The colorful versatility of adipocytes: white-to-brown transdifferentiation and its therapeutic potential in humans

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Brown and brite adipocytes contribute to energy expenditure through nonshivering thermogenesis. Though these cell types are thought to arise primarily from the de novo differentiation of precursor cells, their abundance is also controlled through the transdifferentiation of mature white adipocytes. Here, we review recent advances in our understanding of the regulation of white-to-brown transdifferentiation, as well as the conversion of brown and brite adipocytes to dormant, white-like fat cells. Converting mature white adipocytes into brite cells or reactivating dormant brown and brite adipocytes has emerged as a strategy to ameliorate human metabolic disorders. We analyze the evidence of learning from mice and how they translate to humans to ultimately scrutinize the relevance of this concept. Moreover, we estimate that converting a small percentage of existing white fat mass in obese subjects into active brite adipocytes could be sufficient to achieve meaningful benefits in metabolism. In conclusion, novel browning agents have to be identified before adipocyte transdifferentiation can be realized as a safe and efficacious therapy.

Introduction

Mammalian adipose tissue is comprised of two main types of adipocytes, white and brown, which inversely contribute to energy balance regulation. White adipocytes possess a large unilocular lipid droplet, reside in white adipose tissue (WAT), and store excess energy as fat. Brown adipocytes, on the other hand, possess a multilocular appearance (multiple small lipids droplets), reside in brown adipose tissue (BAT), consume energy reserves, and produce heat. Brown adipocytes have an enormous capacity for substrate oxidation conferred by a very high abundance of mitochondria. These mitochondria are equipped with uncoupling protein 1 (UCP1), a 32 kDa protein residing in the inner mitochondrial membrane. When activated by sympathetic nerves that control the lipolytic release of activating fatty acids and the degradation of inhibitory purine nucleotides [1,2], UCP1 induces a proton leak that uncouples oxygen consumption from ATP production, facilitating macronutrient catabolism. This adaptive mechanism increases energy expenditure and

Abbreviations

AC, adenylyl cyclase; ANP, atrial natriuretic peptide; BAT, brown adipose tissue; BMP, bone morphogenic protein; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CIDEA, cell death-inducing DFFA-like effector a; cPGI2, carbaprostacyclin; EBF2, early B-cell factor 2; FGF21, fibroblast growth factor 21; FGFR, fibroblast growth factor 21 receptor; FNDC, fibronectin type III domain-containing protein; GC, guanylyl cyclase; hMADS cells, human multipotent adipose-derived stem cells; IP, prostaglandin I2 receptor; KLF11, Kruppel-like factor 11; LXR, liver X receptor; NPRA, natriuretic peptide receptor-A; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α; PKA, protein kinase A; PKG, protein kinase G; PPAR, peroxisome proliferator-activated receptor; PRDM16, PR domain containing 16; RIP140, receptor-interacting protein 140; RXR, retinoid X receptor; T3, triiodothyronine; TLE3, transducin-like enhancer of split 3; TWIST1, twist basic helix–loop–helix transcription factor 1; TZD, thiazolidinedione; UCP1, uncoupling protein 1; WAT, white adipose tissue; ZFP423, zinc finger protein 423; β3, β3-adrenoreceptor.
makes BAT an important heater organ, especially in small mammals [3,4]. The same mechanism is found in brown-like adipocytes which have been given multiple names such as ‘inducible’, ‘beige’, or ‘brite’ (brown-in-white) referring to their brown adipocyte-like appearance and function but are found in WAT depots. Brown and brite adipocytes are distinct cell types, yet their transcriptomic signature and cellular function become remarkably similar under conditions that enforce adaptive heat production [5–8]. Brite adipocyte recruitment (a process called ‘browning of WAT’) is enhanced upon BAT loss, suggesting that these cells complement brown adipocyte functions [9,10].

The abundance of mature adipocytes is controlled by the balance between preadipocyte expansion, differentiation, and eventual cell death. Canonically, adipocytes are thought to arise from the de novo differentiation of precursor cells committed to white, brown, or brown adipocyte lineages. However, terminally differentiated mature adipocytes exhibit phenotypic plasticity, and the morphological and functional conversion of a fully differentiated mature adipocyte into another type of fat cell has been termed ‘adipocyte transdifferentiation’ [11]. Thus, mature adipocytes can dynamically alter their phenotype from white to brown/brite and vice versa to adopt to changing environmental conditions and energy availability and demand. Moreover, white, brite, and brown adipocytes can transdifferentiate into ‘pink adipocytes’ and contribute to milk secretion in lactating mice [12–14]. In humans, white adipocytes are far more abundant than brown and brite adipocytes [15,16], and the absolute number of an individuals’ adipocytes is kept rather constant throughout adulthood [17,18]. Thus, transdifferentiation constitutes an important mechanism in the control of brown, brite, and white adipocyte quantity. We here analyze recent findings to reconcile de novo differentiation and transdifferentiation as complementary origins of brown and brite adipocytes, summarize our current understanding on the regulation of adipocyte transdifferentiation in human adipose tissue, and finally scrutinize the relevance of WAT browning as a therapeutic concept in man.

The phenotypic versatility of mammalian adipose tissue

Brown and brite adipocyte origins: de novo adipogenesis versus transdifferentiation

Brite adipocytes can derive both from a distinct precursor population residing in WAT and from the transdifferentiation of mature white adipocytes upon thermogenic stimulation [19–24]. It is now quite clear that both are complementary mechanisms controlling brite adipocyte quantity throughout life and in response to different environmental conditions. During postnatal browning, some murine WAT depots transiently increase the abundance of brite adipocytes soon after birth with a subsequent decline after weaning [25–28]. In posterior subcutaneous (inguinal) WAT, this peak in the number of brite adipocytes around weaning is influenced by both cell-intrinsic mechanisms and sympathetic innervation, resulting in a greater browning response of newborn pups at lower housing temperature [26,29,30]. In adult mice, white adipocytes with a history of Ucp1 expression can be found in inguinal WAT, and a significant proportion of adipocytes in this depot is capable of switching their phenotype from white to brite upon cold exposure [21,26,31,32]. This proportion is considerably higher when mice were born and raised at subthermoneutral temperature or temporarily subjected to cold in the adolescent stage [31]. Moreover, ablation of postnatally formed brite adipocytes results in impaired WAT browning in adult mice [33]. Thus, brite adipocytes initially develop from committed precursor cells, a process that can be enhanced by an initial phase of cold exposure (either during the perinatal period or later). In the absence of a thermogenic stimulus, newly differentiating brite adipocytes can become ‘camouflaged’ as white adipocytes, but retain their ability to rapidly undergo white-to-brite transdifferentiation upon cold exposure [5,21,24,31,33]. This coordinated sequence of postnatal browning and subsequent brite adipocyte camouflage is influenced by lipid species found in breast milk, suggesting maternal nutrition and rearing behavior as crucial determinants of browning capacity [34]. Collectively, when assessing transdifferentiation in mice, UCP1-positive cells may not exclusively originate from the transdifferentiation of a white adipocyte, but rather from the rerecruitment of a primed, quiescent brite cell (Fig. 1).

As described above, the housing temperature history of a mouse considerably influences the proportion of brite adipocytes that emerge via mature adipocyte transdifferentiation in response to a new cold challenge. Interestingly, upon selective agonism of the β3-adrenoreceptor, which is primarily expressed by mature murine adipocytes and pharmacologically mimics a cold exposure challenge, the browning response of WAT is different. The majority of the emerging brite adipocytes is then derived from mature adipocytes regardless of a prior cold exposure history [24,31,35,36], suggesting that white-to-brite transdifferentiation can occur via both the rerecruitment of a
quiescent brite cell and the transdifferentiation of a mature white fat cell that did not exhibit a brite phenotype previously (Fig. 1). In line with this view, bipotent WAT-resident precursor populations have been identified that give rise to both brite and white adipocytes, the latter of which bears a transdifferentiation potential [37,38].

The presence of brite adipocytes in murine WAT depends on factors such as age and environment. Yet, camouflage is not a unique property of brite adipocytes. Aging or feeding mice a high-calorie diet chronically in a thermoneutral environment (‘physiologically humanized mice’) not only favors the absence of brite adipocytes but also enhances lipid storage in brown adipocytes [39–41]. This ‘BAT whitening’ results in the transformation of mature brown adipocytes into cells with a white adipocyte-like appearance with attenuated UCP1 expression. Thus, BAT whitening can be roughly considered as the opposite of WAT browning (Fig. 1).

Adipocyte transdifferentiation in the human adipose organ

Browning of WAT in humans in vivo has been observed in different anatomical locations as a secondary effect of pathophysiological conditions (such as paraganglioma, pheochromocytoma, burn injury, and cancer-associated cachexia), but also in response to change of season and repeated localized cold exposure [42–50]. Brite adipocytes are, however, largely absent in the WAT of most adult humans under normal conditions, possibly due to living in thermoneutral conditions. In human subcutaneous WAT, distinct precursor pools with brite adipogenic potential have been identified [51–53], and we have recently shown that mature adipocytes also have the ability to transdifferentiate into brite cells in vitro [19,54,55]. Thus, browning of WAT may be of a similar nature in humans as it has been shown to occur in rodents, with brite cells emerging from both differentiation of precursor cells and transdifferentiation from existing white adipocytes (Fig. 1). Treating primary human mature adipocytes or preadipocytes with a browning compound during or after differentiation results in equal UCP1 mRNA induction (Fig. 2), suggesting that the potential of human adipocytes to brown can be exploited at any stage of maturation.

Rodents possess a significant amount of BAT distributed across different anatomical locations. The interscapular BAT depot is the largest and most studied. A corresponding depot is found in human infants, which disappears after the first decade of life, but is found as a remnant in some individual adults [56–59]. Adult humans possess most BAT in the cervical, supracleavicular, axillary, mediastinal, paraspinal, and abdominal region [15]. However, thermogenic activity is only detected in a portion of the total depot volume [15], suggesting attenuated overall BAT function due to whitening. Indeed, the vast majority of adipocytes in perirenal adipose tissue seems to exist as dormant brown adipocytes camouflaged as UCP1-expressing, unilocular fat cells [60,61], a phenotype that somewhat resembles the BAT in ‘physiologically humanized mice’
In line with the reversible nature of BAT whitening in mice [39], intermittent cold exposure of adult humans increases cold-induced BAT activity [62–64]. Given the substantial browning capacity of human adipocytes in vitro (Figs 2 and 4) and the evidence that dormant brown and brite adipocytes appear to be recruitable by thermogenic stimulation throughout the human body [42,44,65,66], achieving meaningful improvements in patient health through thermogenic activation appears to be a realistic and viable opportunity.

**Mechanisms of white-to-brown/brite transdifferentiation**

**Transcriptional regulators**

Mechanistic insight into the transcriptional regulation of a brown fat transdifferentiation program has been gleaned through years of study on predominantly in vitro-differentiated brown preadipocytes and the brown fat of genetically modified animals. Many transcription factors and other signaling proteins have been found to regulate browning (the breadth of this topic has been extensively reviewed elsewhere [67–71]). However, only a small number of factors including peroxisome proliferator-activated receptor-γ (PPARγ) coactivator 1α (PGC1α), PR domain containing 16 (PRDM16), and early B-cell factor 2 (EBF2) have been deemed ‘master regulators’ of browning for their ability to dictate brown adipocyte lineage specificity and/or an ability to orchestrate a complete browning program when overexpressed in adipocytes [72–74]. These proteins, like many other regulators of brown adipogenesis, interact with PPARγ [72,75–87], a member of the PPAR family of nuclear receptors that is required for adipogenesis in vivo and in vitro [88–91]. Upon ligand binding, PPARγ functions as a heterodimeric transcription factor regulating the expression of PPAR-responsive genes. Selective agonism of PPARγ by thiazolidinediones (TZDs) is sufficient to drive a brown fat program [92–94]. TZDs drive a sirtuin 1-dependent deacetylation of PPARγ promoting its interaction with PRDM16, which facilitates the interaction of PPARγ with the mediator complex involved in chromatin looping [87,95–97]. Although PPARγ can directly bind DNA, it is thought that it is recruited to brown fat-specific enhancers and promoters via interactions with its binding partners and transcriptional coactivators such as EBF2 [79] (Fig. 3). Through this mechanism, PPARγ is recruited and bound to multiple enhancers near and proximal to the promoters of brown fat-selective genes (i.e., genes enriched in brown and brite adipocytes). Through chromatin looping, many enhancers can be brought together forming clusters of high transcriptional activity known as super-enhancers [98–100]. These super-enhancers are found predominately at genes that are responsible for cell identity, and a loss of them can convert BAT into a white-like fat depot [100].

It is presumed that many mechanisms of the transcriptional cascade occurring in a differentiating brown adipocyte also hold true in an adipocyte precursor cell undergoing brite adipogenesis in WAT [101,102].
However, brown and brite adipocytes are distinct cell types with inherent plasticity, and although they possess similar gene expression profiles [5,23,103,104], differences in the regulation of these two cell types are likely to exist. For instance, the transcription factors transducin-like enhancer of split 3 (TLE3) and zinc finger protein 423 (ZFP423) are enriched in WAT and, in the absence of a browning stimulus, repress a brown fat-like transcriptional program through EBF2 inhibition [105–107]. Similarly, other transcriptional coregulators have the capacity to prevent browning by repressing the levels and transcriptional activity of PGC1α and by disrupting its interaction with PPARγ [84,108–113] (Fig. 3). Conversely, overexpression of PGC1α and PPARγ agonism sufficient to drive browning in human mature white adipocytes [19,54,76]. Thus, key transcriptional regulators of browning seem to be conserved in mouse and man, and have similar functions in preadipocytes and transdifferentiating adipocytes (Fig. 3). Human adipocyte transdifferentiation has been further associated with transcription factors enhancing the transcriptional activity of PPARγ. While Kruppel-like factor 11 (KLF11) stabilizes the TZD-induced expression of brite adipocyte genes, cell

Fig. 3. Cell-extrinsic mediators and intracellular signaling pathways involved in the white-to-brite conversion of human mature adipocytes. In the unstimulated state, the activity of transcription factors involved in brown/brite adipogenesis (displayed in green and blue) is attenuated by corepressors (displayed in red) to maintain white adipocyte identity. Different cell-extrinsic mediators are able to overcome this repression, resulting in the suppression of corepressors, and the formation and stabilization of transcriptional complexes in the enhancer regions of brown-selective genes such as UCP1. Rosiglitazone, tesaglitazar, and cPGI2 activate this process via a direct interaction with the transcription factor PPARγ. FGF21 enhances the effect of rosiglitazone via an unknown mechanism presumably involving an activation of the FGF21 receptor. Noradrenaline (released via sympathetic nerve fibers), CL-316,243, and mirabegron activate the β3-adrenoreceptor, while cPGI2 signals via the IP receptor. Both receptors elicit adenylyl cyclase activation leading to elevated cAMP levels and PKA activation, the disinhibition of brown-selective gene transcription, and the lipolytic release of free fatty acids from intracellular stores. The same effects occur after ANP-mediated activation of the NPR-A receptor, which signals via guanylyl cyclase and cGMP to activate PKG. Fatty acids serve as thermogenic substrates and as direct activators of UCP1 in mitochondria. AC, adenylyl cyclase; ANP, atrial natriuretic peptide; β3, β3-adrenoreceptor; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CIDEA, cell death-inducing DFFA-like effector a; cPGI2, carbaprostacyclin; EBF2, early B-cell factor 2; FGF21, fibroblast growth factor 21; FGFR, fibroblast growth factor 21 receptor; GC, guanylyl cyclase; IP, prostaglandin I2 receptor; KLF11, Kruppel-like factor 11; LXR, liver X receptor; NPR-A, natriuretic peptide receptor-A; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α; PKA, protein kinase A; PKG, protein kinase G; PPARγ, peroxisome proliferator-activated receptor-γ; PRDM16, PR domain containing 16; RIP140, receptor-interacting protein 140; RXR, retinoid X receptor; TLE3, transducin-like enhancer of split 3; TWIST1, twist basic helix-loop-helix transcription factor 1; UCP1, uncoupling protein 1; ZFP423, zinc finger protein 423.
death-inducing DFFA-like effector a (CIDEA) seemingly shuttles from the cytosol to the nucleus and acts as a transcriptional coregulator directly modulating UCP1 expression in white adipocytes via the suppression of liver X receptor (LXR) [82,114,115] (Fig. 3). The recent identification of key regulatory factors involved in murine adipocyte browning [116] will certainly help to further characterize the transcriptional circuitry involved in the white-to-brite conversion of mature human adipocytes.

**Cell-extrinsic mediators**

The last decade has revealed a plethora of substances and stimuli associated with the recruitment and activation of brown and brite adipocytes, which have been extensively reviewed elsewhere [117–120]. However, only several of these have been reported to act directly on human adipocytes and act via a transdifferentiation mechanism.

In mice, cold exposure is perhaps the most potent and physiologically relevant stimulus that drives the recruitment of thermogenic capacity and activity in BAT and WAT. In human WAT, changes in brown-selective gene expression have been observed after a single exposure to locally restricted cold (ice pack application) [48]. Such rapid changes suggest the recruitment of human brite adipocytes in vivo to be influenced by transdifferentiation. During cold exposure, sympathetically released noradrenaline acts on adrenergic receptors on the plasma membrane of adipocytes to orchestrate the activation and recruitment of thermogenic capacity [2] (Fig. 3). In mice and humans, these effects can be mimicked by CL-316,243 and mirabegron, selective agonists of the β3-adrenoceptor, which triggers the direct white-to-brite conversion of mature adipocytes [31,49,114–123]. The G protein-coupled β3-receptor signals via cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) increasing lipolysis, UCP1 activation, and the transcriptional recruitment of thermogenic capacity [2] (Fig. 3). Similarly, the heart-derived hormone atrial natriuretic peptide (ANP), which is an endogenous ligand of the natriuretic peptide receptor-A (NPRA) that signals via cyclic guanosine monophosphate (cGMP) and protein kinase G (PKG), mediates the white-to-brite conversion of human adipocytes [114,124] (Fig. 3). We assume that in vivo, many stimuli have the potential to affect human adipocyte transdifferentiation via increasing the sympathetic tone and noradrenaline release.

Among the several members of the TZDs, rosiglitazone is well known for its effect on brown and brite adipogenesis, especially in murine cells [125,126], but also in human adipocytes [19,54,94,114,127,128]. Interestingly, fibroblast growth factor 21 (FGF21) enhances the transdifferentiation effect of rosiglitazone on cultured human adipocytes, while FGF21 itself seems to have only minor effects on this conversion [54]. This liver-derived, PPARγ-responsive hormone confers the dual PPARα/γ agonist tesaglitazar a superior efficacy to induce WAT browning in vivo in mice compared to rosiglitazone [54]. While TZDs constitute synthetic agonists, oxygenated derivatives of fatty acids (i.e., oxylipins, commonly referred to as eicosanoids) serve as an endogenous class of PPARγ ligands. These molecules have recently emerged as novel regulators of adipocyte-based thermogenesis [129]. Oxylipins are produced via several distinct pathways including cyclooxygenase, which has been implicated in adipose tissue browning in mice [130,131]. Carbaprostacyclin (cPGI2), a stable analog of the naturally occurring cyclooxygenase derivative prostaglandin I2, induces the formation of a brite adipocyte phenotype in human multipotent adipose-derived stem cells (hMADS cells) [132]. When applied to mature adipocytes during the final stage of the adipogenic differentiation, cPGI2 induces UCP1 mRNA and protein expression accompanied by the recruitment of mitochondrial capacity. These effects seem to originate from a combined activation of the G protein-coupled prostaglandin I2 receptor of the plasma membrane (the IP receptor) and an interaction with PPARγ (Fig. 3). Many compounds among the plethora of known oxylipins interact with membrane-bound receptors or PPAR transcription factors [133]. Thus, the ability of oxylipins to mediate adipocyte transdifferentiation may not be restricted to prostaglandin I2. Interestingly, prostaglandin E2 acutely increases UCP1 mRNA levels in tissue explants and primary mature adipocytes isolated from human WAT, while it inhibits rosiglitazone-mediated white-to-brite transdifferentiation of hMADS cells [134,135]. Thus, the presumed function of oxylipins on adipocyte transdifferentiation requires validation and more detailed investigations.

Collectively, the current state of the art suggests the existence of endogenous and exogenous mediators capable of mediating a direct phenotypic conversion of human adipocytes. In addition to the above-mentioned factors, several others have been reported to influence human adipocyte browning in different cell models and experimental settings, including triiodothyronine (T3), bone morphogenic proteins (BMP) 4 and 7, and fibronectin type III domain-containing proteins (FNDC) 4 and 5, as well as the FNDC5 cleavage product irisin [136–141]. It remains to be determined how
robust these effects are in the context of the transdifferentiation of human mature white adipocytes [19]. Nevertheless, future research will undoubtedly reveal further cues with translational relevance.

**Therapeutic potential of white-to-brite transdifferentiation**

**How much browning do we need?**

In the past decade, considerable progress has been made to estimate and quantify the amount of active BAT in humans. As discussed above, BAT can be recruited and activated acutely by both cold exposure and treatment with the β3-agonist mirabegron [15,63,121,142,143]. Importantly, prolonged treatment with either mirabegron or mild cold exposure leads to a significant improvement in metabolism (although this might not be mediated entirely by BAT activation), providing support to the notion that safe and efficacious BAT activation, or converting WAT into BAT, is a promising strategy for the treatment of metabolic diseases in human [123,144,145]. However, it is extremely difficult to accurately determine BAT mass in subjects, and most studies rely on the uptake of radiolabeled tracers (radiolabeled glucose in particular), which reflects BAT activity more than mass. Thus, total BAT mass is likely underestimated in many studies, especially in overweight or obese subjects and in insulin-resistant states [15]. However, detectable BAT mass/activity decreases with age and obesity [146–149], raising concerns that there may not be enough recruitable BAT to treat patients with age-related and obesity-associated diabetes. On the other hand, there is no shortage of WAT in a typical diabetic person and converting white fat cells into brite adipocytes may represent a more attractive strategy to increase energy expenditure for the treatment of metabolic diseases.

It has been demonstrated recently that the contribution of brite adipocytes to systemic energy expenditure in mice is significantly less compared to brown adipocytes [150], raising questions about the quantity of brite adipocytes required for a significant therapeutic benefit. Determining how much browning of WAT would be required to achieve a meaningful improvement in metabolism in humans is a difficult question to answer with a good level of confidence, but one can try to, with some approximations in spite of many unknown parameters. A moderate weight loss of about 5% in obese patients has considerable health benefits, including decreased intra-abdominal and intrahepatic fat, and increased multi-organ insulin sensitivity and β-cell function [151]. Assuming an average energy intake of ~2850 kcal-day⁻¹, increasing energy expenditure by 175 kcal-day⁻¹ is predicted to give ~4% body weight decrease in one year for an obese individual [152]. Although estimates about BAT-related energy expenditure can vary greatly across studies, 200 g of activated BAT has been hypothesized to lead to an increase of ~175 kcal-day⁻¹ [15,121]. If one estimates the WAT mass in an overweight subject to be >20 kg, the full conversion of just 1% of this WAT would lead to an additional 200 g of brite fat equivalent. Expression of the unequivocal brown/brite adipocyte marker UCP1 is considerably higher in BAT compared to WAT (Fig. 4), also in humans [153,154]. Considering that UCP1 mRNA levels are ~1000-fold higher in BAT compared to WAT, we estimate that a therapeutically relevant browning agent should elevate UCP1 levels in WAT by at least 10-fold in order to raise total UCP1 expression in WAT to ~1% of the levels present in BAT. This would represent the equivalent of doubling the existing BAT mass considering a ratio of WAT/BAT of 100. This is a strict minimum estimated under the assumption that the brite cells formed would be fully activated, and with large uncertainties in estimating the exact thermogenic potential of BAT. UCP1 has been shown to function either as monomer or as oligomer [155,156], and there are many UCP1-independent effects of brown and brite adipocytes, as discussed below. Therefore, UCP1 levels likely do not linearly correlate with thermogenic capacity. It is also important to note that UCP1 itself does not possess intrinsic basal uncoupling activity, which prevents proton conductance in the absence of an activating stimulus [157]. A clear distinction should be made between thermogenic capacity and thermogenic activity. Thus, the extent of WAT browning required for a therapeutic benefit largely depends on, and must be inversely proportional to, the level of activation expected in vivo [158]. A better understanding of sympathetic nervous system activity in the different adipose tissue depots in obese and diabetic conditions is required. A combination therapy consisting of a browning agent and an activator (mirabegron, cold or other) is likely to result in synergistic metabolic benefits. Interestingly, the simple overexpression of UCP1 in human adipocytes in vitro leads to increased basal glucose uptake [159]. Moreover, the browning agent tesaglitazar significantly increases energy expenditure in mice in vivo even in thermoneutral conditions [54]. This suggests that the recruitment of brown and brite adipocytes may lead to some degree of metabolic improvements due to the endogenous basal activity of these cells, even in the absence of an activating stimulus.
Heterogeneity in WAT browning

Estimations on the degree of browning required to induce metabolic benefits are rendered more complex by the large heterogeneity among WAT depots. It appears clear that a simple classification of adipocytes as white, brite, or brown is insufficient. Different WAT depots, but also adipocytes within a single WAT or BAT depot, display a large range of characteristics with varying gene expression profiles, different developmental origins, and differences in adipocyte function [51,52,160–168]. This appears to be the case in both murine and human fat, with a subset of human adipocytes surprisingly lacking the β2-adrenoreceptor, particularly in metabolically impaired obese patients [169]. It is therefore not surprising that different adipose tissue depots have different capacities to undergo browning and that brite adipocytes are not homogeneously dispersed within a single depot, as characterized in detail in mice [170–174]. Recent evidence for the existence of a novel, natural ‘brite fat depot’ in mice further underlines that the structure of the adipose organ is more complex than previously anticipated [175,176]. Among the murine WAT depots, the subcutaneous inguinal fat seems to have the largest capacity to brown, while visceral and mesenteric fat have the lowest [170–172]. A corresponding difference in brown/brite adipocyte marker gene expression between these depots is even found in the absence of a browning stimulus [171,172]. Interestingly, humans and mice display opposing patterns of browning genes, with human visceral adipose depots having significantly higher expression of BAT markers compared to subcutaneous fat [177–179]. Still, human mature subcutaneous white adipocytes have the capacity to transdifferentiate into brite adipocytes when treated ex vivo with PPAR ligands (Fig. 2) [19,54]. It is tempting to speculate that adipocytes from other human WAT depots would have an even greater capacity to brown. A better characterization of human adipose

Fig. 4. Absolute levels of UCP1 transcript in white, brown, and brite fat of different species. Transcription levels were quantified by quantitative real-time PCR using species-specific standard curves with known UCP1 cDNA copy numbers. Murine brown and white adipose tissue were obtained from lean and diet-induced obese mice kept at room temperature (22 °C) or acclimated to thermoneutrality (30 °C) [54]. Nonhuman primate brown and white adipose tissues were obtained from the axillar/supraclavicular and subcutaneous abdominal region of Rhesus monkeys, respectively. Mature adipocytes were isolated from human subcutaneous abdominal white fat and exposed to vehicle or rosiglitazone (1 μM) for 1 week to obtain white or brite adipocytes, respectively. Fold differences (brown/brite versus white) in UCP1 absolute transcript levels are indicated for each model. UCP1, uncoupling protein 1.
tissue heterogeneity and their respective capacity to respond to different browning stimuli will certainly advance the field.

UCP1 is not everything

The determination of browning capacity of adipose tissue depots is often quantified as changes in UCP1 expression. However, browning of murine WAT in vivo can occur even in the absence of UCP1 [180–183] and the conversion of human white into brite adipocytes in vitro results in metabolic reprogramming and an increase in UCP1-independent mitochondrial uncoupling [184,185]. Thus, it is overly simplified to restrict the therapeutic potential of browning agents solely to their ability to induce UCP1-dependent energy expenditure. Several distinct mechanisms of adipocyte-based nonshivering thermogenesis have been described, including a mitochondrial creatine/creatinine-phosphate futile cycle controlled by creatine kinase, ATP-dependent calcium cycling by the sarcoplasmic/endoplasmic reticulum calcium ATPase 2b and the ryanodine receptor 2, the circulating enzyme peptidase M20 domain containing 1 (PM20D1) catalyzing the formation of N-acetyl amino acids that function as endogenous uncouplers, AMP-activated protein kinase-dependent thermogenesis, and the proton transport function of the mitochondrial ADP/ATP carrier [182,186–192]. Whether these pathways are all present and significantly contribute to thermogenesis in human adipose tissue will require further studies.

Browning of WAT for diabetes or obesity treatment?

Transplantation studies in mice support a beneficial effect of thermogenic adipocytes on body weight control [193,194]. However, we believe that the weight loss that can be achieved by adipocyte-based thermogenesis alone is unlikely to be sufficient to qualify as an obesity treatment in humans. Firstly, as described above, there are uncertainties about the amount of BAT present in diabetic/obese patients, and browning of WAT may not lead to a massive increase in energy expenditure considering the sedentary and thermoneutral environment most humans live in. Moreover, BAT-centered therapies in humans have failed to show effects on body weight thus far [62,123,195]. Secondly, while body weight loss can be achieved acutely, it is much harder to maintain in the long term due to compensatory mechanisms and a drive of the organism to come back to the original body weight set point. Similar to weight loss induced by lifestyle changes, recent antidiabetes drugs cause a certain amount of weight loss initially, which progressively decreases over time [196]. This is due to a decrease in resting metabolic rate and a concomitant compensatory increase in food intake, which prevents further weight loss and contributes to weight regain. Interestingly, it has been hypothesized that meal-induced but not cold-induced BAT activation is accompanied by a limitation of energy intake [197]. This view is, on the one hand, based on the essential role of food energy to ensure the maintenance of BAT thermogenesis for body temperature regulation upon prolonged cold exposure. On the other hand, the gastrointestinal hormone secretin, which is released upon food consumption and is able to acutely activate BAT in both mice and humans, was recently shown to be a mediator of a novel gut–BAT–brain axis that controls prandial satiation [198]. Although food intake stimulates human BAT activity to a similar degree as cold exposure, it is unlikely that chronic secretin is sufficiently capable of achieving significant weight loss [197,199]. Combination strategies of drugs acting on increasing energy expenditure with drugs acting on reducing food intake may act more potently to achieve synergistic weight loss [200].

At least some of the beneficial metabolic effects of increasing thermogenesis appear to be weight reduction-independent. In fact, BAT has a high capacity to clear circulating glucose, lipids, and triglycerol-rich lipoproteins [181,201,202]. Accordingly, an acute increase in BAT activity in human subjects can result in immediate effects on glucose and lipid homeostasis [145,203], which are likely to improve metabolic health in the long term [123,195]. Moreover, transplantation of human brite adipocytes into mice results in an improvement of glucose homeostasis [204]. These effects may originate, for instance, from ‘BATokines’, that is, factors with autocrine, paracrine, and endocrine actions secreted by brown and brite adipocytes [205–209]. Overall, browning of WAT and BAT activation can cause both body weight-dependent and body weight-independent improvements in metabolism, but the case for antidiabetic effects appears stronger than for the treatment of obesity in humans. However, browning of WAT and BAT activation may be useful in combination with other weight-reducing agents to achieve greater weight reduction, or as an add-on therapy to help prevent weight regain.

Availability of pharmacological browning inducers

To date, there are no approved drugs for the treatment of diabetes or obesity whose main mode of action is
WAT browning. As described above, the ligand-activated nuclear receptor PPAR\(\gamma\) is a central hub in the regulation of brown and brite adipogenesis and thus may be expected to serve as promising pharmacological target for that purpose. Indeed, TZDs such as rosiglitazone or pioglitazone, which have been approved as drugs for the treatment of diabetes as insulin sensitizers, induce browning of human adipocytes with significant efficacy (Figs 2 and 4). Yet, this effect has not been reported in patients using TZDs for the treatment of diabetes. The clinical development of the dual PPAR\(\alpha/\gamma\) agonist tesaglitazar, which promotes browning of WAT in mice in vivo with superior efficacy than rosiglitazone [54], was stopped due to safety concerns. It is not known whether some of the beneficial effects of tesaglitazar on glucose and lipid metabolism in humans are mediated at least in part via WAT browning.

In recent years, efforts have been made to identify and develop approved substances or novel molecules as pharmaceutical effectors of human adipocyte browning. For instance, a screen of molecules identified Janus kinase inhibitors as novel browning agents in stem cell-derived human adipocytes [210]. As an alternative to classical agonism, post-translational modifications may be targeted by novel drugs acting through PPAR\(\gamma\). Phosphorylation of PPAR\(\gamma\) at serine 273, mediated by cyclin-dependent kinase 5 and extracellular signal-regulated kinase, is increased in obesity and insulin-resistant states resulting in a dysregulation of adipocyte gene expression [211,212]. A screen to identify compounds inhibiting this phosphorylation revealed Gleevec, a well-known anticancer drug, as modulator of WAT browning in mice [213]. Roscovitine, an inhibitor of cyclin-dependent kinase 5, is also able to mediate WAT browning in mice via the prevention of PPAR\(\gamma\) phosphorylation at serine 273 [214]. It is currently unknown whether these compounds induce WAT browning in human adipocytes. Interestingly, short-term application of the phosphodiesterase inhibitor sildenafil, used for the treatment of pulmonary arterial hypertension and erectile dysfunction, initiates the formation of brite adipocytes in WAT of overweight subjects, but this effect does not appear to be mediated via a direct action on adipose tissue [215].

Based on the effect of cold exposure to both increase browning of WAT and activate BAT, it is likely that at least some substances with the ability to acutely activate BAT would also be suitable to recruit further thermogenic capacity when applied chronically. Mirabegron is (besides cold stimulation) probably the most potent and advanced pharmaceutical activator of human BAT identified to date. Chronic mirabegron administration to humans can recruit thermogenic capacity in BAT and modestly elevate protein expression of brown adipocyte markers in subcutaneous WAT suggesting a browning potential [49,123]. Although mirabegron is an approved drug, it is currently not intended for the treatment of diabetes and obesity. In fact, the high doses required to achieve a significant increase in energy expenditure are also associated with increased heart rate and blood pressure [121,123,216,217]. Thus, mirabegron may be unsuitable to treat obese and diabetic patients for which a negative impact on the cardiovascular system would not be tolerated in this at-risk population. Still, antidiabetic effects may be achieved at lower doses [195]. Future studies will be required to explore the full potential of this BAT activator to improve metabolism.

Concluding remarks

The differentiation of preadipocytes and the transdifferentiation of mature adipocytes are complementary mechanisms in the control of thermogenic capacity as browning and whitening affect the quantity of white, brite, and brown adipocytes in both WAT and BAT. This transdifferentiation potential likely confers the organism a greater flexibility to quickly adapt to nutritional and environmental changes without inducing major alterations in adipocyte turnover and cell number. Pro-adipogenic, sedentary lifestyle habits cause brown and brite adipocytes to exist quiescently and become camouflaged as white fat cells. The development of drugs triggering their reconversion holds promise for the treatment of obesity-associated metabolic diseases maybe more so than obesity itself. Future investigations will certainly help to further explore this potential.

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Conflict of interest

The authors all are employees of AstraZeneca.

Author contributions

All authors wrote, edited, and reviewed the manuscript. SM prepared figures. All authors approved the final version of the manuscript.
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References

1 Fromme T, Kleigrew K, Dunkel A, Retzler A, Li Y, Maurer S, Fischer N, Diezko R, Kanzleiter T, Hirschberg V et al. (2018) Degradation of brown adipocyte purine nucleotides regulates uncoupling protein 1 activity. Mol Metab 8, 77–85.

2 Klingenspor M, Bast A, Bolze F, Li Y, Maurer S, Schweizer S, Willershauer M, Fromme T (2017) Brown adipose tissue. In Adipose Tissue Biology (Symonds ME eds), pp. 91–147. Springer International Publishing.

3 Enerback S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper M-E & Kozak LP (1997) Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. Nature 387, 90–94.

4 Golozoubova V, Hohtola E, Matthias A, Jacobsson A, Cannon B & Nedergaard J (2001) Only UCP1 can mediate adaptive nonshivering thermogenesis in the cold. FASEB J 15, 2048–2050.

5 Roh HC, Tsai LTY, Shao M, Tenen D, Shen Y, Kumari M, Lyubetskaya A, Jacobs C, Dawes B, Gupta RK et al. (2018) Warming induces significant reprogramming of beige, but not brown, adipocyte cellular identity. Cell Metab 27, 1121–1137 e5.

6 Perdikari A, Leparc GG, Balaz M, Pires ND, Lidell ME, Sun W, Fernandez-Albert F, Muller S, Akchiche N, Dong H et al. (2018) BATLAS: deconvoluting brown adipose tissue. Cell Rep 25, 784–797 e4.

7 Li Y, Fromme T, Schweizer S, Schöttl T & Klingenspor M (2014) Taking control over intracellular fatty acid levels is essential for the analysis of thermogenic function in cultured primary brown and brite/beige adipocytes. EMBO Rep 15, 1069–1076.

8 Shabalina IG, Petrovic N, de Jong JMA, Kalinovich AV, Cannon B & Nedergaard J (2013) UCP1 in brite/beige adipose tissue mitochondria is functionally thermogenic. Cell Rep 5, 1196–1203.

9 Schulz TJ, Huang P, Huang TL, Xue R, McDougall LE, Townsend KL, Cypess AM, Mishina Y, Gussoni E & Tseng Y-H (2013) Brown-fat paucity due to impaired BMP signalling induces compensatory browning of white fat. Nature 495, 379–383.

10 Hoffmann JM, Grünberg JR, Church C, Elias I, Palsdottir V, Jansson J-O, Bosch F, Hammarstedt A, Hedjazifar S & Smith U (2017) BMP4 gene therapy in mature mice reduces BAT activation but protects from obesity by browning subcutaneous adipose tissue. Cell Rep 20, 1038–1049.

11 Cinti S (2002) Adipocyte differentiation and transdifferentiation: plasticity of the adipose organ. J Endocrinol Invest 25, 823–835.

12 Morroni M, Giordano A, Zingaretti MC, Boiani R, De Matteis R, Kahn BB, Nisoli E, Tonello C, Pisoschi C, Luchetti MM et al. (2004) Reversible transdifferentiation of secretory epithelial cells into adipocytes in the mammary gland. Proc Natl Acad Sci USA 101, 16801–16806.

13 De Matteis R, Zingaretti MC, Murano I, Vitali A, Frontini A, Giannulis I, Barbatelli G, Marcucci F, Bordicchia M, Sarzani R et al. (2009) In vivo physiological transdifferentiation of adult adipose cells. Stem Cells 27, 2761–2768.

14 Li L, Li B, Li M, Niu C, Wang G, Li T, Król E, Jin W & Speakman JR (2017) Brown adipocytes can display a mammary basal myoepithelial cell phenotype in vivo. Mol Metab 6, 1198–1211.

15 Leitner BP, Huang S, Brychta RJ, Duckworth CJ, Baskin AS, Megee S, Tai I, Dieckmann W, Gupta G, Kolodny GM et al. (2017) Mapping of human brown adipose tissue in lean and obese young men. Proc Natl Acad Sci USA 114, 8649–8654.

16 Gerngross C et al. (2017) Active brown fat during (18) F-FDG PET/CT imaging defines a patient group with characteristic traits and an increased probability of brown fat redetection. J Nucl Med 58, 1104–1110.

17 Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Näslund E, Britton T et al. (2008) Dynamics of fat cell turnover in humans. Nature 453, 783–787.

18 Salans LB, Horton ES & Sims EA (1971) Experimental obesity in man: cellular character of the adipose tissue. J Clin Invest 50, 1005–1011.

19 Harms MJ, Li Q, Lee S, Zhang C, Kull B, Hallen S, Thorell A, Alexandersson I, Hagberg CE, Peng X-R et al. (2019) Mature human white adipocytes cultured under membranes maintain identity, function, and can transdifferentiate into brown-like adipocytes. Cell Rep 27, 213–225, e5.

20 Berry DC, Jiang Y & Graff JM (2016) Mouse strains to study cold-inducible beige progenitors and beige adipocyte formation and function. Nat Commun 7, 10184.

21 Rosenwald M et al. (2013) Bi-directional interconversion of brite and white adipocytes. Nat Cell Biol 15, 659–667.

22 Wang QA, Tao C, Gupta RK & Scherer PE (2013) Tracking adipogenesis during white adipose tissue development, expansion and regeneration. Nat Med 19, 1338–1344.

23 Wu J, Boström P, Sparks LM, Ye L, Choi JH, Giang A-H, Khandekar M, Virtanen KA, Nuutila P, Schaart G et al. (2012) Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150, 366–376.

24 Cattaneo P et al. (2020) Parallel lineage-tracing studies establish fibroblasts as the prevailing in vivo adipocyte progenitor. Cell Rep 30, 571–582, e2.

25 Lasar D, Julius A, Fromme T & Klingenspor M (2013) Browning attenuates murine white adipose
tissue expansion during postnatal development. *Biochim Biophys Acta* 1831, 960–968.

26 Wu Y, Kinnebrew MA, Kutyavin VI & Chawla A (2020) Distinct signaling and transcriptional pathways regulate peri-weening development and cold-induced recruitment of beige adipocytes. *Proc Natl Acad Sci USA* 117, 6883–6889.

27 Xue B, Rim J-S, Hogan JC, Coulter AA, Koza RA & Kozak LP (2007) Genetic variability affects the development of brown adipocytes in white fat but not in interscapular brown fat. *J Lipid Res* 48, 41–51.

28 Birnbacher L, Maurer S, Scheidt K, Herzen J, Pfeiffer F & Fromme T (2018) Electron density of adipose tissues determined by phase-contrast computed tomography provides a measure for mitochondrial density and fat content. *Front Physiol* 9, 707.

29 Chabowska-Kita A, Trabczynska A, Korytko A, Kaczmarek MM & Kozak LP (2015) Low ambient temperature during early postnatal development fails to cause a permanent induction of brown adipocytes. *FASEB J* 29, 3238–3252.

30 Wu R, Yu W, Fu L, Li F, Jing J, Cui X, Wang S, Cao Q, Xue B & Shi H (2020) The postnatal leptin surge is critical for the transient induction of the developmental beige adipocytes in mice. *Am J Physiol Endocrinol Metab* 318, E453–E461.

31 Shao M, Wang QA, Song A, Vishvanath L, Bushboso NC, Scherer PE & Gupta RK (2019) Cellular origins of beige fat cells revisited. *Diabetes* 68, 1874–1885.

32 Lee YH, Petkova AP, Konkar AA & Granneman JG (2015) Cellular origins of cold-induced brown adipocytes in adult mice. *FASEB J* 29, 286–299.

33 Wang Y, Paulo E, Wu D, Wu Y, Huang W, Chawla A & Wang B (2017) Adipocyte liver kinase b1 suppresses beige adipocyte renaissance through class IIa histone deacetylase 4. *Diabetes* 66, 2952–2963.

34 Yu H, Dilbaz S, Colßmann J, Hoang AC, Diedrich V, Herwig A, Harauma A, Hoshi Y, Moriguchi T, Landgraf K et al. (2019) Breast milk alkylglycerols sustain beige adipocytes through adipose tissue macrophages. *J Clin Invest* 129, 2485–2499.

35 Jiang Y, Berry DC & Graff JM (2017) Distinct cellular and molecular mechanisms for beta3 adrenergic receptor-induced beige adipocyte formation. *Elife* 6, e30329.

36 Contreras GA, Lee Y-H, Mottillo EP & Granneman JG (2014) Inducible brown adipocytes in subcutaneous inguinal white fat: the role of continuous sympathetic stimulation. *Am J Physiol Endocrinol Metab* 307, E793–E799.

37 Lee YH et al. (2012) In vivo identification of bipotential adipocyte progenitors recruited by beta3-adrenoceptor activation and high-fat feeding. *Cell Metab* 15, 480–491.
white adipose tissue in humans: evidence for thermogenic gene induction. *J Clin Endocrinol Metab* 99, E2772–E2779.

49 Finlin BS, Memetimin H, Confides AL, Kasza I, Zhu B, Vekaria HJ, Harffmann B, Jones KA, Johnson ZR, Westgate PM et al. (2018) Human adipose beiging in response to cold and mirabegron. *JCI Insight* 3, e121510.

50 Finlin BS, Zhu B, Confides AL, Westgate PM, Harffmann BD, Dupont-Versteegden EE & Kern PA (2017) Mast cells promote seasonal white adipose beiging in humans. *Diabetes* 66, 1237–1246.

51 Min SY, Desai A, Yang Z, Sharma A, DeSouza T, Genga RMJ, Kucukural A, Lifshitz LM, Nielsen S, Scheele C et al. (2019) Diverse repertoire of human adipocyte subtypes develops from transcriptionally distinct mesenchymal progenitor cells. *Proc Natl Acad Sci USA* 116, 17970–17979.

52 Raajendiran A, Ooi G, Bayliss J, O’Brien PE, Schittenhelm RB, Clark AK, Taylor RA, Rodheffer MS, Burton PR & Watt MJ (2019) Identification of metabolically distinct adipocyte progenitor cells in human adipose tissues. *Cell Rep* 27, 1528–1540, e7.

53 Xue R, Lynes MD, Dreyfuss JM, Shamsi F, Schulz TJ, Zhang H, Huang TL, Townsend KL, Li Y, Takahashi H et al. (2015) Clonal analyses and gene profiling identify genetic biomarkers of the thermogenic potential of human brown and white preadipocytes. *Nat Med* 21, 760–768.

54 Kroon T, Harms M, Maurer S, Bonnet L, Alexandersson I, Lindblom A, Ahnmark A, Nilsson D, Gennemark P, O’Mahony G et al. (2020) PPARγ and PPARα synergize to induce robust browning of white fat in vivo. *Mol Metab* 10, 100964.

55 Alexandersson I, Harms MJ & Boucher J (2020) Isolation and culture of human mature adipocytes using membrane mature adipocyte aggregate cultures (MAAC). *J Vis Exp* 156. https://doi.org/10.3791/60485.

56 Aherne W & Hull D (1964) The site of heat thermogenic gene induction. *Proc R Soc Med* 57, 1172–1173.

57 Heaton JM (1972) The distribution of brown adipose tissue in the human. *J Anat* 112 (Pt 1), 35–39.

58 Lidell ME, Betz MJ, Leinhard OD, Heglund M, Elander L, Slawik M, Muscack T, Nilsson D, Romu T, Nuutila P et al. (2013) Evidence for two types of brown adipose tissue in humans. *Nat Med* 19, 631–634.

59 Fletcher LA, Kim K, Leitner BP, Cassimatis TM, O’Marra AE, Johnson JW, Halprin MS, McGehee SM, Brychta RJ, Cypess AM et al. (2020) Sexual dimorphisms in adult human brown adipose tissue. *Obesity (Silver Spring)* 28, 241–246.

60 Jespersen NZ, Feizi A, Andersen ES, Heywood S, Hattel HB, Daugaard S, Peijs L, Bagi P, Feldt-Rasmussen B, Schultz HS et al. (2019) Heterogeneity in the perirenal region of humans suggests presence of dormant brown adipose tissue that contains brown fat precursor cells. *Mol Metab* 24, 30–43.

61 Svensson PA, Lindberg K, Hoffmann JM, Taube M, Pereira MJ, Moens-Kanson T, Hafner A-L, Rizell M, Palming J, Dani C et al. (2014) Characterization of brown adipose tissue in the human perirenal depot. *Obesity (Silver Spring)* 22, 1830–1837.

62 Yoneshiro T, Aita S, Matsushita M, Kayahara T, Kameya T, Kawai Y, Iwanaga T & Saito M (2013) Recruited brown adipose tissue as an antiobesity agent in humans. *J Clin Invest* 123, 3404–3408.

63 van der Lans AA, Hoeks J, Brans B, Vijgen GHEJ, Visser MGV, Vosselman MJ, Hansen J, Jorgensen JA, Wu J, Mottaghy FM et al. (2013) Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. *J Clin Invest* 123, 3395–3403.

64 Blondin DP, Labbé SM, Tingelstad HC, Noll C, Kunach M, Phoenix S, Guérin B, Turcotte EE, Carpentier AC, Richard D et al. (2014) Increased brown adipose tissue oxidative capacity in cold-acclimated humans. *J Clin Endocrinol Metab* 99, E438–E446.

65 Efremova A, Senzacqua M, Venema W, Isakov E, Di Vincenzo A, Zingaretti MC, Protasoni M, Thomski M, Giordano A & Cinti S (2019) A large proportion of mediastinal and perirenal visceral fat of Siberian adult people is formed by UCP1 immunoreactive multilocular and paucilocular adipocytes. *J Physiol Biochem* 76, 185–192.

66 Ogawa Y, Abe K, Sakoda A, Onizuka H & Sakai S (2018) FDG-PET and CT findings of activated brown adipose tissue in a patient with paraganglioma. *Eur J Radiol Open* 5, 126–130.

67 Inagaki T, Sakai J & Kajimura S (2016) Transcriptional and epigenetic control of brown and beige adipose cell fate and function. *Nat Rev Mol Cell Biol* 17, 480–495.

68 Seale P (2015) Transcriptional regulatory circuits controlling brown fat development and activation. *Diabetes* 64, 2369–2375.

69 Loft A, Forss I & Mandrup S (2017) Genome-wide insights into the development and function of thermogenic adipocytes. *Trends Endocrinol Metab* 28, 104–120.

70 Harms M & Seale P (2013) Brown and beige fat: development, function and therapeutic potential. *Nat Med* 19, 1252–1263.

71 Shapiro SN & Seale P (2019) Transcriptional control of brown and beige fat development and function. *Obesity (Silver Spring)* 27, 13–21.

72 Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scime A, Devarakonda S, Conroe HM, Erdjument-Bromage H et al. (2008) *PRDM16* controls
a brown fat/skeletal muscle switch. *Nature* **454**, 961–967.

73 Wang W, Kissig M, Rajakumari S, Huang L, Lim H, Won K-J & Scale P (2014) Ebf2 is a selective marker of brown and beige adipogenic precursor cells. *Proc Natl Acad Sci USA* **111**, 14466–14471.

74 Uldry M, Yang W, St-Pierre J, Lin J, Scale P & Spiegelman BM (2006) Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation. *Cell Metab* **3**, 333–341.

75 Puigserver P, Wu Z, Park CW, Graves R, Wright M & Spiegelman BM (1998) A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* **92**, 829–839.

76 Tiraby C, Tavernier G, Lefort C, Larrouy D, Bouillaud F, Ricquier D & Langin D (2003) Acquisition of brown fat cell features by human white adipocytes. *J Biol Chem* **278**, 33370–33376.

77 Kajimura S et al. (2009) Initiation of myoblast to brown fat switch by a PRDM16-C/EBP-beta transcriptional complex. *Nature*** **460**, 1154–1158.

78 Seale P, Kajimura S, Yang W, Chin S, Rohas LM, Uldry M, Tavernier G, Langin D & Spiegelman BM (2007) Transcriptional control of brown fat determination by PRDM16. *Cell Metab* **6**, 38–54.

79 Rajakumari S, Wu J, Ishibashi J, Lim H-W, Giang A-H, Won K-J, Reed RR & Scale P (2013) EBF2 determines and maintains brown adipocyte identity. *Cell Metab* **17**, 562–574.

80 Wang L et al. (2013) Histone H3K9 methyltransferase G9a represses PPARgamma expression and adipogenesis. *EMBO J* **32**, 45–59.

81 Abe Y, Rozqie R, Matsumura Y, Kawamura T, Nakaki R, Tsurutani Y, Tanimura-Inagaki K, Shiono A, Magoori K, Nakamura K et al. (2015) JMJD1A is a signal-sensing scaffold that regulates acute chromatin dynamics via SW1/SNF association for thermogenesis. *Nat Commun* **6**, 7052.

82 Loft A et al. (2015) Browning of human adipocytes requires KLF11 and reprogramming of PPARgamma superenhancers. *Genes Dev* **29**, 7–22.

83 Lee JE, Wang C, Xu S, Cho Y-W, Wang L, Peng X, Baldrige A, Sartorelli V, Zhuang L, Peng W et al. (2013) H3K4 mono- and di-methyltransferase MLL4 is required for enhancer activation during cell differentiation. *Elife* **2**, e01503.

84 Hansen JB et al. (2004) Retinoblastoma protein functions as a molecular switch determining white versus brown adipocyte differentiation. *Proc Natl Acad Sci USA* **101**, 4112–4117.

85 Zhou H, Wan B, Grubisic I, Kaplan T & Tjian R (2014) TAF7L modulates brown adipose tissue formation. *Elife* **3**, e02811.

86 Gupta RK, Arany Z, Scale P, Mepani RJ, Ye L, Conroé HM, Roby YA, Kulaga H, Reed RR & Spiegelman BM (2010) Transcriptional control of preadipocyte determination by Zfp243. *Nature* **464**, 619–623.

87 Quang L et al. (2012) Brown remodeling of white adipose tissue by SirT1-dependent deacetylation of Ppargamma. *Cell* **150**, 620–632.

88 Wang F, Mullican SE, DiSpirito JR, Peed LC & Lazar MA (2013) Lipotoxicity and severe metabolic disturbance in mice with fat-specific deletion of Ppargamma. *Proc Natl Acad Sci USA* **110**, 18656–18661.

89 Rosen ED et al. (2002) C/EBPalpha induces adipogenesis through PPARgamma: a unified pathway. *Genes Dev* **16**, 22–26.

90 Kubota N et al. (1999) PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol Cell* **4**, 597–609.

91 Barak Y et al. (1999) PPAR gamma is required for placental, cardiac, and adipose tissue development. *Mol Cell* **4**, 585–595.

92 Petrovic N et al. (2008) Thermogenically competent nonadrenergic recruitment in brown preadipocytes by a PPARgamma agonist. *Am J Physiol Endocrinol Metab* **295**, E287–E296.

93 Bartesaghi S, Hallen S, Huang L, Svensson P-A, Momo RA, Wallin S, Carlsson EK, Forsløw A, Scale P & Peng X-R (2015) Thermogenic activity of UCPI in human white fat-derived beige adipocytes. *Mol Endocrinol* **29**, 130–139.

94 Elabd C, Chielini C, Carmona M, Galitzky J, Cochet O, Petersen R, Pénicaud L, Kristiansen K, Bouloumié A, Casteilla L et al. (2009) Human multipotent adipose-derived stem cells differentiate into functional brown adipocytes. *Stem Cells* **27**, 2753–2760.

95 Ohno H et al. (2012) PPARgamma agonists induce a white-to-brown fat conversion through stabilization of PRDM16 protein. *Cell Metab* **15**, 395–404.

96 Harms MJ, Ishibashi J, Wang W, Lim H-W, Goyama S, Sato T, Kurokawa M, Won K-J & Scale P (2014) Prdm16 is required for the maintenance of brown adipocyte identity and function in adult mice. *Cell Metab* **19**, 593–604.

97 Iida S et al. (2015) PRDM16 enhances nuclear receptor-dependent transcription of the brown fat-specific Ucp1 gene through interactions with mediator subunit MED1. *Genes Dev* **29**, 308–321.

98 Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, Rahl PB, Lee TI & Young RA (2013) Master transcription factors and mediator establish super-enhancers at key cell identity genes. *Cell* **153**, 307–319.

99 Siersbaek R, Rabiee A, Nielsen R, Sidoli S, Traynor S, Loft A, Poulsen LLC, Rogowska-Wrzesinska A, Jensen ON & Mandrup S (2014) Transcription factor cooperativity in early adipogenic hotspots and super-enhancers. *Cell Rep* **7**, 1443–1455.
100 Harms MJ, Lim H-W, Ho Y, Shapira SN, Ishibashi J, Rajakumari S, Steger DJ, Lazar MA, Won K-J & Seale P (2015) PRDM16 binds MED1 and controls chromatin architecture to determine a brown fat transcriptional program. *Genes Dev* **29**, 298–307.

101 Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, Cohen P, Cinti S & Spiegelman BM (2011) Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. *J Clin Invest* **121**, 96–105.

102 Stine RR, Shapira SN, Lim H-W, Ishibashi J, Harms M, Won K-J & Seale P (2016) EBF2 promotes the recruitment of beige adipocytes in white adipose tissue. *Mol Metab* **5**, 57–65.

103 Shinoda K, Luijten IHN, Hasegawa Y, Hong H, Sonne SB, Kim M, Xue R, Chondronikola M, Cypress AM, Tseng Y-H et al. (2015) Genetic and functional characterization of clonally derived adult human brown adipocytes. *Nat Med* **21**, 389–394.

104 Sharp LZ, Shinoda K, Ohno H, Scheel DW, Tomoda E, Ruiz L, Hu H, Wang L, Pavlova Z, Gilsanz V & et al. (2012) Human BAT possesses molecular signatures that resemble beige/brite cells. *PLoS One* **7**, e49452.

105 Villanueva CJ et al. (2013) Adipocyte subtype-selective recruitment of TLE3 or Prdm16 by PPARgamma specifies lipid storage versus thermogenic gene programs. *Cell Metab* **17**, 423–435.

106 Pearson S, Loft A, Rajbhandari P, Simcox J, Lee S, Tontonoz P, Mandrup S & Villanueva CJ (2019) Loss of TLE3 promotes the mitochondrial program in beige adipocytes and improves glucose metabolism. *Genes Dev* **33**, 747–762.

107 Shao M, Ishibashi J, Kusminski CM, Wang QA, Hepler C, Vishvanath L, MacPherson KA, Spurgin SB, Sun K, Holland WL et al. (2016) Zfp423 maintains white adipocyte identity through suppression of the beige cell thermogenic gene program. *Cell Metab* **23**, 1167–1184.

108 Picard F, Géhin M, Annicotte J-S, Rocchi S, Champy M-F, O’Malley BW, Chambon P & Auwerx J (2002) SRC-1 and TIF2 control energy balance between white and brown adipose tissues. *Cell* **111**, 931–941.

109 Christian M, Kiskinis E, Debevec D, Leonardsson Gran, White R & Parker MG (2005) RIP140-targeted repression of gene expression in adipocytes. *Mol Cell Biol* **25**, 9383–9391.

110 Powelka AM et al. (2006) Suppression of oxidative metabolism and mitochondrial biogenesis by the transcriptional corepressor RIP140 in mouse adipocytes. *J Clin Invest* **116**, 125–136.

111 Leonardsson G, Steel JH, Christian M, Pocock V, Milligan S, Bell J, So P-W, Medina-Gomez G, Vidal-Puig A, White R et al. (2004) Nuclear receptor corepressor RIP140 regulates fat accumulation. *Proc Natl Acad Sci USA* **101**, 8437–8442.

112 Wang H et al. (2008) Liver X receptor alpha is a transcriptional repressor of the uncoupling protein 1 gene and the brown fat phenotype. *Mol Cell Biol* **28**, 2187–2200.

113 Pan D et al. (2009) Twist-1 is a PPARdelta-inducible, negative-feedback regulator of PGC-1alpha in brown fat metabolism. *Cell* **137**, 73–86.

114 Jash S, Banerjee S, Lee M-J, Farmer SR & Puri V (2019) CIDEA transcriptionally regulates UCPI for browning and thermogenesis in human fat cells. *Science* **20**, 73–89.

115 Kulyte A, Pettersson AT, Antonson P, Stenson BM, Langin D, Gustafsson J-A, Stuels B, Rydén M, Arner P & Laurencikiene J (2011) CIDEA interacts with liver X receptors in white fat cells. *FEBS Lett* **585**, 744–748.

116 Li Y, Schwalie PC, Bart-Habersbrunner A, Moeck S, Russeil J, Fromme T, Deplancke B & Klingenspor M (2019) Systems-genetics-based inference of a core regulatory network underlying white fat browning. *Cell Rep* **29**, 4099–4113, e5.

117 Herz CT & Kiefer FW (2019) Adipose tissue browning in mice and humans. *J Endocrinol* **241**, R97–R109.

118 Bargut TCL, Souza-Mello V, Aguila MB & Mandarim-de-Lacerda CA (2017) Browning of white adipose tissue: lessons from experimental models. *Horm Mol Biol Clin Investig* **31**, http://dx.doi.org/10.1515/hmbci-2016-0051.

119 Kaisanlahti A & Glumoff T (2019) Browning of white fat: agents and implications for beige adipose tissue to type 2 diabetes. *J Physiol Biochem* **75**, 1–10.

120 Montanari T, Posic N & Colitti M (2017) Factors involved in white-to-brown adipose tissue conversion and in thermogenesis: a review. *Obes Rev* **18**, 495–513.

121 Cypess AM et al. (2015) Activation of human brown adipose tissue by a beta3-adrenergic receptor agonist. *Cell Metab* **21**, 33–38.

122 Hao L, Scott S, Abbasi M, Zu Y, Khan MSH, Yang Y, Wu D, Zhao L & Wang S (2019) Beneficial metabolic effects of mirabegron in vitro and in high-fat diet-induced obese mice. *J Pharmacol Exp Ther* **369**, 419–427.

123 O’Mara AE et al. (2020) Chronic mirabegron treatment increases human brown fat, HDL cholesterol, and insulin sensitivity. *J Clin Invest* **130**, 2209–2219. http://dx.doi.org/10.1172/jci131126.

124 Bordicchia M, Liu D, Amri E-Z, Ailhaud G, Dess 1184.

125 Merlin J et al. (2018) Rosiglitazone and a beta3-adrenoceptor agonist are both required for functional
browning of white adipocytes in culture. *Front Endocrinol (Lausanne)* **9**, 249.
126 Petrovic N et al. (2010) Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. *J Biol Chem* **285**, 7153–7164.
127 Markussen LK, Isidor MS, Breining P, Andersen ES, Rasmussen NE, Petersen LI, Pedersen SB, Richelsen B & Hansen JB (2017) Characterization of immortalized human brown and white pre-adipocyte cell models from a single donor. *PLoS One* **12**, e0185624.
128 Pisani DF et al. (2011) Differentiation of human adipose-derived stem cells into “brite” (brown-in-white) adipocytes. *Front Endocrinol (Lausanne)* **2**, 87.
129 Maurer SF et al. (2019) Fatty acid metabolites as novel regulators of non-shivering thermogenesis. *Handb Exp Pharmacol* **215**, 183–214.
130 Vegtiooulos A, Muller-Decker K, Strzoda D, Schmitt I, Chichelnitskiy E, Ostertag A, Diaz MB, Rozman J, Hrabe de Angelis M, Nusing RM et al. (2010) Cyclooxygenase-2 controls energy homeostasis in mice by de novo recruitment of brown adipocytes. *Science* **328**, 1158–1161.
131 Madsen L, Pedersen LM, Lillefosse HH, Fjære E, Bronstad I, Hao Q, Petersen RK, Hallenborg P, Ma T, De Matteis R et al. (2010) UCP1 induction during recruitment of brown adipocytes in white adipose tissue is dependent on cyclooxygenase activity. *PLoS One* **5**, e11391.
132 Ghindour RA, Giroud M, Vegtiooulos A, Herzig S, Ailhaud G, Amri E-Z & Pisani DF (2016) IP-receptor and PPARs trigger the conversion of human white to brite adipocyte induced by carbaprostacyclin. *Biochim Biophys Acta* **1861**, 285–293.
133 Barquissau V, Ghindour RA, Ailhaud G, Klingenspor M, Langin D, Amri E-Z & Pisani DF (2017) Control of adipogenesis by oxylipins, GPCRs and PPARs. *Biochimie* **136**, 3–11.
134 Garcia-Alonso V, Titos E, Alcaraz-Quiles J, Rius B, Lopez-Vicario C, Jakobsson P-J, Delgado S, Lozano J & Claria J (2016) Prostaglandin E2 exerts multiple regulatory actions on human obese adipose tissue remodeling, inflammation, adaptive thermogenesis and lipolysis. *PLoS One* **11**, e0153751.
135 Pisani DF et al. (2014) The omega-6-fatty acid, arachidonic acid, regulates the conversion of white to brite adipocyte through a prostaglandin/calciun-mediated pathway. *Mol Metab* **3**, 834–847.
136 Frühbeck G, Fernández-Quintana B, Paniauga M, Hernández-Pardos AW, Valenti V, Moncada R, Catalán V, Becerril S, Gómez-Ambrosi J, Portincasa P et al. (2020) FNDC4, a novel adipokine that reduces lipogenesis and promotes fat browning in human visceral adipocytes. *Metabolism* **108**, 154261.
137 Kluczek A et al. (2019) Differentiating SGBS adipocytes respond to PPARgamma stimulation, irisin and BMP7 by functional browning and beige characteristics. *Sci Rep* **9**, 5823.
138 Elsen M, Raschke S, Tennagels N, Schwahn U, Jelenik T, Roden M, Romacho T & Eckel J (2014) BMP4 and BMP7 induce the white-to-brown transition of primary human adipose stem cells. *Am J Physiol Cell Physiol* **306**, C431–C440.
139 Lee YJ, Takahashi N, Yasubuchi M, Kim Y-I, Hashizaki H, Kim M-J, Sakamoto T, Goto T & Kawada T (2012) Triiodothyronine induces UCP-1 expression and mitochondrial biogenesis in human adipocytes. *Am J Physiol Cell Physiol* **302**, C463–C472.
140 Zhang Y, Xie C, Wang H, Foss RM, Clare M, George EV, Li S, Katz A, Cheng H, Ding Y et al. (2016) Irisin exerts dual effects on browning and adipogenesis of human white adipocytes. *Am J Physiol Endocrinol Metab* **311**, E530–E541.
141 Gustafson B, Hammarsstedt A, Hedjazifar S, Hoffmann JM, Svensson P-A, Grimsby J, Rondinone C & Smith U (2015) BMP4 and BMP antagonists regulate human white and beige adipogenesis. *Diabetes* **64**, 1670–1681.
142 Hanssen MJ, van der Lans AJJ, Brans B, Hoeks J, Jardon KMC, Schaart G, Mottaghy FM, Schrauwen P & van Marken Lichtenbelt WD (2016) Short-term cold acclimation recruits brown adipose tissue in obese humans. *Diabetes* **65**, 1179–1189.
143 Orava J, Nuutila P, Lidell ME, Oikonen V, Nononen T, Viljanen T, Scheinin M, Taïttonen M, Niemi T, Enerbäck S et al. (2011) Different metabolic responses of human brown adipose tissue to activation by cold and insulin. *Cell Metab* **14**, 272–279.
144 Hanssen MJ, Hoeks J, Brans B, van der Lans AJJ, Schaart G, van den Driessche JJ, Jongens JA, Boekschoten MV, Hesselink MKC, Havekes B et al. (2015) Short-term cold acclimation improves insulin sensitivity in patients with type 2 diabetes mellitus. *Nat Med* **21**, 863–865.
145 Chondronikola M, Volpi E, Borsheim E, Porter C, Saraf MK, Annamalai P, Yfantis C, Chao T, Wong D, Shinoda K et al. (2016) Brown adipose tissue activation is linked to distinct systemic effects on lipid metabolism in humans. *Cell Metab* **23**, 1200–1206.
146 Vijgen GH, Bouvy ND, Teule GJJ, Brans B, Schrauwen P & van Marken Lichtenbelt WD (2011) Brown adipose tissue in morbidly obese subjects. *PLoS One* **6**, e17247.
147 van Marken Lichtenbelt WD, Vanhomerig JW, Smulders NM, Drossaerts JMAFL, Kemerink GJ, Bouvy ND, Schrauwen P & Teule GJJ (2009) Cold-
activated brown adipose tissue in healthy men. *N Engl J Med* **360**, 1500–1508.

Cypress AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng Y-H, Doria A et al. (2009) Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* **360**, 1509–1517.

Ouellet V et al. (2011) Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. *J Clin Endocrinol Metab* **96**, 192–199.

Challa TD, Dupito DH, Kullenkampff E, Kiehlmann E, Moser C, Straub L, Sun W & Wolfrum C (2020) A genetic model to study the contribution of brown and beige adipocytes to metabolism. *Cell Rep* **30**, 3424–3433, e4.

Magkos F, Fraterrigo G, Yoshino J, Luecking C, Kirbach K, Kelly SC, de las Fuentes L, He S, Okunade AL, Patterson BW et al. (2016) Effects of moderate and subsequent progressive weight loss on metabolic function and adipose tissue biology in humans with obesity. *Cell Metab* **23**, 591–601.

Hall KD, Sacks G, Chandramohan D, Chow CC, Wang YC, Gortmaker SL & Swinburn BA (2011) Quantification of the effect of energy imbalance on bodyweight. *Lancet* **378**, 826–837.

Virtanen KA, Lidell ME, Orava J, Hegland M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto N-J, Enerbäck S et al. (2009) Functional brown adipose tissue in healthy adults. *N Engl J Med* **360**, 1518–1525.

Dieckmann S, Maurer S, Fromme T, Colson C, Virtanen KA, Amri E-Z & Klingenspor M (2020) Fatty acid metabolite profiling reveals oxylipins as markers of brown but not brite adipose tissue. *Front Endocrinol (Lausanne)* **11**, 73.

Crichton PG, Lee Y & Kunji ER (2017) The molecular features of uncoupling protein 1 support a conventional mitochondrial carrier-like mechanism. *Biochimie* **134**, 35–50.

Hoang T, Smith MD & Jelokhani-Niaraki M (2013) Expression, folding, and proton transport activity of human uncoupling protein-1 (UCP1) in lipid membranes: evidence for associated functional forms. *J Biol Chem* **288**, 36244–36258.

Shabalina IG, Ost M, Petrovic N, Vrbacky M, Nedergaard J & Cannon B (2010) Uncoupling protein-1 is not leaky. *Biochim Biophys Acta* **1797**, 773–784.

Johann K, Cremer AL, Fischer AW, Heine M, Pensado ER, Resch J, Nock S, Virtue S, Harder L, Oelkrug R et al. (2019) Thyroid-hormone-induced browning of white adipose tissue does not contribute to thermogenesis and glucose consumption. *Cell Rep* **27**, 3385–3400, e3.

Tews D, Pula T, Funcke JB, Jastroch M, Keuper M, Debatin KM, Wabitsch M & Fischer-Posovszky P (2019) Elevated UCP1 levels are sufficient to improve glucose uptake in human white adipocytes. *Redox Biol* **26**, 101286.

Lee KY, Luong Q, Sharma R, Dreyfuss JM, Ussar S & Kahn CR (2019) Developmental and functional heterogeneity of white adipocytes within a single fat depot. *EMBO J* **38**, http://dx.doi.org/10.15252/embj.201899291.

Lee KY et al. (2017) Tbx15 defines a glycolytic subpopulation and white adipocyte heterogeneity. *Diabetes* **66**, 2822–2829.

Chau YY, Bandiera R, Serrels A, Martínez-Estrada OM, Qing W, Lee M, Slight J, Thornburn A, Berry R, McHaffie S et al. (2014) Visceral and subcutaneous fat have different origins and evidence supports a mesothelial source. *Nat Cell Biol* **16**, 367–375.

Sanchez-Gurmaches J & Guertin DA (2014) Adipocytes arise from multiple lineages that are heterogeneously and dynamically distributed. *Nat Commun* **5**, 4099.

Sanchez-Gurmaches J, Hsiao WY & Guertin DA (2015) Highly selective in vivo labeling of subcutaneous white adipocyte precursors with Prx1-Cre. *Stem Cell Reports* **4**, 541–550.

Chen Y, Ikeda K, Yoneshiro T, Scaramozza A, Tajima K, Wang Q, Kim K, Shinoda K, Sponton CH, Brown Z et al. (2019) Thermal stress induces glycolytic beige fat formation via a myogenic state. *Nature* **565**, 180–185.

Lee YH, Kim S-N, Kwon H-J & Granneman JG (2017) Metabolic heterogeneity of activated beige/brite adipocytes in inguinal adipose tissue. *Sci Rep* **7**, 39794.

Long JZ, Svensson KJ, Tsai L, Zeng X, Roh HC, Kong X, Rao RR, Lou J, Lokurkar I, Baur W et al. (2014) A smooth muscle-like origin for beige adipocytes. *Cell Metab* **19**, 810–820.

Song A, Dai W, Jang MJ, Medrano L, Li Z, Zhao H, Shao M, Tan J, Li A, Ning T et al. (2020) Low- and high-thermogenic brown adipocyte subpopulations coexist in murine adipose tissue. *J Clin Invest* **130**, 247–257.

Hagberg CE, Li Q, Kutschke M, Bhowmick D, Kiss E, Shabalina IG, Harms MJ, Shiklova O, Kozina V, Nedergaard J et al. (2018) Flow cytometry of mouse and human adipocytes for the analysis of browning and cellular heterogeneity. *Cell Rep* **24**, 2746–2756, e5.

Zhang F, Hao G, Shao M, Nham K, An Y, Wang Q, Zhu Y, Kusminski CM, Hassan G, Gupta RK et al. (2018) An adipose tissue atlas: an image-guided identification of human-like BAT and beige depots in rodents. *Cell Metab* **27**, 252–262, e3.

Walden TB, Hansen IR, Timmons JA, Cannon B & Nedergaard J (2012) Recruited vs. nonrecruited
molecular signatures of brown, “brite,” and white adipose tissues. *Am J Physiol Endocrinol Metab* **302**, E19–E31.

172 de Jong JM, Larsson O, Cannon B & Nedergaard J (2015) A stringent validation of mouse adipose tissue identity markers. *Am J Physiol Endocrinol Metab* **308**, E1085–E105.

173 Barreau C, Labit E, Guissard C, Rouquette J, Boizeau M-L, Gani Koumassi S, Carriére A, Jeanson Y, Berger-Müller S, Dromard C et al. (2016) Regionalization of browning revealed by whole subcutaneous adipose tissue imaging. *Obesity (Silver Spring)* **24**, 1081–1089.

174 Chi J, Wu Z, Choi CHJ, Nguyen L, Tegegne S, Ackerman SE, Crane A, Marchildon F, Tessier-Lavigne M & Cohen P (2018) Three-dimensional adipose tissue imaging reveals regional variation in beige fat biogenesis and PRDM16-dependent sympathetic neurite density. *Cell Metab* **27**, 226–236, e3.

175 Wang H, Willershäuser M, Karlas A, Gorpas D, Reber J, Ntzachristos V, Maurer S, Fromme T, Li Y & Klingenspor M (2019) A dual Ucp1 reporter mouse model for imaging and quantitation of brown and brite fat recruitment. *Mol Metab* **20**, 14–27.

176 Chan M, Lim YC, Yang J, Namwanje M, Liu L & Qiang L (2019) Identification of a natural beige adipose depot in mice. *J Biol Chem* **294**, 6751–6761.

177 Zuriaga MA, Fuster JJ, Gokee N & Walsh K (2017) Humans and mice display opposing patterns of “browning” gene expression in visceral and subcutaneous white adipose tissue depots. *Front Cardiovasc Med* **4**, 27.

178 Lim J, Park HS, Kim J, Jang YJ, Kim J-H, Lee YJ & Heo Y (2020) Depot-specific UCP1 expression in human white adipose tissue and its association with obesity-related markers. *Int J Obes (Lond)* **44**, 697–706.

179 Tarabra E, Nouws J, Vash-Margita A, Nadzam GS, Goldberg R, Van Name M, Pierpont B, Knight JR, Shulman GI & Caprio S (2020) The omentum of obese girls harbors small adipocytes and browning transcripts. *JCI Insight* **5**, http://dx.doi.org/10.1172/jci.insight.135448

180 Ukropec J et al. (2006) UCP1-independent thermogenesis in white adipose tissue of cold-acclimated Ucp1-/ - mice. *J Biol Chem* **281**, 31894–31908.

181 Maurer SF, Fromme T, Mocek S, Zimmermann A & Klingenspor M (2020) Uncoupling protein 1 and the capacity for nonshivering thermogenesis are components of the glucose homeostatic system. *Am J Physiol Endocrinol Metab* **318**, E198–E215.

182 Pollard AE, Martins L, Muckett PJ, Khadayate S, Bornot A, Clausen M, Admyre T, Bjursell M, Fiodeiro R, Wilson L et al. (2019) AMPK activation protects against diet induced obesity through Ucp1-independent thermogenesis in subcutaneous white adipose tissue. *Nat Metab* **1**, 340–349.

183 Liu X, Rossmeisl M, McClane J & Kozak LP (2003) Paradoxical resistance to diet-induced obesity in Ucp1-deficient mice. *J Clin Invest* **111**, 399–407.

184 Nyman E, Bartesaghi S, Melin Rydkfalk R, Eng S, Pollard C, Gennemark P, Peng X-R & Cedersund G (2017) Systems biology reveals uncoupling beyond UCP1 in human white fat-derived beige adipocytes. *NPJ Syst Biol Appl* **3**, 29.

185 Barquissau V, Beuzelin D, Pisani DF, Beranger GE, Mairal A, Montagner A, Roussel B, Tavernier G, Marques M-A, Moro C et al. (2016) White-to-brite conversion in human adipocytes promotes metabolic reprogramming towards fatty acid anabolic and catabolic pathways. *Mol Metab* **5**, 352–365.

186 Kazak L, Chouchani ET, Jedrychowski MP, Erickson BK, Shinoda K, Cohen P, Vetrivelan R, Lu GZ, Laznik-Bogoslavski D, Hasenfuss SC et al. (2015) A creatine-driven substrate cycle enhances energy expenditure and thermogenesis in beige fat. *Cell* **163**, 643–655.

187 Kazak L, Rahbani JF, Samborska B, Lu GZ, Jedrychowski MP, Lajoie M, Zhang S, Ramsay LA, Dou FY, Tenen D et al. (2019) Ablation of adipocyte creatine transport impairs thermogenesis and causes diet-induced obesity. *Nat Metab* **1**, 360–370.

188 Bertholet AM, Kazak L, Chouchani ET, Bogaczyńska MG, Paranjpe I, Wainwright GL, Bétourné A, Kajimura S, Spiegelman BM & Kirichok Y (2017) Mitochondrial patch clamp of beige adipocytes reveals UCP1-positive and UCP1-negative cells both exhibiting futile creatine cycling. *Cell Metab* **25**, 811–822, e4.

189 Ikeda K, Kang Q, Yoneshio T, Camporez JP, Maki H, Homma M, Shinoda K, Chen Y, Lu X, Marettich P et al. (2017) UCP1-independent signaling involving SERCA2b-mediated calcium cycling regulates beige fat thermogenesis and systemic glucose homeostasis. *Nat Med* **23**, 1454–1465.

190 Long JZ, Svensson KJ, Bateman LA, Lin H, Kamenecka T, Lokurkar IA, Lou J, Rao RR, Chang MR, Jedrychowski MP et al. (2016) The secreted enzyme PM20D1 regulates lipidosed amino acid uncouplers of mitochondria. *Cell* **166**, 424–435.

191 Bertholet AM, Chouchani ET, Kazak L, Angelin A, Fedorenko A, Long JZ, Vidoni S, Garrity R, Cho J, Terada N et al. (2019) H(+) transport is an integral function of the mitochondrial ADP/ATP carrier. *Nature* **571**, 515–520.

192 Roesler A & Kazak L (2020) UCP1-independent thermogenesis. *Biochem J* **477**, 709–725.
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193 Tharp KM, Jha AK, Kraiczy J, Yesian A, Karateev G, Sinisi R, Dubikovskaya EA, Healy KE & Stahl A (2015) Matrix-assisted transplantation of functional beige adipose tissue. *Diabetes* **64**, 3713–3724.

194 Stanford KI, Middelbeek RJW, Townsend KL, An D, Nygaard EB, Hitchcox KM, Markan KR, Nakano K, Hirshman MF, Tseng Y-H et al. (2013) Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. *J Clin Invest* **123**, 215–223.

195 Finlin BS et al. (2020) The beta3-adrenergic receptor agonist mirabegron improves glucose homeostasis in obese mice. *J Clin Invest.* **130**, 2319–2331. http://dx.doi.org/10.1172/jci134892

196 Brown E et al. (2019) Weight loss variability with SGLT2 inhibitors and GLP-1 receptor agonists in type 2 diabetes mellitus and obesity: mechanistic possibilities. *Obes Rev* **20**, 816–828.

197 Schnabl K, Li Y & Klingenspor M (2020) The gut hormone secretin triggers a gut-brown fat-brain axis in the control of food intake. *Exp Physiol*. http://dx.doi.org/10.1113/ep087878

198 Li Y, Schnabl K, Gabler S-M, Willershäuser M, Reber J, Karlas A, Laurila S, Lahesmaa M, Din MU, Bast-Habersbrunner A et al. (2018) Secretin-activated brown fat mediates prandial thermogenesis to induce satiation. *Cell* **175**, 1561–1574, e12.

199 Din MU, Saari T, Raiko J, Kudomi N, Maurer SF, Lahesmaa M, Fromme T, Amri E-Z, Klingenspor M, Solin O et al. (2018) Postprandial oxidative metabolism of human brown fat indicates thermogenesis. *Cell Metab* **28**, 207–216, e3.

200 Clemmensen C et al. (2018) Coordinated targeting of cold and nicotinic receptors synergistically improves obesity and type 2 diabetes. *Nat Commun* **9**, 4304.

201 Bartelt A, Bruns OT, Reimer R, Hohenberg H, Ittrich H, Peldschus K, Kaul MG, Tromsdorf U, Weller H, Waurisch C et al. (2011) Brown adipose tissue activity controls triglyceride clearance. *Nat Med* **17**, 200–205.

202 van den Berg R, Kooijman S, Noordam R, Ramkisosening A, Abreu-Vieira G, Tambryajalh LL, Dijk W, Ruppert P, Mol IM, Kramar B et al. (2018) A diurnal rhythm in brown adipose tissue causes rapid clearance and combustion of plasma lipids at waking. *Cell Rep* **22**, 3521–3533.

203 Chondronikola M, Volpi E, Borsheim E, Porter C, Annamalai P, Enerback S, Lidell ME, Saraf MK, Labbe SM, Hurren NM et al. (2014) Brown adipose tissue improves whole-body glucose homeostasis and insulin sensitivity in humans. *Diabetes* **63**, 4089–4099.

204 Min SY, Kady J, Nam M, Rojas-Rodriguez R, Berkenwald A, Kim JH, Noh H-L, Kim JK, Cooper MP, Fitzgibbonbs T et al. (2016) Human ‘brute/beige’ adipocytes develop from capillary networks, and their implantation improves metabolic homeostasis in mice. *Nat Med* **22**, 312–318.

205 Deshmukh AS, Peis L, Beaudry JL, Jespersen NZ, Nielsen CH, Ma T, Brunner AD, Larsen TJ, Bayarri-Olmos R, Prabhakar BS et al. (2019) Proteomics-based comparative mapping of the secretomes of human brown and white adipocytes reveals EPDR1 as a novel batokine. *Cell Metab* **30**, 963–975, e7.

206 Villarroya F, Cereijo R, Villarroya J & Giralt M (2017) Brown adipose tissue as a secretory organ. *Nat Rev Endocrinol* **13**, 26–35.

207 Svensson KJ, Long JZ, Jedychowski MP, Cohen P, Lo JC, Serag S, Kir S, Shinoda K, Tartaglia JA, Rao RR et al. (2016) A secreted Slt2 fragment regulates adipose tissue thermogenesis and metabolic function. *Cell Metab* **23**, 454–466.

208 Lynes MD, Leiria LO, Lundh M, Bartelt A, Shamsi F, Huang TL, Takahashi H, Hirshman MF, Schlein C, Lee A et al. (2017) The cold-induced lipokine 12,13-diHOME promotes fatty acid transport into brown adipose tissue. *Nat Med* **23**, 631–637.

209 Leiria LO, Wang C-H, Lynes MD, Yang K, Shamsi F, Sato M, Sugimoto S, Chen EY, Bussberg V, Narain NR et al. (2019) 12-Lipoxygenase regulates cold adaptation and glucose metabolism by producing the omega-3 lipid 12-HEPE from brown fat. *Cell Metab* **30**, 768–783, e7.

210 Moisan A, Lee Y-K, Zhang JD, Hudak CS, Meyer CA, Prummer M, Zoffmann S, Truong HH, Ebeling M, Kjaalainen A et al. (2015) White-to-brown adipose tissue conversion of human adipocytes by JAK inhibition. *Nat Cell Biol* **17**, 57–67.

211 Choi JH et al. (2010) Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARgamma by Cdk5. *Nature* **466**, 451–456.

212 Banks AS et al. (2015) An ERK/Cdk5 axis controls the diabetogenic actions of PPARgamma. *Nature* **517**, 391–395.

213 Choi SS et al. (2016) PPARgamma antagonist gleevec improves insulin sensitivity and promotes the browning of white adipose tissue. *Diabetes* **65**, 829–839.

214 Wang H, Liu L, Lin JZ, Aprahamian TR & Farmer SR (2016) Browning of white adipose tissue with roscovitine induces a distinct population of UCP1(+) adipocytes. *Cell Metab* **24**, 835–847.

215 Li S, Li Y, Xiang L, Dong J, Liu M & Xiang G (2018) Sildenafil induces browning of subcutaneous white adipose tissue in overweight adults. *Metabolism* **78**, 106–117.

216 Baskin AS et al. (2018) Regulation of human adipose tissue activation, gallbladder size, and bile acid metabolism by a beta3-adrenergic receptor agonist. *Diabetes* **67**, 2113–2125.

217 Loh RKC, Formosa MF, La Gerche A, Reutens AT, Kingwell BA & Carey AL (2019) Acute metabolic and cardiovascular effects of mirabegron in healthy individuals. *Diabetes Obes Metab* **21**, 276–284.