[Frontiers in Bioscience 16, 1233-1260, January 1, 2011]

Brown fat biology and thermogenesis

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TABLE OF CONTENTS

1. ABSTRACT

Brown fat (brown adipose tissue, BAT) primary function is to produce heat. There is now compelling evidence to indicate that brown fat cells in some BAT depots share their predecessor cells with myocytes. Brown adipocyte (trans)differentiation depends on various receptors / transcription factors that include peroxisome proliferator-activated receptor γ (PPARγ), PPARγ-coactivator-1α (PGC1α), PRD1-BF1-RIZ1 homologous domain-containing 16 (PRDM16), CCAAT/enhancer-binding protein β (C/EBP-β) and bone morphogenetic protein 7 (BMP7). Such mediators also help BAT to acquire its thermogenic phenotype, which is essentially conferred by uncoupling protein 1 (UCP1). UCP1 uncouples adenosine-5'-triphosphate (ATP) synthesis from substrate oxidation in brown adipocytes. Its activity depends on the availability of fatty acids delivered upon BAT's β-adrenergic activation, which, physiologically, ensues from the sympathetic nervous system (SNS) activation of the tissue. SNS-mediated thermogenesis is largely controlled by the hypothalamus and brainstem. Recently, positron emission tomography / computed tomography (PET/CT) scanning investigations have revealed the presence in adult humans of important neck and shoulder BAT depots. That finding has contributed to reinstate a strong interest for brown adipocyte biology and thermogenesis. This review aims at the unique biology of BAT with the emphasis put on

the recent discoveries regarding the brown adipocyte development and function.

2. INTRODUCTION: OVERVIEW OF BROWN FAT

BAT is a specialized tissue whose function is to produce heat (1). BAT is found in relative abundance in small eutherian mammals, in which it appears distributed in discrete small depots. In rats and mice, BAT depots are found in the interscapular, subscapular, axillary, perirenal, and periaortic regions. The thermogenic potential of BAT is such that it allows small mammals to live in cold environments without relying on the shivering process to produce heat (2, 3). Early studies have demonstrated that BAT contributes to more than 60% of nonshivering thermogenesis in cold-adapted rats (4) and hamsters (5).

Histologically, BAT cells differ from white adipose tissue (WAT) adipocytes, which are mainly fat reservoirs. In contrast to white fat cells, which contain a single large lipid droplet and few mitochondria, brown adipocytes contain numerous small lipid vacuoles surrounded by a large amount of well-developed mitochondria packed with cristae. It is the presence of those numerous mitochondria containing iron pigmented-cytochromes (and perhaps other oxidative pigments associated with electron transfer) that gives BAT its brownish color. Recent investigations (6-9) have provided compelling evidence that brown and white fat cells are even more distinct than originally thought as they can origin from different precursor cells (see below). BAT cells in so-called classical brown fat depots (for instance interscapular brown fat) would share their origin with myogenic-factor 5 (Myf5)-expressing cells, which are also primordial cells for myocytes.

The outstanding thermogenic potential of BAT in conferred to by UCP1 (1, 10). UCP1, the archetypical UCP, is found in the inner membrane of brown adipocyte mitochondria. UCP1 uncouples mitochondrial respiration from ATP synthesis (11). When activated, it causes a leak that dissipates the electrochemical proton gradient ($\Delta\mu H^+$) that builds up across the inner mitochondrial membrane during BAT fatty acid oxidation. $\Delta\mu H^+$ represents the proton motive force used in most tissues to drive the conversion of adenosine-5′-diphosphate (ADP) to ATP by ATP synthase. Because it is uniquely found in brown adipocyte, UCP1 constitutes the ultimate marker of these cells.

The control of BAT activity depends on the adrenergic stimulation of brown adipocytes (1, 12). BAT depots are densely innervated by efferent branches of the SNS. SNS nerve endings reach individual brown adipocytes and release noradrenaline, which stimulates BAT thermogenic activity by binding to adrenoreceptors receptors, such as the β3 adrenergic receptor. The latter is a prominent actor in driving the cascade of events necessary for heat production in rodent BAT (13). When stimulated, adrenergic receptors trigger a series of metabolic events leading to the activation of adipose triacylglycerol lipase (ATGL), a rate-limiting step in the lipolytic release of fatty acids. In BAT, fatty acids act as substrates for β-oxidation and as stimulators of UCP1 activity (1).

The thermogenic capacity of BAT, which is set by the expression of numerous genes encoding proteins involved in UCP1 expression / activity, substrate oxidation, mitochondriogenesis, and brown fat cell differentiation / trandifferentiation, is primarily determined by the adrenergic stimulation of the tissue. Non-adrenergic activators such as PPARγ agonists (15, 16) can also increase BAT capacity.

The physiological control on SNS-mediated BAT thermogenesis is insured by the integrated and coordinated activity of various brain regions. It involves numerous populations of neurons whose individual solicitation might depend on whether BAT thermogenesis is influenced by environmental temperature (17) or variations in energy balance and nutritional status (18).

The notion that brown adipocyte thermogenesis be a component of energy expenditure has been debated for years (1, 18, 19). However, one can hardly refute some implication of BAT in energy balance, based on the facts that BAT represents a major thermogenesis effector and that BAT receives a major innervation emerging from several brain regions involved in energy balance regulation. Indeed, studies in laboratory rodents have accumulated to demonstrate that the major neuromediators and circulating hormones or nutrients that affect energy balance engage BAT thermogenesis (18).

Up until lately, it was almost unanimously thought that BAT was not present in adult humans. Recently however, nuclear medicine investigations (20-24) have strongly challenged this view that adult humans only possess traces of brown adipocytes. PET/CT scanning explorations, mainly to diagnose cancers have revealed important neck and shoulder BAT depots, whose prevalence could largely be determined by environmental temperature, age, sex, body fat mass and diabetes status (25-27).

In recent years, major progress has been made in understanding the molecular biology / physiology of the brown adipocyte (14, 28, 29) and in deciphering the neural and hormonal control of SNS BAT and WAT thermogenesis (17, 30). Those findings, together with the recent demonstration that BAT can be present in substantial abundance in man, have created a renewed interest for this unique tissue. This chapter aims at reviewing the unique biology and physiology of BAT with an emphasis put on the recent discoveries regarding BAT development and thermogenesis.

3. THE BIOLOGY OF THE BROWN ADIPOCYTE

In contrast to white fat cells, brown adipocytes are not primarily committed to fat storage, but mostly adapted to dissipate chemical energy in the form of heat (1). Histologically, brown fat cells contain a high

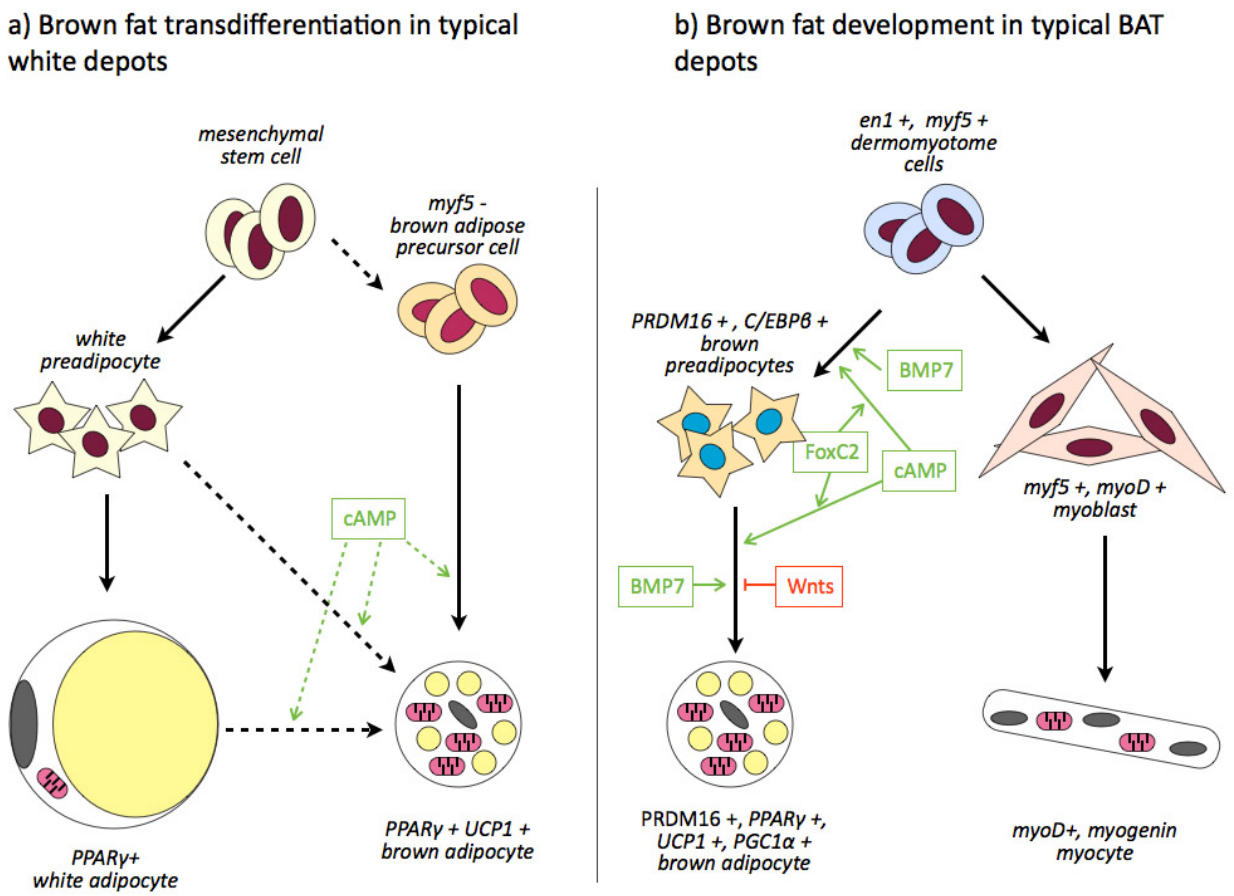

Figure 1. Brown adipocyte origins (26, 29, 32, 38). Brown fat cells development in so-called classical WAT (panel a) and BAT (panel b) have different origins. In WAT depots, brown adipocytes would either originate from the maturation of a pre-existing brown adipocyte precursor cells, differentiate from white preadipocytes or transdifferentiate from mature white adipocytes. In brown depots, brown adipocytes would emerge from a myoblastic lineage. Dashed lines = unsettled pathways; green = stimulating factors; red = inhibitory factors.

mitochondrial density, multilocular lipid inclusions and UCP 1 in their mitochondrial inner membrane (31). Recent comprehensive reviews have clearly exposed the molecular pathways that distinguish brown from white adipogenesis (29, 32, 33). The role of proteins such as PPARγ and PGC1α, which represent major actors not only controlling adipogenesis but also dictating the heat-producing phenotype of BAT, have also been largely clarified (34).

There is now compelling evidence (6-9, 35) to suggest that brown adipocytes from classical BAT depots share their precursor cells with myocytes (Figure 1). Molecules such as PRDM16 (8) and C/EBP-β (36) are regarded as critical molecules involved in the development of brown adipocytes from myoblastic lineage. Meanwhile, there is still uncertainty regarding the formation of brown fat cells in WAT depots. It appears that brown adipocytes in typical WAT (for instance gonadal fat) would either originate from the maturation of a pre-existing brown adipocyte precursor cell (37), differentiate from white preadipocytes, or transdifferentiate from mature white adipocytes (29, 38, 39) (Figure 1).

3.1. PPARγ and PGC1α are key regulators of brown fat cell differentiation

PPARγ is regarded as a master protein for adipocyte differentiation from pre-adipocyte (32-34). This evidence has emerged from various types of studies including murine genetic studies that have decisively confirmed that PPARγ controls the formation of all adipocytes, be they white or brown (40, 41). Genetic mutations lowering PPARγ activity induce a reduction in BAT development and its thermogenic function (42). Importantly, PPARγ appears not to be required for the early establishment of BAT, but rather for its elaboration (41). PPARγ stimulation by specific agonists enhances the thermogenic machinery of brown adipocytes, even in the absence of the adrenergic drive that promotes fatty acid oxidation (43, 44).

PGC1α was discovered in 1998 and described as a transcriptional switch that stimulates mitochondrial genes in BAT in response to cold exposure (45). PGC1α is a transcriptional co-activator that highly contributes to provide BAT with its energy-burning phenotype. Its expression is induced by activation of the protein kinase A (PKA)-cyclic-AMP response element-binding protein (CREB) pathway after adrenergic stimuli (46). It is also elicited by stimulation of adipocyte determination and differentiation-dependent factor 1 (ADD1) / sterol regulatory element-binding protein-1c (47) or by repression of the Wnt signaling cascade (48, 49). Deacetylation of PGC1α by Sirtuin 1 (Sirt1) has emerged as an important regulatory mechanism in BAT, as Sirt1 agonism stimulates PGC1α transcriptional activity (50, 51). Post-translational modifications also play a significant role in the production of PGC1α. For example, p38-mitogen-activated protein kinase (MAPK)-mediated phosphorylation of the residues threonine (Thr) 262, serine (Ser) 265 and Thr 298 disrupts the repression on PGC1α expression induced by p160 myb binding-protein found in normal states (52). Supporting the concept that PGC1α is crucial to brown adipocyte function are also results showing that mice with genetic ablation of leucine-rich protein 130 (Lrp130), which interacts with and modulates PGC1α effects on BAT development, display a reduced UCP1-induced proton leak (53).

3.2. Brown adipocytes in classical BAT depots emerge from dermomyotome cells

Despite their different ultrastructural and metabolic morphologies, it was long believed that white and brown fat cells derived from common cell precursors. This concept was based on the ability of both fat cells to not only store and release lipids but also rely on PPARγ at specific times during the cell evolution to maturity (32, 34, 54). However, recent observations have greatly challenged the above belief. Atit *et al* (35), using a genetic fate mapping approach, demonstrated that engrailed 1 (En1)- and Myf5-expressing cells of the dermomyotome are precursors of not only the dermis and muscle but also interscapular BAT. Concomitantly and based on mRNA microarray analyses, Timmons *et al* (6) made the seminal discovery that brown preadipocytes (from classical brown fat depots) demonstrated a myogenic transcriptional profile, suggesting that brown adipocytes and myocytes emerged from a common cell lineage. Meanwhile Seale *et al* (8, 55), Tseng *et al* (7) and Kajimura *et al* (36) decoded the sequence of events leading to brown fat. Seale *et al* (8, 55) identified PRDM16 as a major transcription factor in BAT triggering brown adipocyte differentiation, mitochondrial biogenesis and expression of UCP1 while Tseng *et al* (7) showed that BMP7 could trigger the commitment of mesenchymal progenitor cells to a brown adipocyte lineage while inducing early regulators of brown fat such as PRDM16 and PPARγ and PGC1α. Kajimura *et al* (36) demonstrated that PRDM16 controls brown adipogenesis from myoblasts by forming a transcriptional complex with the active form of C/EBPβ. Further supporting the notion that brown adipocytes and myocytes may derive from a common cell lineage are the studies by Forner *et al* (56) and Walden *et al* (57). Forner *et al* (56) demonstrated that the proteomics of brown fat corresponded more to that of muscle than to that of white fat. Walden *et al* (57) showed that the muscle microRNAs (myomiR) miR-1, miR-133a and miR-206 were expressed in brown but not white adipocytes. The term "adipomyocyte"' has been coined by Cannon *et al* (58) to designate the brown adipocytes in classical BAT depots.

3.3. PRDM16 induces brown fat from a myoblastic lineage

PRDM16 is a gene selectively, but not exclusively, expressed in brown adipocytes (55). Its transcription is induced during brown fat differentiation, and overexpression of PRDM16 stimulates the transcriptional program of brown adipogenesis. Overexpressors of the gene also enhance BAT mitochondrial density and function (55). PRDM16 transactivates PGC1α and docks it (55). Similarly, PRDM16 also binds to and activates PPARγ (8).

Seale *et al* (8) reported that skeletal myoblasts that overexpress PRDM16 differentiated into brown adipocytes. Meanwhile, Seale *et al* (8) reported that shRNA-induced loss of PRDM16 not only blunted brown fat characteristics, but also caused a switch to promote myogenic differentiation (8, 55) in line with the initial observations made by Timmons *et al* (6). Additionally, mouse embryos deprived from the PRDM16 gene had an altered BAT tissue, in which expression of brown-fat genes was reduced and expression of muscle-specific genes was robustly enhanced (8). More recently, it was demonstrated (36) that PRDM16 forms a transcriptional complex with the active form of C/EBPβ to enhance the establishment of a brown fat lineage through sustained transactivation of PPARγ and other BAT genes. Interestingly, mice with engineered replacement of C/EBPα with C/EBPβ have decreased white fat while exhibiting higher energy expenditure (59), which may be due to a reprogramming of white fat expression profile, including enhanced transactivation of PGC1α (60). Kajimura *et al* (36) demonstrated that the forced induction of PRDM16 and C/EBPβ was sufficient to yield a fully functional brown fat program from naive fibroblastic cells.

3.4. UCP1-expressing cells in WAT could emerge from varied origins

Not all UCP1-expressing adipocytes share their progenitor cells with myocytes (8). In fact, cells in classical WAT that exhibit characteristics of brown fat cells do not originate from dermomyotome cells but are more closely related to white fat cells. The lack of a pure white adipocyte marker has hindered cellular tracking of brown adipocytes in WAT (37, 54). However, there is evidence that white and brown adipocytes in WAT can emerge from a common Myf5-negative cell precursors expressing Hoxc9 (37, 62). It is also possible that brown fat cells in classical WAT might also derive from white preadipocytes or mature white adipocytes that could differentiate or transdifferentiate into brown fat cells (29, 38, 39). Differentiation / transdifferentiation of white adipocytes in brown fat cells might be induced by cold or β-adrenergic agonism (63-65), treatment with retinoic acids (66), and PPARγ activation (15, 16). The brown adipocytes in WAT depots, which do

not abundantly express PRDM16, have recently been referred as "brite" (brown-in-white) adipocytes (37).

The hypothesis that mature white adipocytes transdifferentiate (rather than strictly differentiating) into brown fat cannot be refuted (38,39). Supporting this hypothesis is a series of experimental observations, which were comprehensively reviewed lately (39). Among those observations are the demonstrations (i) that the total number of adipocytes (white plus brown) in a given WAT depot does not change after cold acclimation while the proportion of brown adipocytes significantly increases (67), (ii) that the newly emerging brown adipocytes in WAT following β3-adrenergic stimulation are 5-bromo-2-deoxyuridine (BrdU)-negative (indicating a low mitotic index) (65), (iii) that the just-formed brown-like adipocytes in WAT after either cold acclimation or β3-adrenergic stimulation exhibit an intermediate phenotype between white and brown adipocytes (39).

There is strong evidence for the implication of PPARγ in brown adipocyte differentiation / transdifferentiation (15, 28, 32, 68, 69). WAT in P465L PPARγ mutant mice does not display brown adipocytes in WAT in response to cold (42). The role of PPARγ in WAT acquirement of BAT-like characteristics is further illustrated in response to PPARγ agonists. Chronic administration with PPARγ ligands in mice induces transcriptional reprogramming in white adipocytes, including upregulation of the brown adipocyte marker UCP1 (44). In human white adipocytes, similar acquirement of UCP1 expression can also be achieved by treatment with PPARγ agonists (70, 71). It is noteworthy that overexpression of PRDM16 does not induce WAT differentiation into BAT (8). This is consistent with the observation that white adipocytes treated with PPARγ agonists to differentiate / transdifferentiate into brown adipocytes barely express PRDM16 (37). Modulation of the activity of PPARγ / PGC1α expression and cooperation by other cofactors such as retinoblastoma protein (Rb) (72) have also been shown to serve as a molecular node that controls the switch between white and brown adipocytes *in vitro* (73) and *in vivo* (74).

Interestingly, acquirement of brown phenotype by white adipocytes has now been established in several animal models in which lipid metabolism in WAT depots is impaired (75, 76). For example, hormone-sensitive lipase (HSL) knockout mice have a 7-fold increase in UCP1 and enlarged mitochondria in WAT compared of wild-type littermates (77). Similarly, mice deficient in the fat specific protein-27 (Fsp27), which targets triglyceride storage functions around lipid droplets in adipocytes, knocked out for the autophagy-related 7 gene (Atg7), or for the activating transcription factor 4 (Atf4) gene, are all characterized by enhanced mitochondrial activity and energy consumption in WAT (78-80).

Other transcription factors or proteins, which could play a major role in the elaboration of brown fat cells from white adipocytes, include FoxC2, a member of the forkhead transcription factor family that has been shown to promote brown fat depot development in mice (81). In fact, mice overexpressing FoxC2 specifically in fat (under the control of the aP2 promoter) exhibit an important reduction in WAT with a robust enlargement of BAT (81). BAT of these mice has increased expression of PKA and many mitochondrial genes, which sensitize to β-adrenergic stimuli, resulting in enhanced energy expenditure and insulin sensitization and resistance to diet-induced obesity. This reprogramming also comprises the C/EBPα-induced repression of white adipocyte genes through recruitment of the nuclear-corepressors carboxy-terminal binding proteins 1 and 2 (CtBP1/2) (82). Adipose tissue-specific deletion of raptor also results in enhanced mitochondrial function in WAT (83).

4. UCP1

UCP1 was discovered and isolated at the end of the nineteen seventies (84-86) and it was further characterized in the nineteen eighties (87-89). UCP1, which has also been referred as thermogenin (prior to the discovery of other UCPs), has a molecular weight that ranges between 32 and 33 kDa depending on the species. It is encoded by a single nuclear gene (6 exons) located on chromosome 8 of the mouse genome and chromosome 4 in humans (90).

4.1. UCP1 has evolved as the only thermogenic UCP

UCP1 is a member of the UCP superfamily, itself derived from the broader mitochondrial carrier protein superfamily, which comprises metabolite transporters of the inner mitochondrial membrane such as the adenine nucleotide translocator (ANT), the phosphate carrier (PiC), the aspartate / glutamate carrier (AGC) (90-92). The UCP superfamily was first described to include UCP2, UCP3, UCP4, UCP5 brain mitochondrial carrier protein 1 (BMCP1), which are all expressed in mammals, and proteins homologous to the mammalian UCPs described in plants. However, a reconstructed phylogeny of the mitochondrial carrier protein superfamily currently argues against the inclusion of UCP4 and UCP5 in the UCP superfamily; UCP4 and UCP5 do not share the ancestor gene common to UCP1-3 and plant UCPs (92). UCP1, UCP2 and UCP3 are thought to have developed from a single ancestor through a series of gene duplications (92). Eutherian UCP1 evolved to be the only UCP with a recognized thermogenic function, which is conferred by a proton conductance attribute blockable by nucleotides and activable by fatty acids. UCP1 in ectotherms and non-eutherian mammals (there exist several orthologs of UCP1), as well as UCP2 and UCP3 do not possess that nucleotide-sensitive proton conductance property, which is apparently determined by the presence in the UCP1 protein of a glutamic acid in position 134, which would be occupied by aspartic acid in all other UCPs (92). UCP1 is a 6-domain transmembrane protein (triplication of dimers across the mitochondrial inner membrane) with the position 134 being part of the hydrophilic loop that connects transmembrane domains 3 and 4 (90, 91).

The physiological function of newly discovered UCP1 orthologs as well as UCP2 and UCP3 remains enigmatic (93, 94). These UCPs, as recently pointed out by Klingenspor *et al* (91), could have retained some UCP ancient functions such as the control of oxygen species, while eutherian UCP1 has evolved as a thermogenic protein. The suggestion has been made that the lack of apparent thermogenic function of UCP2 and UCP3 might

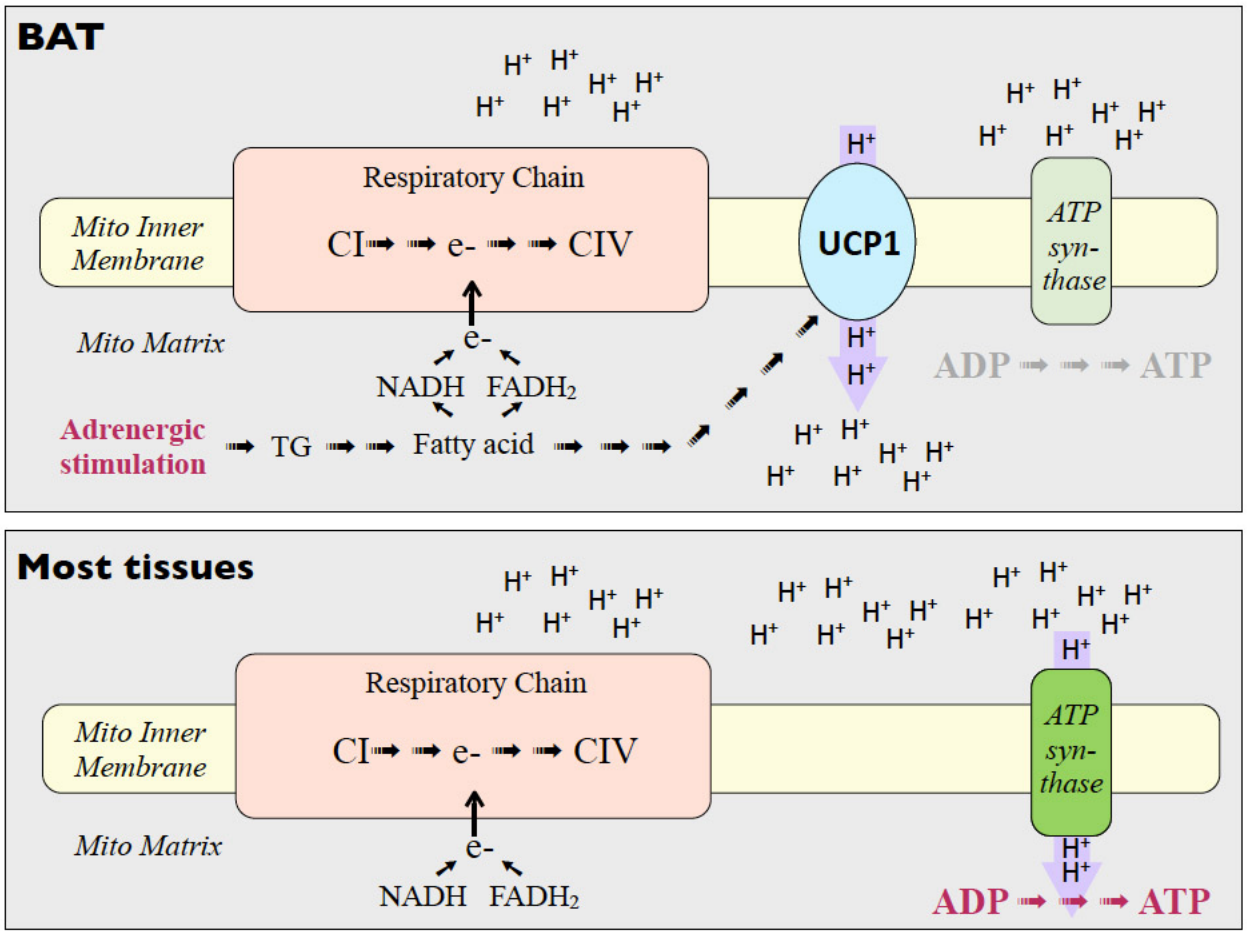

Figure 2. Mechanisms of heat production in BAT. UCP1 confers BAT its outstanding thermogenic power. This protein is found in the inner mitochondrial membrane and serves as a proton translocator. Once activated UCP1 leads to the dissipation of the $\Delta\mu H^+$ that builds up across the mitochondrial inner membrane in BAT during substrate oxidation. $\Delta\mu H^+$ represents the proton-motive force that drives phosphorylation of ADP to ATP by ATP synthase. In stimulated brown adipocytes, ATP synthesis is low and ATP accumulation does therefore not represent a constraint to decelerate the activity of heat releasing catabolic pathways. UCP1 is activated by fatty acids, which override the inhibitory action of purine nucleotides on UCP1. Fatty acids also constitute the energy substrate for BAT heat production while generating the electron donors, NADH and $FADH_2$. The passage of NADH- and $FADH_2$-derived electrons through the various protein complexes of the respiratory chain (electron transport chain) is coupled with the pumping of protons from the mitochondrial matrix to the intermembrane space to establish the $\Delta\mu H^+$ across the inner mitochondrial membrane.

relate to their low tissue concentration (95), assuming that those proteins are true UCPs. However, it is far from being certain that UCP2 and UCP3 are indeed uncouplers (96, 97). Bouillaud *et al* (97) have proposed that UCP2 could serve as a pyruvate transporter insuring the export of pyruvate from mitochondria to the cytosol. This export would limit glycolytic-derived pyruvate utilization and favors lipid oxidation.

4.2. UCP1 confers BAT its extraordinary thermogenic power

Studies conducted in UCP1-ablated mice have demonstrated the importance of UCP1 in non-shivering thermoregulatory thermogenesis (98). In absence of UCP1, mice become cold-sensitive and utilize alternative mechanisms to produce heat (99). UCP1-deficient mice rely on shivering to produce heat (99), a process that is seemingly energetically more costly than BAT nonshivering thermogenesis to insure body temperature maintenance (99). The extent to which UCP-ablated mice are cold sensitive depends on their genetics background. UCP1-KO mice of inbred backgrounds, in contrast to UCP1-KO mice of hybrid background, cannot tolerate low temperature (4ºC) unless they are gradually adapted to cold (100). It is the current general consensus that UCP1 is the sole UCP physiologically involved in thermogenesis (94, 101, 102).

4.3. UCP1 is a nucleotide-sensitive metabolic uncoupler

UCP1 serves as a proton translocator across the mitochondrial inner membrane (1, 11, 90, 103) (Figure 2). Once activated, UCP1 leads to the dissipation of the $\Delta\mu H^+$

that builds up across the mitochondrial inner membrane during BAT oxidation of fatty acids. $\Delta\mu H^+$ represents the proton-motive force that drives phosphorylation of ADP to ATP by ATP synthase. In stimulated brown adipocytes, ATP synthesis is low and ATP accumulation does therefore not represent a constraint to decelerate the activity of heat-releasing catabolic pathways (11).

UCP1 is activated by fatty acids, which override the inhibitory action of purine nucleotides on UCP1 (1, 11, 90, 103) (Figure 2). Fatty acids also constitute the energy substrate for BAT heat production while generating the electron donors, nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$). The passage of NADH- and $FADH_2$-derived electrons through the various protein complexes of the respiratory chain (electron transport chain) is coupled with the pumping of protons from the mitochondrial matrix to the intermembrane space to establish the $\Delta\mu H^+$ across the inner mitochondrial membrane. The production of fatty acids in BAT largely emerges from the breakdown of intracellular triglycerides by the stimulation of AGTL in response to the β-adrenergic activation of the brown adipocyte. The β3-adrenergic receptor is regarded, at least in laboratory rodents, as the key adrenergic receptor triggering BAT activity (13). Fatty acids are transformed into acyl-CoA and transported into mitochondria after being transformed into acyl-carnitine through the action of the enzymes carnitine palmitoyltransferase 1b (CPT1b).

The mechanism underlying both the UCP1-mediated-uncoupling process and the activating action of fatty acids on UCP1 has been the object of debates and series of elegant and complex investigations (103-105). The currently privileged hypothesis is that UCP1 acts as a proton translocator whose basal activity is inhibited in non-thermogenic conditions by purine nucleotide and activated upon adrenergic stimulation. According to that hypothesis (103, 105), the fatty acid carboxyl groups could serve as proton catalysts, binding protons and delivering them to a site on UCP1 from which they would be translocated to the matrix side of the mitochondrial inner membrane. This model described as the proton-buffering model seems currently preferred over the fatty-acid-cycling hypothesis (104). According to the latter, UCP1 would translocate fatty acid anions from the matrix to the intermembrane side as one step of a protonophoric cycle whose other step would be the flip-flop of protonated fatty acids within the lipid layer of the inner membrane to insure the passage of the protons from the intermembrane space to the matrix. A fundamental argument against the fatty-acid-cycling hypothesis is the demonstration that compounds such as long-chain alkylsulfonates, which share resemblance with fatty acids, readily allow for proton conductance, despite the fact that they cannot act as a mobile proton carrier as they cannot be protonated at physiological pH (103, 106).

4.4. UCP1 expression is controlled at the transcriptional level

The knowledge of the molecular mechanisms regulating the transcription of UCP1 has rapidly progressed over the last two decades. Several groups have described an enhancer region that is, in humans, about 3.7 kb upstream of the start codon and that contains several putative sites for direct binding and transactivation by nuclear receptors, transcription factors, and nuclear cofactors for fine tuning (107). A 350 bp human enhancer region shares very significant homology with enhancers regions found in either mice or rats (108). Studies on the promoter of the mouse UCP1 gene have also found the presence of *cis*-acting cAMP, thyroid hormone receptor (TR) and PPARγ response elements, referred to as CRE, TRE, and PPRE, respectively, which are comprised in a 221 bp enhancer sequence about 2.5 kb upstream of the transcription initiation site (109-115). Other nuclear receptors such as estrogen-related-receptor α (ERRα) could also be anchored on UCP1 promoter region to mediate actions on UCP1 expression (116). PGC1α is directly involved in the stimulation of UCP1 expression through its ability to interact with TR and PPARγ (45). PGC1α also interacts with MED1 subunit of the mediator complex, which is an important step into the recruitment of the basal transcriptional machinery (117). The importance of the TR pathway has yet to be fully ascertained, as deiodinase iodothyronine type 2 (Dio2) knockout mice, which have reduced triiodothyronine (T3) levels because of altered conversion of the hormone in BAT, are characterized by a robust reduction in thermogenesis despite normal UCP1 induction upon cold exposure (118, 119).

For obvious reasons, several groups have used cold exposure and adrenergic stimulation to study the early molecular events that are involved in UCP1 transcription. Extensive work by Robidoux *et al* (120) showed that the β-adrenergic pathway triggers PKA activation, transmitting to MAPK kinase 3 and then to p38 MAPK (121), which phosphorylates and activates PGC1α and transcription factor 2 (ATF-2) (110). It was also demonstrated that the induction of PGC1α and UCP1 expression can be dissociated, as cold exposure, in mice lacking β-adrenergic receptors, activates PGC1α whereas, in sharp contrast, does not stimulate UCP1 (122). Recent evidence also showed that as UCP1, PGC1α is required for cold-induced thermogenesis (123). In addition, cold exposure upregulates levels of CREB (114), PPARγ, TRβ, and PPARα (124), which all contribute to increase UCP1 promoter transactivation. In addition, a recent study showed that orphan nuclear receptor NOR-1 (125) bound a sequence 5.6 kb upstream of the starting codon that augmented the activity of the 3.7 enhancer induced by β-adrenergic / cyclic AMP stimulation, indicating a complex interplay in different regions in the UCP1 promoter. Other transcription factors that are able to stimulate UCP1 expression, but whose levels are not upregulated upon cold exposure, are the retinoic acid receptor (RAR), retinoic X receptor (RXR), nuclear factor erythroid-derived 2-like 2 (NFE2L2) (114) and AP-1 (124).

Examples of negative regulation of UCP1 expression are scarce but exist. In mice, genetic ablations of the vitamin D receptor (126), the nuclear coactivator TIF2 (127) or the nuclear corepressor RIP140 (128) lead to

a robust upregulation of UCP1 expression in BAT. TIF2 was shown to compete with its family member steroid-receptor coactivator 1 (SRC-1) for docking and stabilization of the PPARγ / PGC1α complex in BAT (127). Because of binding motifs in the TIF2 protein sequence, the TIF2 / PPARγ / PGC1α is less active and, as a result, SRC-1 knockout mice have lower UCP1 levels (127, 129). Altered access of transcription factors to the UCP1 promoter through modulation of histone status (acetylation or methylation) has been suggested to contribute to the inhibitory impact of RIP140 (130). As a corepressor, RIP140 also blunts the transcriptional activity of PPARγ (128). Moreover, recruitment of RIP140 to the nuclear factor liver X receptor α (LXRα) has been shown to dislocate the PPARγ / PGC1α complex off the PPRE by physically interacting with an overlapping LXRE binding site, thus reducing UCP1 expression (131). Another gene exerting a negative effect on BAT thermogenesis is TWIST-1, a nuclear basic helix–loop–helix (bHLH) transcription factor known to be crucial for embryonic development. A recent study by Pan *et al* (132) showed that TWIST-1 interacts physically with and inhibits PGC1α-induced expression of mitochondrial transcriptional program, including UCP1 expression, through repression of PPARδ. As part of a feedback loop, PPARδ can bind to the TWIST-1 promoter and activates its transcription (132). Consistent with this concept, mice overexpressing TWIST-1 and TWIST-1 knockout mice display reduced and increased brown fat metabolism, respectively. Interestingly, TWIST-1 does not appear to be involved in the differentiation program of the brown adipocyte per se (132).

5. BAT, ENERGY BALANCE REGULATION AND OBESITY

Given the stability of body energy stores in response to energy homeostasis challenges such as weight loss and overfeeding, it has been argued that energy balance is regulated (133-135). Energy stores (in the form of fat, in particular) seem fiercely "defended", which unquestionably sets hurdles on any attempts to tackle obesity. Fat losses do not only promote compensatory increases in hunger and appetite but also lead to a reduction in energy expenditure (18).

The role of energy expenditure in energy balance has received a rather mitigated attention mainly due to difficulty in clearly establishing the existence of adaptive / regulatory thermogenesis in humans. Evidence that energy expenditure could play a role in energy balance regulation has been advocated by some investigators (136, 137) but resolutely questioned by others (138). The current consensus is nonetheless that thermogenesis can play a role in energy balance in humans (136, 137), and that its presence appears easier to demonstrate in energy-deficit conditions than in energy-surfeit paradigms (139).

5.1. SNS-mediated thermogenesis is a determinant of energy expenditure

From animal studies, there is evidence that BAT thermogenesis participates to energy balance (18, 140, 141). In young growing rats and mice, palatable or energy-dense food items induce concomitant increases in energy expenditure and BAT thermogenic activity / capacity to limit excess energetic deposition (142-145). Conversely, fat loss reduces BAT thermogenesis so that energy can be spared (146). A reduction in BAT thermogenesis also contributes to the positive energy balance of several obese mutants such as the leptin deficient *ob/ob* mouse (147), the leptin resistant *db/db* mouse (148) or *fa/fa* rat (149), and the melanocortin-4 receptor (MC4R)-ablated obese mice (150, 151). Also supporting the role of BAT in energy balance regulation, are reports demonstrating increases in metabolic efficiency leading to obesity in mice lacking BAT (152) or UCP1 (153).

Unpredictably, it was not until lately that the role of UCP1 in energy balance was ascertained in UCP1-deficient mice (153). Noteworthily, UCP1-ablated mice showed a marked increase in their fat gain associated with a reduced BAT adaptive thermogenic response only when they were housed at a temperature insuring thermoneutrality (153). From trials done in mice housed below their heat-neutral temperature (< 29°C), there has been no reported demonstration that UCP1-ablated mice (99) could manifest signs of increased metabolic efficiency except when the mice were old (154) or subjected to prolonged cafeteria feeding (155). When subjected to temperatures causing thermal stress, UCP1-KO mutants would resist high-fat induced obesity through exhibiting a reduced energetic efficiency (156); being unable to count on UCP1-mediated thermogenesis to combat cold, UCP1-deficient mice must rely on heat-producing processes, which are apparently energetically more costly than BAT nonshivering thermogenesis (99).

6. THE CONTROL OF SNS-MEDIATED BAT THERMOGENESIS

BAT is richly innervated by SNS efferent fibers. The release by these fibers of noradrenaline not only enhances BAT thermogenic activity but also increases the capacity of the tissue to produce heat (Figure 3). While BAT thermogenic capacity (brown adipogenesis, mitochondriogenesis, synthesis of UCP1 and other BAT thermogenic proteins) can be pharmacologically stimulated by non-adrenergic agents (15, 16), BAT thermogenic activity (heat production as such) quite strictly requires adrenergic stimulation. Physiologically, SNS activation can be considered as the ultimate route of BAT stimulation (1). Conditions such as cold exposure or overfeeding, which stimulate both thermogenic activity and thermogenic capacity, enhance noradrenaline turnover in BAT (157) and have no thermogenic activity in mice lacking β-adrenoreceptors (β-less mice) (12, 158, 159).

The activity of SNS-mediated BAT thermogenesis is controlled by several brain regions, which are roughtly sketched in Figure 4. Transneuronal retrograde viral tract tracing using the pseudorabies virus (PRV) has been of paramount importance in determining those regions as well as the precise neuronal circuits supplying BAT through the SNS outflow (30, 160). When injected into

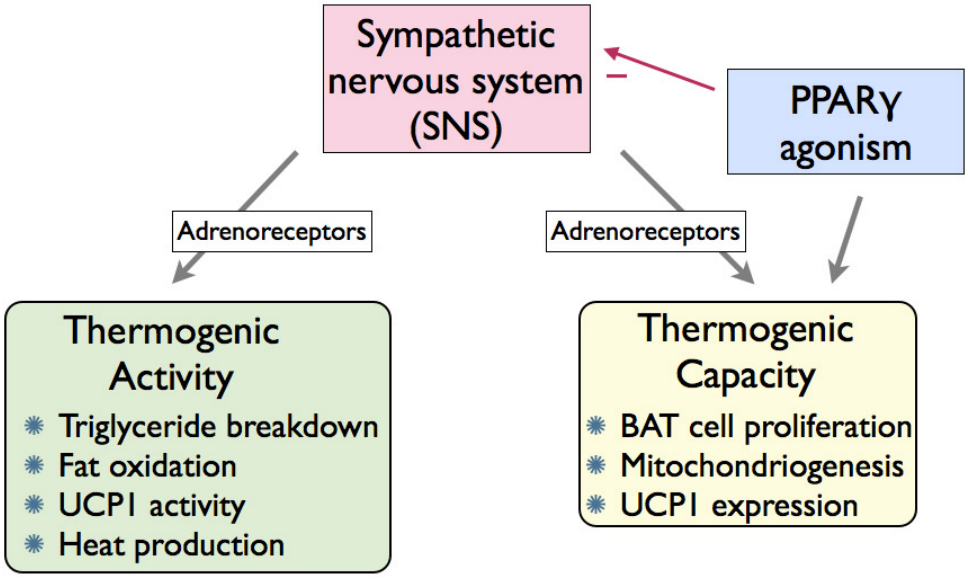

Figure 3. Control of BAT thermogenic capacity and activity. BAT thermogenic activity and capacity is stimulated by the SNS. BAT received a strong adrenergic innervation. While BAT capacity can be enhanced by PPARγ agonism. BAT thermogenic activity requires adrenergic activation. PPARγ activation can even blunt the SNS drive to BAT.

BAT, PRV is within a few days retrogradely transported to the brain while labeling the chain of neurons that make up the SNS outflow to the tissue (160). PRV crosses synapses, and labels neuronal pathways up to their brain origins, where PRV-contaminated neurons can be immunostained (160).

6.1. The preoptic area (POA), dorsomedial hypothalamus (DMH), rostral ventromedial medulla (RVM) govern thermoregulatory thermogenesis

The control of BAT thermoregulatory thermogenesis is insured by a network of circuits controlling the SNS outflow to BAT in response to changes in body temperature. The POA, DMH and RVM, including the raphe pallidus (RPa), are currently seen as major structures in controlling SNS-mediated BAT thermoregulatory thermogenesis (17, 161). Once informed about a cold stimulus sensed by cutaneous thermal receptors, gamma-aminobutyric-acid (GABA)-containing interneurons of the median preoptic nucleus are activated and release the inhibitory action of the POA on DMH thermogenesis-stimulating neurons. These DMH neurons project down to the RVM, where they activate BAT SNS premotor neurons, which include the neurons of the rostral RPa (162). Once activated RPa neurons in turn stimulate the SNS preganglionic neurons at the level of the spinal intermediolateral column (IML).

The role of the ventromedial hypothalamus (VMH) in BAT thermoregulatory thermogenesis, once presented as key (1), has been reevaluated and it currently is much less than certain that this structure is genuinely implicated in thermoregulation (17, 161). As pointed by Dimicco and Zaretsky (161), the methodologies used in the studies investigating the VMH in thermoregulation often included large injection volumes and used methodological approaches (electrical stimulation, electrolytic lesion) that affected not only cell bodies in the VMH but also fibers of passage. The observation that the VMH does not demonstrate any immunostained PRV neurons following the injection of PRV in BAT, in transneuronal retrograde viral tract tracing protocols (163) also questions the genuine role of the VMH in BAT thermogenesis.

Recent data tend to suggest that paraventricular PVH neurons inhibit sympathetic outflow to BAT (164). Microinjection of N-methyl-d-aspartate (NMDA) in the PVH appears able to reverse the increase in BAT SNS activity evoked by body cooling, likely by activating the inhibitory GABAergic input to BAT sympathetic premotor neurons in the RPa.

6.2. Most energy-balance-regulation centers modulate SNS-mediated BAT thermogenesis

The brain pathways involved in energy homeostasis have been the objects of numerous studies in recent years. Altogether these studies have contributed to determine the main neuronal circuits from the hedonic and autonomic brain systems that govern the process of energy balance regulation. The hedonic brain system is made of pathways linking the orbital prefrontal cortex, insula, extended amygdala, ventral pallidum, ventral tegmental area and other structures related to reward, emotion and pleasure (165, 166). On the other hand, the autonomic brain essentially comprises hypothalamic and brainstem circuits supplying the sympathetic and parasympathetic branches of the autonomic nervous system. The control of food intake is insured by both the hedonic and autonomic brain systems, whereas the control of regulatory BAT thermogenesis is essentially insured by the autonomic brain

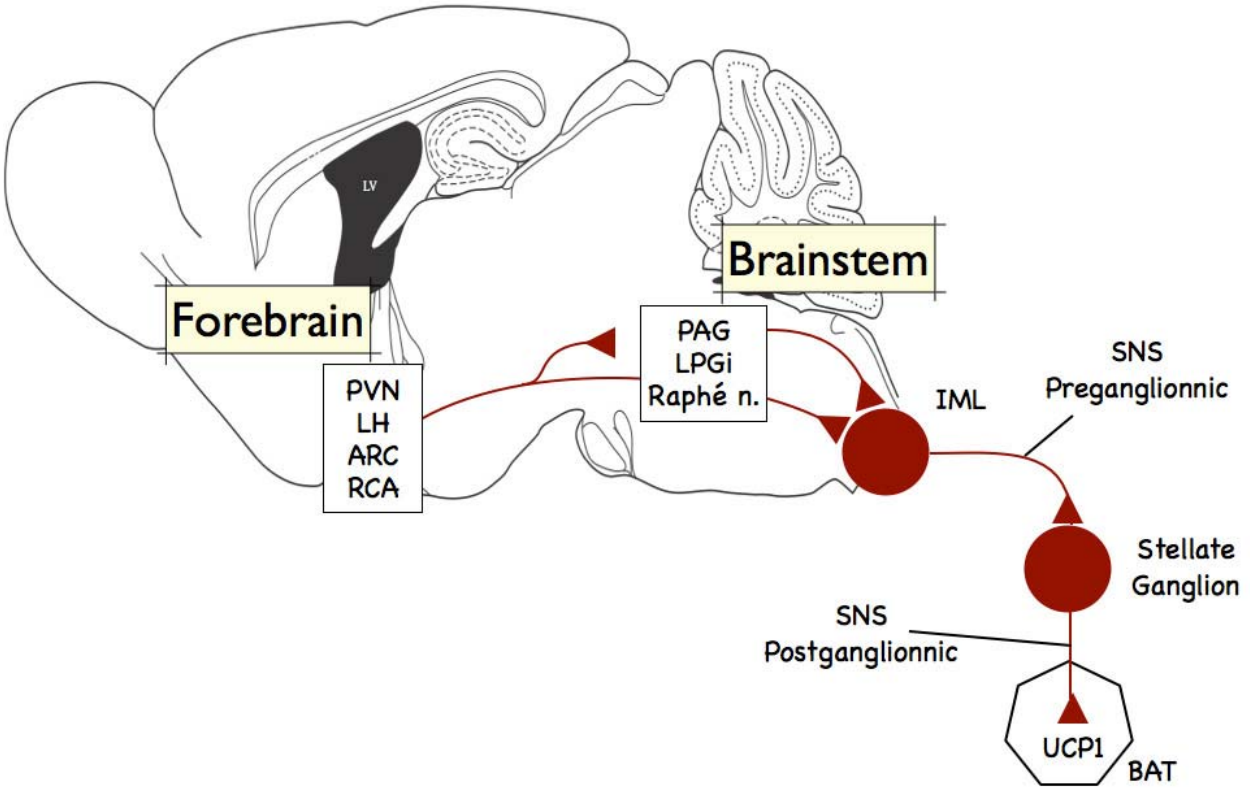

Figure 4. Brain regions governing BAT activity. The hypothalamic regions such as the PVH, LH, ARC and RCA as well as the brainstem regions including the PAG, LPGi (lateral paragigantocellular nucleus), raphe nuclei are connected to BAT and influence (negatively or positively) its activity.

system and involved nuclei such as the hypothalamic arcuate nucleus (ARC), retrochiasmatic area (RCA), PVH, lateral hypothalamus (LH) and brainstem (18, 166, 167).

6.3. The ventral hypothalamus coordinates BAT-mediated energy expenditure

The ARC and RCA appear to be key structures in the autonomic regulation of energy balance and SNS-mediated BAT thermogenesis (168-173). The ARC comprises two groups of neurons strongly involved in the control of energy intake and energy expenditure. One group synthesizes proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), whereas the other produces neuropeptide Y (NPY) and agouti-related peptide (AgRP) (171). POMC and CART are catabolic (favoring energy loss) peptides whereas NPY and AgRP are anabolic (favoring energy gain) molecules (174-177). POMC / CART neurons are also found in the RCA from where they project to the IML.

The POMC / CART and NPY / AgRP neurons innervate several brain regions including both the descending and parvocellular neuroendocrine divisions of the PVH (178). The neurons of the descending division, with axonal output to the brainstem and spinal regions controlling autonomic responses and somatomotor behaviors (179), appear of major importance in energy balance regulation. The descending division also innervates the parabrachial nucleus, periaqueductal gray and RVM as well as nearby areas (179).

6.4. The melanocortin system is a major actor in SNS-mediated BAT thermogenesis

POMC / CART neurons exert their thermogenic effect mainly via α-melanocyte-stimulating hormone (α-MSH), a peptidergic fragment ensuing from the POMC cleavage. Within the brain, α-MSH binds to melanocortin 3 receptor (MC3R) and MC4R with which it constitutes, together with AgRP, the brain metabolic melanocortin system (180-183). The functional significance of both the MC3R and MC4R in energy homeostasis and thermogenesis has been validated in *mc3r*- (184) and *mc4r*- (185) knockout mice. The MC4R deficiency, in particular, causes a massive and widespread body fat deposition, which has been demonstrated in genetically engineered mice (183, 185) to result from not only an increase in energy intake but also a decrease in BAT thermogenesis (150, 183). MC4R activation is endogenously blocked by AgRP (AgRP would also reverse the constitutive activity of the receptor being seen as an inverse agonist). AgRP is solely expressed in ARC neurons where it is co-synthesized with NPY (186). It is overexpressed in obese mice (175) and, when injected centrally, reduces BAT thermogenesis (187) while increasing food intake (187, 188). NPY also is a recognized inhibitor of BAT thermogenesis (189). It

exerts effects on its own receptors but also inhibits POMC neurons (190).

The MC4R is expressed in the PVH (191) and investigations have clearly established a connection between the MC4R-containing neurons of the PVH descending division and BAT (192, 193). In one study, it has been demonstrated that more than 80% of the PVH neurons expressing the MC4R receptors are connected to the sympathetic outflow to BAT (192). In the same study, the link between the PVH and BAT was demonstrated to be functional; injection of the MC4R agonist melanotan 2 in the PVH caused an increase in BAT temperature (192). The signature of the neurons expressing the MC4R in the PVH has yet to be identified but these neurons could be oxytocin neurons, which also have been shown to connect to the SNS outflow to BAT (194). It is noteworthy that the PVH descending neurons also arbor the cannabinoid 1 (CB1) receptor, which has been hypothesized to control the activity of the MC4R descending neurons (195).

There is evidence that the leptin receptor long form (LepRb) and MC4R could be part of the same homeostatic pathway controlling SNS activity in adipose tissues. Absence of the MC4R has been shown to compromise the ability of leptin (be it injected centrally or peripherally) to increase UCP1 expression in BAT and WAT (196). At least three potential leptin-mediated pathways could depend on the MC4R (Figure 5), emphasizing the importance of the leptin-melanocortins partnership in brown adipocyte activity. A first pathway would consist of LepRb / POMC / CART neurons in the ARC that project to the PVH to form synapse with MC4R-expressing neurons that directly descend to the IML in the lateral horn of the spinal cord to synapse with SNS preganglionic neurons (168). As just mentioned, the descending division of the PVH comprises a very high percentage of neurons connected to BAT and WAT (192, 197). A second pathway would consist of LepRb / POMC / CART neurons in the RCA that project to the IML to form synapse with MC4R-expressing SNS preganglionic neurons (198). The MC4R is indeed expressed at the level of the IML on SNS preganglionnic neurons (191). A third pathway would consist of LepRb / POMC / CART neurons in the ARC that project directly or indirectly [through a neuronal relay in the periaqueductal Gray (PAG)] to the RPa to ultimately form synapse with MC4R-expressing SNS premotor neurons, possibly serotonin (5-HT) neurons (199) involved in the control BAT (and potentially WAT) thermogenesis (200). Melanotan 2 (MT2) injections in the RPa increased BAT SNS activity (201).

6.5. Does brain UCP2-expressing neurons modulate BAT UCP1 activity?

UCP2 is the only UCP found in the brain, where its mRNA is widely distributed (202-204). It is expressed (i) in the neuroendocrine division of the PVH, (ii) in ARC neurons expressing NPY / AgRP (205) or POMC / AgRP (206), (iii) in the brainstem within neurons controlling the sympathetic and parasympathetic nervous system (202-204), and (iv) in the hippocampus (207) and other regions sensitive to excitotoxicity.

Animal studies have provided evidence for a role for brain UCP2 in energy homeostasis (205, 206, 208). UCP2 was demonstrated to be involved in the rebound feeding induced by fasting (205). Indeed, compared to wild-type mice, UCP2 KO mice exhibited reduced eating following fasting that likely resulted from a decreased activity of the NPY / AgRP neurons. Also supporting a function for brain UCP2 in energy metabolism was the recent work of Parton and colleagues (206), suggesting that the genetic deletion of UCP2 prevented obesity-induced loss of glucose sensing in POMC / CART neurons. By modulating the activity of ARC neurons UCP2 could likely modulate BAT thermogenesis.

6.6. The activity of the ventral hypothalamus neurons is influenced by peripheral hormones

The adipostatic hormone leptin and the gastrointestinal peptide ghrelin, in particular, appear to be genuine BAT thermogenesis controllers through SNS actions (209). Leptin increases SNS activity in BAT (210) likely by acting at the ARC level, where it is probably actively transported to bind its long-form receptor (Ob-Rb). It exerts its thermogenic action through the signal transducer and activator of transcription 3 (STAT3) signaling cascade, by reducing the production of NPY and AgRP while stimulating synthesis of POMC (211). Leptin also prevents the strong suppressing effects of glucocorticoids on BAT UCP1 expression (212). Similarly, ghrelin inhibits SNS-mediated BAT thermogenesis (213), which is consistent with the recent demonstration that the deficiency either in ghrelin (214) or in growth-hormone secretagogue receptor (GHSR) (215) led to a reduced efficiency in mice fed a high fat diet. Ghrelin reduces UCP1 expression (216, 217) and noradrenaline release (213) in BAT. The central mechanism whereby ghrelin could alter BAT thermogenesis likely involves the ARC-PVN axis (213). The ARC NPY / AgRP neuron is activated by ghrelin (211). UCP2 could be critical to the central action of ghrelin (208). The UCP2-dependent action of ghrelin on ARC NPY / AgRP neurons is seemingly driven by a fatty acid oxidation pathway involving AMP kinase and reactive oxygen species (ROS) that are scavenged by UCP2 (208).

6.7. The melanin-concentrating-hormone (MCH) system suppresses BAT thermogenesis

Evidence keeps accumulating to suggest that the MCH system is implicated in the regulation of energy balance through effects not only exerted on food intake but also on energy expenditure (218, 219). Intracerebral injection of MCH increases food intake while reducing energy expenditure and lipid oxidation (220). Chronic treatment with MCH (221) and MCH overexpression (222) lead to obesity and to an increased susceptibility to high-fat feeding. In the leptin deficient *ob/ob* mouse, deletion of

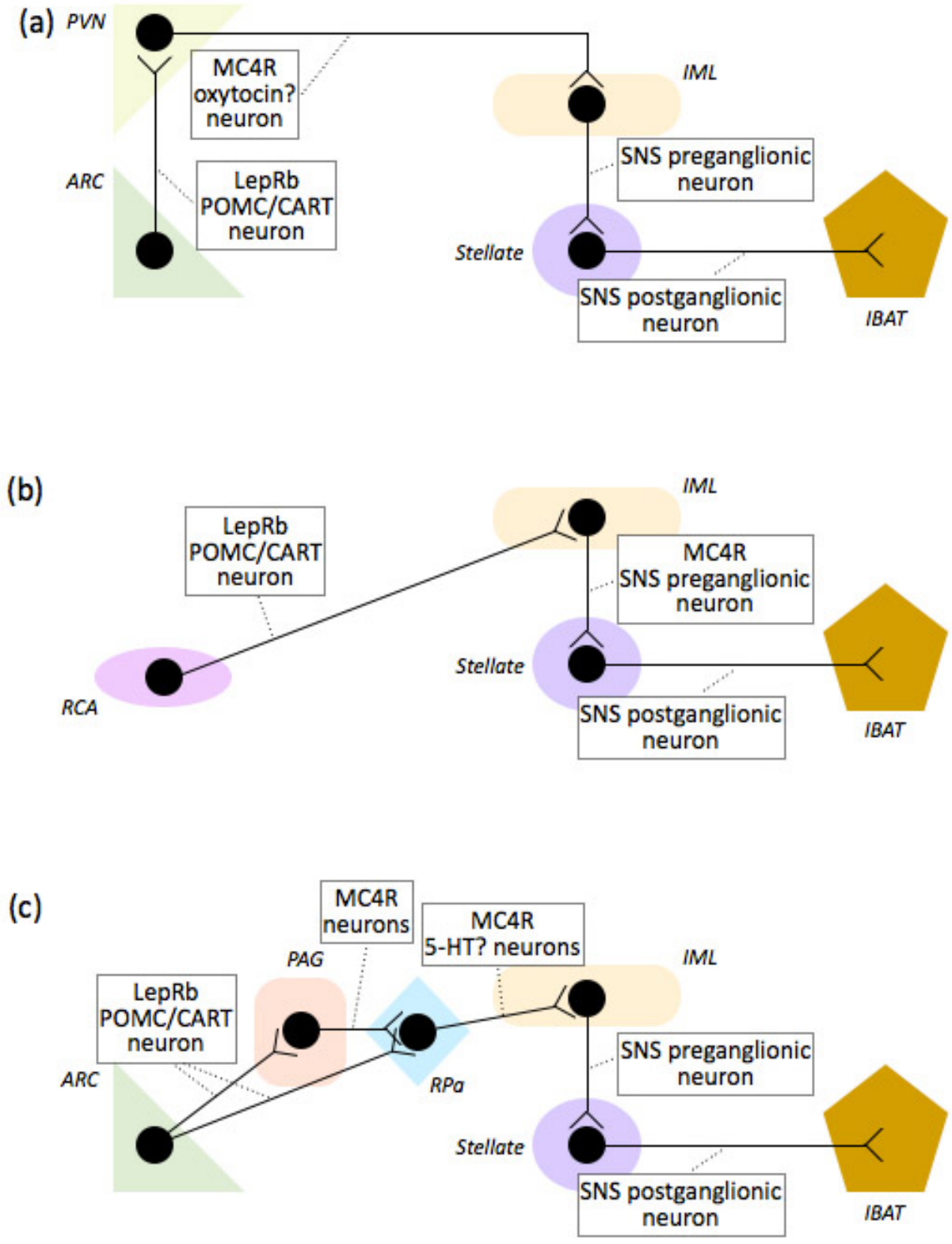

Figure 5. The leptin-melanocortin pathways in brown adipocyte thermogenesis. See section 6.4 for more details.

MCH induces a dramatic fat loss without any food intake reduction (223). Besides, MCH receptor 1 (MCHR1) disruption (224, 225) also leads to leanness despite hyperphagia. In rats and mice, all the effects of MCH, including those on energy metabolism, are mediated through the MCHR1. Other species including humans (226, 227) also express a second MCH receptor, the MCHR2, whose metabolic role has yet to be revealed.

The increase in energy expenditure produced by ablation of the MCH gene is accompanied by an increase in BAT expression of UCP1 (223), suggesting an implication of BAT thermogenesis in MCH-ablation-induced energy expenditure. That MCH can influence BAT is also supported anatomically. Transneuronal viral retrograde tract tracing experiments have indeed demonstrated that MCH neurons are (poly) synaptically linked to BAT (194, 228). MCH neurons are found in the LH and the adjacent zona incerta, where they project to the rest of the brain (229). More than 50% of the LH MCH neurons surrounding the fornix (bundle of axons crossing the LH) are connected to BAT.

6.8. The endocannabinoid system also controls BAT thermogenesis

The endocannabinoid system, which is essentially composed of two receptors, the cannabinoid 1 and 2 (CB1 and CB2) receptors, and of on-demand produced endocannabinoids [the most notable being anandamide and 2-arachidonoylglycerol (2AG)] (230), is involved in energy balance mainly through effects exerted on the CB1 receptor. CB1-receptor-deficient mice resist to diet-induced obesity and the CB1 receptor antagonism, which expectedly has no influence on energy homeostasis in CB1KO mice (231, 232), reduces weight gain significantly in numerous models of obesity (232-234).

The metabolic effect of endocannabinoids is not only attributable to effects on food intake but also to effects on energy expenditure. The CB1 receptor antagonist SR 141716 (rimonabant) reduces weight gain below pair-fed controls (232). Recent investigations have reported increases in oxygen consumption following treatment with rimonabant (235-237). The stimulating effect of the CB1 receptor antagonists on energy expenditure appears to largely result from an increase in SNS-mediated BAT thermogenesis. In fact, it has recently been demonstrated in rats that rimonabant could markedly increase BAT temperature at night and that such effect was blunted in sympathetically denervated rats (238). Rimonabant was also shown to enhance UCP1 expression (238, 233).

The neuronal circuits whereby the endocannabinoids controls SNS-mediated BAT thermogenesis have yet to be fully determined. The CB1 receptor mRNA is expressed in various brain regions and neuron types (195, 239-241). It is for instance found in the hypothalamic CART and MCH neurons as well as in the neurons of PVH descending division. Recently, the proposition was made that endocannabinoids could control SNS-mediated BAT thermogenesis via the PVH neurons expressing the MC4R as well as via the LH and RCA CART neurons (195). All these neurons (poly) synaptically connect to BAT via the SNS efferent neurons. Virtually all CART-containing cells of the LH perifornical area express CB1 receptor mRNA and up to 20% of CART neurons within the RCA expresses CB1 receptor mRNA (239). The CB1 is also expressed in the PVH subdivisions that expressed the MC4R (195). That the endocannabinoids can control the PVH neurons expressing the MC4R appears plausible (242).

7. BAT IN HUMAN

Up until recently, the contention was that BAT was solely detected in newborn infants (243, 244) or in adult individuals bearing hibernomas (245) or catecholamine-secreting tumors such as pheochromocytomas (246, 247). BAT was indeed thought to quickly regress following birth. The few (nonetheless valuable) existing indications for the presence of BAT in healthy adult humans (244, 248, 249) were essentially ignored.

Recently however, nuclear medicine provided evidence to challenge the belief that adult humans carry only vestiges of BAT (27). Indeed, PET/CT scanning investigations aimed at detecting tumoral tissue with the glucose analogue ^{18}F-fluorodeoxyglucose (^{18}F-FDG) — based on the fact that tumors are metabolically active, thereby abundantly taking up glucose — revealed symmetrical ^{18}F-FDG-labelled fat depots in the cervical, supraclavicular and paraspinal regions of the body, which were demonstrated to have all the characteristics of brown fat (20-24).

7.1. Cervical and supraclavicular fat areas taking up ^{18}F-FDG correspond to true BAT depots

PET/CT ^{18}F-FDG scanning reveals in humans a pattern of BAT distribution that differs from that seen in small mammals (Figure 6). In man, the main BAT depots are supraclavicular, cervical, and paraspinal whereas they are interscapular, subscapular and renal in laboratory rodents such as rats and mice (1, 27). The supraclavicular depot appears to not only be the most apparent in humans but also the one with the highest ^{18}F-FDG uptake activity following exposure to cold (22). This depot was previously described as USA-fat (Uptake in Supraclavicular Area Fat) in one of the seminal studies indentifying BAT from ^{18}F-FDG PET/CT scanning (250).

As just mentioned, there is now sound histological and biochemical evidence to suggest that the fat tissue taking up ^{18}F-FDG is indeed BAT (20-23). ^{18}F-FDG fat expresses UCP1 (mRNA and protein) as well as mRNAS encoding other BAT proteins such as DIO2, PGC1α, PRDM16 and β3-adrenergic receptor (23), which are all key molecules in BAT thermogenesis. The cervical / supraclavicular UCP1 positive cells display the classical morphology of brown fat cells with numerous cytoplasmic uniform fat vacuoles and abundant mitochondria (20, 21). They are in addition highly vascularized and densely innervated with nerve fibers immunopositive to tyrosine hydroxylase indicating a rich SNS innervation (20).

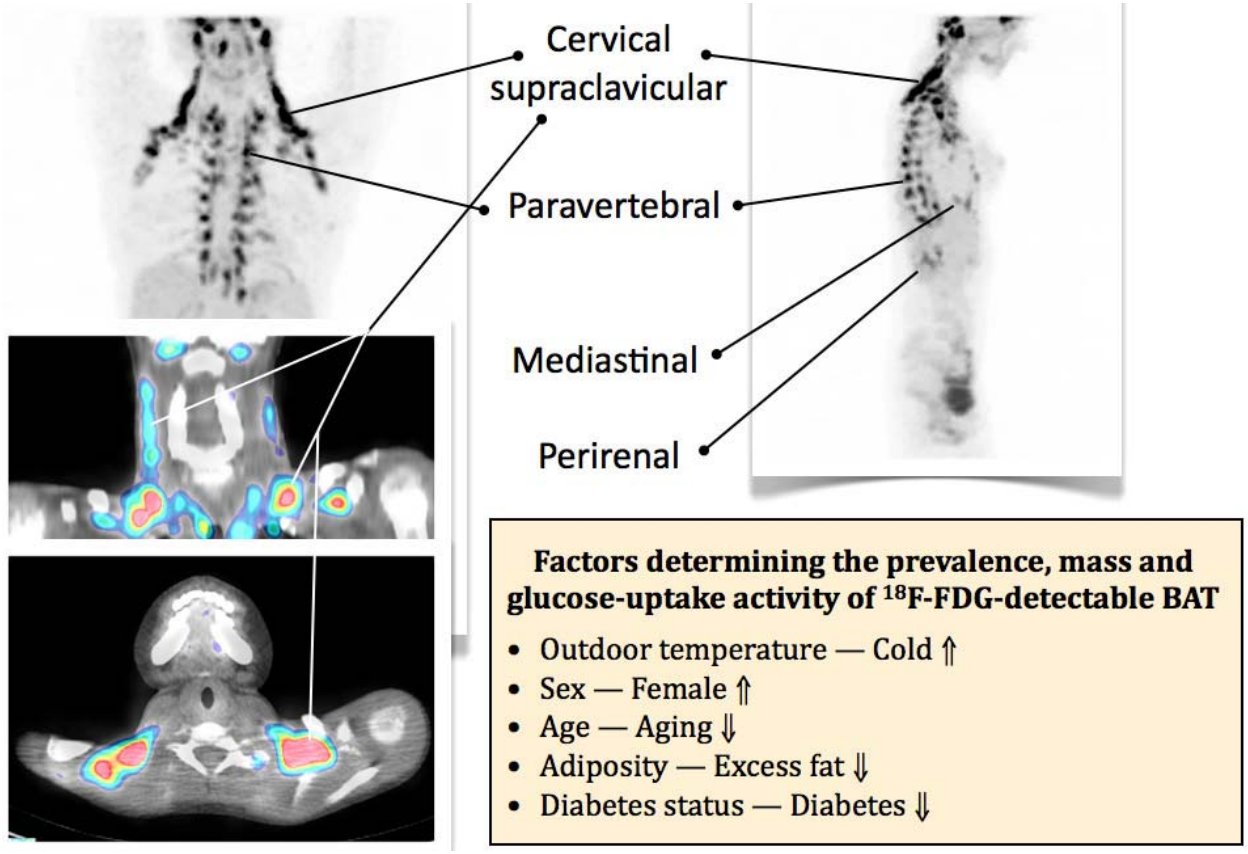

Figure 6. BAT ^{18}F-FDG uptake sites in humans. The PET and PET/CT fused images demonstrate important ^{18}F-FDG uptake sites in the neck and shoulder area. The different colours on the PET/CT images reveal the ^{18}F-FDG uptake activity (blue, low to moderate; yellow, moderate to intense; red, intense to very intense). The main determinants of the ^{18}F-FDG uptake sites are outdoor temperatures, age, sex, adiposity and diabetes status.

7.2. Outdoor temperature, age, sex, body mass index, and diabetic status determine the detection / prevalence of ^{18}F-FDG uptake in BAT in humans

The reported prevalence of ^{18}F-FDG uptake in BAT (^{18}F-FDG BAT) in humans seems to be determined by the environmental temperature at the time of PET / CT scanning procedures as well as by sex, age, and body mass index (BMI) (Figure 6). It could also be influenced by diabetes and the medication. The proportion of subjects/patients with ^{18}F-FDG BAT (i) increases with exposure to low temperature (21, 24, 251-253), (ii) is higher in woman than in man (21), (iii) decreases with age (20, 21, 24), and (iv) is inversely correlated with BMI and fat content (20, 21, 24). The proportion falls in diabetic patients (21) and decreases in patients taking β-blockers (21). Depending on the cohorts and protocols, the prevalence of ^{18}F-FDG BAT has been reported to be either low (around 5% and below), for instance in large cohorts of patients evaluated for cancer (positively or negatively) (21) (Ouellet V, *et al*, submitted 2010) or very high (close to 100%), for instance in small cohorts of cold-exposed young subjects (22). It is clear that the reported prevalence of ^{18}F-FDG BAT in all investigations aimed at detecting tumors represents an underestimation of the true prevalence of BAT since BAT can be present while left undetected. In fact, BAT needs to be activated for ^{18}F-FDG detection and, in most conditions into which ^{18}F-FDG PET/CT scans are performed for cancer, BAT metabolic activity may not be present.

7.3. Low environmental temperature increases BAT ^{18}F-FDG uptake

Expectedly, the environmental temperature to which subjects are exposed prior to and during ^{18}F-FDG PET/CT scanning investigation proves to be a major factor for detecting ^{18}F-FDG BAT; the colder the temperature at the time of scanning, the higher the prevalence of ^{18}F-FDG BAT (251, 253). Accordingly, detection of ^{18}F-FDG BAT seems to vary with seasons, being facilitated in winter (21, 24, 251, 254). Winter seems to even boost the stimulating effect of acute cold exposure on BAT ^{18}F-FDG uptake (24), which is not surprising considering that winter likely contributes to enhance BAT capacity. That the seasonal effect on BAT be linked to change in photoperiod has been suggested (254). However, it appears that the temperature on the day of testing is a stronger determinant of the presence of ^{18}F-FDG BAT in humans than photoperiod (Ouellet V, *et al*, submitted 2010). In one recent study

conducted in male individuals aged between 18-32 (22), acute exposure to mild cold for 2 hours brought the proportion to ^{18}F-FDG-detectable BAT to 96% when only one out of 24 young individuals did not show traces of ^{18}F-FDG BAT after cold exposure. Besides, cold-induced ^{18}F-FDG uptake in BAT was interrupted by a post-cold return to thermoneutral environment (22).

7.4. Sex influences the detection of ^{18}F-FDG BAT

The prevalence of ^{18}F-FDG BAT, in particular in investigations aimed at cancer detection, has been reported to be higher in women than in men (21, 250, 255-257). Recently, Cypess *et al* (22) reported positive scans for ^{18}F-FDG BAT in 76 out of 1,013 women (7.5%) and 30 out of 959 men (3.1%), corresponding to a female / male ratio greater than 2. Compared to men, women also exhibit a greater mass of ^{18}F-FDG BAT, and their detected BAT appears to additionally demonstrate an enhanced ^{18}F-FDG uptake activity (21). Our own data from a cohort of 4,842 patients (out of which 328 individuals were ^{18}F-FDG BAT positive) (Ouellet V, *et al* submitted 2010) reveal that the factors age and sex interact on ^{18}F-FDG BAT prevalence; the sex effect (i.e. the proportion of women exhibiting ^{18}F-FDG BAT was higher than that of men) tends to disappear with age. The possibility that the ovarian hormones can impact on the detection of ^{18}F-FDG BAT cannot be excluded given the trend for more cases with ^{18}F-FDG BAT in premenopause than after menopause (255). That the higher ability of women to exhibit ^{18}F-FDG BAT be due to an increased sensitivity to cold cannot be excluded and is supported by animal studies (258-261). Female rats are more sensitive to cold than males as their thermogenic response is set at a higher value (260). Consistently, there is no more sex difference between male and female rats in their thermogenic capacity / activity when they are all exposed to cold (4^{o}C) (260). The later finding is in agreement with the results of Saito *et al* (24), who did not report any sex difference in the prevalence of ^{18}F-FDG BAT when the subjects were exposed to cold.

7.5. ^{18}F-FDG BAT is more prevalent in young subjects

Age also appears to be a significant determinant for the prevalence ^{18}F-FDG BAT (21, 24, 257, 262). In the various cohorts so far studied, people exhibiting ^{18}F-FDG BAT sites were seen to be on average younger than those showing no ^{18}F-FDG BAT. In one study in which the tested subjects were below 35, van Marken Lichtenbelt *et al* (22) reported the highest so far reported prevalence (96%) of ^{18}F-FDG BAT after cold exposure. Similarly, for subjects aged between 23 and 35, Saito *et al* (24) reported a prevalence of cold-induced ^{18}F-FDG BAT of 52% (16/31) compared to 8% (2/24) for subjects aged between 38 and 65. In line with those results and others (21), Zingaretti *et al* (20) reported that UCP1-positive subjects were younger (39 years old) than UCP-negative subjects (55 years old).

7.6. The prevalence, mass and glucose uptake activity of ^{18}F-FDG BAT decreases with the increase in adiposity

The prevalence of ^{18}F-FDG BAT is inversely correlated to BMI and body fat (21, 24). van Marken Lichtenbelt *et al* (22) reported inverse relationships between cold-induced ^{18}F-FDG-uptake activity in ^{18}F-FDG BAT and both BMI and the percentage of fat. Interestingly, the sole subject who resisted cold-induced BAT ^{18}F-FDG uptake in the van Marken Lichtenbelt *et al*'s study (22) was the individual displaying the largest BMI (38.7) and the largest percentage of body fat (41.8%). It is also worthy of mention that the only two subjects bearing BAT ^{18}F-FDG uptake sites in a subgroup of subjects, described by Saito *et al* (24) as elderly (38-65 years old), were very lean with BMI of 22.2 and 20.6 kg/m^2. The later study (24) as well as that of Zingaretti *et al* (20) did not allow for clear dissociation between the effects of age and BMI on the presence BAT ^{18}F-FDG uptake sites.

7.7. ^{18}F-FDG BAT is less prevalent in diabetic patients and barely detectable following the ß̃-adrenergic-blockade

In line with the recent data published by Cypess *et al* (21), our own data (Ouellet V, *et al*, unpublished data) show that the prevalence of ^{18}F-FDG BAT is much lower in diabetic patients (1.1%) than in non-diabetic subjects (7.5%). The reason for this is not known. The possibility that diabetic patients be more sensitive to cold than non-diabetic subjects cannot be excluded. Meanwhile the detection of ^{18}F-FDG BAT is markedly reduced by β-blockers (21, 263-266), which is not surprising given the necessity for a β-adrenergic activation in BAT thermogenesis (see above). In addition, β-blockers reduce both the mass and the glucose-uptake activity of ^{18}F-FDG BAT (21). A single dose of 80 mg of propranolol given orally 2 hours before ^{18}F-FDG administration may lead to an almost complete disappearance of BAT ^{18}F-FDG uptake (264). The stimulating effect of catecholamines on BAT in humans has been demonstrated in patients with phaeochromocytoma, who incidentally show an intense BAT ^{18}F-FDG uptake (267-269), which disappear following the resection of the catecholamine-secreting tumors (267, 269).

7.8. BAT thermogenesis could be an important energy-dissipating process in humans

BAT ^{18}F-FDG uptake has become a surrogate of BAT thermogenic activity even though BAT is not primarily a glucose user. BAT thermogenesis is essentially dependent on lipolysis and only approximately 10% of BAT metabolism is insured by glucose (270). The glucose taken up by BAT is in large part used for *de novo* lipogenesis. However, stimulated BAT takes up glucose abundantly and the uptake largely depends on UCP1 activity (271).

The presence of BAT sites taking up ^{18}F-FDG is supportive of a metabolically active BAT. In the recent study by van Marken Lichtenbelt (22), it was reported that the subjects demonstrating the highest BAT ^{18}F-FDG uptakes in response to cold exposure were also those exhibiting the lowest cold-induced drop in skin temperature, which indicates a correlation between BAT and heat production. Resting metabolism was also correlated with BAT activity while there was however no correlation between BAT activity and cold-induced thermogenesis (22). The latter finding does not necessarily refute a role for BAT in thermogenesis but rather indicate, as suggested by van Marken Lichtenbelt (22), that BAT is

not the only tissue producing heat in an acute cold response. It is noteworthy that the subjects in the van Marken Lichtenbelt's study (22) were manifestly not acclimated to cold and therefore had probably not enough BAT capacity to react to cold without shivering. The same is also true for small mammals acutely exposed to cold, which also rely on muscle and BAT to produce heat.

The studies carried out so far in humans have not brought the ultimate demonstration that BAT may affect energy balance. The existence of adaptive thermogenesis has long been a matter of controversy in humans (19, 136-138). However, the fact that BAT ^{18}F-FDG uptake is stimulated by cold and inhibited by aging, fatness as well as β-blockers speaks in favor of a BAT participation to energy expenditure. The relative difficulty in detecting adaptive thermogenesis in humans (138) does not necessarily argue against BAT thermogenesis. In fact, the low prevalence (often less than 5%) of ^{18}F-FDG-detectable BAT in humans (based on large cohorts of cold-unstimulated people) possibly both prevents detection of adaptive thermogenesis and accounts for the large individual variability in energy expenditure measurements. Metabolic studies conducted in cold-adapted young individuals would help to delineate the role of BAT thermogenesis in influencing energy expenditure in humans. Based on reasonable assumptions, Virtanen *et al* (23) recently estimated the energy expenditure associated to the submaximal stimulation of an estimated supraclavicular BAT depot of 63 g could be around 4.1 kg of fat per year. Such an estimate is in line with that of Rothwell and Stock (272), who pioneered the view that active brown fat could have a profound influence on energy metabolism.

8. CONCLUDING REMARKS

BAT represents a unique tissue, whose function is primarily to produce heat to maintain body temperature constant in a cold environment. Its exceptional heat producing capacity is attributable to UCP1, which has evolved in eutherian mammals as a nucleotide-sensitive proton translocator dissipating the proton gradient that builds up across the mitochondrial inner membrane during fat oxidation and that is behind ATP synthesis (1, 11, 90-92,103).

Recent series of investigations have demonstrated that brown adipocytes from classical BAT depots share their predecessor cells with myocytes (6-8, 29, 36, 56) while brown fat cells in typical WAT depots can either differentiate from a yet to be described precursor, differenciate / transdifferentiate from white preadipocytes, or transdifferentiate from mature white adipocytes (29, 38, 39). BAT differentiation / transdifferentiation depends on a plethora of proteins that include PPARγ, PGC1α and newly discovered molecules such as PRDM16 (8) and C/EBP-β (36). Those chemical mediators also help BAT to acquire its outstanding thermogenic phenotype conferred to by UCP1.

SNS-mediated thermogenesis is controlled by brain autonomic circuits that could differ depending on whether BAT thermogenesis is triggered by cold or by energy homeostatic alterations (16, 18). Recent investigations have clearly established anatomical links between BAT and populations of neurons key to energy balance regulation. These neurons include Ob-Rb-expressing neuronal cells of the ARC (210), MC4R-expressing neurons of the PVH (192), MCH-containing neurons of the lateral hypothalamus (194, 228) and hypothalamic CART-expressing neurons (194). The possibility that other systems such as the endocannabinoid system be anatomically linked to BAT is also probable (195).

Finally, certainly one of the most interesting breakthroughs regarding BAT in recent years has been the demonstration from PET/CT scanning investigations that BAT can be present in substantial amounts in human adults. It is noteworthy that it took almost a decade of nuclear medicine investigations and a timely and decisive review by Ian Nedergaard, Tore Bengtsson, and Barbara Cannon (27) to highlight the key finding and to recognize the value of PET/CT as a mean to further explore BAT in humans. Recently, major contributions (20-23) added to seminal papers (250, 251) to establish that the fat tissue taken ^{18}F-FDG was BAT and to identify the various factors (environmental temperature, age, sex, body fat mass and diabetes status) determining the presence of BAT in humans. One might now expect that the future studies will decipher the potential role that BAT may have in energy metabolism in humans and whether the tissue can be seen again as a target for obesity treatment. Considering the thermogenic potential of BAT, there are unquestionably sound reasons to believe that BAT may participate to energy metabolism in humans at least under certain circumstances.

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Key Words: Brown Adipose Tissue, White Adipose Tissue, Uncoupling Protein 1, Energy Balance, Energy Expenditure, Obesity, Human, Rat, Mouse, Brain, Review

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