REVIEW

Adipose tissue browning in mice and humans

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Abstract

In the midst of an obesity epidemic, the promotion of brown adipose tissue (BAT) function and the browning of white adipose tissue (WAT) have emerged as promising therapeutic targets to increase energy expenditure and counteract weight gain. Despite the fact that the thermogenic potential of *bone fide* BAT in rodents is several orders of magnitudes higher than white fat containing brite/beige adipocytes, WAT browning represents a particularly intriguing concept in humans given the extreme amount of excess WAT in obese individuals. In addition, the clear distinction between classic brown and beige fat that has been proposed in mice does not exist in humans. In fact, studies of human BAT biopsies found controversial results suggesting both classic brown and beige characteristics. Irrespective of the true 'color', accumulating evidence suggests the induction of thermogenic adipocytes in human WAT depots in response to specific stimuli, highlighting that WAT browning may occur in both, mice and humans. These observations also emphasize the great plasticity of human fat depots and raise important questions about the metabolic properties of thermogenically active adipose tissue in humans and the potential therapeutic implications. We will first review the cellular and molecular aspects of selected adipose tissue browning concepts that have been identified in mouse models with emphasis on neuronal factors, the microbiome, immune cells and several hormones. We will also summarize the evidence for adipose tissue browning in humans including some experimental pharmacologic approaches.

Key Words

- ▶ adipose tissue
- ▶ metabolism
- ▶ obesity
- ▶ diabetes mellitus

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Adipose tissue browning and whitening

A hallmark feature of adipose tissue browning is the induction of thermogenically active adipocytes in white fat depots. These adipocytes are often referred to as beige or brite (brown in white) cells and express high amounts of the key thermogenic factor uncoupling protein 1 (UCP1). UCP1 is located in the inner mitochondrial membrane and uncouples the proton motive force from oxidative phosphorylation leading to the dissipation of the energy as heat (Cannon & Nedergaard 2004). Beige cells are inducible and possess characteristics of both, white and brown fat cells. The number of beige adipocytes increases substantially in mice in response to certain environmental, genetic or pharmacological stimuli (Kiefer 2016, Jankovic

et al. 2017, Vargas-Castillo *et al.* 2017). One of the most potent physiologic inducers of adipose tissue browning is chronic cold exposure. Low temperatures are sensed by the skin and central signals result in the release of the neurotransmitter norepinephrine via sympathetic neurons (Cannon & Nedergaard 2004). Through the activation of β -adrenergic receptors (predominantly subtype β_3) and downstream cyclic adenosine monophosphate signaling, a thermogenic gene program is turned on that defines a BAT-like molecular phenotype (Himms-Hagen *et al.* 1994, Rosell *et al.* 2014).

The appearance of newly formed beige adipocytes within white fat raises the question of where these cells

come from. Two general concepts have been proposed: one is the precursor model which suggests that these beige cells are derived from distinct progenitor cells upon certain stimuli such as cold exposure or β -adrenergic stimulation. The other concept is the transdifferentiation model or more precisely the interconversion model because it proposes that mature white adipocytes can convert into beige adipocytes when appropriately challenged. There is high-quality evidence from elegant lineage tracing studies for both theories (Barbatelli *et al.* 2010, Lee *et al.* 2012, 2015, Rosenwald *et al.* 2013, Wang *et al.* 2013, Kiefer 2016). It is possible that additional factors such as the genetic background, the environment or fat depot-specific differences contribute to the decision how beige cells are ultimately formed.

Recently, a new hallmark of beige adipocytes has been identified using reporter mouse models that allow to tag white, beige and brown adipocytes. Warm temperatures confer a white fat program in both, beige and brown adipocytes, a process coined 'whitening'. However, only beige adipocytes undergo temperature-dependent chromatin reprogramming from brown toward white, while retaining an epigenomic memory of prior cold exposure. In other words, beige cells remember when they have previously been exposed to cold and can therefore quickly reboot their thermogenic machinery when re-challenged. These findings also emphasize the great molecular and metabolic plasticity of beige adipocytes and illustrate why adipose tissue browning has been linked to a lean phenotype and improved energy metabolism (Roh *et al.* 2018).

Neuronal control of WAT beiging

WAT is innervated by sympathetic noradrenergic nerve fibers with various innervation densities depending on the anatomical location (Youngstrom & Bartness 1995). WAT denervation leads to an increased fat pad mass and fat cell number due to impaired centrally regulated lipolysis (Youngstrom & Bartness 1998). Correspondingly, cold acclimation leads to increased tyrosine hydroxylase-positive nerve fibers indicative of higher innervation density and a higher fraction which is paralleled by the emergence of beige adipocytes in different WAT depots (Murano *et al.* 2009). Using retrograde neuronal viral tracing it has been demonstrated that BAT and WAT share both, common and distinct central neuronal projections, suggesting coordinated sympathetic nervous system control of BAT thermoregulation and beige cell recruitment (Nguyen *et al.* 2017).

A central regulator of BAT thermogenesis and WAT browning is hypothalamic AMP-activated protein kinase (AMPK). Activated by low intracellular energy stores, AMPK reduces ATP-consuming and promotes ATP-producing processes. Inactivation of AMPK in the ventromedial hypothalamus (VMH) increases sympathetic output to BAT and WAT which conveys BAT activation and WAT browning (Lopez *et al.* 2016). Several hormonal regulators of adipocyte browning act through AMPK. Central administration of liraglutide, a glucagon-like peptide 1 receptor agonist, thyroid hormones and estradiol increase thermogenesis through suppression of AMPK in the VMH (Lopez *et al.* 2010, Beiroa *et al.* 2014, Martinez De Morentin *et al.* 2014, Martinez-Sanchez *et al.* 2017a,b). While the liraglutide and thyroid hormones enhance both BAT function and WAT browning, estradiol only affected BAT thermogenesis (Beiroa *et al.* 2014, Martinez De Morentin *et al.* 2014, Martinez-Sanchez *et al.* 2017a). Deletion of the AMPK subunit AMPK α 1 in steroidogenic factor 1 neurons in the VMH promotes a thermogenic program in BAT and WAT and thus could represent a potential treatment target for metabolic disease (Martinez-Sanchez *et al.* 2017b, Seoane-Collazo *et al.* 2018). Whereas decreasing hypothalamic AMPK activity seems to confer some metabolic benefits, the loss of adipocyte AMPK exacerbates insulin resistance and hepatic steatosis through impaired brown and beige fat function (Mottillo *et al.* 2016). Potential therapies targeting AMPK activity must thus be tissue specific in order to maximize metabolic benefit.

Hypothalamic endoplasmic reticulum stress is an important contributing factor leading to diminished thermogenesis and WAT browning which is induced, e.g. through ceramide-mediated lipotoxicity and alleviated through the chaperone GRP78/BiP (glucose-regulated protein 78 kDa/binding immunoglobulin protein) (Contreras *et al.* 2014, 2017a). The mechanisms by which estradiol and thyroid hormones positively regulate energy balance also include the suppression of VMH endoplasmic reticulum (Martinez-Sanchez *et al.* 2017b, Gonzalez-Garcia *et al.* 2018).

The arcuate nucleus is another important hypothalamic structure controlling adipose tissue thermogenesis. It harbors orexigenic neuropeptide Y (NPY)/agouti-related protein (AgRP) neurons which are counteracted by anorexigenic pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) neurons. In addition to their established role in feeding control, arcuate nucleus neurons are involved in the central regulation of energy expenditure

through their canonical modulators such as the pro-thermogenic leptin or the anti-thermogenic ghrelin (Dodd *et al.* 2015, Contreras *et al.* 2017b, Wu *et al.* 2017). POMC neuron-specific deletion of inositol-requiring enzyme α , a regulator of the unfolded protein response, leads to impaired BAT thermogenesis and reduced WAT browning due to increased hypothalamic endoplasmic reticulum stress and leptin resistance (Yao *et al.* 2017). O-linked β -N-acetylglucosamine (O-GlcNAc) signaling in AgRP neurons suppresses thermogenesis and conserves energy in response to fasting, whereas inactivation of the modifying enzyme O-GlcNAc transferase induces WAT browning and protects against obesity and insulin resistance (Ruan *et al.* 2014).

Activation of hypothalamic guanylyl cyclase 2C through the intestinal hormone uroguanylin has been shown to regulate feeding and to induce BAT thermogenesis as well as WAT browning in mice, in part possibly through hypothalamic POMC signaling (Valentino *et al.* 2011, Figueira *et al.* 2016). However, some studies question the metabolic relevance of this new proposed gut–brain axis (Begg *et al.* 2014, Fernandez-Cachon *et al.* 2018).

Beiging and the gut microbiome

Among the different factors that have been linked to adipose tissue browning, the gut microbiota deserves particular attention. Previously, cold exposure has been shown to alter the microbial composition in the gut and transfer of microbiota from cold-adapted mice into germ-free mice induced WAT browning in the recipients with consecutively improved cold tolerance and insulin sensitivity (Chevalier *et al.* 2015, Zietak *et al.* 2016). More recently intermittent fasting has been demonstrated to have similar effects. Every other day fasting (EODF) compared to *ad libitum* feeding resulted in a shift in the gut microbiota composition, selectively stimulated beige fat development within WAT and dramatically attenuated obesity, insulin resistance and hepatic steatosis in mice. Importantly, also the transplantation of microbiota from EODF-treated mice to germ-free mice activated beiging and improved glucose metabolism, whereas microbiota-depleted mice were resistant to EODF-induced adipose browning. The underlying mechanism seems to involve increased intestinal lactate production by the newly formed microbiome after intermittent fasting (Li *et al.* 2017). Lactate production was also increased in response to cold exposure and lactate has previously been established as a potent inducer of thermogenic gene expression in mouse

and human adipocytes (Carrière *et al.* 2014). Recently, short-term caloric restriction was demonstrated in mice to confer a very similar phenotype as intermittent fasting. Thirty days of 40% caloric restriction significantly altered the gut microbial composition and promoted adipose tissue browning. Mechanistically, these effects were linked to lower expression of the key bacterial enzymes necessary for the lipid A biosynthesis, a critical lipopolysaccharide (LPS)-building component. Decreased LPS production was associated with anti-inflammatory M2 macrophage polarization and eosinophil infiltration in WAT (Fabbiano *et al.* 2018). Both cell types have previously been shown to contribute to the thermogenic reprogramming of WAT (Qiu *et al.* 2014). Together, these findings not only established a microbiota–fat signaling axis but linked innate immune responses with adipose tissue browning.

Immune cells and thermogenic programming

First evidence for a direct contribution of immune cells toward adaptive thermogenesis came in 2011 when it was reported that cold exposure rapidly promoted interleukin 4 (IL4)-dependent alternative activation of adipose tissue macrophages. It was postulated that these M2 type macrophages release catecholamines to induce thermogenic gene expression in BAT and lipolysis in WAT (Nguyen *et al.* 2011). However, these findings have been contradicted by others showing that specific deletion of the rate-limiting enzyme of catecholamine synthesis, tyrosine hydroxylase, in hematopoietic cells does not affect adipose browning or cold tolerance in mice. In addition, bone marrow–derived macrophages did not release norepinephrine in response to stimulation with IL4, and conditioned media from IL4-stimulated macrophages failed to induce the expression of thermogenic genes in isolated adipocytes (Fischer *et al.* 2017). The controversy of this topic is further highlighted by a recent report showing that a newly identified macrophage population – so called sympathetic neuron-associated macrophages (SAMs) – import and degrade norepinephrine via the transporter solute carrier family 6 member 2 and the degradation enzyme monoamine oxidase A, that are not present in other adipose tissue macrophages. They also demonstrated that SAM-mediated clearance of extracellular norepinephrine has detrimental effects on adipose tissue browning, thermogenesis and obesity (Pirzgalska *et al.* 2017).

Eosinophils are another critical immune cell population involved in adipose tissue thermogenesis. Genetic loss of

eosinophils or IL4/13 signaling impaired cold-induced biogenesis of beige fat through lack of alternative activation of M2 macrophages (Qiu *et al.* 2014). Another study identified the circulating muscle-derived factor meteorin-like that was induced in response to exercise and stimulated WAT browning due to eosinophil-dependent type 2 cytokine production and anti-inflammatory macrophage activation (Rao *et al.* 2014).

Exercise and browning

Accumulating evidence in rodents suggests that exercise contributes to adipose browning mainly through hormonal factors including myocyte-secreted molecules called myokines. The first myokine that has been linked to WAT browning was irisin, a cleavage product of the membrane-bound protein fibronectin type III domain-containing protein 5 (FNDC5). Many of the beneficial metabolic effects of exercise on muscle are mediated by PPAR γ coactivator-1 α (PGC1A). Transgenic expression of PGC1A in muscle stimulated FNDC5 expression and induced WAT browning. Also, treatment of primary adipocytes with recombinant irisin promoted thermogenic gene expression (Bostrom *et al.* 2012). Even though elevated circulating irisin levels were reported in exercised mice, results in humans are much more controversial (Crujeiras *et al.* 2015). Several human studies failed to show an increase in systemic irisin concentrations after acute or chronic endurance training despite enhanced PGC1A expression in muscle (Hecksteden *et al.* 2013, Moraes *et al.* 2013, Kurdiova *et al.* 2014, Norheim *et al.* 2014, Qiu *et al.* 2015).

Another exercise-induced circulating factor secreted from skeletal muscle is IL6. Accumulating evidence implicates IL6 in exercise-dependent WAT browning. Indeed, IL6-deficient mice were resistant to exercise-induced thermogenic gene expression in white fat (Knudsen *et al.* 2014). Both, *in vivo* and *in vitro* treatment with IL6 stimulated UCP1 expression in white adipocytes (Wan *et al.* 2012, Knudsen *et al.* 2014). However, skeletal muscle does not seem to be the only source of exercise-induced IL6 production, given that muscle-specific IL6-knockout mice displayed increased IL6 levels in response to exercise (Gudiksen *et al.* 2016).

Although fibroblast growth factor 21 (FGF21) is not a classic myokine (it is mainly secreted by the liver), it is also increased after acute and long-term exercise (Staiger *et al.* 2017). FGF21 has become a focus of clinical trials for obesity and diabetes because it has many beneficial

effects on metabolism including increased glucose uptake, improved insulin sensitivity and weight reduction (Gaich *et al.* 2013, Talukdar *et al.* 2016). Some of these FGF21 actions may be mediated, at least in part, by stimulating fatty acid oxidation and energy dissipation through browning of adipocytes (Xu *et al.* 2009, Fisher *et al.* 2012, Schlein *et al.* 2016). Mice deficient in FGF21 displayed an impaired ability to adapt to chronic cold exposure with diminished browning of WAT (Fisher *et al.* 2012). Administration of FGF21 lowered plasma triglycerides in mice through reduced hepatic very low-density lipoprotein secretion and increased lipoprotein disposal in adipose tissue (Schlein *et al.* 2016). FGF21 and PRDM16 have been reported to compensate for the reduced sympathetic response to cold exposure in a tyrosine hydroxylase haplodeficient mouse model suggesting a mechanism independent of the classic adrenergic pathway (Vazquez *et al.* 2018). In humans, circulating FGF21 correlated with brown fat activity determined by FDG uptake in PET/CT studies and in human brown adipocytes FGF21 and FNDC5 had additive effects on norepinephrine-stimulated thermogenesis (Lee *et al.* 2014a, Hanssen *et al.* 2015a).

Endogenous modulators of WAT browning

Other hormones with significant browning potential are parathyroid hormone (PTH) and PTH-related peptide (PTHrP). In a mouse model of kidney failure, secondary hyperparathyroidism promoted muscle wasting and cachexia through WAT browning (Kir *et al.* 2016). A similar phenotype was observed in a murine lung carcinoma model with excess PTHrP production (Kir *et al.* 2014). These effects were abolished in adipose tissue PTH receptor-knockout mice arguing for a fat-specific PTH receptor signaling pathway (Kir *et al.* 2016). These findings also suggested potential detrimental effects of increased energy wasting through adipose tissue browning, particularly in diseases associated with a catabolic state. Extending previous findings of PTH action in mouse WAT, our lab recently found that PTH stimulation in human primary adipocytes isolated from subcutaneous fat triggered a thermogenic program with increased UCP1 expression and enhanced cellular respiration (Hedesan *et al.* 2018). This suggests a previously unknown parathyroid-adipose crosstalk in humans and raises important questions about the regulation of energy expenditure in medical conditions associated with high PTH or PTHrP levels. The physiological connection between increased PTH receptor signaling and catabolic states is unclear;

however, it is possible that PTH and PTHRP oversecretion trigger maladaptive processes that result in enhanced lipolytic activity and energy wasting.

There is also accumulating evidence for a crosstalk between heart and adipose tissue. Heart-derived hormones such as the natriuretic peptides can control fat function including non-shivering thermogenesis and fatty acid oxidation (Sengenès *et al.* 2000, 2003, Bordicchia *et al.* 2012). For instance, infusion of the brain natriuretic peptide, which is usually elevated in heart failure patients, caused increased UCP1 expression in subcutaneous fat and enhanced energy expenditure in mice. This effect was mainly mediated by natriuretic peptide receptors through the activation of p38 MAPK (Bordicchia *et al.* 2012). Recently, cardiotrophin-1, a heart-derived cytokine, was also found to improve glucose and lipid metabolism, with enhanced mitochondrial biogenesis and browning of WAT (Moreno-Aliaga *et al.* 2011).

Bile acids are another group of important signaling molecules with potent action within adipose tissue. Most of the metabolic effects of bile acids are mediated through the nuclear receptor farnesoid X receptor (FXR) and the G protein-coupled bile acid receptor TGR5 (Li & Chiang 2015). In mice, cold exposure induced hepatic conversion of cholesterol to bile acids via the alternative bile acid synthesis pathway that involves cytochrome P450, family 7, subfamily b and polypeptide 1 (CYP7B1). As a result, plasma levels and fecal excretion of bile acids were increased in response to cold, whereas this effect was blunted in CYP7B1-deficient mice. CYP7B1 deficiency also mitigated cold-induced thermogenic gene expression in WAT and BAT with reduced core body temperatures (Worthmann *et al.* 2017). Selective activation of intestinal FXR induced fibroblast growth factor 15, leading to altered bile acid composition with enhanced WAT thermogenesis and protection against diet-induced weight gain (Fang *et al.* 2015). In addition, pharmacologic administration of chenodeoxycholic acid promoted thermogenic gene expression and weight loss in mice and stimulated BAT activity and energy expenditure in humans (Teodoro *et al.* 2014, Broeders *et al.* 2015).

Recent data suggest that BAT serves also as a secretory organ and thereby contributes to its own activation and/or browning of WAT. The chemokine CXCL14 has recently been identified as a novel BAT-derived adipokine that is involved in the recruitment of alternatively activated macrophages, WAT browning and improved glucose homeostasis in obese mice. Impairment of type 2 cytokine signaling, as seen in *Stat6*-deficient mice,

blunted the action of CXCL14 (Cereijo *et al.* 2018). These findings suggested again a potential crosstalk between immune cells and adipocytes, controlling the thermogenic remodeling of adipose tissue.

Using a lipidomic approach, Lynes *et al.* identified 12,13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME) as a cold-induced lipokine in humans which correlates with BAT activity and a favorable metabolic risk profile. Brown adipocytes have been identified as the primary source of cold-induced circulating 12,13-diHOME in mice. 12,13-diHOME increases fatty acid uptake into brown/beige adipocytes by increasing the expression of the fatty acid transporters FATP1 and CD36 within the cell membrane which promotes triglyceride clearance and thus facilitates a thermogenic program (Lynes *et al.* 2017).

Adipocyte browning in humans

Since the discovery of functional BAT in human adults through the advent of the widespread use of ^{18}F -FDG PET imaging (Nedergaard *et al.* 2007), almost a decade of research has been committed toward the importance of BAT for human energy metabolism. In several cross-sectional clinical studies using cold exposure to activate BAT, the presence of BAT ^{18}F -FDG uptake was associated with reduced adiposity, younger age and a more favorable metabolic profile (Saito *et al.* 2009a, b, Van Marken Lichtenbelt *et al.* 2009, Virtanen *et al.* 2009, Yoneshiro *et al.* 2011b, Matsushita *et al.* 2014). Biopsy studies confirmed that these ^{18}F -FDG-positive fat depots contained multilocular UCP1-expressing brown adipocytes (Cypess *et al.* 2009, Zingaretti *et al.* 2009). Active BAT depots were mainly discernable in cervical, supraclavicular as well as axillary and sometimes in mediastinal, paraspinal and abdominal fat depots (Leitner *et al.* 2017). BAT activity was positively associated with the cold-induced increase in non-shivering thermogenesis (Yoneshiro *et al.* 2011a, Chen *et al.* 2013). However, in a larger representative cohort in a healthy Japanese population only 48% showed detectable cold-induced BAT activity, all measured during winter when BAT ^{18}F -FDG uptake was highest (Saito *et al.* 2009b, Matsushita *et al.* 2014, Bahler *et al.* 2016).

The idea of positively influencing energy balance in obesity by increasing BAT thermogenesis is hampered by reduced BAT activity in obesity (Van Marken Lichtenbelt *et al.* 2009, Matsushita *et al.* 2014). Even in subjects with high BAT volume, the overall small BAT-specific increase in daily energy expenditure under cold stimulation

accounts only for a small proportion of non-shivering thermogenesis (Muzik *et al.* 2013, Din *et al.* 2016). Alternative therapeutic approaches could therefore include the browning of large classical WAT depots such as the abdominal subcutaneous fat and the visceral fat in order to achieve a larger volume of thermogenically active adipose tissue. Numerous cold exposure studies in mice found that that subcutaneous adipose tissue (SAT) depots are more susceptible to browning than the metabolically unfavorable visceral fat (Seale *et al.* 2011, Van Der Stelt *et al.* 2017). In general, very little is known about the anatomical differences in the browning susceptibility of human WAT. However, one study found that in obese subjects, omental fat expressed higher transcript levels of browning markers and genes involved in mitochondrogenesis compared to abdominal subcutaneous fat (Zuriaga *et al.* 2017). Visceral adipose tissue (VAT) has consistently been associated with an adverse cardio-metabolic risk profile (Fox *et al.* 2007, Liu *et al.* 2010, Neeland *et al.* 2013, Abraham *et al.* 2015). Excess VAT has also been implicated in the pathogenesis of non-alcoholic fatty liver disease through the drainage of pro-inflammatory adipokines as well as lipotoxicity mediated by free fatty acids in the portal circulation (Baranova *et al.* 2007, Verrijken *et al.* 2010, Eguchi *et al.* 2011, Wree *et al.* 2011). Hence, the combustion of excess fatty acids and decreasing the inflammatory state through the browning of VAT could provide manifold benefits in the prevention of metabolic disease. Based on numerous animal studies it is possible that browning of both SAT and VAT may have beneficial effects on global energy metabolism.

Is human WAT susceptible to browning?

There is also some evidence that human SAT can undergo browning at least under very extreme conditions. In a study in intensive care patients the authors found that severe burn injuries led to an increase of UCP1-positive beige cells within the subcutaneous fat with a maximum several weeks after the injury. This was linked to the severe adrenergic stress and the prolonged elevation of norepinephrine levels seen after such burn injuries (Sidossis *et al.* 2015). Endogenous catecholamine excess can also trigger browning of the visceral fat compartment as observed in a study in pheochromocytoma patients. Whereas 50% of pheochromocytoma patients undergoing cholecystectomy had UCP1-positive brown adipocytes in the greater omentum, none of the control patients

had any signs of WAT browning (Frontini *et al.* 2013). Another study in pheochromocytoma patients, however, found brown/beige adipocytes only in periadrenal but neither omental nor subcutaneous fat (Vergnes *et al.* 2016). WAT browning also seems to underlie seasonal variations since the expression of thermogenic marker genes in SAT was increased during winter compared to biopsies obtained during summer. In the same study, the application of an ice pack to the skin of one thigh for 30 min resulted in a 1.9-fold and 2.7-fold increase in *UCP1* and *PGC1A* mRNA levels, respectively. This effect was not present during winter which was attributed to the higher baseline levels.

In primary adipocyte cell cultures derived from femoral SAT, the direct exposure of cells to 16°C for 30 min led to a delayed increase in thermogenic gene expression which became noticeable only 4 and 24 h after recovery from the cold stimulus (Kern *et al.* 2014). Interestingly, in murine adipocytes, this thermo-sensory capacity was only observed in WAT and beige adipocytes but not in BAT (Ye *et al.* 2013).

Interventional approaches targeting BAT activity and WAT browning

Cold exposure studies of variable duration have repeatedly demonstrated that *bone fide* BAT can be successfully activated and recruited in humans through cold stress (van der Lans *et al.* 2013, Yoneshiro *et al.* 2013, Blondin *et al.* 2014, Lee *et al.* 2014b, Hanssen *et al.* 2015b, 2016). However, none of the studies found abdominal WAT browning using abdominal subcutaneous biopsies of SAT/VAT or ¹⁸F-FDG PET scans (van der Lans *et al.* 2013, Hanssen *et al.* 2015b, 2016) suggesting that moderate cold exposure may not be sufficient to promote WAT browning in humans.

β -adrenergic receptor agonists are among the most widely studied pharmacological agents that induce BAT function and browning of WAT. Drugs highly selective for human β_3 adrenergic receptors (AR) with a good oral bioavailability were developed but they failed in the clinic due to their β_1 - and β_2 -AR-mediated cardiovascular effects in humans (Arch 2011). However, recently the selective β_3 -agonist mirabegron, previously approved for the treatment of overactive bladder, was shown to increase BAT activity and energy expenditure in humans without causing severe cardiovascular side effects. After a single dose of 200 mg, BAT ¹⁸F-FDG uptake and resting

metabolic rate but also blood pressure were increased in healthy young lean men (Cypess *et al.* 2015). Daily intake of 50 mg mirabegron for 10 weeks induced the beiging of abdominal SAT in obese participants, demonstrated by increased protein content of thermogenic markers such as UCP1 (Finlin *et al.* 2018). Long-term studies in obese and insulin-resistant individuals are warranted to investigate the effectiveness and cardiovascular safety of mirabegron treatment to induce weight loss and improvements in metabolic health.

Another pharmacological approach involved the phosphodiesterase type-5 inhibitor sildenafil which is marketed for the treatment of erectile dysfunction. It was recently tested in a double-blind placebo-controlled trial after animal studies had demonstrated promising effects of sildenafil as a browning agent. Indeed a 7-day treatment with 100 mg sildenafil daily resulted in the induction of UCP1-expressing beige cells within the SAT. This was accompanied by an increase in the resting metabolic rate and elevated catecholamine and cGMP concentrations. However, this study does not allow to draw any conclusions about the effect of sildenafil on classical human BAT because only three participants underwent ^{18}F -FDG PET/CT scans (Li *et al.* 2018). Interestingly, previous preclinical work had already suggested that cGMP and consecutive mTOR activation are also mechanistically involved in the induction of a thermogenic gene program (Liu *et al.* 2018).

Inhibitors of the sodium-glucose cotransporter 2 (SGLT2) are potent antidiabetic drugs with additional body weight-lowering effects due to increased urinary glucose excretion. However, a recent study in mice identified another potential mechanism that may contribute toward body weight loss seen with SGLT2 inhibition. Oral administration of empagliflozin in mice on a high-fat diet reduced M1-polarized macrophage accumulation in adipose tissue while favoring an anti-inflammatory M2 macrophage phenotype. As a consequence, WAT browning and fat utilization were increased which attenuated weight gain and insulin resistance (Xu *et al.* 2017).

The anticancer drug imatinib was found to block cyclin-dependent kinase 5-mediated peroxisome proliferator-activated receptor γ (PPARG) phosphorylation in obese mice while acting as a non-classical PPARG ligand. Similar to the classical PPARG ligands thiazolidinediones, imatinib improved insulin sensitivity and hepatic steatosis and increased energy expenditure through WAT browning (Choi *et al.* 2016).

Increasing whole body nitric oxide availability through oral L-arginine supplementation has the potential to promote adipocyte thermogenesis through increased mitochondrogenesis in mice (Nisoli *et al.* 2003, 2004). A recent study in humans, however, did not show any effect of L-arginine on BAT activity or energy expenditure while suggesting an improvement in cold-induced glucose oxidation (Boon *et al.* 2019).

Tissue grafting procedures could represent an alternative strategy to conventional pharmacological approaches due to the lack of potential systemic side effects. An initial study in mice revealed that transplantation of subcutaneous fat into the visceral cavity of the host mice exerted beneficial metabolic effects while transplantation of epididymal fat into any of the two depots did not. A salutary secretory profile of subcutaneous fat was suggested to contribute to its beneficial effects (Tran *et al.* 2008). Transplantation of BAT into the visceral cavity of mice has also been shown to improve glucose metabolism and body composition dependent on the presence of BAT-derived interleukin 6 (Stanford *et al.* 2013). We recently reported a concept that involved autologous beige fat transplantation. First, murine SAT fragments were exposed to a single-step browning procedure using a distinct culture medium. After 3 weeks of *ex vivo* culture, the fragments were re-implanted and explanted again 8 weeks later. Not only was the auto-transplant well integrated and vascularized, those tissues that were *ex vivo* treated with browning media showed sustained UCP1 expression, whereas the tissues cultured in control media retained a WAT-like appearance. Most importantly, this protocol was also successfully applied in human fat. By incubating small fragments from abdominoplastic surgeries with browning media for 2-week conversion of WAT into UCP1-expressing multilocular beige fat was achieved (Blumenfeld *et al.* 2018).

Conclusion and future outlook

Targeting BAT and browning of WAT have emerged as potential strategies to treat obesity and its related metabolic disorders. Particularly, the transformation of WAT into a thermogenically active fat depot holds great promise as a therapeutic intervention given the excess amount of white fat in obese conditions. Increasing research efforts have identified a large number of molecular pathways controlling the reprogramming of white into beige adipocytes.

Figure 1

Summary of proposed mechanisms involved in the browning of white adipose tissue. 12,13 diHOME, 12,13-dihydroxy-9Z-octadecenoic acid; AgRP, agouti-related protein; AMPK, adenosine monophosphate-activated protein kinase; ANP, atrial natriuretic peptide; AC, adenylate cyclase; ADRB3, β 3-adrenergic receptor; BA, bile acid; BNP, B-type natriuretic peptide; cAMP, cyclic adenosine monophosphate; CART, cocaine and amphetamine-regulated transcript; CD36, cluster of differentiation 36; cGMP, cyclic guanosine monophosphate; CXCL14, C-X-C Motif Chemokine Ligand 14; EO, eosinophil; ER, endoplasmic reticulum; FFA, free fatty acid; FGF21, fibroblast growth factor 21; FGFR, fibroblast growth factor receptor; GC, guanylyl cyclase; GLP1, glucagon-like peptide 1; GRP78/BiP, 78-kDa glucose-regulated protein/binding immunoglobulin protein; HSL, hormone-sensitive lipase; IL, interleukin; IRE1A, inositol-requiring enzyme 1 α ; LPS, lipopolysaccharide; M1, M1 macrophage; M2, M2 macrophage; MAOA, monoamine oxidase A; NPY, neuropeptide Y; OGT, O-linked N-acetylglucosaminyltransferase; PDE5, phosphodiesterase type 5; PKA, protein kinase A; PKG, protein kinase G; POMC, pro-opiomelanocortin; PTH, parathyroid hormone; PTHR, parathyroid hormone receptor; PTHRP, parathyroid hormone-related peptide; SAM, sympathetic neuron-associated macrophage; SkM, skeletal muscle; SNS, sympathetic nervous system; T3, triiodothyronine; TGR5, G protein-coupled bile acid receptor; UCP1, uncoupling protein 1; VMH, ventro-medial hypothalamus.

These pathways as discussed in this review are summarized in Figure 1. In addition, accumulating evidence suggests that browning of WAT can also occur in humans either under extreme medical conditions such as severe adrenergic stress or in response to some pharmacological stimuli. However, it remains to be resolved whether WAT browning in humans can be harnessed therapeutically to increase energy expenditure and reduce body fat mass. Additional research is needed to identify the distinct cell populations in human fat depots that can give rise to thermogenically active beige fat cells. Another area of investigation concerns the development of novel radioligands that can be used as alternative tracers to detect and quantify BAT activity and potentially beige fat within classic WAT depots. Together, all these research efforts will improve our understanding of BAT function and WAT browning with the ultimate goal of developing new therapeutics for the treatment of obesity and related sequelae.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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