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STATE-OF-THE-ART REVIEW

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

Stefanie Maurer[1], Matthew Harms[1] and Jeremie Boucher[1,2,3]

1 Bioscience Metabolism, Research and Early Development, Cardiovascular, Renal and Metabolism, BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden

2 Lundberg Laboratory for Diabetes Research, University of Gothenburg, Gothenburg, Sweden

3 Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, Gothenburg, Sweden

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Correspondence

J. Boucher, AstraZeneca R&D, 431 83 Mölndal, Sweden

Tel: +46 31 776 2385

E-mail: jeremie.boucher@astrazeneca.com

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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.

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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, brite, 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

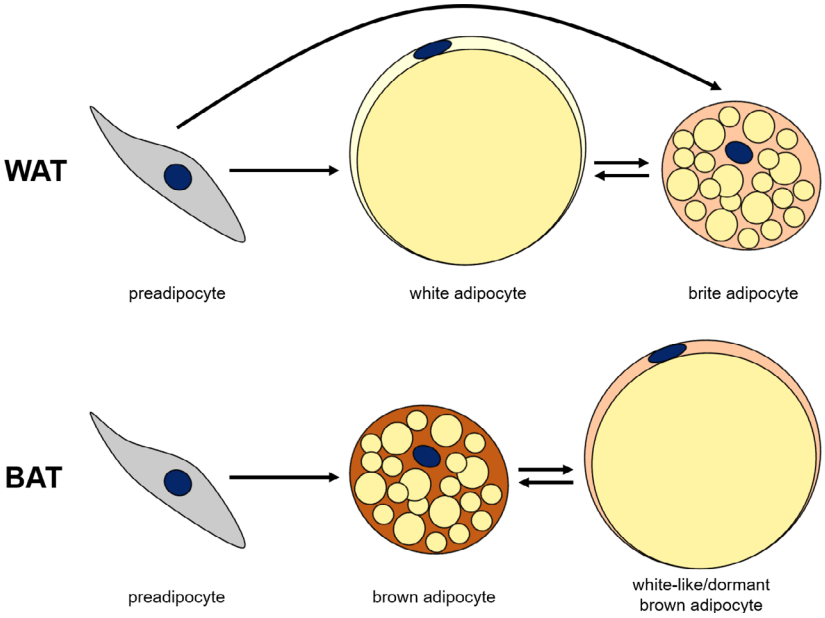

Fig. 1. Adipocyte plasticity in WAT and BAT. Brite and brown adipocytes can originate from the differentiation of preadipocytes in WAT and BAT, respectively. Additionally, preadipocytes in WAT give rise to mature white adipocytes with the potential to become brite adipocytes at a later point of time. In the absence of thermogenic stimulation or in response to an obesogenic diet, brown and brite adipocytes can become camouflaged as white adipocytes ('whitening'). Upon thermogenic stimulation, these quiescent cells can be rerecruited and undergo a phenotypical change from white to brown/brite ('browning'). BAT, brown adipose tissue; WAT, white adipose tissue.

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, supraclavicular, 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'

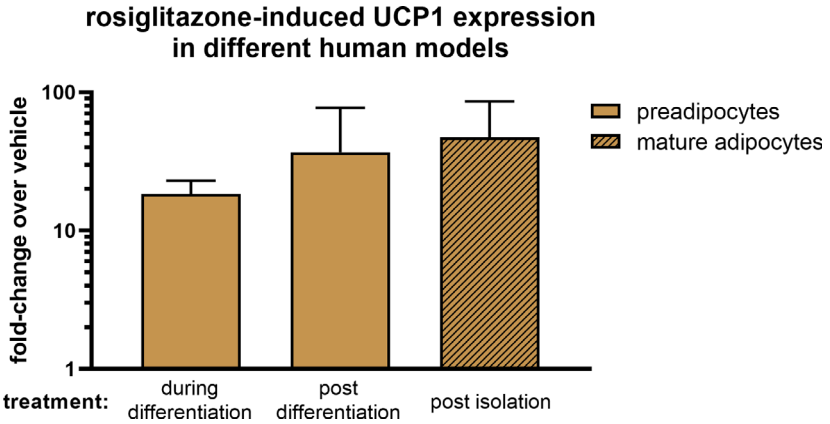

Fig. 2. Brite adipogenic potential of human adipocytes. Human primary preadipocytes were isolated from subcutaneous abdominal white adipose tissue, grown to confluence, and differentiated *in vitro*. The cells were exposed to vehicle or rosiglitazone (1 μM) during (day 0 to 12) or after (day 12 to 20) the differentiation. Human mature adipocytes were freshly isolated from subcutaneous abdominal white fat and cultured in the presence of vehicle or rosiglitazone (1 μM) for 7 days. The expression of UCP1 was determined by quantitative real-time PCR and normalized to the expression of TATA-box-binding protein. Data are expressed as fold change compared to the vehicle-treated controls. UCP1, uncoupling protein 1.

[39]. 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 transcriptional 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 PGC1a and by disrupting its interaction with PPARγ [84,108–113] (Fig. 3). Conversely, overexpression of PGC1a 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

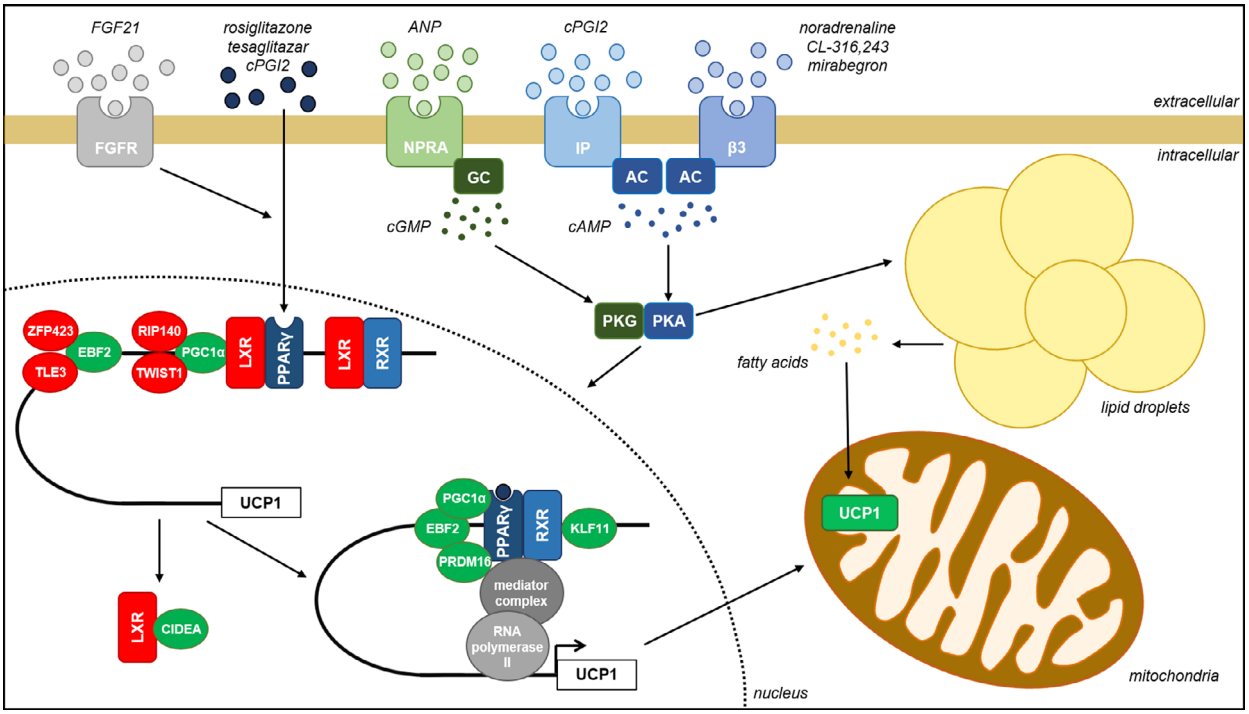

Fig. 3. Cell-extrinsic mediators and intracellular signaling pathways involved in the white-to-brite conversion of human mature adipocytes. In the unstimulated state, the activity of transcription factors involved in brown/brite adipogenesis (displayed in green and blue) is attenuated by corepressors (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 NPRA 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; NPRA, 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-adrenoreceptor, which triggers the direct white-to-brite conversion of mature adipocytes [31,49,114,121–123]. 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, PPARα-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

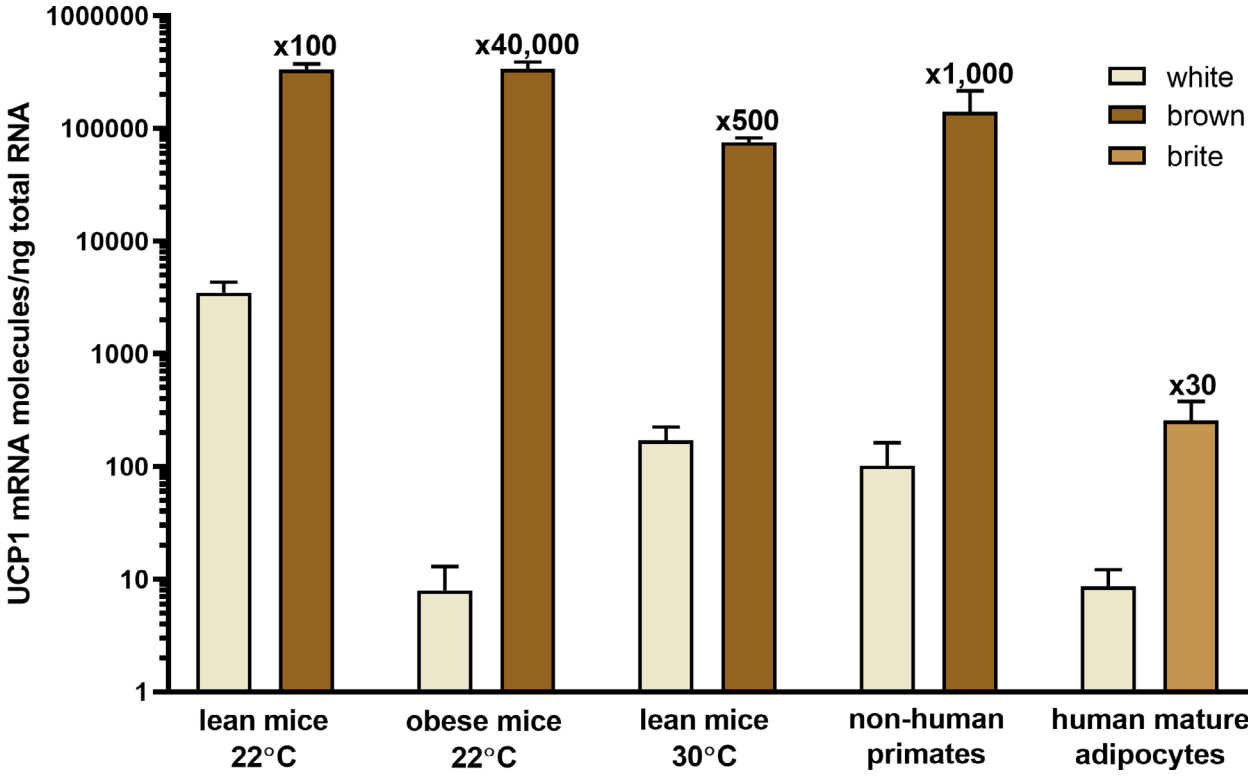

Fig. 4. Absolute levels of UCP1 transcript in white, brown, and brite fat of different species. Transcript levels were quantified by quantitative real-time PCR using species-specific standard curves with known UCP1 cDNA copy numbers. Murine brown and white adipose tissue were obtained from lean and diet-induced 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/creatine-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-acyl 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 PPARγ 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 PPARα/γ 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 PPARγ. Phosphorylation of PPARγ 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 PPARγ 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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