Review
OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

Obesity

The Critical Period for Brown Adipocyte Development: Genetic and Environmental Influences

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Objective: The current review summarizes recent advances in the origin of brown adipocytes in rodents and humans.

Methods: This review describes recent insights into induction of the brown adipocyte phenotype (BAP) in white fat (WAT) revealed by murine studies during the early postnatal period and reversible temperature transitions. The origin of adipocytes and identity of progenitors as indicated by lineage tracing experiments are reviewed.

Results: We describe a genetic model for brown adipocyte development that involves the appearance of brown adipocytes in WAT at 21 days of age and a mechanism of post-weaning involution relevant for acquisition of the BAP in fully functional WAT in mice. Under normal physiological conditions, the BAP is dormant with the potential to be stimulated by changes in the external environment. Current evidence for the acquisition of brown adipocytes by interconversion of mature adipocytes versus de novo recruitment of progenitors suggests that mechanisms for acquisition of the BAP in WAT in mice are depot-specific and controlled by allelic variation.

Conclusions: Although the BAP is highly variable among mice, there is no information on genetic variability in the expression of brown adipocytes in humans. Thus, deeper understanding of genetic mechanisms underlying development of functional brown adipocytes is crucial.

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Introduction

Obesity develops as a result of chronic energy imbalance, when energy consumption exceeds energy expenditure (EE). Most of the currently available pharmacological treatments of obesity reduce food intake or decrease the efficiency of food absorption in the intestines. Recent identification of metabolically active brown adipose tissue (BAT) in adult humans (1-4) has markedly increased the interest in the therapeutic potential of BAT and its role in the regulation of energy homeostasis. BAT increases EE in response to sympathetic stimulation in murine models of obesity. Thus, increasing BAT activity in individuals with obesity could be an effective therapeutic alternative for patients who are incapable of physical activity or fail to follow a dietary regimen.

This review summarizes recent advances in brown adipocyte (BA) development and/or activation in rodents and humans. The goal being to identify effective strategies for induction of BAs that can serve as an anti-obesity therapy based on maximizing the capacity for thermogenesis. Using murine models we outline the critical time for development of brown and white adipose tissue (WAT), including their lineages, molecular signatures, and depot-specific differences. We describe the progression in the development of functional BAs in WAT from the early postnatal period that first involves a biosynthetic phase from birth until 21 days of age, followed by development of a mechanism of involution in the post-weaning period that is essential for the dynamic brown adipocyte phenotype (BAP) that waxes and wanes with the requirements for thermogenesis. We propose that the BAP in WAT is dormant and can be stimulated as soon as WAT develops its fully functional structure. Then we discuss current knowledge on the origin of BAs in WAT and acquisition of the BAP in adult WAT, evaluating mechanisms for the interconversion of mature adipocytes versus de novo recruitment of progenitors, including depot and genetic variability. Finally, we review the molecular identity of human BAT and the recruitment and/or activation of BAT in humans.

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Structural and Functional Features of WAT Versus BAT

Adipose tissue in mammals is classified into two major types serving opposite functions in energy balance regulation. WAT is responsible for storing excess energy as triacylglycerols, de novo synthesis of triacylglycerols from glucose and mobilization of energy in the form of free fatty acids. As an endocrine organ, WAT contributes to the regulation of energy homeostasis by secretion of molecules active in the control of food intake, insulin sensitivity and inflammatory responses (5). The primary role of BAT is to uncouple oxidative phosphorylation for defense against hypothermia. Non-shivering thermogenesis is essential for thermoregulation in small animals and neonates, characterized by a large surface-to-volume ratio, and the arousal from hibernation in mammals (6). The majority of a cell volume of white adipocytes (WAs) is occupied by a single, large lipid droplet, with a ring of cytoplasm compacted to a thin layer surrounding the lipid particle and a nucleus displaced to the periphery of the cell. In contrast, BAs contain multiple small lipid droplets and mitochondria, dispersed within the cytoplasm, that contain the uncoupling protein 1 (UCP1), which dissipates the electrochemical gradient driving ATP synthesis, increases the activity of the respiratory chain and generates heat.

Development of Classical BAs

Interscapular BAT (iBAT) in rodents develops during late embryogenesis with the first morphological evidence for BAT depot observed by 15-16 days of gestation and the maximal mitotic activity of BAs reported during 17-19 days of fetal development (7). Studies in rats revealed a progressive increase in *Ucp1* and *CoII* mRNA levels from 18 days of embryogenesis until after birth (8). *Ucp1* expression in iBAT was first detected in 18-day-old fetuses of C57Bl/6 (B6) and A/J mice with a subsequent increase immediately before birth and peak values for Ucp1 mRNA and protein levels occurring on postnatal day 1 and 10, respectively (9). Although iBAT *Ucp1* mRNA levels remain relatively stable throughout life in mice reared at 23°C (9), its expression increases slightly in response to acute cold stimulation (10,11). Chronic cold exposure induces proliferation of interscapular BAs (12), while a transfer from cold to 28°C enhances iBAT apoptosis (13), indicating a physiological balance between proliferation, survival and degradation of BAs. Recent studies showed that cold exposure induced $Pdfra^{+}$ BA progenitors only in the narrow dorsal region of iBAT (14). Interscapular BAs in mice arise within the central dermomyotome from $En1^{+}$ progenitors on embryonic day 9.5 (15) and $Pax7^{+}$ precursors between 9.5 to 11.5 days of gestation (16).

The expression of *Zic1*, that differentiates interscapular BAs from BAs in WAT in mice (17,18), was over 100-, 10-, and 6-fold greater in iBAT than in supraclavicular, periadrenal, or perirenal regions in infants, respectively, indicating that classical BAT is located in the same anatomical region in mice and humans (19). Human iBAT was most active during infancy and early childhood and disappeared progressively with increasing age (20). Additionally, BAs were found in axillary and perirenal fat pads and *Ucp1* expression was highest in infants and children between 1-15 years of age, respectively, and decreased significantly during adult life (21). The main locations for BAT in adult humans were found in the supraclavicular region, around neck, vasculature, epicardium, and solid organs such as kidney, adrenal, pancreas, and liver (22,23).

Depot-Specific Differences in White Adipose Tissue Development

The subcutaneous WAT (sWAT) in mice appears first during the last days of gestation while lipids accumulate during the first days of life. WAs from subcutaneous and retroperitoneal WAT (rWAT) contained lipid droplets already on postnatal day 1; however, they were smaller than mature adipocytes in adult mice (24). Lipid-filled subcutaneous WAs were identified by postnatal day 2, with a uniocular structure detected on postnatal day 5, while WA biomarkers (*Pparg*, *Cebpa, Fabp4)* were expressed on the embryonic day 17.5, prior to lipid accumulation (25). There was little fat deposition in inguinal WAT (iWAT) from 2-day-old B6 mice, whereas lipid accumulation increased from 5 until 10 days of age (26). Studies using a doxycycline-inducible system revealed that differentiation of sWAT began between the 14th and 18th day of embryogenesis and the number of subcutaneous WAs remained relatively stable during life while visceral WAs were formed during the first weeks of postnatal development over a relatively long period of time (27). Epididymal WAT (eWAT) contained a small number of fibroblast-like cells between postnatal day 1 and 3 while lipid accumulation occurred in a 3-day-old mouse (28). Lipid droplets in eWAT were evident in 7-day-old mice with mature WAs being observed on postnatal day 14 (24). Although traces of lipid and biomarker expression of WAs from subcutaneous and visceral fat (vWAT) depots are detected during late gestation, mature adipose tissue appears post-natally. In contrast, mature WAT in humans appears during fetal development at 14 weeks of gestation and develops progressively until 24 weeks of embryogenesis (29).

Despite differences in the specific time frame for development, visceral, and sWAT are characterized by distinct secretion of adipokines, insulin sensitivity and rates of lipolysis (30). Adipocyte precursors from sWAT and vWAT have different molecular signature, capacity for differentiation and responsiveness to growth factors as well as genetic and environmental stimuli (31). The analysis of inguinal, retroperitoneal, mesenteric, and epididymal fat collected from adult B6 mice revealed differences in total amount of protein, level of proteins associated with ATP synthesis, glycolysis and glyceroneogenesis, as well as average adipocyte size (32). It was shown that iWAT and eWAT have different developmental origins which could underlie differences between these two fat depots (33).

WAT expansion occurs by increased number (hyperplasia) and/or the average volume of adipocytes (hypertrophy). Feeding a high-fat diet (HFD) to B6 mice rapidly increased the expression of *Mest* and *Sfrp5* (34), the biomarkers for WAT expansion in adult mice, in eWAT and iWAT indicating that in response to a positive energy balance mice accumulate fat by hypertrophy rather than hyperplasia. Hypertrophy is the main contributor to the growth of vWAT in response to feeding a HFD while the average number of adipocytes is strain-specific (35). Both the number and mean size of WAs in rodents reared under standard conditions is established by the end of adolescence (36). Recent studies further indicated that the amount (27) and the average volume (37) of subcutaneous WAs remained relatively constant in adult mice fed a standard diet. Human studies showed that fat mass gain in people with obesity results from hypertrophy, while the mean number of WAs is stable and characteristic for each individual (38). In addition, approximately 10% of adipocytes are renewed each year indicating

an active cell turnover. Therefore, cell proliferation in response to a HFD regimen might be associated with WAT remodeling including cell death and renewal necessary to sustain a stable level of adipocytes.

Early Postnatal Development of BAs in WAT

In addition to interscapular BAs, clusters of BAs, called "brown in white" - brite (39) or beige (40), are dispersed within WAT. In mice raised at 23°C BAs appear spontaneously in rWAT during early postnatal development with a peak of expression observed at 21 days of age (9). BAs were transiently induced in WAT from 20-day-old B6 and 129S6sv/ev (129) mice reared at 23°C (41). We demonstrated that development of BAs in iWAT and rWAT occurs independently of the ambient temperature, between 10 and 21 days of age, with a greater BAP reported in 21-day-old mice raised at 17°C compared to animals reared at 29°C from birth until weaning (11). Neither 17°C nor administration of thyroid hormone stimulated precocious induction of BAs in WAT suggesting that their appearance is determined genetically. Consistent with this genetic model for development, iWAT in 10-day-old B6 and AxB8 mice can induce the BAP in response to a β3-adrenergic receptor agonist treatment indicating that at 10 days of age WAT in mice has the molecular machinery to induce the BAP. Therefore, the low *Ucp1* expression observed in 10-day-old mice raised at 17°C is due to the absence of input from the sympathetic nervous system in WAT. In contrast, under-nutrition during the lactation period decreased the BAP in 21-day-old mice (42), suggesting that nutritional status during the early postnatal period affects the development of functional WAT and its thermogenic capacity. However, similar to transient effects of ambient temperature, the suppression of the BAP from under-nutrition between birth and 21 days of age was not retained in adult mice; that is, BAP could be fully induced in adult mice when exposed to 4°C.

Involution of the BAP in WAT

A vital characteristic of BA development is the spontaneous disappearance of the BAP. Involution of the BAP in rWAT occurred at approximately 35 and 56 days of age in B6 and A/J mice, respectively (9). BAs strongly induced in WAT from 21-day-old mice reared at 17°C, 23°C and 29°C in rWAT and iWAT, disappeared in 56-day-old B6 and AxB8 mice (11,42). Furthermore, the BAP was reduced in rWAT and iWAT in 30-day-old B6 and 129 mice compared with WAT from 20-day-old mice (41). This BAP, which includes a differentiated phenotype at weaning followed by spontaneous involution, is determined by a fixed genetic program in mammals subjected to transient modulation in response to nutrition and ambient temperature. We propose that fully-functional adipocytes able to acquire the BAP in response to sympathetic stimulation develop in a two-step process, first, a biosynthetic phase involving the appearance of BAs in WAT at weaning, and second, a phase called involution, in which BAs are degraded when an environment with elevated ambient temperature precludes the need for thermogenesis (Figure 1). Acquisition and disappearance of the BAP in WAT re-occurs in adult mice during cold exposure and re-adjustment to the warmth. The BAP was strongly induced in iWAT from adult AxB8 mice in response to 10 days at 4°C and decreased substantially within 14 days of re-acclimation to thermoneutrality with a complete involution of BAs observed after 21 days at 29°C (43). This indicates that under normal physiological conditions the BAP in WAT is dormant and can be activated, e.g., upon cold exposure, when the need for thermogenesis is increased and is ceased upon termination of external cues.

Genetic Variability in Acquisition of the BAP in WAT

The number of BAs in WAT is highly variable within inbred strains of mice and distinct WAT depots (44). The adenylyl cyclase activity in cell membranes collected from eWAT was markedly greater in A/J than B6 mice (45). The expression of BA biomarkers, *Ucp1* and *Cidea,* was markedly higher in sWAT from 129-S1 and A/J strains compared to B6 mice (46). A/J mice were characterized by significantly greater *Ucp1* expression in rWAT and eWAT compared to B6 mice, while strain-dependent differences in the BAP in iWAT were less definitive (9,44). After 7 days of cold exposure (4°C) *Ucp1* expression in rWAT was more prominent in AxB8 mice, while BAs were equally induced in iWAT from AxB8 and B6 mice (11). The BAP was greater in vWAT and sWATof 129 than in B6 mice; however, these differences were more profound in rWAT than iWAT (41). Similarly, sWAT from 129SVE mice reared under standard conditions was characterized by higher expression of BA biomarkers, e.g., *Ucp1*, *Pgc1a* and *Cidea*, compared to eWAT (40).

Generation and analysis of recombinant inbred strains of mice revealed that induction of the BAP in WAT depends on synergistic interaction among multiple genetic loci and new recombination of alleles present in the parental strains causes transgressive variation and increases *Ucp1* expression much beyond the maximal levels reported for A/J and B6 mice (44,47,48). Upon cold exposure *Ucp1* mRNA levels varied over 70-fold between AxB8 mice, characterized by the highest number of BAs in WAT, and AxB10 mice, in which *Ucp1* expression was even lower than that reported for B6 mice (44). Variation in *Ucp1* mRNA levels in rWAT between A/J and B6 mice was determined by a genetic interaction between nine quantitative trait loci (QTLs), mapped to eight different chromosomes, which also regulated the expression of other BA biomarkers, e.g., *Pgc1a*, *Ppara,* and *Dio2* (49). Thus, these three factors, regulated by QTLs described previously, are components of a regulatory system for the induction of BAs by sympathetic signaling. Therefore, as in mice, variation in the amount and activity of human BAT might be determined genetically and points to the need for comprehensive genetic analyses that could facilitate the development of an effective strategy to induce BAs and increase thermogenic capacity.

WA and Classical BA Progenitors in Adult Mice

The origin of BAs and WAs has been extensively studied in adult mice. Genetic lineage-tracing studies indicated that *Myf5*$^+$ cells give rise to BAs and myocytes indicating a common origin for iBAT and the skeletal muscle in mice (50). All BAT-derived

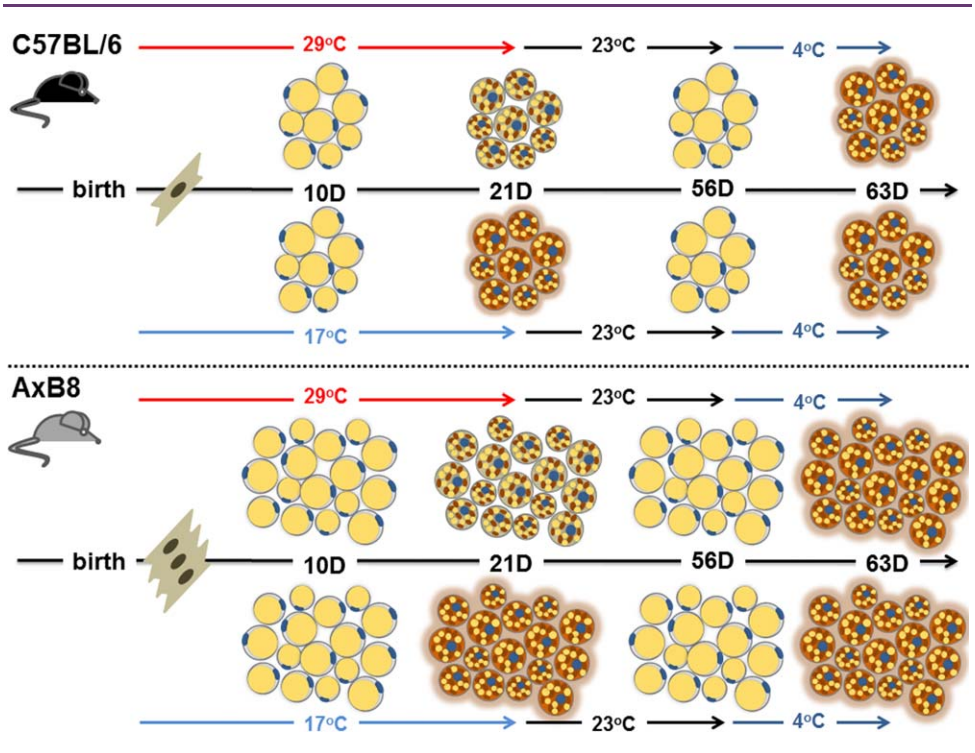

Figure 1 A model for development of functional BAs in WAT. Thermogenic capacity determined by the number of adipocytes capable of activating the BAP in WAT is distinct among different strains of mice. The BAP in WAT is induced at 21 days of age and is transiently greater in mice reared at 17°C. The BAP disappears post-weaning. Induction of the BAP in adult life is dependent on activation of the sympathetic nervous system, e.g., by cold exposure independently of environmental temperature during the lactation period.

adipocytes cultured *in vitro* originated from progenitors expressing *Pax3*, an upstream regulator of *Myf5* expression during embryonic development of the skeletal muscle (51). The vast majority of adipocyte precursors and all mature interscapular BAs from 6-week-old mice expressed both *Myf5* and *Pax3* (52). In addition, stem cells residing in skeletal muscle of adult mice differentiate into BAs *in vitro*, again suggesting that BAs and myocytes originate from the same precursors (53). Transcriptome and lineage-tracing analyses in $PTEN^{myf5cKO}$ mice showed that $Myf5^{+}$ precursors gave rise to a subset of interscapular and retroperitoneal WAs with fewer $Myf5^{+}$ cells found in inguinal and perigonadal WAT (54). 31%, 11%, and 14% of stromal vascular fraction (SVF) cells from anterior subcutaneous, inguinal, and epididymal fat, respectively, derived from *Myf5,Sca1*-expressing progenitors residing in WAT (55). In addition, around 60% of WAs in rWAT arise from the $Pax3^{+}$ cells, while less than 15% of inguinal WAs originated from the $Pax3^{+}$ lineage (56) indicating that the expression of *Pax3* and *Myf5* is not sufficiently specific to discriminate between BAs, myocytes, and WAs.

Experiments tracking VE-cadherin expression at different developmental stages indicated that BAs and WAs in adult WAT originate from endothelial precursors located in interscapular, subcutaneous, and epididymal fat depots (57). Cells expressing *Zfp423* were located in the vicinity of blood vessels and perivascular cells, suggesting that brown and white preadipocytes arise from endothelial lineage *in vivo* (58). Another lineage-tracing study showed that retroperitoneal and inguinal WAs originate from a pool of proliferating $PDFR\beta^{+}$ progenitors residing within mural cells of WAT vasculature (59). A close anatomical relationship between clusters of differentiated adipocytes and WAT vasculature was reported already in 1982 (28). A well-organized network of blood vessels in eWAT was developed already on postnatal day 4 and preceded the appearance of lipid-containing adipocytes, which occurred first on the proximal branches of vasculature in 7-day-old mice. Clusters of lipid-filled adipocytes covered the entire network of blood vessels on postnatal day 11 to 14 while inhibition of the VEGFA signaling pathway delayed the development of eWAT (24). Given a tight anatomical and functional association between growing vascular system and adipose tissue, it might be difficult to accurately distinguish whether endothelial progenitors located within WAT gives rise specifically to blood vessels or might also differentiate into WAs. Recently, genetic tracing studies using *Cdh5*-Cre:mT/mG, *Tie2*-Cre:mT/mG and *Vav1*-Cre:mT/mG mice in which GFP fluorescence is detected only in membranes of cells expressing endothelial or hematopoietic lineage markers, respectively, showed that mature adipocytes under normal conditions were derived from a population of CD24+;$PdfR\alpha^{+}$ adipocyte progenitors residing within WAT, not from endothelial or hematopoietic precursors (60). In addition, an inducible Cre-LoxP system led to identification of bipotential $PDGFR\alpha^{+}$ stromal stellate-like cells able differentiate to BAs or WAs depending on the external signals (61).

Although identification of the origin for BAs and WAs in adult tissues is relevant it seems to have little practical aspect at present. Given that master transcription factors and signaling molecules exert

their functions in multiple cell types, e.g., adipose, muscle, vasculature, targeting them to increase cell proliferation in a tissue-specific manner seems problematic. While designing a strategy for BA induction as an anti-obesity therapy, one should consider when adipose tissue acquires its functional structure that could be stimulated to make a difference to the overall metabolism. Although iBAT is fully active upon birth, lipid storage and thermogenic capacity of WAT develop post-natally. Since results obtained from lineage-tracing studies used to identify BA- and/or WA-specific progenitors in adult tissues are often contradictory, it seems crucial to determine the exact time and molecular mechanisms underlying development of functional WAT. Given variation in the genetic predisposition to diet-induced obesity in humans this could lead to the development of an effective therapeutic strategy in which thermogenic activity of BAs in WAT would be stimulated as soon as WAT function was established.

Identification of Progenitors Specific for BAs in WAT in Adult Mice

The origin of BAs in adult WAT remains inconclusive. BAs stimulated in WAT cultures *in vitro* or in eWAT from cold-exposed adult mice did not express BA biomarkers, e.g., *Zic1*, *miR-206,* and *Lhx8*; nevertheless they were characterized by a distinct molecular signature including the expression of *Hoxc9* and *Shox2* (17,39). A global microarray analysis of gene expression in inguinal BA cell cultures identified *Fgf21*, *Car4,* and *Cited1* genes as specific for BAs in WAT in contrast to *Zic1*, *Lhx8,* and *Epstl1*, characteristic for interscapular BAs (62). Forskolin-differentiated BAs from sWAT were characterized by a unique molecular signature, e.g., *Tbx1*, *Slc27a1*, *Cd40* and unlike other SVF cells expressed *Cd137* and *Tmem26* (40). Recent validation of putative biomarkers for BAs demonstrated that among all proposed genes only *Zic1* and *Hoxc9* expression could accurately distinguish between interscapular BAs, BAs in WAT, and WAs (18).

A population of Sca1$^+$/CD45$^-$/Mac1$^-$ precursors in sWAT expressed *Ucp1* in response to BMP7 treatment *in vitro* (46). The expression of interscapular brown (*Ucp1*, *Cidea*, *Prdm16*, *Pgc1a*) and brown in WAT (*Tmem26*, *Tbx1*) adipocyte biomarkers were significantly lower in *Myf5*$^+$ precursors compared to *Myf5*$^-$ cells in sWAT (55), suggesting that BAs in iWAT arise from *Myf5*$^-$ cells. The expression of *Ucp1*, *Cidea*, *Cox7a*, *Cox8b,* and *Pgc1a* in *Pax3*$^-$ cells, which comprised 85% of WAs in iWAT, was 45-, 30-, 8-, and 3-fold greater compared to *Pax3*$^+$ cells indicating that BAs in WAT might arise from *Pax3*$^-$ lineage (56). In response to 10 days of cold stimulation, a subset of subcutaneous BAs arose from *Myh11*$^+$ cells leading the authors to suggest a smooth-muscle like origin (63). Chemical and genetic tracing studies showed that 82% of the BAs in eWAT originated from precursors that expressed *Pdfrα*, *CD34,* and *Sca1*, while only 5.8% of BAs in iWAT originated from de novo proliferation in response to a β3-adrenergic receptor agonist administration (61). Using a doxycycline-inducible tagging system it was shown that upon adrenergic stimulation the majority of BAs in sWAT arose from de novo differentiation, while β3-adrenergic stimulation significantly increased white adipogenesis in eWAT (27). Basal and stimulated *Ucp1* expression in eWAT is significantly lower compared to iWAT (44,61) indicating a greater number of BAs in sWAT. Therefore, proliferation of epididymal WAs in response to sympathetic stimulation might increase the total number of adipocytes and stimulate the overall thermogenic capacity of vWAT.

These observations explain the existence of precursors specific for BAs in WAT that argue against a mechanism for acquisition of the BAP based upon the temperature-modulated mature WA-to-BA interconversion. However, any *in vitro* experiment does not reflect physiological conditions. Freshly isolated SVF cells from anterior sWAT did not express *Cd137* and *Tmem26* while their expression was induced during proliferation *in vitro* (56), suggesting that these cell surface biomarkers do not mark BA progenitors in WAT *in vivo*. Since gene expression patterns of most of the BA-specific biomarkers *in vitro* were not replicated under physiological conditions (18), the origin of BAs in WAT cannot be determined exclusively based on *in vitro* studies which need a stringent validation *in vivo*.

WA-to-BA Conversion

Transdifferentiation is commonly defined as a conversion of one differentiated cell type to another cell type, usually from the same lineage, without returning to a pluripotent state (64). It may occur as a result of epigenetic changes, somatic mutation, and/or environmental cues that affect the expression of master control genes necessary to maintain a physiological function of mature cells. Transdifferentiation represents one of the mechanisms by which mature cells adapt to changes in the external environment. Therefore, as an adaptation to cold exposure mature WAs might acquire the BAP, but as opposed to known examples of transdifferentiation this adaptive mechanism is reversible.

The evidence that WAT acquires the BAP in response to cold exposure and re-adapt to the warmth by involution of the BAP was reported over 20 years ago (65,66). Since then, multiple studies indicated that BAs in WAT appear through a direct WA-to-BA conversion accompanied by molecular and morphological changes, from unilocular to multilocular structure, and increased thermogenic activity (67,68); however, this mechanism seems to be depot-specific. BAs in iWAT appear as a result of conversion of pre-existing unilocular adipocytes that acquired the BAP upon a β3-adrenergic receptor agonist treatment (61) or cold stimulation (14). Similarly, overexpression of *Prdm16* activated the BAP in iWAT while there were no morphological and/or molecular changes in eWAT (69). Deletion of the cannabinoid receptor type 1 induced conversion of inguinal WAs into thermogenically active BAs, evident by increased expression of BA biomarkers, mitochondrial biogenesis and oxygen consumption, while *Ucp1* and *Pgc1a* mRNA levels were decreased in eWAT (70). Cold exposure up-regulated the expression of BA biomarkers in iWAT but not eWAT; however, it was only a transient effect lost after 5 weeks of re-acclimation at 23°C (10). During adaptation to the warmth BAs converted into WAs, which re-acquired the BAP upon repeated cold exposure, suggesting that a mechanism of physiological adaptation to variation in ambient temperature in iWAT occurs in both directions. The induction of *Ucp1* mRNA in iWAT reaches half of its maximal levels after only 12 h of cold exposure and decreases by 40% after 1 day at 29°C (43). Therefore, it is likely that the BAP, at least in sWAT from adult mice, is acquired during WA-to-BA conversion rather than recruitment of BAs from progenitors.

TABLE 1 Anatomical and molecular characterization of murine and human classical and BAs dispersed within WAT

	Mice			Humans	
	Cellular origin	Anatomical distribution	Molecular biomarkers	Anatomical distribution	Molecular biomarkers
Classical BAs	*En1*$^+$, *Pax7*$^+$ *Myf5*$^+$, *Pax3*$^+$	Interscapular	*Zic1* (18) *Zic1, miR-206, Lhx8* (17,39) *Zic1, Lhx8, Epstl1* (17,39,62) *Eva1, Pdk4* (40,62)	Interscapular Deep neck	*ZIC1* (19) *ZIC1, LHX8* (23)
BAs dispersed in WAT	*Myf5* $^-$, *Pdfr*α^+ rWAT: *Myf5*$^+$, *Pax3*$^+$	Subcutaneous WAT Visceral WAT	*Hoxc9* (18) *Hoxc9, Shox2* (17,39) *Fgf21, Car4, Cited1* (62) *Cd40, Tbx1, Cd137, Tmem26, Slc27a1* (40)	Supraclavicular Periadrenal	Supraclavicular: *miR-206, miR-133b, LHXB8, ZIC1, TBX1, TMEM26* (74) *MTUS1, KCNK3* (73) *TMEM26, SHOX2, TBX1* (19) Periadrenal: HOXC9, EVA1, ZIC1 (19)

According to current knowledge on the origin of BAs in WAT we cannot determine a definite and uniform mechanism for acquisition of the BAP. In support of morphological and kinetic studies described above, any mechanism for induction of BAs in WAT based on progenitors needs to be consistent with kinetics of induction of *Ucp1* (43) and *Pgc1a* (47), for iWAT and rWAT, respectively, that becomes maximally expressed within 12 h after cold stimulus. Two independent studies suggest that BAs in iWAT arise from direct conversion of preexisting WAs (10,61) while one demonstrated cold-induced de novo proliferation of BAs in sWAT (27). Since the average number of adipocytes is characteristic for a WAT depot and each individual, de novo proliferation of BAs needs to be accompanied by corresponding cell degradation. RFP$^+$ labeling in iWAT was evident even after re-adaptation to warm environment suggesting the disappearance of the BAP not dead cell removal (10). Upon re-adaptation to thermoneutrality mitochondria of adipocytes in iWAT undergo dynamic structural changes (43) further indicating a phenotypic BA-to-WA switch rather than adipocyte death. Since eWAT consists of bi-potential progenitor cells able to increase thermogenic or storage capacity in response to distinct external signals (61) the existence of one mechanism for the induction of the BAP does not necessarily exclude the other; however, it seems to be depot-specific.

The Molecular Signature of Human BAT

Two murine experiments demonstrated that BAs in WAT versus classical BAs were similar in terms of thermogenic potential in response to cold stimulation (43,71); however, only mitochondria from iBAT retained their normal function in response to changes in ambient temperature and were not degraded at thermoneutrality (43). While, the activity of iBAT is relatively similar among distinct strains of mice the number of BAs and UCP1 content within WAT is a highly variable genetic trait (9,11). Although, thermogenic activity of human BAT varies enormously with respect to sex, age, adiposity, and within individuals from the same age and ethnic group (1,3,72), it is unknown whether this variation is specifically restricted to BAs in WAT or also includes classical BAs.

The molecular identity of human supraclavicular BAs resembled murine BAs in WAT rather than iBAT (40), while human neck BAT was similar to murine iBAT (23) (Table 1). Supraclavicular, retroperitoneal or intra-abdominal human BAT, expressed biomarkers specific for BAs in WAT, e.g., *HOXC9* or *CITED1* (62). Human brown preadipocytes expressed multiple genes of the smooth muscle lineage (73) similar to BAs in mouse WAT (63). Recent analysis of clonal brown preadipocyte cell lines generated from SVF obtained during biopsies of supraclavicular BAT confirmed molecular resemblance between human BAT and murine BAs in WAT; however, several genes encoding molecules that control thermogenesis, mitochondrial function and fatty acid oxidation were expressed in human BAT biopsies as well as mouse iBAT and BAs in WAT (73). An independent study showed that supraclavicular BAT in humans expressed biomarkers for classical BAs and markers specific for BAs in WAT (74) indicating that human BAT might be composed of a mixed population of BAs. Two types of BAs, those in the interscapular region and those dispersed within unilocular WAs, have been distinguished in neonatal and adult humans (19).

β-Adrenergic Stimulation of BAT in Humans

Current research utilizing ^{18}fluoro-2-deoxyglucose positron emission tomography (^{18}FDG-PET) scans showed that the amount of BAT is negatively correlated with adiposity in adult humans (1-3). Short-term exposure (2 h) to 16-17°C increased resting metabolic rate in 10/10 lean and 13 out of 14 overweight or individuals with obesity, whose BAT activity was, in general, significantly lower than in healthy subjects (2). Daily exposure to 15.5°C for 2.5 h increased

glucose uptake, oxidative metabolism and blood flow in the supraclavicular BAT and enhanced EE in 9 individuals characterized by high basal BAT activity (75). Long-term exposition to 15-16°C for 2 h per day significantly increased EE through non-shivering thermogenesis, glucose uptake and the amount of BAT in 16 out of 17 individuals indicating the recruitment of human BAT upon chronic cold stimulation (76). Exposure to 17°C for 2 h per day for 6 weeks resulted in a two fold increase in BAT activity, stimulation of EE and a significant decrease in fat mass (77) indicating that long-term mild cold stimulation exerts favorable effects on adiposity in humans.

In contrast to rodent studies, isoprenaline treatment in humans had no effect on BAT activity, despite an increase in EE (78). Administration of ephedrine did not alter BAT activity as measured by PET/CT scans, while it significantly increased heart rate and blood pressure, as opposed to cold exposure which increased BAT thermogenesis, induced EE and oxygen capacity measured by indirect calorimetry, decreased heart rate and had little effect on circulating blood metabolites (79). Administration of mirabegron increased BAT thermogenesis and resting metabolic rate with fewer side-effects on the cardiovascular system as compared to other sympathomimetic drugs (80). While developing an effective and safe pharmacological approach to stimulate BAT in humans is a time-consuming process, there is promising evidence that environmental stimuli, e.g., mild cold-exposure, represent an alternative and feasible way to increase EE in individuals with obesity (76).

Conclusion

Since thermogenic potential of iBAT in mice remains relatively stable throughout life, maintaining iBAT in adult humans might provide a constant level of BAT activity; however, adult human BAT resembles murine BAs in WAT. Therefore, development of a powerful strategy to fight obesity epidemics would be to increase the number and/or thermogenic capacity of BAs in WAT, rather than target the activity of iBAT. The mean number of adipocytes in mice (27) and humans (38) is relatively constant, indicating that the total number of adipocytes is determined during the early postnatal period (11). Strain- and depot-specific differences in the ability to acquire the BAP in WAT in mice and enormous interindividual variation in the amount and activity of BAT in humans indicate that this trait is determined genetically. Therefore, the maximal amount and thermogenic capacity of BAs in humans might be strictly regulated and fixed for a depot and each individual. Thus, it is crucial to determine when BAs in humans acquire their functional structure that could be stimulated to have a significant effect on adiposity. O

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