Review Article

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Hormonal factors in the control of the browning of white adipose tissue

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Abstract:
Adipose tissue has been historically classified into anabolic white adipose tissue (WAT) and catabolic brown adipose tissue (BAT). Recent studies have revealed the plasticity of WAT, where white adipocytes can be induced into 'brown-like' heat-producing adipocytes (BRITE or beige adipocytes). Recruiting and activating BRITE adipocytes in WAT (so-called 'browning') is believed to provide new avenues for the treatment of obesity-related diseases. A number of hormonal factors have been found to regulate BRITE adipose development and activity through autocrine, paracrine and systemic mechanisms. In this mini-review we will discuss the impact of these factors on the browning process, especially those hormonal factors identified with direct effects on white adipocytes.

Keywords: beige adipocyte, BRITE adipocyte, brown adipose, hormonal regulation, thermogenesis, white adipose

DOI: 10.1515/hmbci-2017-0017

Received: April 3, 2017; **Accepted:** May 11, 2017

Introduction

Adipose tissue has historically been classified into energy-storing white adipose tissue (WAT) and energy-dissipating brown adipose tissue (BAT) [1]. In contrast to WAT, BAT is a highly oxidative tissue containing abundant mitochondria that oxidize fatty acids and generate heat. Positron emission tomography (PET) scans have revealed the existence of canonical BAT in humans [2], however, there is still controversy as to whether it serves important roles in thermoregulation and energy balance for adult humans [3]. Recent studies have highlighted the plasticity of WAT, where brown adipocyte-like cells emerge upon sustained cold exposure or β-adrenergic activation [1], [4]. This process is termed "browning" of WAT [5]. Studies indicate that the newly formed brown adipocyte-like cells stemming from existing WAT are not traditional myf5+ preadipocytes found in BAT [6]. A recent study has suggested that BRITE adipocytes contain comparable amounts of uncoupling protein 1 (UCP1) as fully stimulated brown adipocytes, suggesting that they may have similar thermogenic capacities [7]. Interestingly, a link between BRITE adipocytes and obesity was also found in humans. The BRITE adipocytes derived from the preadipocytes of subcutaneous WAT of obese humans contain reduced amounts of UCP1 [8]. Several lines of evidence even suggested that human BAT is mainly composed of BRITE adipocytes [9]. The discovery of BRITE adipocytes in humans has also attracted research interest in identification of browning activators for metabolic benefits [10]. These recent findings raise biomedical interest in the pharmacological browning of WAT for treating obesity and related metabolic disease in humans [11].

UCP1 expression is a representative marker of canonical brown adipocytes, which is also a distinct marker to identify the process of 'browning' of white adipocytes [7]. The UCP1 protein locates at the inner mitochondrial membrane, serving as a channel transporting protons from the mitochondrial intermembrane space to the mitochondrial matrix [12]. This UCP1-mediated proton leak uncouples the respiratory chain, allowing energy to be released as large amounts of heat instead of generating ATP [13]. UCP1 biosynthesis is largely controlled at the level of transcription [14]. The 5′ flanking region of the *UCP1* gene shares a common genomic structure in mouse, rat and human: a proximal regulatory region near the transcriptional start site containing CCAAT-enhancer binding protein (C/EBP)-binding sites and a cyclic adenosine monophosphate (cAMP) regulatory element [15]. In addition, a highly conserved distal enhancer is present, containing two additional cAMP regulatory elements and a complex organization of nuclear receptor binding sites which mediate the transcriptional

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activation of the *UCP1* gene by peroxisome proliferator-activated receptor (PPAR) agonists, thyroid hormones and retinoids [16]. To date, the majority of known browning agents are found to have the capacity to directly or indirectly promote *UCP1* gene transcription. Although *UCP1* expression is a main characteristic of BRITE cells and commonly used for identifying browning of WAT, other essential events occur during this process, such as mitochondrial biogenesis and increases in the cellular capacity for glucose and fatty acid uptake and oxidation [17], [18]. Besides, it is also widely believed that BRITE and brown adipocytes still may have distinct cell-type-specific functions that have yet to be studied [7].

The potential for inducing BRITE adipocytes as an anti-obesity strategy has attracted extensive study over the last decade, which has already enhanced our understanding of the underlying mechanisms of WAT browning. It is suggested that a complex interplay of hormonal factors are involved in the browning process. These factors include molecules synthesized locally within adipose tissue as well as hormones released by other metabolically active organs. In this mini-review, an overview of the current understanding of the impact of these factors on the browning process will be summarized.

Norepinephrine – the key molecular control of WAT browning

In canonical brown adipocytes, the most striking characteristic is the capacity to generate heat by non-shivering adaptive thermogenesis [19]. UCP1 is responsible for this process [20]. The most significant and the most studied activator of adaptive thermogenesis is norepinephrine. It is released from sympathetic nerve endings and acts on β-adrenergic receptors on the surface of brown adipocytes to elicit a myriad of thermogenic events, including induction of UCP1 transcription [21]; enhancing intracellular lipolysis and mitochondrial oxidation; and stimulation of circulating triglyceride uptake [22]. The β3-adrenergic receptor is the most important adrenergic receptor involved in BAT activation, although both β1- and β2-adrenergic receptor activations are able to compensate for the loss of the β3-adrenergic receptor in knock-out mice models for BAT activation [23].

Along with its critical roles in BAT activation and recruitment, norepinephrine also occupies a central role in WAT browning. Noradrenergic regulation of WAT browning is considered to act mainly through the β3-adrenergic receptor. Indeed, some studies indicated that the β3-adrenergic receptor may play an indispensable role in WAT browning, as the loss of the β3-adrenergic receptor in knock-out mice almost abolishes the cold-induced browning in WAT [23], [24]. However, a recent study has revealed that the β3-adrenergic receptor is not required for browning of WAT due to cold exposure [25]. The inconsistency in these findings may be attributed to the different mouse strains used in the studies. Regardless of the receptor requirements, the promoting effect of noradrenergic stimulation on browning was also demonstrated in humans. In a clinical study, the subcutaneous WAT of severely burned patients was found to adopt a BAT-like phenotype after prolonged and severe adrenergic stress, with increased multilocular UCP1-positive adipocytes, mitochondrial density and respiratory leak capacity being observed, which demonstrates that human subcutaneous WAT also can transform into energy-dissipating tissue [26]. Due to the potential importance of the β3-adrenergic receptor in WAT browning, agonists to β3-adrenergic receptors are promising therapeutic drug candidates to curb obesity. Although a number of β3-adrenergic receptor agonists have failed in clinical trials due to significant side effects, a recent report claimed that the mirabegron, a β3-adrenergic stimulator approved for the treatment of overactive bladder, can activate BAT in healthy humans [27]; however, its therapeutic utility in obese or diabetic humans remains to be established.

Thyroid hormones in the control of WAT browning

Both BAT and WAT express a wide range of nuclear receptors that have the potential to regulate the expression of genes involved in brown/BRITE adipocyte biology [28]. Thyroid hormones act through the thyroid receptor and are considered non-sympathetic activators of BAT. Animal studies revealed that hypothyroid mice had significantly decreased and hyperthyroid mice had significantly increased interscapular BAT activities compared to euthyroid controls [29]. Thyroid hormones can both directly and indirectly stimulate UCP1 gene transcription in BAT. The thyroid hormones can enhance sympathetic nervous system (SNS) activity through the induction of AMP-kinase in the hypothalamus to activate BAT [30]. In addition, the thyroid response elements located in the distal enhancer of the UCP1 gene also allow the binding of the thyroid receptor to regulate UCP1 expression [31]. Furthermore, there is extensive crosstalk between the norepinephrine and thyroid hormone actions in controlling BAT activities. UCP1 levels vary with triiodothyronine (T3) concentration in BAT, which can be converted from thyroxine (T4) by type II iodothyronine 5′-deiodinase (DIO2). In BAT, DIO2 can be activated by the

SNS and, therefore, adrenergic signals can be amplified by thyroid hormones to reach a maximal thermogenic response [32].

Thyroid hormones also induce the thermogenic program in WAT, leading to the browning of white fat depots with a restoration of cold tolerance in cold-intolerant mice [33], [34]. The thermogenic genes [UCP1, PR domain containing 16 (PRDM16), fibroblast growth factor 21 (FGF21), cell death-inducing DFFA-like effector A (Cidea), PPARγ coactivator 1α (PGC-1α)] and BRITE adipocyte markers [Cd137, transmembrane protein 26 (Tmem26)] were significantly increased in epididymal WAT in hyperthyroid mice compared to hypothyroid and euthyroid mice [29], [33]. Thyroid receptor agonist-induced browning was also observed in white adipocytes in vitro, indicating that thyroid hormones may directly mediate browning in a cell-autonomous manner [34]. Notably, the DIO2 expression level in mature white adipocytes is much lower than in brown adipocytes, suggesting that thyroid hormones may promote BAT activation and WAT browning through different mechanisms [35].

Recent data also reported that liver X receptor β (LXRβ) controls thyroid hormone feedback in the brain and regulates browning of subcutaneous WAT. LXRs, especially LXRβ, serve to repress the browning process of subcutaneous WAT. The knock-out of LXRs increases the production of thyroid hormones through the hypothalamic-pituitary-thyroid axis. Consequently, the circulating thyroid hormone level and browning of WAT are upregulated. As LXR is the receptor for cholesterol and fatty acids, these findings demonstrate a possible mechanism of thyroid hormones regulating energy expenditure in response to cholesterol and fatty acid metabolism [36].

FGF21 and irisin – signals from shivering and non-shivering thermogenesis to WAT

In response to cold stimulation, the body will try to maintain body temperature homeostasis via both shivering (skeletal muscles) and non-shivering (BAT activation) thermogenesis. Interestingly, it was also found that cold exposure also increases secretion of the myokine irisin and the brown adipokine FGF21 from muscle and BAT, respectively. Both irisin and FGF21 promote browning of white adipocytes in adaptive thermogenesis [37], [38]. These effects represent connections between muscle, BAT and WAT, orchestrating cold-induced adaptive thermogenesis.

Irisin is a cleaved version of fibronectin type III domain-containing protein (*FNDC5*), which is a target gene regulated by PGC-1α. As exercise increases expression of PGC-1α in muscle, *FNDC5* was first identified as an exercise-induced activator of browning. Primary subcutaneous white adipocytes treated with *FNDC5* show a significant increase in UCP1 mRNA [38]. Further studies suggested that *FNDC5* will be cleaved at the C-terminal, glycosylated and secreted in the form of irisin. Accumulating evidence indicates that irisin is the active form of *FNDC5* in the circulation, playing an important role in converting white adipocytes to BRITE adipocytes [38], [39], [40]. The secretion of irisin correlates with shivering intensity, suggesting that irisin may amplify heat production by passing on the signals from shivering thermogenesis to non-shivering thermogenesis [38]. This effect may be mediated by the irisin-induced phosphorylation of the p38 mitogen-activated protein kinase (p38 MAPK) and the extracellular signal-related kinase (ERK) signaling pathways in white adipocytes [39]. Recently, it was further demonstrated that irisin has a dual role in pre- and mature white adipocytes. Irisin only promotes "browning" of mature white adipocytes, whereas it inhibits adipogenic differentiation of preadipocytes [40].

Similarly, FGF21, a cytokine with the capacity for WAT browning, was also significantly augmented after cold stimulation. Cold-stimulation mainly increases FGF21 secretion from BAT [41]. Experimental studies have demonstrated that the central effects of FGF21 on sympathetic activation and the direct effects mediated by the FGF receptor on white adipocytes both contribute to the browning of WAT [37], [42], [43]. Additionally, FGF21 also activates BAT in a cell-autonomous way [37]. FGF21 regulates this process, at least in part, by enhancing adipose tissue PGC-1α protein levels independently of mRNA expression [37]. FGF21, as well as its analogs, have shown anti-diabetic and anti-obesogenic effects in rodent models [44], [45], [46]. Moreover, FGF21-deficient mice display an impaired cold response in inguinal WAT but not in canonical BAT, indicating an important role in metabolic regulation. However, a recent study indicates that FGF21 is still efficacious in UCP1-null mice, suggesting that FGF21's anti-diabetic and anti-obesogenic effects are independent of thermogenesis in brown and BRITE adipocytes [47].

Notably, adipose-derived FGF21 expression is not only regulated by cold stimulation. Nutritional signals and physical activity also play important roles in the regulation of FGF21 expression [48], [49], suggesting that FGF21 may be an important molecular regulator of thermogenesis in response to environmental metabolic conditions. Furthermore, FGF21 is also released by the liver and muscle [50], [51], [52]. FGF21 secreted by the liver directly activates BAT heat production as well as browning of WAT depots, hinting that this liver-to-adipose tissue regulatory loop also contributes to the thermogenic program [50].

Recently, another exercise- and cold-induced myokine hormone (meteorin-like) was discovered. Increasing circulating levels of meteorin-like stimulates the expression of BRITE adipocyte marker genes associated with thermogenesis [53]. In light of these findings, cold-induced hormonal changes may be the potent and relevant physiological regulators of thermogenesis.

Effect of insulin and glucagon on WAT browning

Insulin is the most important regulator of glucose metabolism, with its major role in promoting the absorption of glucose from the circulation into adipose tissue, liver and skeletal muscle. In addition, insulin is also crucial for the development of canonical brown adipocytes as well as for the development of inducible BRITE adipocytes. Insulin deficiency was found to impair the differentiation of BRITE adipocytes, although this inhibitory effect could be overcome by stronger stimuli such as adrenergic activation [54]. Insulin produces its effect on browning through its action on pro-opiomelanocortin (POMC) neurons together with leptin [55].

Interestingly, although glucagon plays the opposing role to insulin in glucose metabolism, it has also been recognized as a strong BAT activator in rodents [56]. In addition, glucagon supplementation was found to induce a significant increase in plasma FGF21 in glucagon-depleted mice via enhanced hepatic FGF21 secretion [57]. As it has been demonstrated that increased circulating FGF21 promotes the browning of WAT, glucagon may also mediate the browning process indirectly. Latest evidence also indicates that glucagon increases energy expenditure by a similar magnitude compared with cold activation, but independently of BAT thermogenesis, also hinting the possible effect of glucagon on WAT browning [58].

Gastrointestinal hormones in the control of browning

The gastrointestinal tract is the organ that digests ingested food and absorbs energy and nutrients; therefore, it can directly sense the fluctuation of nutrient status and secrete a range of hormones to maintain energy homeostasis. Many gastrointestinal hormones can send hunger or satiety signals to the central nervous system (CNS) and SNS via the gut-brain axis and further regulate feeding behavior and metabolic processes involving the activation of BAT and the browning of WAT. In general, anorexigenic gut hormones normally stimulate BAT while orexigenic gut hormones often inhibit BAT activity.

For example, glucagon-like peptide-1 (GLP-1), an anorexigenic polypeptide, is able to regulate BAT activity by hypothalamic action. Although the principal effect of GLP-1 is to stimulate insulin secretion and to reduce glucagon secretion, studies also demonstrated the control of GLP-1 on BAT thermogenesis through the GLP-1 receptor in the hypothalamus, which is independent of nutrient intake and insulin responsiveness but correlated with increased activity of sympathetic fibers innervating BAT [59], [60]. Furthermore, central injection of a GLP-1R agonist also stimulates WAT browning through the hypothalamic AMPK. The mechanism controlling these actions is located in the hypothalamic ventromedial nucleus (the ventromedial hypothalamus, VMH) [60].

Similarly, several other anorexigenic hormones were found to strengthen BAT activity via the SNS, although there is no clear evidence concerning their effect on browning. For example, amylin, an anorexigenic peptide secreted into the circulation by pancreatic β-cells, was also found to act in the CNS to increase sympathetic nerve activity to BAT and elicit a thermogenic response [61]. Injection of anorexigenic cholecystokinin (CCK) into the third ventricle or into selected hypothalamic sites was also found to increase interscapular BAT sympathetic nerve activity, BAT temperature and expired CO_2 in anesthetized rats. Bilateral cervical vagotomy prevented these CCK-evoked effects, indicating that peripheral CCK acts via vagal afferents to increase BAT activity [62], [63].

In contrast, ghrelin, as an orexigenic gastrointestinal hormone, stimulates the appetite and increases food motivation [64], [65]. Ghrelin acts on the hypothalamus by stimulating secretion of agouti-related protein (AgRP) [66]. Centrally administered ghrelin into the third cerebral ventricle suppresses sympathetic nerve activity innervating BAT and decreases BAT temperature in rats [67].

Interestingly, evidence also emerged that certain anorectic and orexigenic hormones may counteract each other's effects on SNS activation. For example, galanin, which stimulates food intake, inhibited sympathetic nerve firing rate to interscapular BAT. In contrast, enterostatin, an anorectic pentapeptide formed in the lumen of the small intestine from the pancreatic procolipase under the influence of digestive enzymes, was reported to transiently increase the sympathetic firing rate of nerves innervating BAT [68]. Importantly, the response to enterostatin was determined by diet. On a high fat diet, enterostatin induced a large and sustained response,

whereas a minimal short-lived response was observed on a chow diet. This study supports the hypothesis that gastrointestinal hormone-regulated food intake and SNS activity have a reciprocal relationship.

There are additional notable metabolic substances with roles in the regulation of browning. For example, bile acids also have metabolic actions in the body resembling those of hormones. They are an important class of lipid-signaling molecules synthesized in the liver. In addition to their major function as detergents facilitating gut lipid digestion, they have actions in other organs including adipose tissues through the specific bile acid receptors farnesoid X receptor-α (FXRα), a nuclear receptor activated by bile acids, and the G-protein-coupled receptor TGR5. The bile acid chenodeoxycholic acid (CDCA) has been found to activate BAT thermogenesis and protect against obesity [69]. Importantly, the bile acid-mediated protection from obesity is lost in UCP1-knockout mice [70], supporting that the effect is through thermogenesis. Furthermore, activation of TGR5 in brown adipocytes by either the bile acids CDCA or lithocholic acid (LCA) or a selective ligand (CpdA) stimulated respiratory uncoupling [71]. As with β-adrenergic receptors, TGR5 couples to adenylate cyclase with cAMP-dependent signaling likely to activate the same processes that elevate energy expenditure. For the browning of WAT, research to date indicates effects of bile acids through FXR. It was reported that selectively activating intestinal FXR by a gut-restricted FXR agonist robustly induced enteric intestinal endocrine hormone fibroblast growth factor 15 (FGF15), leading to reduced diet-induced weight gain, body-wide inflammation and hepatic glucose production, as well as enhanced thermogenesis and browning of WAT [72].

Taken together, gastrointestinal hormones play crucial roles in WAT browning in response to the fluctuation of dietary nutrients or the energy state in the body.

Hypothalamic hormones in the control of WAT browning

Great efforts have been made to elucidate the neural circuits responsible for governing the sympathetic outflow to BAT. Current evidence indicates that cold signals sensed by thermoreceptors, together with other peripheral signals such as hormones and nutrients, are integrated in the brain, especially the arcuate nucleus (ARC) of the hypothalamus. Two populations of neurons in the ARC function in opposing ways to express orexigenic AgRP/neuropeptide Y (NPY) and anorexigenic POMC, respectively. The second-order neurons located largely in the lateral hypothalamus (LH) and the paraventricular nucleus (PVN) of the hypothalamus receive projections from the ARC as well as direct inputs from peripheral signals. Together with the ARC, the LH and PVN function as a metabolic integrator and regulator by projecting to high-order neurons in the CNS and secreting various neuropeptides such as orexin, melanin-concentrating hormone (MCH), cocaine- and amphetamine-regulated transcript (CART) and corticotropin-releasing hormone (CRH). Indeed, both BAT and WAT are extensively innervated by sympathetic fibers that can be tracked back to the hypothalamus [73]. The details of this neuroanatomical and neurotransmitter/hormonal organization of the core thermoregulatory network have been systematically reviewed previously [74].

Although it has not been confirmed that these neuronal circuits play the same role in the control of WAT browning, it is reasonable to hypothesize that they exert their functions in a similar way. Several studies have revealed that activation of AgRP neurons in the hypothalamus suppresses the browning process [75], while stimulation of POMC neurons promotes WAT browning [55]. These two opposing effects demonstrate that the AgRP/NPY and POMC neurons in the ARC could sense the body's energy state and regulate whole-body metabolism, including the browning of fat. Additional studies also support that hypothalamic hormones play crucial roles in the regulation of browning, depending on energy states. For example, neuron and adipocyte co-culture studies indicate that NPY secreted from sympathetic neurons inhibit β-adrenergic-mediated signaling [76]. Viral-mediated knockdown of orexigenic NPY in the dorsomedial hypothalamus (DMH) promotes BAT activation and WAT browning through the SNS [77].

Oxytocin is recognized as an anorexigenic neuropeptide, which has effects including reducing gastric emptying and GI transit, as well as by suppressing the feeding reward circuit [78], [79]. Knockout mice for oxytocin or its receptor (OXTR) were shown to develop late-onset obesity [80], without alterations in food intake, indicating that oxytocin also controls metabolic homeostasis by modulating energy expenditure. Oxytocin treatment of db/db mice resulted in a decrease in adipocyte size, reduction in fat pad mass as well as induction of BRITE adipocytes in WAT [81].

CRH is a peptide hormone and neurotransmitter involved in stress response. The ability of CRH to stimulate BAT thermogenesis has been extensively studied. Central injection of CRH leads to the activation of BAT thermogenesis in rats [82]. CRH functions mainly through two G protein-coupled receptors: CRH receptors types 1 (CRH-R1) and 2 (CRH-R2) [83]. Both receptors have been identified in brown adipocytes as well as in white adipocytes. Using the T37i cell in vitro model, CRH was found to promote brown adipocyte differentiation through inhibition of Fyn kinase. Pharmacological inhibition of Fyn kinase also enhanced brown

adipocyte differentiation [82]. Recently, greater insight into the underlying mechanisms by which CRH regulates adipocyte differentiation was revealed, with the balance between CRH-R1 and CRH-R2 important in the control of adipocyte plasticity. Activation of CRH-R2 or inhibition of CRH-R1 in 3T3-L1 preadipocytes was able to induce morphological and biochemical characteristics of BRITE adipocytes [84]. An in vivo study also supported the corticotrophin-releasing factor/urocortin system in regulating browning through paracrine mechanisms in mice. Increased CRH-R2 activity in adipocytes induces browning of WAT, differentiation of BAT and is associated with a favorable metabolic phenotype in mice lacking CRH-R1 [85]. Furthermore, CRH also affects energy metabolism via its cross-talk with other hormones. For example, CRH works downstream of the PVN to increase secretion of the neurotransmitter CART, which also induces the expression of UCP1 in both BAT and WAT [86]. CRH was also found to be a component of the pathway of leptin-dependent regulation of UCP1 expression [87]. It has been recognized that chronic stress may be a contributor to the increased risk for obesity; therefore, these findings indicate that CRH is a key hormone which mediates the links between stress and energy metabolism.

Numerous studies in a variety of species, including humans, showed that growth hormone (GH) levels are negatively correlated with adipose tissue mass. Adults and children with GH deficiency have increased fat mass, while treatment of GH-deficient patients with recombinant human GH decreases fat mass [88], [89], [90]. In mouse models, loss of the GH receptor or treatment with a GH antagonist increases interscapular BAT mass and UCP1 expression [91], [92], while a decrease in interscapular BAT was found after bovine GH overexpression with decreased UCP1 expression [92]. Similarly, the knockout of GHR or GH antagonist treatment also increased the UCP1-expressing adipocytes within subcutaneous WAT depots, suggesting that GH also affects the browning process [92]. Knockout of GH-releasing hormone, a hormone required for GH secretion in mice, also leads to browning of WAT and elevated expression of UCP1 [93].

An enriched environment was also proved to promote browning via induction of hypothalamic brain-derived neurotrophic factor (BDNF) expression. Hypothalamic BDNF has been shown to increase thermogenesis and energy expenditure by acting on neurons in the PVN and VMH [94], [95], which further upregulates the brown adipocyte fate-determining gene PRDM16, specific marker UCP1 and genes involved in thermogenesis and β-adrenergic signaling in mouse WAT. These findings suggested a mechanism whereby a response to environmental stimuli leads to selective sympathoneural modulation of WAT to induce 'browning' and increased energy dissipation [96].

Taken together, the hypothalamus, as an important organ responsible for maintaining homeostasis (including body temperature and body weight), can integrate signals received from the body, to then make the appropriate changes in hormone secretion to activate or inhibit thermogenesis and browning of WAT.

Adipose-derived hormones in the control of browning

WAT is not only a site of energy storage, but is also an endocrine organ that secretes a wide range of adipokines [97]. Therefore, WAT-derived hormones have the potential to regulate the browning process through autocrine mechanisms. Leptin represents a good model of how WAT sends signals related to energy status to the CNS (hypothalamus) to impact on whole-body metabolism and also directly affects the browning of white adipocytes. Leptin injection was demonstrated to reduce the body weight with a 4–5-fold increase in UCP1 expression in both interscapular BAT and retroperitoneal WAT in ob/ob mice [98]. This enhancement of UCP1 expression in BAT seems to be through a β3-adrenoceptor independent mechanism [99]. A leptin-knockout mouse model confirmed that leptin stimulates UCP1 expression as UCP1 expression was decreased in BAT, muscle, inguinal and gonadal fat [98].

Bone morphogenetic protein 4 (BMP-4) is another important adipose-derived factor that promotes browning. Transgenic expression of BMP-4 stimulates the conversion of mesenchymal precursors specifically to BRITE adipocytes, suggesting an important role for BMP-4 in browning [100]. β3-Adrenergic receptor activation also augments BMP-4 expression in an organ-autonomous manner in adipocytes [100]. BMP-4 belongs to the BMP family which influences the differentiation of mesenchymal stem cells. Several other members of this protein family are also identified as key regulators of canonical BAT differentiation and activation. For example, BMP-7 promotes the differentiation of brown preadipocytes by inducing the expression of PRDM16 and PPARγ [101], while BMP-8b directly regulates thermogenesis in mature brown adipocytes by increasing their responsiveness to noradrenaline and upregulating intracellular lipase activity via the protein kinase A (PKA)-MAPK pathway [102]. The activity of BMPs in adipose tissue is counteracted by gremlin-1 which is secreted by WAT preadipocytes [103]. However, the precise roles of the BMP family members and their regulators in browning still needs further validation.

In response to β3-adrenergic receptor activation, prostaglandins are also produced in an organ -autonomous manner in WAT to promote browning. Prostaglandin synthesis in WAT is mainly controlled by prostaglandin-endoperoxide synthase (PTGS) activity [104], which could be activated by cold exposure. Thereby, prostaglandin levels will be augmented after cold stimulation, which further stimulates the expression of PGC-1α in WAT- resident mesenchymal progenitors [105].

During the activation of browning, WAT undergoes a series of morphological changes to adapt to energy expenditure and heat production [106]. For example, cold stimulates the branching and recruitment of sympathetic nerves as well as the sprouting and growth of blood vessels during the browning of WAT [107]. Therefore, hormones regulating these events also regulate browning [108], [109]. For example, VEGF secreted by adipose tissue, a hormone with an angiogenic effect, also enhances the recruitment of brown and BRITE adipocytes [110], [111], suggesting a complexity in signaling pathways involved in the browning process. Another EFG-like factor, neuregulin 4, expressed by adipocytes, is known to be secreted by brown adipocytes and has the capacity to promote neuronal innervation [1]. As neuregulin 4 is upregulated in WAT in response to cold exposure, it may have an important role in promoting innervation in this tissue during browning.

Recently, it was also revealed that white adipocytes, but not brown adipocytes, could directly sense cold stimulation to activate thermogenesis and UCP1 expression. These findings provide an unusual insight into the role of WAT in its browning, as well as an alternative way to target non-shivering thermogenesis [112]. The autocrine effects of WAT-derived hormones may play important roles in this process.

Hormones from other peripheral organs in the regulation of WAT browning

Catecholamines play important roles in regulating browning. Interestingly, besides sympathetic nerve synapses, alternatively activated macrophages (M2) in adipose tissue have been reported to also secrete norepinephrine for BAT activation [113]. The adrenal gland is also a key organ releasing epinephrine and norepinephrine into the circulation for systemic effects on multiple organs including adipose tissue. Furthermore, several observational studies demonstrated that pheochromocytoma patients have increased F18-fluorodeoxyglucose (FDG) uptake activity in BAT, which is diminished following surgical treatment [114], [115]. Hence, these catecholamine-releasing cells and organs may also regulate BAT activity and WAT browning through adrenergic receptors.

Recently, heart-derived hormones such as natriuretic peptides (NPs) were also found to enhance BAT thermogenesis as well as WAT browning. Treatment with brain NP increases UCP1 expression in both BAT and WAT. This effect was mainly mediated by NP receptors through the activation of p38 MAPK [116]. Mice lacking the NP clearance receptor, the negative regulator of nitric oxide (NO) activity, also showed enhanced browning of WAT. Recently, cardiotrophin-1, a heart-derived cytokine, was also found to improve glucose and lipid metabolism, with enhanced mitochondrial biogenesis and browning phenotype in WAT [117]. A summary of the hormonal factors implicated in the control of the browning of white adipose tissue is illustrated in Figure 1.

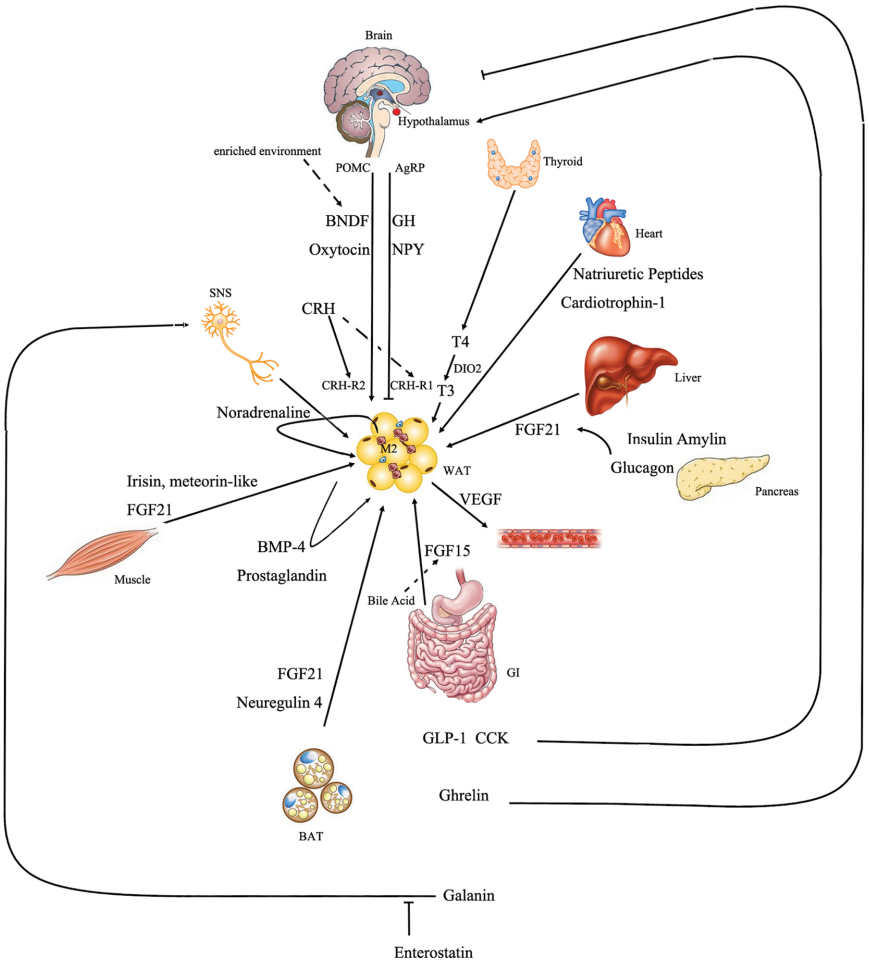

Figure 1: Hormonal factors in the control of browning of white adipose tissue.
A number of hormonal factors secreted from different organs and tissues have been found to regulate the browning of white adipose tissue through systemic, autocrine and paracrine mechanisms. AgRP, agouti-related protein; BAT, brown adipose tissue; BMP-4, bone morphogenetic protein 4; BNDF, brain-derived neurotrophic factor; CCK, cholecystokinin; CRH, corticotropin-releasing hormone; CRH-R1, corticotropin-releasing hormone receptor 1; CRH-R2, corticotropin-releasing hormone receptor 2; DIO2, type II iodothyronine 5′-deiodinase; FGF15, fibroblast growth factor 15; FGF21, fibroblast growth factor 21; GH, growth hormone; GI, gastrointestinal tract; GLP-1, glucagon-like peptide-1; M2, alternatively activated macrophages; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; T4, thyroxine; T3, triiodothyronine; VEGF, vascular endothelial growth factor; WAT, white adipose tissue.

With the discovery of these novel links between other organs and WAT during the browning process, the comprehensive regulation network for thermogenesis will further be elucidated in future investigations.

Conclusion

In summary, given the scarcity of BAT in human adults, pharmacological and nutritional induction of BRITE cells is a promising anti-obesity and anti-diabetic strategy to treat metabolic disorders. Indeed, the interest to reveal hormonal pathways to trigger WAT browning has been ignited in the last decade. As the β-adrenergic pathway appears to be the most important mediator of BRITE induction and common to many of the different browning activators, it is a key focus of therapeutic interventions. Several strategies such as upregulating sympathetic input into WAT, increasing sensitivity and/or the amount of adrenergic receptors in WAT and manipulating key transcription factors in the browning process (e.g. PPARγ) have been explored as pragmatic approaches to induce browning. For example, human studies have shown that highly selective β3-selective adrenergic agonists such as mirabegron and L-796568 may increase energy expenditure with minor or no cardiovascular side effects in overweight men [27], [118], [119]. Furthermore, re-examining the browning effects of already approved drugs seems to also be a viable option. For example, exenatide and sildenafil, originally designed for type 2 diabetes and erectile dysfunction treatment, respectively, are subject to testing their brown-

ing effects in phase 4 clinical trials (http://clinicaltrials.gov/show/NCT02524184 and http://clinicaltrials.gov-/show/NCT03002675).

Additionally, identifying nutritional factors with positive effects on WAT browning also seems to be an attractive approach. Based on current evidence, hormonal factors also play important roles in mediating the browning effects of many nutritional factors. For example, capsaicin and capsinoids with well-documented WAT browning properties can activate the transient receptor potential cation channel, subfamily V, member 1 (TRPV1) to stimulate Ca^{2+} influx, which then activates the Ca^{2+}/calmodulin-dependent protein kinase II and AMP-activated kinase, with the consequent stimulation of sirtuin-1-dependent deacetylation of PPARγ and PRDM16 to increase synthesis of BMP8b and UCP1 [120], [121]. Similarly, fucoxanthin and fish oil [rich in ω3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid and docosahexaenoic acid] can also induce UCP1 expression in WAT via upregulating β3-adrenergic receptor expression and consequently enhancing WAT sensitivity to adrenergic stimulation in adipocytes [94], [95], [122].

Notably, the levels of WAT browning may also represent whole-body energy status instead of being decided only by hormonal pathways. For instance, although exercise has been reported to increase browning of WAT and increase energy expenditure [38], a study in athletes and non-athletes showed that BAT volume and activity in athletes tended to be lower. This demonstrates that brown fat may undergo adaptive reductions in cases where there is an energy deficit, such as with chronic exercise [123]. In conclusion, due to the high degree of integration and redundancy of metabolic regulation by numerous hormones, elucidating the signaling pathway networks controlling BRITE cell recruitment is still a complex task but crucial to lay the foundation for the exploration of novel molecules promoting browning and development of a new generation of anti-obesity drugs.

Author statement

Research funding: Authors state no funding involved.

Conflict of interest: Authors state no conflict of interest.

Informed consent: Informed consent is not applicable.

Ethical approval: The conducted research is not related to either human or animal use.

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