism (glycolysis, glyconeogenesis, lipogenesis, oxidation, and thermogenesis) are still unclear (Townsend and Tseng, 2014). On the other side, a portion of glucose can enter the hexosamine biosynthetic pathway to generate uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), which is then used for O-linked β-N-acetylglucosamine modification (O-GlcNAcylation) of intracellular proteins (Ruan et al., 2013). Protein O-GlcNAcylation has been also shown to modulate adipose tissue thermogenesis via cell autonomous and non-autonomous mechanisms (Ruan et al., 2014; Ohashi et al., 2017). In spite of these knowledge, it is desired to determine whether there are heterogenous adipocyte populations that prefer to use certain nutrient substrate(s) than others.

Conclusion
Obesity is a major risk factor for many diseases, including type 2 diabetes, cardiovascular disease, and some types of cancers (Taksler et al., 2017). The activity of BAT in adult humans is inversely associated with body mass index (Betz and Enerback, 2011; Tam et al., 2012), indicating its potential involvement in obesity development and therapeutic function. There are many outstanding questions regarding the heterogeneity of lineage development and metabolic function within thermogenic adipose tissue. Supraclavicular, cervical, and axillary BAT in adult humans appears to contain mixed brown and beige adipocytes; however, their developmental origins, even in mouse models, have not been identified. What are the cellular and molecular mechanisms governing the age-dependent involution of BAT? Does the diversity in fuel utilization, gene expression, and thermogenic activity observed in brown and beige adipocytes stem from the lineal heterogeneity of adipocyte precursors during development? Do beige and white adipocytes arise from distinct precursor populations and do these precursors still possess plasticity in their cellular identity? What is the ‘master’ switch that regulates metabolic flexibility of thermogenic adipocytes and whether the fuel infllexibility within the brown and beige fat contributes to systemic metabolic dysfunction? How do thermogenic adipocytes utilize the two-way communication with tissue-resident immune, fibrogenic, and nerve cells to maintain tissue homeostasis and control whole-body metabolism? Answering these questions will pave the way for activating, rejuvenating, and regenerating BAT to combat obesity, diabetes, and related chronic conditions.

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