Adipose tissue browning and metabolic health

Alexander Bartelt and Joerg Heeren

Abstract | Accumulation of excess white adipose tissue (WAT) has deleterious consequences for metabolic health. The activation of brown adipose tissue (BAT), the primary organ for heat production, confers beneficial effects on adiposity, insulin resistance and hyperlipidaemia, at least in mice. As the amount of metabolically active BAT seems to be particularly low in patients with obesity or diabetes mellitus who require immediate therapy, new avenues are needed to increase the capacity for adaptive thermogenesis. In this light, we review the findings that BAT in human adults might consist of not only classic brown adipocytes but also inducible brown adipocytes (also called beige, brown-in-white, or brite adipocytes), which are phenotypically distinct from both white and brown adipocytes. Stimulating the development of beige adipocytes in WAT (so called 'browning') might reduce adverse effects of WAT and could help to improve metabolic health. This article focuses on the development and regulatory control of beige adipocytes at the transcriptional and hormonal levels. Emerging insights into the metabolic role of beige adipocytes are also discussed, along with the developments that can be expected from these promising targets for therapy of metabolic disease in the future.

Bartelt, A. & Heeren, J. *Nat. Rev. Endocrinol.* advance online publication 22 October 2013; doi:10.1038/nrendo.2013.204

Introduction

During evolution, animal species have been repeatedly challenged by two major threats to which they had to adapt: limited food supplies and cold temperatures. The adipose organ is an important tissue that responds to both changes in nutrient supply and ambient temperature. In higher vertebrates, white adipose tissue (WAT) stores vast amounts of nutrients as lipids in unilocular white adipocytes, which can then be released as fatty acids when food is scarce.[1] Endothermic birds and mammals, moreover, can maintain their body core temperature through basal metabolism or muscle-shivering thermogenesis.[2] In mammals, brown adipose tissue (BAT) can both store nutrients as lipids and dissipate their energy as heat in a process called nonshivering thermogenesis.[3]

Classic brown adipocytes are characterized by a multilocular lipid droplet structure, high amounts of mitochondria and production of the mitochondrial brown fat uncoupling protein 1 (UCP1) which is located in the inner mitochondrial membrane (Box 1). When activated by sympathetic tone, brown adipocytes dissipate chemical energy stored as triglycerides by channelling fatty acids into β-oxidation.[3] UCP1 uncouples electron transport from ATP production, which in turn leads to controlled exothermic resolution of the electrochemical gradient and generation of heat to maintain body core temperature. Consequently, BAT is highly metabolically active and can be detected as hot spots of glucose uptake on PET or by MRI (Box 2). BAT activity (as determined by PET–CT) is positively correlated with the amount of BAT,[4,5] its activation status[4,6] and environmental factors, such as low temperatures.[7] In humans, repeated cold exposure leads to increased BAT activity,[8,9] which is associated with a self-reported decrease in sensitivity to cold. Increases in BAT activity have also been tightly correlated with cold-induced increases in energy expenditure by nonshivering thermogenesis,[8,9] but not with the contribution of shivering thermogenesis to energy expenditure.

As nutrient shortage and cold can occur transiently and independently from each other, both WAT and BAT undergo adaptive and dynamic changes in response to starvation or overfeeding, as well as in response to cold or thermoneutrality.[3,10] Initially (within 24 h), these changes might only involve altered expression of proteins, but after 2–3 days, these stimuli induce marked tissue remodelling, which results in altered adipose tissue morphology and possibly also modified functional properties.[10]

Another intensively studied cell type is the beige (also called inducible brown, brown-in-white, or brite) adipocyte (Box 1).[11] The accumulation of beige adipocytes in WAT is often referred to as 'browning' of WAT (Figure 1). The idea of using beige adipocytes and browning of WAT therapeutically has gained a lot of attention, because the actual amount of BAT found in adults is typically quite low and correlates inversely with BMI and age.[4,5,7,12–16] However, whether a decline in BAT activity is causally involved in the development of obesity in humans remains unclear. Experiments in mice show that obesity develops when BAT is ablated genetically.[17] By contrast, mice lacking UCP1 are lean when kept at room temperature, as they compensate by shivering thermogenesis,[18] but gain weight when kept at thermoneutral conditions (that is, in the absence of thermal stress).[19] In line with these results, transgenic expression of *Ucp1* in adipose tissue prevents obesity in mice.[20] The excess WAT accumulation that occurs in obesity is a major risk factor for

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Competing interests
The authors declare no competing interests.

Key points

- The term browning describes the emergence of beige adipocytes in white adipose tissue—a reversible process that represents adaptation to increased thermogenic demand and exercise
- Human brown adipose tissue is diverse and consists of both brown and beige adipocytes, in proportions that differ according to the fat depot's anatomical location and the age of the person
- Beige adipocytes are generated by both *de novo* recruitment from progenitor cells and transdifferentiation from white adipocytes—independent processes that might coexist
- Cellular energy sensing, in addition to sympathetic tone, are the driving forces that regulate the transcriptional networks controlling browning
- Cold exposure and other metabolic challenges elicit complex hormonal responses that facilitate communication between tissues and prepare the body for adaptive thermogenesis
- Brown adipose tissue is a critical regulator of metabolic health in mice; yet, whether induction of browning will be a promising avenue to treat metabolic disorders in humans remains unclear

Box 1 | Adipocytes and adipose tissue browning

Adipose tissue consists of three types of adipocytes: white, brown and beige. In humans, reliable markers for brown adipocytes are *LHX8* and *ZIC1* and for beige adipocytes are *TNFRSF9*, *TBX1* and *TMEM26*.

White adipocytes
UCP1-negative, predominant in white adipose tissue, store chemical energy as lipids and contribute to metabolic disease in obesity.

Brown adipocytes
UCP1-positive, predominant in brown adipose tissue, dissipate chemical energy for heat production in response to cold, counteract obesity and metabolic disease.

Beige adipocytes
UCP1-positive, predominant in white adipose tissue under conditions of increased energy expenditure, such as cold and exercise; these cells might contribute to heat production as well as counteract obesity and metabolic disease.

developing insulin resistance, type 2 diabetes mellitus and cardiovascular disease.[21,22] Increased understanding of the morphology, development and metabolism of classic brown as well as beige adipocytes is, therefore, important, as stimulation of the activity of these cell types in patients could potentially facilitate weight loss and improve metabolic health.[23]

In this Review, we discuss the origin of beige adipocytes in the context of the gene expression profiles of different types of adipocytes. Furthermore, we consider the link between exercise, energy sensing and browning, and describe how browning affects metabolic health.

Characterization of adipocyte types

Cellular origin

Both beige and brown adipocytes express UCP1. Identifying adult human UCP1-positive cells as either beige or brown adipocytes is a major task, as herein lies important information regarding their putative therapeutic potential. Several studies of human biopsy samples have addressed the question of whether human BAT consists mainly of classic brown adipocytes or beige adipocytes. The answer seems to depend on which anatomical location is investigated.[24] For many years, only infants and rodents were thought to have substantial amounts of BAT, predominantly in the interscapular region. However, by 2009, UCP1-positive multilocular adipocytes had been found in cervical and supraclavicular regions in human adults.[5,12] At about the same time, classic brown adipocytes in mice were shown to derive from a Myf-5$^+$ myogenic lineage rather than adipogenic precursors, adding another layer of complexity to their origin.[25,26] In 2012, endothelial and perivascular cells were identified as a source of beige adipocytes in WAT of mice.[27–29] Thus, in mice, beige adipocytes are not derived from the Myf-5$^+$ brown adipocyte lineage,[26] and their source is also distinct from that of white adipocyte precursors.[30] Overall, these studies provide evidence that beige adipocytes arise from *de novo* generation.[28,30–33]

The coexistence of an alternative process—in which beige adipocytes arise from transdifferentiation of white adipocytes[34,35]—has been supported by an elegant *in vivo* lineage-tracing study in transgenic mice, using transient and permanent fluorescent cell labelling.[36] The results showed that beige adipocytes in inguinal WAT, which arose from precursor cells during cold adaptation, lost their morphology and gained white-adipocyte-specific gene expression profiles after a period of warm adaptation. Importantly, the same cells regained their multilocular morphology and beige-adipocyte-specific gene expression profiles after a second period of cold adaptation, in a process resembling transdifferentiation. Taken together, *de novo* differentiation and transdifferentiation could coexist and are timely independent processes.[36]

Further evidence for the coexistence of both concepts of adipocyte plasticity was provided by the identification of a population of adipocyte precursor cells that express platelet-derived growth factor receptor α[28] and can differentiate into either beige or white adipocytes (Figure 2). Specifically, these bipotential precursor cells developed into beige adipocytes under β_3-adrenergic receptor activation or into white adipocytes in mice fed a high-fat diet. Interconversion of beige and white adipocytes, in response to alternating periods of cold and warmth, has also been reported in another mouse study.[36] This same population of bipotential precursors, therefore, is probably the source of the beige and white adipocytes identified in this study.[36]

Surprisingly, yet another distinct set of beige adipocyte precursors has been identified in skeletal muscle,[32] which further underlines the complexity of adipogenic lineages. To sum up, at least three distinct types of precursors exist that give rise to mature brown, white and beige adipocytes, respectively (Box 1, Figure 3).

Gene expression profiles

Another way to investigate whether human BAT consists of brown or beige adipocytes is to compare their gene expression profiles. Beige adipocytes from inguinal WAT in mice have been isolated and cloned, after which their gene expression signatures were compared with those of adipocytes in human BAT biopsy samples

Box 2 | Imaging of brown and beige adipocytes

PET–CT can be used for detecting uptake of radiolabelled glucose, fatty acids or their respective metabolites, but, given its rather low resolution and sensitivity, this imaging modality might not accurately measure glucose uptake in widely dispersed beige adipocytes. Moreover, PET–CT does not provide information on the actual amount (in terms of mass) or function (in terms of thermogenesis) of BAT. Fat–water MRI offers advantages over PET–CT for fat mass quantification in mice[155] and humans, and does not involve exposure to hazardous ionizing radiation. Functional MRI[156,157] and dual-energy CT[158] can be used to measure blood flow in BAT.[156,157] Another functional, dynamic and sensitive MRI-based method for assessing BAT activity relies on the incorporation of nontoxic superparamagnetic iron oxide nanocrystals within the core of recombinant lipoproteins (as Trojan horses).[137,159,160]

Abbreviation: BAT, brown adipose tissue.

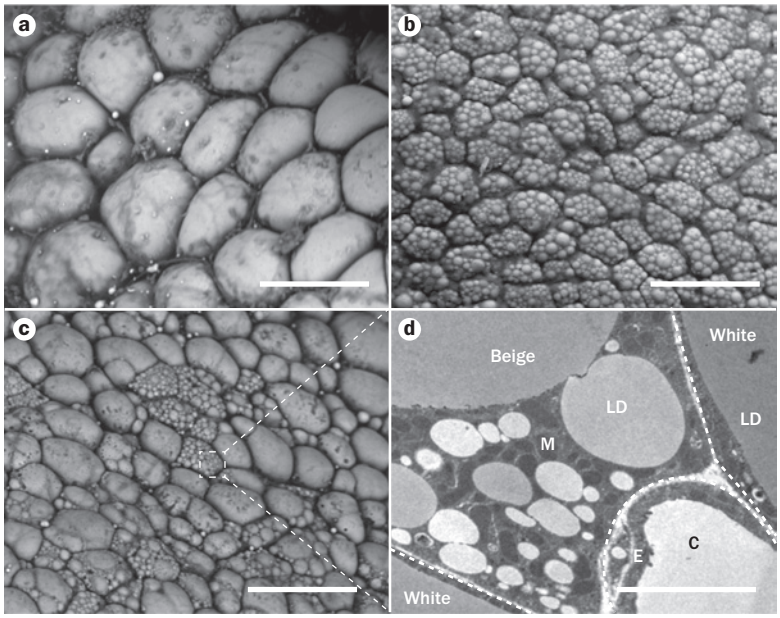

Figure 1 | Phenotypes of adipose tissue depots. Environmental scanning electron micrographs demonstrate the characteristic appearance of mouse adipose tissue. **a** | White adipocytes in inguinal WAT contain a single lipid droplet. **b** | Brown adipocytes in interscapular brown adipose tissue contain multilocular lipid droplets. **c** | Browning of WAT (induced by sustained pharmacological activation of β_3-adrenergic receptors) leads to formation of islets of multilocular beige adipocytes within inguinal WAT. **d** | Transmission electron micrograph of beige adipocytes within WAT, showing their high mitochondrial content. Scale bars: a–c, 50 μm; d, 5 μm. Abbreviations: C, capillary; E, endothelial cell; LD, lipid droplet; M, mitochondria; WAT, white adipose tissue.

isolated from the supraclavicular region (in this study, BAT was identified from cold-induced PET signals).[33] Expression of several mouse genes was identified *in vitro* and *in vivo* as being specific to either beige adipocytes (for example, *Tnfrsf9* and *Tmem26)* or brown adipocytes (such as *Eva1, Hspb7* and *Pdk4*).[33] Importantly, human UCP1-positive cells also had high levels of expression of the human orthologues of mouse genes specific to beige adipocytes, implying that human UCP1-positive cells more closely resemble mouse beige adipocytes than mouse classic brown adipocytes.[33] Moreover, human beige adipocytes initially expressed low levels of UCP1, but expression of *UCP1* increased dramatically after mimicking cold exposure by stimulation with cAMP. Although basal expression of UCP1 was apparently low, the high increase supports the idea that beige adipocytes are morphologically flexible and can dynamically respond to environmental cues as outlined above. These findings were supported by another study that compared gene expression profiles from mouse adipocytes differentiated *in vitro* with those of adipocytes in postmortem human biopsy samples obtained from infants and young adults.[37] The researchers identified expression of several genes as being specific to beige adipocytes (including *HOXC8, HOXC9* and *CITED1*) in addition to *UCP1,* whereas expression of genes specific to brown adipocytes (*EPSTI1, LHX8* and *ZIC1*) was almost undetectable in this set of human samples.[37]

Anatomical sites

Differences in the proportions of brown and beige adipocytes reported in published studies could reflect either the specific anatomical site from which they were obtained, or a general heterogeneity of human adipose tissue. Analysis of human adult neck fat biopsy samples, from the most superficial to the deepest depots, revealed that subcutaneous fat contained only white-adipocyte-like cells, whereas intermediate (possibly beige-adipocyte-like) cells were located at the carotid sheath and brown-adipocyte-like cells were sited at the *musculus longus colli*.[38] Interestingly, the deeper the neck location from which the biopsy sample was taken, the higher was the expression of *UCP1* and other brown-adipocyte-specific genes (*LHX8* and *ZIC1*). In another large study, in which samples of supraclavicular human BAT were analysed, the researchers divided the samples into two subgroups defined by high or low *UCP1* expression.[39] However, in both groups, expression of brown-adipocyte-specific genes (such as *LHX8* and *ZIC1)* was accompanied by low levels of expression of beige-adipocyte-specific genes (*HOXC8* and *HOXC9)*.[39] The brown-adipocyte-specific profiles were also strongly associated with expression of the beige-adipocyte-specific markers *TBX1* and *TMEM26*, which suggests that both brown and beige adipocyte lineages coexist within one location. Interestingly, one study that assessed browning of abdominal subcutaneous adipose tissue found no differences in the expression of several marker genes (*TNFRSF9*, *TMEM26*, *PPARGC1A* and *UCP1*).[8] However, such changes in gene expression probably would have been detected in regions that displayed increases in BAT activity, and might shed light on whether the recruitment process involved brown or beige adipocytes.

In 2013, the presence of interscapular BAT (which resembles classic mouse interscapular BAT in both anatomical location and gene expression) was confirmed in infants.[40] The researchers pointed out that *ZIC1* is a marker of interscapular BAT, whereas *TBX1* is a beige adipocyte marker.[40] Beige adipocytes and their molecular signatures were originally identified and defined as a population that arises after chronic stimulation of primary adipocyte cultures with rosiglitazone, an agonist of peroxisome proliferator-activated receptor γ (PPARγ).[30]

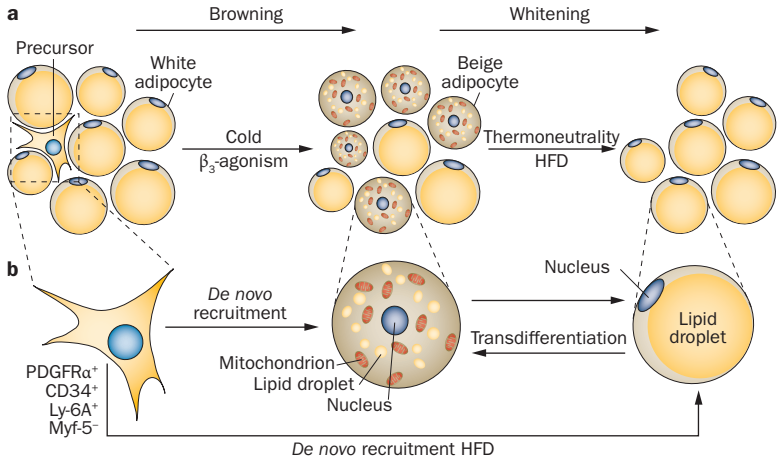

Figure 2 | Browning of adipose tissue is an adaptive and reversible response to environmental challenges. **a** | Specific WAT depots can either develop high numbers of beige adipocytes (browning) or increase lipid storage in cells that morphologically resemble classic white adipocytes (whitening). These processes are dependent on environmental challenges, such as cold temperatures for browning or a high-fat diet for whitening. Adaptation to cold can also be pharmacologically mimicked by treatment with a β_3-adrenergic receptor agonist, whereas the absence of heat stress (thermoneutrality) reduces the rate of thermogenesis and leads to deposition of excess calories as lipids. **b** | A bipotential precursor population resides within these depots that expresses PDGF receptor α, CD34 and Ly-6A, but not Myf-5. These precursors can differentiate into beige or white adipocytes, depending on the environmental demand (*de novo* recruitment). However, once differentiated, these cells are morphologically flexible and might acquire beige or white phenotypes, respectively, when challenged with whitening or browning stimuli (transdifferentiation). Abbreviations: HFD, high-fat diet; Ly-6A, lymphocyte antigen 6 complex locus A; Myf-5, myogenic factor 5; PDGF, platelet-derived growth factor; WAT, white adipose tissue.

Studies of gene expression in adipose tissue depots of mice adapted to either 4 °C or 30 °C (representing maximal versus minimal stimulation of adaptive thermogenesis) revealed strong induction of *Ucp1* expression in both classic BAT depots and inguinal WAT at 4 °C. Surprisingly, none of the genes previously identified as being specific to beige adipocytes displayed a robust correlation with either cold challenge or *Ucp1* expression.[41] However, increased expression of beige-adipocyte-specific genes correlated strongly with the location of the fat depot, an association that was also observed for expression of brown-adipocyte-specific genes.[41] In other words, *Hoxc9, Shox2, Lhx8* and *Zic1* might represent static markers that are characteristic of adipose depots containing brown or beige adipocyte lineages (Figure 3) but do not indicate the actual cells undergoing dynamic functional and morphologic changes that drive adaptive thermogenesis.

Whether these markers will ultimately be useful for assessing the functional significance of brown, beige and white adipocytes remains unclear. The formation and activity of beige adipocytes (in terms of increased metabolism and heat production) cannot be delineated by beige-adipocyte-specific gene expression profiles. Instead, useful information might be provided by the assessment of ubiquitously expressed genes that are essential for the transcription of crucial metabolic genes (such as *Ucp1*) found in brown or beige adipocytes.

Transcriptional regulation of beige adipogenesis

Many of the genes described above as specifically expressed in brown or beige adipocytes encode proteins involved in the control of transcription; yet, whether beige adipocytes can be identified on the basis of a unique transcription factor profile remains unclear. Among the many transcriptional regulators of adipocyte differentiation and function, PPARγ and CCAAT/enhancer-binding protein α (C/EBPα) are the two master regulators of adipogenesis. PPARγ is necessary and sufficient for adipocyte formation and function *in vitro* and *in vivo*.[42] C/EBPα has an important role in cellular insulin sensitivity[43] but is not essential for brown adipocyte differentiation.[44]

SHOX2 (which encodes short stature homeobox protein 2) is highly expressed in inguinal WAT in mice and humans[45] and represses C/EBPα activity and lipolysis in an adipocyte cell line.[46] In mice, adipocyte-specific deletion of *Shox2* increased browning and levels of β_3-adrenergic receptors and lipolysis in inguinal WAT.[46]

The actions of PPARγ and C/EBPα are modulated by a large set of proadipogenic or antiadipogenic transcriptional cofactors, such as members of the Krüppel-like family, GATA transcription factors, liver X receptors and sterol regulatory element-binding protein 1c.[42] Certain transcription factors are particularly important for brown adipocyte differentiation and functional maintenance, such as forkhead box protein C2,[47] retinoblastoma protein,[48,49] retinoblastoma-like 1,[50] eukaryotic translation initiation factor 4E-binding protein 1,[51] nuclear receptor-interacting protein 1,[52] and nuclear receptor coactivators 1 and 2.[53] However, the contribution of these transcription factors to browning of WAT is less clear.

Regulation of *UCP1* expression

UCP1 is crucial to brown and beige adipocyte function, and transcription factors that stimulate *UCP1* expression are, therefore, thought to control both browning and the metabolic functions of these cells.[54] However, only a few identified *UCP1* transcription factors are specific to brown or beige adipocytes.[54] Adaptive thermogenesis and *Ucp1* expression are mainly regulated by sympathetic tone through β-adrenergic signalling and cAMP levels,[3] which can be directly sensed by protein kinase A (PKA) and thereby lead to activation of mitogen-activated protein kinases (MAPKs).[55] The MAPK signalling pathway is linked to phosphorylation of a set of important transcription factors, including cAMP-response element binding protein (CREB), which controls expression of type 2 iodothyronine deiodinase (DIO2).[56,57] DIO2, in turn, converts inactive tetraiodothyronine (T_4) to tri-iodothyronine (T_3) in brown adipose tissue and thereby increases the activation of thyroid hormone receptors as well as *Ucp1* expression. The MAPK pathway also phosphorylates cAMP-dependent transcription factor ATF-2, which in turn initiates transcription of *Ucp1* and *Ppargc1a* (encoding the PPARγ cofactor PGC-1α).[55] Surprisingly, primary white adipocytes isolated from inguinal and epididymal fat depots, but not primary brown adipocytes, increase *Ucp1* and *Ppargc1a* mRNA levels *in vitro* at temperatures

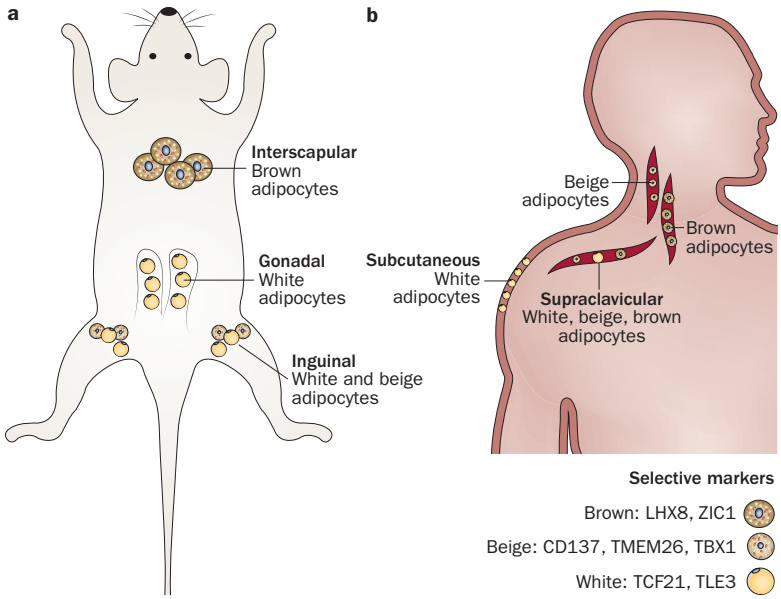

Figure 3 | Anatomical sites of brown, white and beige adipocytes in mice and humans. **a** | In mice, interscapular brown adipose tissue contains classic brown adipocytes. Epididymal white adipose tissue predominantly contains white adipocytes. Inguinal white adipose tissue contains a mixed population of white and beige adipocytes, the proportions of which depend on environment and diet. **b** | In human adults, the situation is far more complex. Subcutaneous fat has characteristics of classic white adipose tissue. Supraclavicular brown adipose tissue is composed of white, brown and beige adipocytes, whereas classic brown adipose tissue can be found deep in the neck, close to muscle tissue. In mice and men, several depot-specific and adipocyte-type-specific markers have been found. Abbreviations: CD137, tumour necrosis factor receptor superfamily, member 9; LHX8, LIM homeobox protein 8; TBX1, T-Box 1; TCF21, transcription factor 21; TLE3, transducin-like enhancer of split 3; TMEM26, transmembrane protein 26; ZIC1, zinc finger protein of the cerebellum 1.

below 37 °C, independently of the CREB pathway.[58] This phenomenon was also observed in cold-exposed mice that lack all three β-adrenergic receptor types,[58] which suggests that a certain proportion of the thermogenesis that occurs in WAT could result from a novel, as yet undefined mechanism.

Many studies that have investigated browning lacked a thorough characterization of the observed beige adipocyte phenotypes, especially as these often occur together with changes in BAT. Importantly, as *Ucp1* expression might change drastically and dynamically with sympathetic tone without necessarily translating into altered Ucp1 protein levels,[59] a standardized method of assessing browning is needed. Very helpful information would be obtained if, in addition to *Ucp1* expression and protein levels, the detailed metabolic contribution of all organs implicated in adaptive thermogenesis was assessed, for example by systematically following the fate of radiolabelled nutrient tracers.

Regulation mediated by the PGC-1α–PPAR complex

PGC-1α was originally identified as being differentially expressed in BAT and skeletal muscle upon cold exposure[60] and is now considered to probably be the most important regulatory protein in thermogenesis. When ectopically introduced into white adipocytes, PGC-1α activated the expression of *Ucp1* and key mitochondrial enzymes of the respiratory chain, such as Cox4, as well as increasing mitochondrial biogenesis.[60] PGC-1α binds to complexes of PPARα or PPARγ and retinoid X receptor (RXR), which both activate *Ucp1* expression by binding to a PPAR response element in the *Ucp1* promoter. Thus, treating adipocytes with PPARα, PPARγ or RXR agonists results in increased transcription of *Ucp1*.[61,62] Of note, PPARδ[63] and retinoic acid receptor (RAR)[64] agonists also increase *Ucp1* expression in adipocytes.

Prolonged treatment with retinoic acid has been used for the induction of browning in mice.[65] However, retinal, a precursor of retinoic acid, seems to be the active intermediate that induces browning; browning still occurs in mice deficient in retinaldehyde dehydrogenase, the enzyme that catalyzes the conversion of retinal to retinoic acid.[66] Importantly, activation of *Ucp1* expression via PPAR response elements in its promoter involves a positive feedback loop, resulting from the induction of *Ppargc1a*.[67] This mechanism might also be fine-tuned by direct negative regulators of PGC-1α, such as twist basic helix-loop-helix transcription factor 1, which is co-induced by PPARδ.[67]

PRDM16-mediated regulation

The activity of the PGC-1α–PPAR complex is modulated by another BAT-specific cofactor, PR domain zinc finger protein 16 (PRDM16). This cofactor is highly enriched in brown adipocytes compared with white adipocytes and is essential for the development of brown adipocytes. In mice, PRDM16 suppresses classic white adipocyte genes by interacting with C-terminal-binding proteins, and stimulates the transcription of several proteins involved in thermogenesis in WAT (including PGC-1α, UCP1 and DIO2).[68,69] When expressed in adipose tissue, PRDM16 contributes to the high levels of UCP1 in inguinal WAT.[70] Notably, PRDM16 is also required for the WAT remodelling induced by β_3-adrenergic signalling.[70] Moreover, chronic rosiglitazone-mediated activation of PPARγ induces PGC-1α[71] and stabilizes PRDM16 protein,[72] which increases browning.

The expression of *Prdm16* is under the control of the microRNA miR-133,[73,74] and manipulation of miR-133 influences the development of both classic brown[73] and beige adipocytes.[74] Deletion of miR-133 increases the levels of the beige-adipocyte-specific markers *Tnfrsf9* and *Tmem26* (but not *Tbx1*) in inguinal WAT,[74] suggesting that miR-133 acts as an early gatekeeper for cells entering the beige adipocyte lineage. PRDM16 itself stimulates expression of miR-193b-365, which leads to repression of myogenesis.[75] By contrast, miR-155 suppresses the activity of classic BAT, as well as browning, by silencing *Cebpb* (the gene encoding C/EBPα),[76] whereas miR-196a stimulates *Cebpb* through silencing of *Hoxc8* (encoding homeobox protein Hox-C8).[77] Hox-C8 itself acts in concert with histone deacetylase 3 (HD3) to modulate browning,[77] which is in line with the results of a study showing that systemic pharmacologic inhibition of HD3 is associated with improved oxidative capacity in BAT and skeletal muscle.[78]

Another modulator of PRDM16 is the cofactor transducin-like enhancer of split 3 (TLE3), which might specifically block the PRDM16–PPARγ interaction in fat depots.[79] Overexpression of this cofactor in WAT promotes lipid storage; conversely, its deletion results in the transcription of genes involved in thermogenesis.[79]

The AMPK–SIRT1–PGC-1α axis

The deacetylation of PPARγ by NAD-dependent protein deacetylase sirtuin-1 (SIRT1) also promotes browning,[80] which mimics the effects of chronic PPARγ activation with rosiglitazone. In hepatocytes, SIRT1 also deacetylates and activates PGC-1α directly.[81] In skeletal muscle, SIRT1 is activated by 5'-AMP-activated protein kinase (AMPK) through modulation of NAD^+ levels.[82] In this tissue, AMPK can also directly phosphorylate PGC-1α, increasing mitochondrial biogenesis.[83] High concentrations of resveratrol—a natural polyphenol found in red wine—activate the AMPK pathway by modulating cellular energy homeostasis.[84] Low concentrations of resveratrol indirectly stimulate AMPK signalling through Rap guanine nucleotide exchange factor 3 (EPAC1), which is a cellular cAMP sensor.[85]

This shared mechanism for energy sensing in the liver and skeletal muscle, involving the AMPK–SIRT1–PGC-1α axis, could have an equivalent in adipose tissue, which should influence browning; however, this suggestion is controversial, and the literature on this topic is conflicting. Chronic administration of resveratrol to mice can prevent diet-induced obesity, increase mitochondrial content in BAT and improve cold tolerance,[86] but the possible effects of resveratrol on browning of WAT were not investigated in this study.

In vitro studies have found disparate results, depending on the sites and types of adipocytes analysed.[54] Activation of AMPK using 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) in rats increases energy expenditure and mitochondrial content but not *Ucp1* expression in subcutaneous and visceral WAT,[87] whereas another study reported browning of gonadal WAT without changes in BAT.[88]

In summary, the balance of evidence suggests an involvement of the AMPK pathway in browning. Further studies should aim to understand the physiological relationship between energy sensing and browning, especially as treatment with salicylate has been described to directly activate AMPK;[89] salicylate might, therefore, represent a putative browning agent.

Lipid droplets in browning

The formation and composition of nuclear receptor complexes containing PPARs that are important for browning is dependent on hydrophobic ligands. The nature and origin of endogenous PPAR ligands is unclear, in addition to a limited understanding of how the generation of ligands is regulated. However, adipose triglyceride lipase (ATGL) is implicated in the generation of PPARα ligands derived from lipolysis of intracellular triglycerides in the heart.[90] Given that PPARα stimulates PGC-1α,[62] and that ATGL deficiency results in severe cold intolerance[91] and BAT malfunction,[92] ATGL could be important for generating PPAR ligands involved in the process of browning. This hypothesis is supported by a study which demonstrated that lipolytic products activate PPARα and PPARδ in brown adipocytes.[93]

Furthermore, deficiency of cell death activator CIDE-3 (formerly known as fat-specific protein-27)[94,95] and transgenic overexpression of perilipin A[96] are both associated with browning and increased levels of UCP1 in WAT, which point to a physiological link between lipid droplet remodelling and adipocyte identity.

The role of autophagy in browning

Energy sensing seems to be a critical regulator of browning; hence, downstream pathways that respond to energy shortage might also influence adipose tissue remodelling. One such pathway is autophagy, a lysosome-dependent catabolic process induced by nutrient deprivation.[97] A direct link between autophagy and lipid metabolism has been established; lipid droplets can be degraded in autophagosomes to yield free fatty acids.[98] Moreover, mice lacking the critical macroautophagy protein, ubiquitin-like modifier-activating enzyme ATG7, in adipocytes are resistant to diet-induced obesity and exhibit marked browning of gonadal WAT.[99]

The transcription factor EB, a master regulator of both lysosomal biogenesis and autophagy, is also a positive regulator of the hepatic fasting response and acts via PGC-1α and PPARα.[100] However, the beneficial metabolic effects of transcription factor EB overexpression are dependent on ATG7; hence, whether transcription factor EB and its effects on PGC-1α and PPARα are directly linked to browning remains unclear.

Hormonal regulation of browning

The development and activation of brown adipocytes in response to physiological and environmental changes are regulated by a complex hormonal interplay. This network comprises factors that are synthesized locally within adipose tissue as well as factors released by other metabolically active organs, including brain, muscle, heart and liver; hence, metabolic demands are linked to tissue remodelling. So far, most studies have focused on the hormonal regulation of classic brown adipocytes,[3,101] but the very same mechanisms might be equally important for the development and activation of beige adipocytes.

Many hormonal factors orchestrate systemic energy metabolism in other organs, as well as promoting browning (Figure 4). Metabolic challenges clearly affect browning, through the factors discussed below, but whether browning contributes to adaptations to these metabolic challenges in other organs is less well understood.

Catecholamines

The release of catecholamines at sympathetic terminals in BAT, WAT and the adrenal medulla is mandatory for the immediate activation of brown and existing beige adipocytes, as well as for the differentiation of beige adipocytes from their precursors.[3] In addition, alternatively activated (M2) macrophages in adipose tissue have

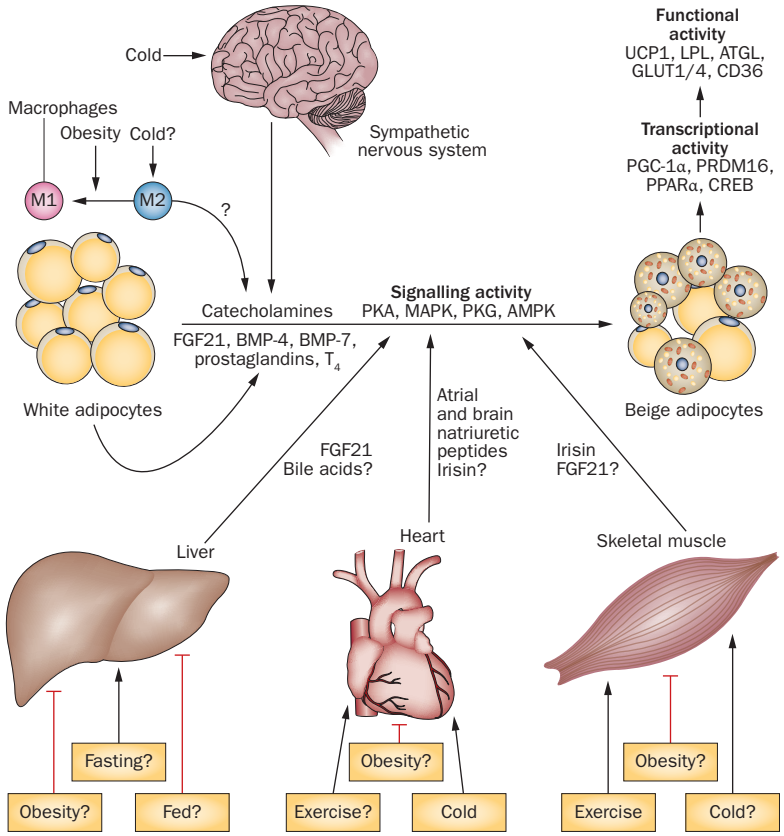

Figure 4 | Hormonal control of browning. Metabolic adaptations to environmental factors, such as cold, or metabolic conditions, including exercise, fasting, feeding and obesity, are regulated by the release of endocrine and paracrine factors from metabolically active organs. In response to cold, catecholamines released by the sympathetic nervous system and possibly also tissue-resident M2 macrophages are of key importance for the activation of energy-sensing pathways in white adipocytes and beige adipocyte precursors. Obesity induces a switch towards proinflammatory M1 macrophages that might impair this process. The beige phenotype is generated by expression of transcription factors that induce activities characteristic of beige adipocytes (increases in energy uptake, energy processing and energy expenditure). Question marks indicate that the proposed relation to browning has not been established yet.

been identified as another source of catecholamines and are involved in the regulation of lipolysis in response to acute cold stress,[102] but whether these macrophages have a role in browning remains speculative. Further studies could reveal whether the proinflammatory M1 macrophages present in hypertrophic adipose tissue of individuals with obesity[103] counteract the increased catecholamine production from alternatively activated macrophages, and thus might prevent browning in these individuals. In general, catecholamine-triggered responses to cold are regulated by neuronal circuits, along with orchestration of the adaptive responses to undernutrition or overnutrition (for example, by fibroblast growth factor 21 [FGF21] or leptin), as well as social and physical behaviour (by brain-derived neurotrophic factor [BDNF] or irisin).[101]

Lipid-derived hormones

In response to β_3-adrenergic receptor activation, prostaglandins, bone morphogenetic protein 4 (BMP-4) and FGF21 are produced in an organ-autonomous manner, and these changes particularly promote browning.[104–107] Prostaglandins are fatty acid derivatives generated cyclooxygenase (COX; also known as prostaglandin G/H synthase) activity. Cold exposure increases COX activity and, thereby, prostaglandin levels, which stimulates the expression of PGC-1α in WAT-resident mesenchymal progenitors by an as yet undefined signalling pathway.[104,105] Notably, transgenic expression of *Ptgs2*, the gene that encodes Cox-2, in mouse adipocytes prevents diet-induced obesity,[105] suggesting that WAT-derived prostaglandins regulate systemic energy homeostasis. Furthermore, a study in mice lacking adipocyte-specific fatty acid synthase—which display increased browning—showed that other fatty acid derivatives (from *de novo* lipogenesis) are linked to thermogenesis via alkyl-ether-lipid-mediated activation of PPARγ.[108] However, an increase in *de novo* lipogenesis in adipocytes—as observed in mice with adipocyte-specific deletion of lipoprotein lipase—does not modulate browning,[109] supporting the notion that the products of *de novo* lipogenesis might have differential effects on transcriptional control depending on the intermediates involved and their intracellular concentrations.

Peptide hormones

FGF21

The expression of *Fgf21* in WAT increases after cold exposure and β_3-adrenergic receptor activation, suggesting a physiological role for FGF21 in the recruitment of beige adipocytes.[107] Moreover, FGF21-deficient mice display an impaired cold response in inguinal WAT but not in classic BAT. Interestingly, FGF21 increases browning by stabilizing PGC-1α protein without affecting PGC-1α mRNA expression.[107] Paradoxically, FGF21 expression increases in WAT in the fed state which, via a feed-forward loop, regulates PPARγ activity and white adipogenesis.[110] This emerging model of FGF21 action is further complicated by the fact that hepatic FGF21 contributes to thermogenesis in neonatal brown adipocytes.[111] Furthermore, FGF21 released from skeletal muscle (as a result of mitochondrial dysfunction) promotes browning of WAT.[112] However, at this time, a physiological model on how browning is affected by increased levels of local or circulating FGF21 is difficult to define.

Bone morphogenic proteins

Bone morphogenic proteins represent a family of secreted molecules that influence the differentiation of mesenchymal stem cells. BMP-7 promotes the differentiation of brown preadipocytes by inducing the expression of *Prdm16* and *Ppargc1a*.[113] In addition, BMP-8b directly regulates thermogenesis in mature brown adipocytes by increasing their responsiveness to noradrenaline and upregulating intracellular lipase activity via the PKA–MAPK pathway.[114] Unlike BMP-7 and BMP-8b, transgenic expression of *Bmp4* stimulates the conversion of mesenchymal precursors specifically to beige adipocytes.[106] However, as BMP-4 also facilitates the commitment of

mesenchymal cells to the adipocyte lineage[115] and drives white adipocyte differentiation *in vitro*,[116] its precise role in browning needs further validation.

Environmental influences on browning

Cold—sensed by central and peripheral thermoreceptors and integrated by neurons of the preoptic area—is by far the strongest sympathogenic signal.[101,117] The levels of several peripheral hormones (including leptin, insulin and ghrelin) as well as those of central factors, such as hypothalamic endocannabinoids, reflect the nutritional conditions that influence sympathetic tone.[101,118–120] Signalling processes stimulated by exploration of objects and socialization are also influenced by the neuronal endocannabinoid system and by certain neuropeptides, such as BDNF. Mice housed in an enriched environment with complex physical and social stimulation have increased levels of BDNF in hypothalamic areas. This phenomenon is associated with marked browning of WAT,[121] which indicates the existence of a signalling pathway from the hypothalamus via the sympathetic nervous system to WAT. In principle, any hormonal signal that can stimulate this pathway sufficiently, for example, PKA, MAPKs or PGC-1α, should be able to induce browning.

Cold exposure promotes thermogenesis and browning but also increases heart rate, blood pressure and muscle strength.[3] Hence, cardiac and skeletal muscle might conceivably initiate a hormonal response to prolonged cold exposure that notifies the body to produce heat, as it does upon exercise.[122] In addition, this hormonal response could help to preserve the key functions of cardiac and skeletal muscle under conditions of chronically increased catecholamine release. In line with these considerations, factors released by cardiac and skeletal muscle display hormonal properties that trigger browning; for example, atrial and brain natriuretic peptides (ANP and BNP, respectively) released upon cold exposure by cardiomyocytes lead to the expression of PGC-1α and UCP1 in WAT.[123] These endocrine factors are also important for the regulation of blood pressure and induce cGMP-dependent protein kinase G signalling in adipocytes.[124] This catecholamine-independent pathway also promotes healthy expansion and browning of WAT via MAPK signalling.[124] From an evolutionary point of view, the release of ANP and BNP might have another beneficial effect, namely to counteract the harmful effects of prolonged cold exposure, such as cardiac hypertrophy and hypertension.[125]

Linkage of exercise and browning

Endurance exercise (and probably also cold-induced shivering) is associated with browning of WAT, arguing for a role of muscle activity in beige adipocyte biology. Similarly to the mechanisms described for brown adipocytes, an increase in energy expenditure induces PGC-1α expression in muscle cells. PGC-1α also mediates beneficial effects of exercise by, for example, inducing mitochondrial biogenesis and muscle fibre-type switching.[126] In addition, PGC-1α stimulates the secretion of muscle-derived factors that could potentially be involved in the regulation of systemic energy expenditure.

Fibronectin type III domain containing 5 (FNDC5), encoded by *Fndc5*, was identified in 2012.[127] This myokine is specifically regulated by exercise and PGC-1α.[127] FNDC5 is membrane-bound and its proteolytic cleavage leads to the release of irisin, an endocrine factor that drives browning of WAT by so far unknown pathways.[127] In line with these observations, myostatin-deficient mice (which have increased muscle mass) showed a concomitant and FNDC5-dependent increase in browning of WAT.[128,129] Notably, PGC-1α_4 (an isoform of PGC-1α that is highly expressed in exercising muscles) represses myostatin-dependent pathways,[130] which provides further evidence for a systemic regulatory network linking muscle workload to browning. Translational studies published in 2012 and 2013 describe a positive correlation between expression of *PPARGC1A* in exercised muscle and plasma levels of irisin.[131–133] However, owing to a mutation in the start codon (ATA instead of ATG), human FNDC5 has been annotated as a transcribed pseudogene which is not effectively translated into full-length FNDC5 protein.[134] Thus, although common genetic polymorphisms in *FNDC5* are associated with insulin sensitivity,[135] the physiological significance of irisin, and possibly other myokines, needs to be validated by interventional studies.

Implications of browning for metabolic health

BAT is a powerful metabolic tissue that controls plasma glucose levels and triglyceride metabolism—at least in mice.[23] The ability of BAT to consume and metabolize nutrients is unequalled by any other tissue, on unit weight basis, in mice.[136] Moreover, BAT activation facilitates weight loss, ameliorates insulin resistance and corrects hyperlipidaemia after 24 h cold exposure in mice.[137]

Systemic effects of BAT transplantation

In transplantation experiments looking at the effects of increased BAT mass, recipient mice kept at room temperature demonstrate improved glucose tolerance, increased insulin action and protection from diet-induced weight gain.[138,139] Interestingly, one study group noted that most of the beneficial systemic effects of BAT transplantation in mice are dependent on interleukin 6 (IL-6) being released from the transplanted BAT,[138] whereas other researchers did not observe systemic changes in IL-6 levels.[139] IL-6 is a promiscuous cytokine that has many apparently contradictory metabolic effects; for example, exercise induces an acute increase in the production of IL-6 in skeletal muscle, but regular exercise is associated with reduced plasma levels of IL-6 levels during the resting state.[122] By contrast, plasma IL-6 levels are increased in individuals with obesity, and the IL-6 produced by white adipocytes has deleterious effects on hepatic insulin sensitivity in mice.[140] These observations raise questions as to whether the continuous increase in IL-6 plasma levels resulting from BAT transplantation is beneficial for metabolic health.

As many molecules, dietary factors and pharmacological interventions have effects on both BAT and

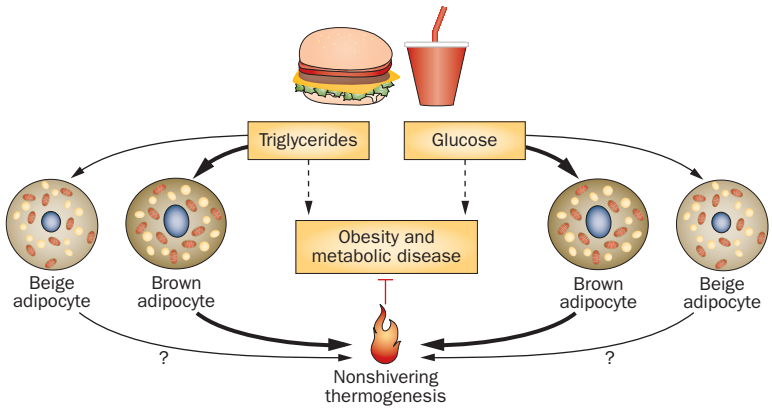

Figure 5 | Contributions of browning to systemic nutrient handling. Excess intakes of triglycerides and glucose ultimately lead to obesity and metabolic disease. Increased clearance and utilization of nutrients by brown and beige adipocytes could reduce this excess of triglycerides and glucose, and confer beneficial metabolic effects or protection from obesity. However, whether nutrients channelled into beige adipocytes are used in nonshivering thermogenesis, as they are in classic brown adipocytes, remains unclear.

browning, the estimation of their respective contributions to the observed metabolic consequences is complex. Furthermore, even when assessing interventions that specifically target BAT or browning, other organs that are important in the regulation and activation of thermogenesis (such as the hypothalamus, liver, skeletal muscle or heart)[3] might have an adaptive role that contributes to the observed effects. The presence of increased energy expenditure, even in conjunction with elevated *Ucp1* expression in WAT, does not necessarily justify the conclusion that the observed phenotype results from browning. Instead, measurement of the intertissue flux and intratissue oxidation of nutrients is important to estimate the contribution of each implicated tissue to the process of adaptive thermogenesis.

An early finding in analyses of the contribution of browning to metabolic health came from comparisons of different inbred mouse strains after β_3-adrenergic receptor agonist treatment. Whereas UCP1 protein content in BAT did not differ between these mouse strains, the UCP1 increase in inguinal WAT and retroperitoneal WAT was higher in A/J mice than in C57BL6/J mice.[141] Another study showed that β_3-agonist-mediated weight loss was most pronounced in A/J mice and was tightly correlated with UCP1 protein content in retroperitoneal WAT, although no difference between the strains was noted in diet-induced obesity. However, in another study, diet-induced obesity was attenuated in 129/Sv mice compared with that in C57BL/6J mice.[142] Taken together, these observations led to the conclusion that an inherited browning capacity modulates adiposity.

Studies of visceral (gonadal) and inguinal WAT transplantation have shown that only inguinal WAT transplantation has beneficial effects on metabolic parameters, such as adiposity and insulin resistance,[143] analogous to those observed in studies of BAT transplantation.[138,139] As the donor mice had been acclimatized to standard laboratory conditions, the observed beneficial effects of inguinal WAT transplantation might be caused by the metabolic activity of beige adipocytes in the grafts.

The interplay of BAT function and browning

The amount of BAT can be reduced by deleting BMP receptor 1a (BMPR-1a) in Myf5$^+$ brown adipocyte progenitors, which results in a compensatory systemic increase in catecholamine levels and marked browning of WAT.[144] In this model, loss of BAT did not increase susceptibility to diet-induced obesity, probably because of this browning—although a definitive evaluation of the metabolic contributions of BAT, WAT and skeletal muscle to thermogenesis was not performed. In that vein, sarcolipin (a regulator of calcium homeostasis in the sarcoplasmic and endoplasmic reticulum) has been implicated in adaptive thermogenesis, especially when BAT function is compromised.[145] However, again, browning was not assessed in this study.

Our research group became interested in the study of browning when we observed that short-term cold exposure at 4 °C in mice not only increased the delivery of triglyceride-rich lipoprotein to BAT but also—albeit to a lesser extent—its delivery to inguinal WAT.[137] This observation indicates that in mice that had been housed at 22 °C, beige adipocytes can be stimulated to contribute to systemic energy handling. Indeed, in a setting that stimulates browning, such as after 7 days of treatment with a β_3-adrenergic receptor agonist, glucose and fatty acid delivery during a meal are increased by about 1.75-fold in both BAT and WAT. In particular, our results show that mouse beige adipocytes can increase their energy uptake to an extent similar to that observed in classic brown adipocytes *in vivo* (A. Bartelt and J. Heeren, unpublished work). In this model (Figure 5), the activation of brown and beige adipocytes leads to improved fat and glucose tolerance in lean animals without major changes in adiposity (A. Bartelt and J. Heeren, unpublished work).

In summary, at least in mice, beige adipocytes are important contributors to metabolic health; whether these cells also produce heat to the same extent that brown adipocytes do remains unclear.[59] Human epicardial fat has been demonstrated to display relatively high levels of *UCP1* expression, as well as high mitochondrial UCP1 content. Notably, UCP1 mRNA levels in epicardial fat biopsies correlated positively with HDL-cholesterol levels, whereas levels of mRNA for *LPL* (which encodes lipoprotein lipase) correlated positively with HDL-cholesterol levels and inversely with plasma triglyceride levels.[146] These observations support the concept that levels of UCP1 protein expression in humans are correlated with metabolic health.

Conclusions

Brown and beige adipocytes can be detected in human BAT using common imaging techniques (Box 2);[33,37,38,40] however, whether nutrient handling and energy dissipation differ between these two types of adipocytes in humans remains unclear. From the metabolic point of view, this uncertainty might not be very important, as

both cell types contribute to handling of glucose and fatty acids in humans.[147,148] However, the observation that, in humans, low BAT activity is correlated with ageing, obesity and measures of metabolic disease is pivotal.[4,6,14,15] This relationship suggests a causal link between decreased BAT activity and weight gain. Several critical processes might contribute to the loss of BAT activity in humans: the functional decline might start with impaired responsiveness because of insulin resistance,[147,149] which could progress to apoptosis of mature brown and beige adipocytes[150–152] and finally damage progenitor cell populations.[153,154] Intervention at these stages might help sustain the functional properties of brown and beige adipocytes in ageing and obesity, thus preserving the metabolic functions of these cells and thereby their beneficial influence on health.

Most of the standard metabolic parameters routinely assessed by physicians, such as blood glucose and lipid levels, are influenced by the activity of brown (and possibly also beige) adipocytes. Hence, a thorough understanding of the mechanisms underlying the formation and decline of brown and beige adipocytes in humans offers the possibility to develop new therapeutic strategies for weight loss, insulin resistance and hyperlipidaemia. In this context, the induction of browning represents one important approach that could potentially convert deleteriously lipid-overloaded WAT into metabolically active and healthy BAT.

Review criteria

A search for original articles from 2009 until 2013 focusing on brown adipose tissue and browning was performed in PubMed. The search terms used were "brown adipose tissue", "browning", "brite" and "beige". All articles selected were English-language, full-text papers. References cited in identified articles were examined separately and used to generate further leads.

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Acknowledgements
The authors thank all members of the Heeren lab for continuous support and enjoyable atmosphere, and are grateful to Rudolph Reimer for providing pictures taken by electron microscopy. A. Bartelt is supported by a Deutsche Forschungsgemeinschaft Research Fellowship (BA 4925/1-1). J. Heeren is supported by a grant from the Fondation Leducq —Triglyceride Metabolism in Obesity and Cardiovascular Disease and by EU FP7 project RESOLVE (FP7-HEALTH-2012-305707).

Author contributions
Both authors contributed equally to all aspects of this article.