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Browning of white adipose tissue: lessons from experimental models

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Abstract: Beige or *brite* (brown-in-white) adipocytes are present in white adipose tissue (WAT) and have a white fat-like phenotype that when stimulated acquires a brown fat-like phenotype, leading to increased thermogenesis. This phenomenon is known as browning and is more likely to occur in subcutaneous fat depots. Browning involves the expression of many transcription factors, such as PR domain containing 16 (PRDM16) and peroxisome proliferator-activated receptor (PPAR)-γ, and of uncoupling protein (UCP)-1, which is the hallmark of thermogenesis. Recent papers pointed that browning can occur in the WAT of humans, with beneficial metabolic effects. This fact indicates that these cells can be targeted to treat a range of diseases, with both pharmacological and nutritional activators. Pharmacological approaches to induce browning include the use of PPAR-α agonist, adrenergic receptor stimulation, thyroid hormone administration, irisin and FGF21 induction. Most of them act through the induction of PPAR-γ coactivator (PGC) 1-α and the consequent mitochondrial biogenesis and UCP1 induction. About the nutritional inducers, several compounds have been described with multiple mechanisms of action. Some of these activators include specific amino acids restriction, capsaicin, bile acids, Resveratrol, and retinoic acid. Besides that, some classes of lipids, as well as many plant extracts, have also been implicated in the browning of WAT. In conclusion, the discovery of browning in human WAT opens the possibility to target the adipose tissue to fight a range of diseases. Studies have arisen showing promising results and bringing new opportunities in thermogenesis and obesity control.

Keywords: *brite* adipocytes; browning; browning induction; white adipose tissue; UCP-1.

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Introduction

Beige or *brite* (brown-in-white) adipocytes were newly reported as adipocytes located in the white adipose tissue (WAT), but that resemble the brown adipocytes phenotype. In the basal state, *brite* adipocytes act as white adipocytes, but under the adequate stimulus they might transform into brown-like adipocytes, in a process called "browning" [1]. Recent studies indicated that human brown adipose tissue (BAT) is a *brite* adipocyte that acquired a brown-like phenotype [2] and that this conversion has beneficial metabolic consequences [3].

The subcutaneous depots of WAT are the most common location for browning as these adipocytes are predominantly smaller and have a greater potential to differentiate [4]. The ectopic expression of uncoupling protein 1 (UCP1) and PR domain containing 16 (PRDM16) are consistent to identify the presence of *brite* adipocytes within the white adipocytes [5, 6].

In the last years, a wide variety of pharmacological and nutritional compounds have been studied as agents of browning in humans and experimental models. In the present study, we focused on discussing recent in vitro and in vivo findings, though some problems in translating animals to human data exist [7].

Characterization of the *brite*/beige adipocyte

The adipose tissue is composed mainly by adipocytes, which are predominantly white adipocytes in the WAT,

and brown adipocytes in the BAT. Consequently, WAT and BAT have different structures and biological roles. White adipocytes have a single large lipid droplet occupying most of the cell volume with few mitochondria, dislocating the nucleus peripherally. Brown adipocytes are polygonal cells containing several small lipid droplets (therefore, called multilocular adipose tissue), with a central nucleus surrounded by a clear cytoplasm and large amounts of mitochondria [8, 9] (Figure 1).

Also, WAT and BAT have different origins and progenitor cells, and many adipogenesis mediators [10]. WAT is found throughout the body, being divided into visceral (around organs – mesenteric, perigonadal, omental) and subcutaneous (under the skin – inguinal) depots. BAT is found in specific regions that comprises interscapular, subscapular, axillary, perirenal and periaortic regions in rodents, and cervical, supraclavicular, paravertebral, mediastinal and perirenal regions in humans [11]. Also, WAT represents the main energy reservoir of the body, while BAT is characterized by energy dissipation through thermogenesis. Both WAT and BAT function as endocrine tissues, signaling to other organs through *adipokines* (WAT) and *batokines* (BAT) [12, 13].

Brite adipocytes were newly reported as a type of adipocytes set in WAT, but resembling brown adipocytes phenotype. In the basal state, *brite* adipocytes act as white adipocytes, but under the adequate stimulus they might transform into brown-like adipocytes [1]. The origin of the *brite* adipocytes is still a matter of debate. When WAT is stimulated, a subset of cells may acquire a thermogenic phenotype (i.e. brown fat-like phenotype), without sharing the genetic markers of BAT, having a single developmental origin and molecular characteristics [14]. Indeed, *brite* adipocytes have a gene expression pattern different of WAT and BAT [1]. The key features of WAT, BAT and *brite* adipocytes are detailed in Table 1.

It is relevant information that humans may have activation of BAT [15, 16]. Recent studies have indicated that human BAT is a *brite* adipocyte that was originally white, but, under stimulation acquired a brown-like phenotype [2]. Thus, human white adipocytes can be converted into *brite* adipocytes with beneficial metabolic consequences [3].

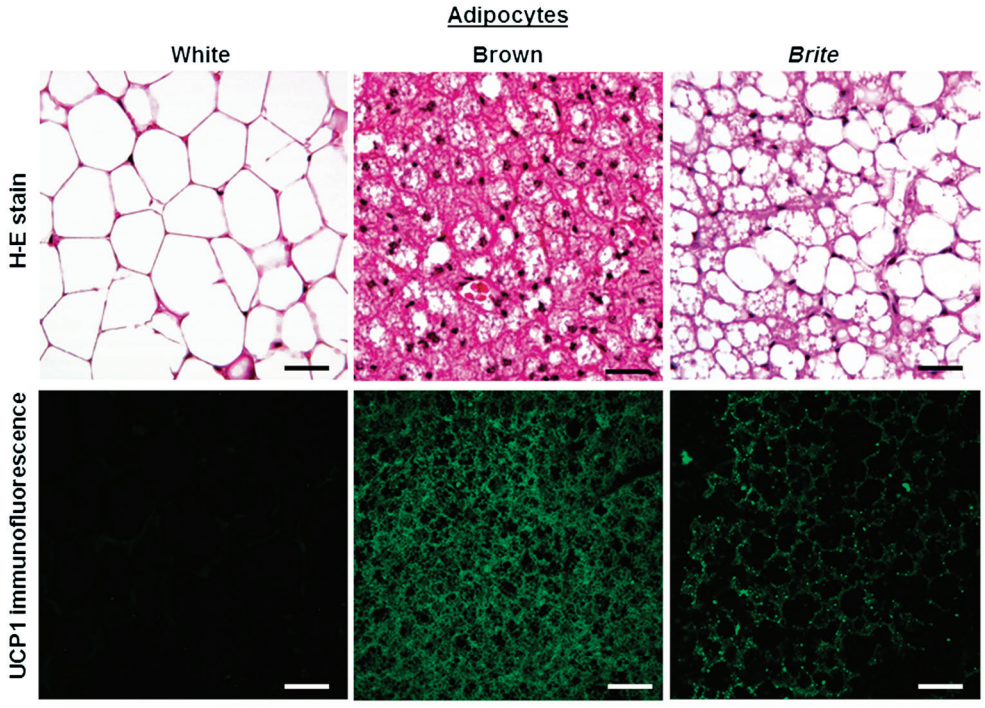

Figure 1: Adipocytes.
White adipocytes have large lipid droplets, surrounded by little cytoplasm and a decentralized nucleus. Brown adipocytes have a polygonal appearance with multiple small lipid droplets and a centralized nucleus surrounded by a clear cytoplasm. *Brite* adipocytes are located in white adipose tissue resembling white adipocytes that under certain stimuli acquire a brown fat-like phenotype (tissues from C57BL/6 mice: light microscopy with hematoxylin and eosin stain or immunofluorescence and confocal microscopy marked with anti-uncoupling protein (UCP) 1 antibody). Same magnification, bar calibration = 50 μm.

Table 1: Comparisons between white, brown and "*brite*" adipose tissue.

Data	WAT	BAT	Brite
Origin	Myf5- cells	Myf5+ cells	Myf5- cells (differentiation or transdifferentiation)
Function	Energy storage and endocrine tissue	Thermogenesis and endocrine tissue	Adaptive thermogenesis (under stimuli)
Phenotype	White-fat phenotype	Brown-fat phenotype	White-fat phenotype that acquires a brown-fat phenotype under stimuli
Mitochondria	Low	Abundant	Present (upon stimulation)
UCP-1 expression	Absent	Present	Present (under stimuli)
Protein markers	LPL, leptin, adiponectin	PGC1α, PRDM16	CD137, PRDM16, Tmem26
Pharmacological induction	PPAR agonists, renin-angiotensin system blockers, thiazolidinediones, among others	Sympathomimetic drugs, thyroid hormones, thiazolidinediones, hormones like FGF21 and irisin, among others	Adrenergic receptor agonist, thyroid hormones, PPARα agonist, FGF21, irisin, BMP7, BMP8, AMPK activator, leptin, insulin, among others
Nutritional induction	n-3 PUFA, polyphenols, vitamin D, vitamin E, vitamin A, carotenoids, among others	PUFA, especially n-3 PUFA, bile acids, among others	Amino acid restriction, capsaicin, bile acids, n-3 PUFA, retinoic acid, among others

AMPK, AMP-activated protein kinase; BAT, brown adipose tissue; BMP, bone morphogenetic protein; CD137, cluster of differentiation 137; FGF21, fibroblast growth factor 21; LPL, lipoprotein lipase; Myf5, myogenic regulatory factor 5; PGC1alpha, PPAR coactivator 1 α; PPAR, peroxisome proliferator-activated receptor; PRDM16, PRD1-BF-1-RIZ1 homologous domain protein containing protein-16; PUFA, polyunsaturated fatty acids; Tmem26, transmembrane protein 26; UCP1, uncoupling protein 1; WAT, white adipose tissue.

Molecular pathways related to browning and thermogenesis

The browning phenomenon gained relevance among scientific community when cold-activated thermogenic adipocytes were accidentally identified in patients subjected to positron emission tomography (PET) CT scans in Sweden [17]. These adipocytes, observed in the supraclavicular region, resembled the *brite* adipocytes seen in mouse models [18].

Before that observation, we believed that thermogenesis could not produce a significant body mass loss in adults [19]. However, in recent years *brite* adipocytes are considered a metabolic benefit: only 63 g of full-activated thermogenic adipocytes can burn approximately 4 kg of WAT a year (an obese adult – BMI > 30 kg/m² – has 27 kg of WAT on average) [20, 21].

Subcutaneous adipocytes are more likely to undergo browning than visceral adipocytes because subcutaneous adipocytes are predominantly smaller and have a greater potential to differentiate [4]. The various stimuli capable of inducing browning are still in discussion. However, there is a consensus that the ectopic expression of UCP1 and PRDM16 is consistent to identify the presence of *brite* adipocytes within the white adipocytes [5, 6].

While UCP1 is the protein that performs thermogenesis itself [22], PRDM16 is a stimulus responsible for maintaining the *brite* adipocyte phenotype. Although PRDM16 is a common gene of BAT, brown adipocytes can perform thermogenesis even with a low expression of PRDM16. However, as recently shown, *brite* adipocytes may turn into white adipocytes again when the PRDM16 expression is low [23, 24]. Thus, browning is a reversible phenomenon and PRDM16 is a pivotal molecule when it comes to browning induction and thermogenic maintenance of the *brite* adipocytes [24].

Experimental evidence and clinical reports agree that sustained adrenergic stimulation is crucial to triggering the thermogenesis pathway [25]. An abundant innervation has always been attributed to BAT, but WAT is also significantly affected by this stimulus [26]. Viral tracking techniques have revealed an intricate sympathetic innervation in both visceral and subcutaneous WAT (sWAT) [27].

Beta-3 adrenergic receptor (β-3AR) is the main receptor involved in the thermogenesis pathway [15]. The p38 mitogen-activated protein kinase (p38 MAPK) stimulates the activating transcription factor 2 (ATF-2), driving the peroxisome proliferator-activated receptor gamma coactivator (PGC) 1-α transcription [28]. Peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1-α) has got significant downstream effects promoting mitochondrial biogenesis and peroxisome proliferator-activated receptors (PPAR) activation [29]. PGC1-α activates nuclear respiratory factor 1 (NRF1), which communicates the nucleus with the mitochondrion and triggers mitochondrial replication by the activation of the mitochondrial transcription factor A (TFAM) [30].

UCP1, the thermogenesis effector, is in the inner mitochondrial membrane, indicating that mitochondrial biogenesis is essential to *brite* adipocytes induction. Moreover, mitochondria are widespread in the larger cytoplasm of the thermogenic-activated *brite* adipocytes [31, 32]. All PPAR isoforms (α, β, and γ) have been associated with UCP1 transcription [33, 34].

PPAR-γ orchestrates UCP1 transcription during brown adipocytes differentiation, but it is repressed in mature activated brown adipocytes [35]. After the differentiation, PPAR-α controls UCP1 levels in mature brown adipocytes [34]. Even though PPAR-γ plays a role in browning, the PPAR-α seems to be indispensable in activating the transcription of genes related to lipid oxidation carnitine palmitoyltransferase 1 (CPT1), which triggers β-oxidation and allows an unilocular adipocyte turn into a multilocular adipocyte [36].

As mentioned, PRDM16 is essential for *brite* adipocyte maintenance, but it also influences the browning process. Once again, PPAR-α controls the transcription of this essential gene, which interacts with PGC1-α to provide the machinery necessary for the transdifferentiation or differentiation of the *brite* adipocyte [29].

Today we accept that *brite* adipocytes stem from mature white adipocyte [low cluster of differentiation 137 (CD137), MYF5-cell progenitor], which under specific stimuli acquire a brown-like phenotype, or still from a beige preadipocyte (high CD137, MYF5-cell progenitor), which differentiates into a multilocular cell capable of performing thermogenesis. The latter originates from a lineage that differs from WAT [37, 38].

Irisin, a newly described adipokine, has a role in the differentiation of the preadipocyte in mature beige adipocyte [6], which express the cluster of differentiation (CD) 137, a beige-lineage selective cell surface protein. The PPAR-α stimulation is accompanied by a high irisin gene level. Also, irisin acts via PGC1-α to enhance UCP1 expression, which is also a PPAR-α target gene, maximizing thermogenesis [39, 40]. The crosstalk between different pathways controlled by PPAR-α suggests that PPAR-α might orchestrate thermogenesis in the mature *brite* adipocytes and has potential to trigger Browning, though the way (transdifferentiation or differentiation) remains to be unraveled. Figure 2 summarizes the main pathways outlined in this section.

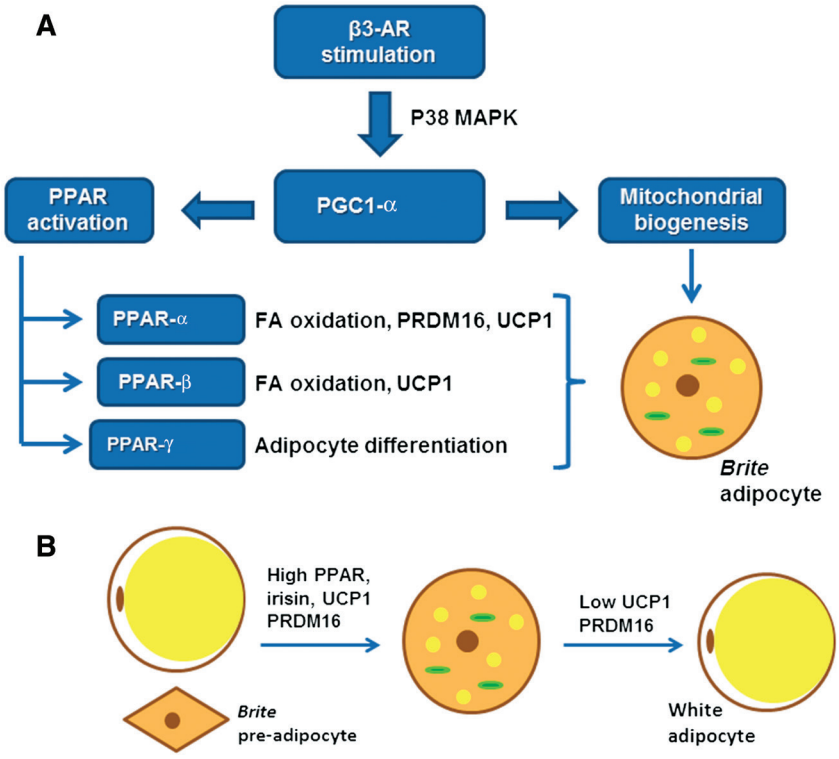

Figure 2: Pathways related to thermogenesis and browning.
Beta 3-adrenergic receptor stimulation leads to PGC1 induction, which drives PPAR activation and mitochondrial biogenesis. These stimuli allow the white adipocyte to acquire brown adipocyte features in an event called "browning", where the enhanced expression of PRDM16 and UCP1 are considered as hallmarks for thermogenic activity in the new beige/*brite* adipocyte (A). An interaction between PPAR-α and irisin stimulates browning as it favors UCP1 and PRDM16 great expressions. Conversely, under reduced expression of PRDM16 and UCP1, the *brite* adipocyte can turn in a white adipocyte, showing the reversible nature of browning phenomenon (B).

Pharmacological induction of Browning

Many pharmacological agents have been linked to a facilitation of *brite*/beige phenotype acquisition by white adipocytes [41]. Despite being a recent issue, we aimed to describe in this section the main pharmacological approaches related to WAT browning as well as the endogenous signals activated by each one of them.

An adrenergic stimulation is essential to trigger thermogenesis. Thus, many strategies to induce WAT browning, converge to the stimulation of β-3AR, with the consequent enhanced lipolysis, which is followed by a greater capacity for lipid oxidation and thermogenesis in the mitochondria [42]. Chronic treatment with β-3AR agonist induces ectopic UCP1 expression in WAT coupled with a significant mitochondrial enhancement. Also, a moderate elevation of β-3AR expression is associated with a significant body mass loss due to WAT browning [43], while a β-3AR depletion in knockout mice reduce WAT multilocular adipocytes and UCP1 expression [42]. The proposed mechanisms are related to the cyclic adenosine monophosphate (c-AMP)-dependent protein kinase A (PKA) activation and the activation of its target gene p38 MAPK with downstream effects such as PGC1-α and PPAR-α activation [44].

A selective PPAR-α activation by fenofibrate makes WAT browning in a diet-induced obesity model [45, 46], with a consequent reduction in the body mass and hepatic steatosis, implying that thermogenesis can metabolize the excessive free fatty acids from lipolysis, mitigating their deposition as fat droplets in the liver [47, 48].

Along with PPAR agonists, the chronic use of AMP-activated protein kinase (AMPK) activators ended up in increased energy expenditure and mitochondrial biogenesis, without a great impact on ectopic UCP1 expression [49]. Although AMPK activators potentially may enhance PGC1-α, the effects on WAT browning is controversial and seem to be species-dependent. The increased UCP1 expression in gonadal white adipocytes has been identified in rats under a long-term treatment with the AMPK activator 5-Aminoimidazole-4-carboxamide ribonucleoside (AICAR) [49].

Irisin might explain the reason why AMPK activators do not always produce WAT browning. This adipokine relies on PGC1-α to trigger WAT browning [1, 39]. Moderate augmentation in irisin level complies with ectopic expression of UCP1 in WAT, followed by obesity and insulin resistance tackling [39]. It seems that the enhanced PPAR-α expression coupled with high levels of irisin acts as an important surrogate of WAT browning [39, 46]. Of note, exercise seems to stimulate WAT browning through an irisin-dependent pathway as irisin is similarly secreted by the skeletal muscles (being also classified as a myokine) [39, 40]. However, it is likely that muscle secretion does not influence WAT browning significantly as sweat production does [50].

The FGF21 is a metabolic regulator. It is secreted predominantly by the liver, but is also secreted by BAT and sWAT after a suitable stimulation (cold exposure or adrenergic stimulation) [51, 52]. Paracrine and autocrine signals induce UCP1 and other thermogenic genes through a PGC-α-dependent mechanism in FGF21-treated mice, being more relevant in sWAT than in BAT [52]. A possible interaction with irisin is thought to cause increased oxygen consumption by adipocytes, which might explain the reduced fat depots following FGF21 treatment [51].

Another synergism happens between natriuretic peptides and β-3AR stimulation. Formerly regarded as a hormone involved in blood pressure regulation through the salt excretion control and renin-angiotensin system modulation, the atrial natriuretic peptide (ANP) is released after exercise and yields increased UCP1 gene and protein levels in human adipocytes in vitro [53]. Energy uncoupling increase by ANP does not rely on adrenergic stimulation, but is maximized by its [27]. A significant overlapping between PKA and cyclic guanosine monophosphate (cGMP)-dependent protein kinase G (PKG) downstream effects has been described and this observation is possibly the reason why ANP stimulates lipolysis in a similar degree of adrenergic stimulation [54].

The nicotine is strongly associated with a decrease in body mass, a small food intake, an increase of both lipolysis and energy expenditure [55]. However, cigarette smoking did not induce browning in sWAT [56], whereas treatment with nicotine caused increased UCP1 gene levels by multilocular adipocytes in WAT [57].

Bone morphogenetic proteins (BMPs) play different roles in adipocyte differentiation and physiology [58]. BMP-7 is associated with enhanced lipid accumulation, UCP1 expression and mitochondrial density in brown adipocytes [59], and browning of murine and human sWAT in vitro [60]. Also, BMP-7 stimulates PRDM16, which, in turn, induces PGC1-α and its downstream effects related to mitochondrial biogenesis and UCP1 activity [61].

BMP-8 acts centrally to intensify the adrenergic signaling, an important triggering stimulus of both browning and thermogenesis [62]. Increased BMP-8 gene levels were detected in obese mice treated with PPAR-α agonist, and BMP-8 augmentation was proportional to β-3AR and the

UCP-1 rise [46]. Conversely, mice lacking BMP-8 showed a greater susceptibility to diet-induced obesity [62].

The adipoinsular axis refers to the interplay between insulin and leptin to control appetite and glucose handling [63]. Insulin and leptin function synergistically in hypothalamic neurons to promote WAT browning. The deletion of tyrosine protein phosphatase 1B (PTP1B) and tyrosine protein phosphatase nonreceptor type 2 (TCPTP) augment insulin and leptin signaling in POMC neurons, which a greater energy expenditure and *brite* adipocytes in WAT [64]. The infusion of leptin and insulin into the central nervous system yielded activated POMC neurons and put forward a central control of WAT browning [64, 65]. Also, the thyroid hormone has an influence on WAT plasticity. After treatment with T3 (triiodothyronine) human adipocytes differentiate from multipotent adipose-derived stem cells, acquiring a multilocular aspect, enhancing mitochondrial density and UCP1 expression [66].

Nutritional induction of Browning

Nutritional elements have effects centrally in the brain, like some amino acids restriction and malnutrition, and capsaicin. Capsaicin is an ingredient of hot pepper, widely used as a spice in food products. Capsaicin is recognized as a target to treat obesity and adipogenesis because it binds to the TRPV1 protein activating neurons, increasing catecholamine secretion and thermogenesis. Capsaicin gave to rats fed a high-fat diet led to an increased UCP1 mRNA expression in WAT [67]. Low doses of capsaicin induce a *brite* phenotype in differentiating 3T3-L1 preadipocytes [68]. Also, Capsaicin activates TRPV1 channels, promoting browning of WAT that counteracts obesity in mice [69].

Dietary methionine restriction induces an increase in energy expenditure with a rise in UCP1 expression in WAT [70], even in *ob/ob* mice [71]. The dietary methionine restriction appears to increase UCP1 and energy expenditure through increased nervous system stimulation of adipose tissue [72]. In maternal rodent undernutrition, there is an enhancement of UCP1 gene expression in WAT of male offspring until postnatal day 21, but this effect is lost after weaning [73].

Fucoxanthin, a carotenoid from edible seaweeds can upregulate UCP1 expression in mice WAT [74], which could partially counteract obesity in KK-Ay mice [75]. These beneficial effects of fucoxanthin in WAT against obesity appear to be related to increases in the expression of β-3AR [76].

In diet-induced obesity in mice supplemented with the flavonoid luteolin, there is increased energy expenditure associated with upregulation of thermogenic genes (e.g. UCP1, PGC1-α, PPAR-α, among others) in sWAT. The effects of luteolin are mediated by AMPK/PGC1-α signaling since AMPK inhibition ablated the effects [77].

Another potential nutrient is the amino acid Citrulline. Citrulline treatment of lean and diet-induced obese mice upregulated UCP1, PPAR-α, and PGC1-α in WAT, resulting in elevated thermogenesis accompanied by a reduced body fat mass [78].

The role of bile acids in upregulating thermogenesis was recently described. Bile acids are essential for lipid absorption in the intestine and may have an involvement in lipid metabolism [79]. There are effects of bile acids (i.e. cholic acid and chenodeoxycholic acid) on BAT increasing energy expenditure and inducting UCP1-mediated thermogenesis [79, 80]. In WAT, stimulation of a bile acid sensor (farnesoid X receptor, FXR) by its agonist (FXR agonist fexaramine) promotes browning, opening a new therapeutic field [81]. At least in BAT, the mechanism of action of bile acid is mediated by G protein-coupled receptor 5 (TGR5) [79].

Another well-studied browning inducer is Resveratrol, a polyphenol present in berries and grapes, among others. Resveratrol supplemented to mouse embryonic fibroblast-derived adipocytes elevated mRNA expression of UCP1 [82]. In vitro, Resveratrol increased gene and protein expressions of brown fat markers including UCP1, PRDM16, and PGC1-α in adipocytes [83, 84]. Resveratrol induces browning of WAT with UCP1 upregulation and enhancement of fatty acid oxidation in vivo, possibly by activating AMPK [83].

Some types of lipids [n-3 polyunsaturated fatty acids – (PUFA)] have the potential to induce browning. The n-3 PUFA is related to a wide variety of beneficial effects in many diseases as immune, inflammatory, and cardiovascular diseases, besides cancer, obesity, and the metabolic syndrome [85]. The eicosapentaenoic acid (EPA, one of the bioactive n-3 PUFA) can promote browning of subcutaneous adipocytes [86]. In accordance, fish oil (rich in n-3 PUFA) given to mice induces browning of subcutaneous WAT, with the presence of several gene markers, including CD137 that is exclusive of *brite* cells [87].

Also, the conjugated linoleic acid (CLA) enhances UCP1 in obese *ob/ob* mice independently of increases in β-3AR, acting against fat deposition [88]. The CLA-induced UCP1 expression in WAT contributes to obesity reversion in a mechanism independent of PPAR-α [89]. The synthetic fatty acid 2-hydroxyoleic acid given to rats resulted

in increased UCP1 expression in WAT, inducing body mass and fat mass losses [90].

The retinoic acid is the carboxylic acid form of vitamin A with action on several nuclear receptors. All-*trans* retinoic acid induces UCP1 expression through its binding to the retinoic acid receptor in white rodent adipocytes, independently of PGC1-α [91]. In obese mice, treatment with all-*trans* retinoic acid induces UCP1 expression, through both retinoic acid receptor and PPAR-β/ǎ [92]. All-*trans* retinoic acid increases mice multilocular adipocytes in inguinal WAT, suggesting browning with concomitant increases in mRNA expression of UCP1, PPAR-α, PGC1-α, CPT-1, among others [93]. Also, mouse embryonic fibroblast-derived adipocytes exposure to all-*trans* retinoic acid showed enhancement of UCP1 mRNA and protein expressions accompanied by increases in PRDM16 [94].

Other nutrients, including plant extracts, may have potential in inducing browning. For example, thymol (5-methyl-2-isopropylphenol), a natural monoterpene phenolic constituent of essential oils produced by plants such as thyme species, induces browning of 3T3-L1 adipocytes, enhancing the expression of many brown fat specific markers [95]. On a diet-induced obese model, β-lapachone (a naphthoquinone) stimulates the browning of WAT, with higher UCP1 expression and lower body mass [96]. The black soybean seed coat extract, a polyphenol-rich food material, also elevates UCP1 protein expression in sWAT and reduces body mass with regularization of glucose intolerance [97]. Berberine, a naturally occurring plant alkaloid present in many Chinese herbal medicines, activates thermogenesis in WAT of *db/db* mice, with the browning of this tissue through AMPK and PGC1-α signaling [98]. Lastly, artepillin C, a typical Brazilian Propolis-derived component, induces brown-like adipocytes in mouse primary inguinal WAT-derived adipocytes due to activation of PPAR-γ and PRDM16 stabilization, independent of β3-adrenergic signaling [99].

Other metabolites may be added to the list of browning inducers. Among them, both lactate and the ketone body β-hydroxybutyrate were shown to increase UCP1 expression in murine WAT cells, therefore promoting browning, possibly as a mechanism to alleviate redox pressure [100]. Besides that, rats given inorganic nitrate in drinking water showed expression of the brown adipocytes genes and proteins and β-oxidation genes in WAT, increasing oxygen consumption. The mechanism of browning appears to be related to the reduction of nitrate to nitric oxide that in turns increase cGMP, activating PKG and, consequently, increasing the expression of PGC1-α and other key browning genes [101].

Cold adaptation: energy dissipation, non-shivering thermogenesis

The cold-induced thermogenesis can be either a non-shivering thermogenesis (NST) or a shivering thermogenesis. Shivering is a repetitive contraction-relaxation process activated by repeated stimulation of the neuromuscular junction that leads to elevation of cytosolic Ca^{++} concentration, thereby activating ATP hydrolysis to produce heat. During shivering, heat is primarily generated by the major ATP-utilizing enzymes, including Na^{+}/K^{+} ATPase, myosin ATPase, and sarcoplasmic/endoplasmic reticulum Ca^{++} transport ATPase (SERCA) [102, 103]. In a cold environment, heat production increases by 10–30 W during the initial first minutes without any increase of muscle activity [104]. Later, extra heat is generated by involuntary contractions of skeletal muscles (shivering). Heat production through muscle shivering is well known as the first line of defense to acute cold exposure. Acute exposure to cold triggers immediate responses with the dual purpose of minimizing heat loss and producing heat. Shivering occurs when the core and skin temperature surpass a certain threshold and may produce heat equivalent to about four times resting metabolism. There are vasoconstriction and furred mammals undergo piloerection.

Heat production is initiated instantly by shivering, the direct form of facultative thermogenesis. Muscle contraction increases heat production. However, facultative shivering thermogenesis is a very high energy cost and disrupts activity [105]. Also, it is hence of limited value and rapidly replaced by non-shivering facultative thermogenesis [106]. The facultative thermogenesis resides in another evolutionary homeostatic advancement to adapt to the cold, the BAT (Figure 3).

People who have adapted to cold environments show some resistance to the development of diabetes, possibly due to the maintenance of larger amounts of BAT [107]. Likewise, the extent of human BAT activity in patients is inversely associated with obesity, age and type II diabetes [108]. In a comparison of overweight and lean subjects on thermogenesis in response to mild cold, the increase in heat production in response to a mild cold stimulus was observed to be three times as large in lean subjects compared with overweight subjects [104]. The mouse strains with higher thermogenic gene expression in WAT depots tended to be more resistant to obesity and insulin resistance than those with lower levels [109].

We know now that skeletal muscle could serve as a place of non-shivering besides BAT in mammals, including humans. During cold acclimation, shivering is gradually

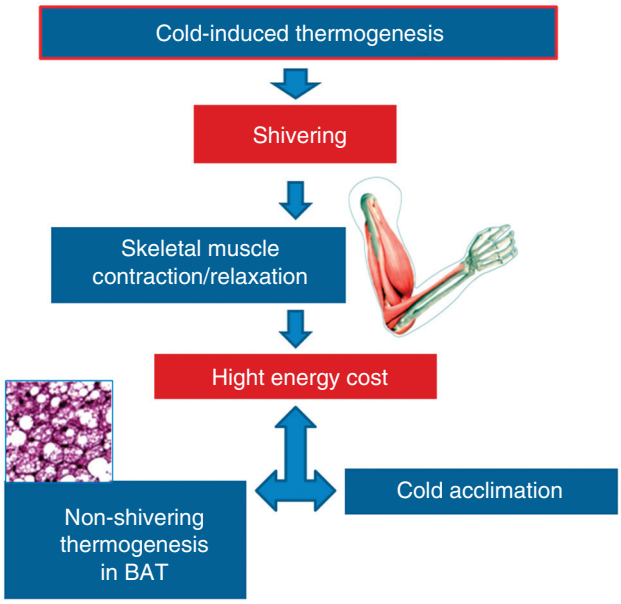

Figure 3: Cold adaptation: energy dissipation, non-shivering thermogenesis.
Heat production is initiated instantly by shivering, the direct form of facultative thermogenesis. Muscle contraction increases heat production. Skeletal muscle could serve as a site of non-shivering besides BAT in mammals, including humans. During cold acclimation, shivering is gradually replaced by non-shivering thermogenesis because repetitive muscle contractions during constant shivering can cause muscle damage.

replaced by NST to save muscle and prevent muscle injury due to repetitive contractions during constant shivering [110]. Moreover, high-intensity shivering relies predominantly on muscle glycogen that can become limiting after a few hours [111].

The question is whether muscle will become a major site of NST when the BAT function is minimized in mice. The interscapular BAT (iBAT, which constitutes approximately 70% of total BAT) has been surgically removed, and mice exposed to prolonged cold (4° C) for nine days. Interestingly, the iBAT-ablated mice have maintained optimal body temperature (approximately 35–37° C) during the entire period of cold exposure. After four days in the cold, both sham controls and iBAT-ablated mice stopped shivering and resumed routine physical activity, indicating that they are cold-adapted. The iBAT-ablated mice showed higher oxygen consumption and decreased body mass and fat mass, showing a raised energy cost of cold adaptation. Moreover, the skeletal muscles in these mice underwent extensive remodeling of both the sarcoplasmic reticulum and mitochondria, including alteration in the expression of the main components of Ca^{++} handling and mitochondrial metabolism. The changes, along with increased sarcolipin expression, provide evidence for the recruitment of NST in skeletal muscle. Therefore, the skeletal muscle becomes the major site of NST when BAT activity is minimized [112]. The heat production in skeletal muscle is tightly associated with sarcolipin, a regulator of SERCA [113].

Expert opinion

The recent discovery that adult humans possess adipocytes capable of performing thermogenesis opened the possibility to target new strategies to fight obesity and its comorbidities. Even though many studies have arisen, showing promising results and bringing new opportunities, the understanding of the browning phenomenon and its metabolic effects configures a new field of study, with many questions to be answered.

Outlook

Browning is regarded as a new potential strategy to fight obesity. The experimental background provides a large body of evidence for body mass control, improved glucose handling and beneficial metabolic outcomes after the induction of *brite* adipocytes formation by nutritional or pharmacological approaches. The main challenge in the upcoming years will be to determine the actual impact of the *brite* adipocyte on human obesity as the translational potential of the experimental evidence remains to be unraveled.

Highlights

- Browning is characterized by the brown-like phenotype acquisition by white adipocytes, mainly from subcutaneous depots;
- The identification of *brite* adipocytes in humans challenged the understanding of the metabolic pathways involved in the browning;
- Adrenergic stimulation is crucial to trigger browning as it initiates the thermogenic pathway;
- PGC1-α is a key factor to drive browning as it stimulates mitochondrial biogenesis and UCP1 transcription;
- PPAR-α activation is linked to irisin induction and enhanced UCP1 transcription and activity;
- In the recent years, many nutritional compounds have been studied as promoters of browning in white adipose tissue;

- Capsaicin, bile acids, Resveratrol, retinoic acid and some classes of lipids are among the most studied nutrients that induce browning;
- The potential of *brite* adipocytes to counter obesity in humans remains to be unraveled.

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