

Review Article

Baskaran Thyagarajan¹ / Michelle T. Foster²

Beiging of white adipose tissue as a therapeutic strategy for weight loss in humans

¹ Department of Pharmaceutics, University of Wyoming School of Pharmacy, HS 279, Dept. 3375, 1000 East University Avenue, Laramie, WY 82071, USA, Phone: +1 (307) 766 6482, Fax: +1 (307) 766 2953, E-mail: Baskaran.Thyagarajan@uwyo.edu

² Department of Food Science and Human Nutrition, Colorado State University, 207 Gifford Building, Fort Collins, CO, USA, Phone: +1 (970) 491-6189, E-mail: Michelle.Foster@colostate.edu

Abstract:

An imbalance between energy intake and expenditure leads to obesity. Adiposity associated with obesity progressively causes inflammation, type 2 diabetes, hypertension, hyperlipidemia and cardiovascular disease. Excessive dietary intake of fat results in its accumulation and storage in the white adipose tissue (WAT), whereas energy expenditure by fat utilization and oxidation predominately occurs in the brown adipose tissue (BAT). Recently, the presence of a third type of fat, referred to as beige or brite (brown in white), has been recognized in certain kinds of WAT depots. It has been suggested that WAT can undergo the process of browning in response to stimuli that induce and enhance the expression of thermogenes characteristic of those typically associated with brown fat. The resultant beige or brite cells enhance energy expenditure by reducing lipids stored within adipose tissue. This has created significant excitement towards the development of a promising strategy to induce browning/beiging in WAT to combat the growing epidemic of obesity. This review systematically describes differential locations and functions of WAT and BAT, mechanisms of beiging of WAT and a concise analysis of drug molecules and natural products that activate the browning phenomenon in vitro and in vivo. This review also discusses potential approaches for targeting WAT with compounds for site-specific beiging induction. Overall, there are numerous mechanisms that govern browning of WAT. There are a variety of newly identified targets whereby potential molecules can promote beiging of WAT and thereby combat obesity.

Keywords: beiging, brite, browning, peroxisome proliferator-activated receptor gamma (PPAR γ), PR domain-containing protein (PRDM-16), sirtuin-1, white adipose tissue (WAT)

DOI: 10.1515/hmbci-2017-0016

Received: March 31, 2017; **Accepted:** April 18, 2017

Introduction

Adipose tissue is a loose connective tissue composed primarily of adipocytes, which are cells distended with stored lipids. Hence, adipose depots are the fundamental storage sites for excess energy, as fat, in the body. These cells play a role in balancing responses to both internal homeostasis and external cues needed to regulate biological function in numerous different ways including, but not limited to, reproduction, inflammation and energy balance. Therefore, perturbation of adipocyte function can lead to disruption of adipose depot homeostasis and metabolic communications that subsequently leads to systemic metabolic dysfunctions. An increase in adiposity associated with obesity is among the most common drivers of adipose depot dysfunction. Excessive adipose can culminate into metabolic disease, progressively leading to type 2 diabetes, dyslipidemia, vascular dysfunctions and cardiovascular diseases [1], [2], [3], [4].

Traditionally, adipose tissue depots are broadly classified into two distinct categories, white and brown adipose tissues (WAT and BAT, respectively). Morphologically, white and brown adipocytes are inherently different, which gives rise to their divergent roles. As discussed previously, white adipocytes are unilocular lipid-laden cells. Brown adipocytes, however, are multilocular cells that contain numerous smaller lipid compartments and an increased number of mitochondria, which gives rise to the brown appearance. Adipocyte morphology translates to functions, where unilocular WAT serve to predominately store energy and cushion and insulate the body, while multilocular BAT is involved in expending the stored energy via lipid oxidation to produce heat by the process of thermogenesis. Last, in response to various activators, WAT can be converted

Baskaran Thyagarajan, Michelle T. Foster are the corresponding authors.

©2017 Walter de Gruyter GmbH, Berlin/Boston.

to “brown-like” adipocytes known as beige cells. There is a growing interest in targeting WAT to be induced toward a beige phenotype. It is postulated that WAT beiging may be used to facilitate increases in energy expenditure, which will result in adiposity losses.

BAT function from infancy to adulthood

Traditionally, BAT was considered to play a more prominent role in neonates and very young children than adults. BAT develops at 5 weeks of gestation and at birth represents 5% of an infant's body weight [5]. Infants, especially those born prematurely, are at a high risk for hypothermia because of their low muscle mass, small surface area and lack of the ability to shiver to generate heat; hence, BAT activation for thermogenesis is critical in the early years post birth [5]. In the past, little attention has been placed on BAT regulation in adults because shivering combats hypothermia as we age; thus, the alternative method of heat production through BAT was predominantly thought to be a method only needed in infants. Indeed, some postulate that BAT remains biologically relevant throughout childhood [6] but regresses as we age by turning into WAT [7], reducing its contribution toward energy metabolism [6], [8].

Positron emission tomography (PET) studies in adult humans, however, shifted traditional thinking by providing evidence that BAT is active in adults [6], [9], [10]. This was first demonstrated in nuclear medicine literature with the use of the intravenously administered radioactive glucose analog ^{18}F -fluorodeoxyglucose (FDG), a non-metabolized glucose analog used to delineate metastatic cancers in PET scans [11], [12]. In these scans, FDG also localized in adipose tissue, specifically BAT that was not associated with tumor tissue, indicating that BAT thermogenesis was still active in adulthood [11], [12]. Subsequently, numerous groups have demonstrated BAT to be located and activated by cold in adults [9], [10], [13].

Anatomical classification of adipose depots

As previously stated, WAT plays a role in insulation and padding protection, while BAT plays a role in thermogenesis. This classification of adipose tissues is based, in part, on their biological functions, which overlaps with the metabolic properties of adipose tissue compartments relative to anatomical regions. Adipose tissue depots primarily consist of WAT. Although the exact proportion is not yet known, it is thus far estimated that BAT constitutes a very small proportion of adipose tissue in the body. BAT and WAT do coexist in certain parts of the body; however, some particular regions of adipose depots stores may consist primarily of one type or the other.

In its role in insulation and protection, WAT is located throughout the body. The largest deposits of adipose tissue in the human can be categorized as intra-abdominal or subcutaneous depots. The adipose tissue stored in our abdominal cavity typically constitutes ~15% of our total body fat, whereas that located subcutaneously is ~85% [14]. In the intra-abdominal cavity, WAT sits among and between organs such as the stomach, liver, intestines and kidneys. Considerable depots include visceral, mesenteric and retroperitoneal WAT. The larger proportion of WAT stores, however, are located subcutaneously between the muscle and skin. Areas of location is general spread underneath the skin; however, larger depots include those located in the hips, thighs, buttocks and the lower abdominal area.

BAT plays a fundamental role in thermogenesis; thus, unlike WAT pads that insulate organs and muscles, BAT is situated within the body to promote survival against cold via hypothermia-induced adaptive thermogenesis [15]. When activated, BAT helps preserve normal body temperature. Studies demonstrate a functional relevance to the anatomical location of human BAT. Although BAT constitutes a small portion of adipose tissue, it is located throughout the human body in numerous distinct regions. The major regions include BAT within the chest, visceral cavity and subcutaneous region. In the chest cavity, BAT is located perivascularly (e.g. around the aorta, common carotid artery, cardiac veins and brachiocephalic artery) and along hollowed tissues (e.g. heart, trachea, lungs and esophagus). In the visceral region, BAT is around hollowed tissues (e.g. colon) and solid organs (pancreas, kidneys, adrenal, liver and spleen) [9], [10], [16], [17], [18]. BAT in the subcutaneous region is located among the interior neck muscles, clavicle region, anterior abdominal wall and inguinal area [18]. In 1969, Smith and Horwitz postulated that the strategic location of BAT and its function in thermogenesis and close association with vasculature allows it to provide internal heating when cooler blood circulates back from the skin surface [19]. Overall, the prominent locations of BAT around vessels, heart, hollow organs, solid organs and the shoulder and groin areas suggest that a major role of BAT is to maintain core temperature and increase temperature in areas where heat may easily be dissipated [18].

BAT activity in lean adults

Similarly to infants, BAT in adults plays a fundamental role in cold-induced thermogenesis. Indeed, studies from Finland demonstrate that working in a cold environment maintains BAT in areas surrounding the neck and heart [20]. Studies support these observations by demonstrating that BAT activity increases as outside temperature decreases [9]. In regard to this study, it is important to note that only 7.5% of women and 3.1% of men were identified to have active BAT. Other studies, however, demonstrate that ~50% of adults have detectable BAT when activated in the laboratory by 2 h of cold exposure [21]. In addition, adults with detectable BAT were characterized as younger with lower HbA_{1c}, cholesterol, low-density lipoprotein (LDL)-cholesterol, glucose, adiposity and body mass index (BMI). In other studies, repeated cold exposure is demonstrated to decrease body fat mass [22], [23] and improve insulin sensitivity in individuals with type 2 diabetes [24]. Cold exposure increases energy expenditure by ~5–20% [10], [25], [26]. Although muscle contributes to this increase in metabolism, BAT is also demonstrated to utilize substrates [22], [23], [27]. Indeed, increases in cold-induced WAT release of non-esterified fatty acids are associated with BAT activation whereas glucose utilization links to muscles that contribute to shivering [28]. Together, these findings indicate that BAT plays a role in the lipid metabolism of healthy lean individuals and, therefore, has the potential to be a novel therapeutic mechanism for the treatment of obesity and, consequently, metabolic disease.

BAT alterations in obesity

BAT locations discussed above are readily identified in infants and children; however, its distribution decreases variably among individuals as age increases, with significant decreases occurring by age ~80 [7]. Although adults have BAT, age remains a factor in its decrease as well as increase in adiposity. Numerous studies demonstrate that BAT activity is inversely associated with BMI [9], [10], [25], [29]. As such, cold-induced thermogenesis is also significantly lower in overweight/obese individuals [30], [31]. It is postulated that increases in adiposity, especially in subcutaneous regions, provides increased insulation against heat loss and protection against cold exposure [30]. Subsequently, increases in adipose insulation enhances heat retention, which consequently decreases BAT responsiveness to cold. Although repeated cold can reduce adiposity in healthy lean individuals [22], [32], this is less likely to occur in obese individuals with greater adipose insulation. Despite these circumstances, there is a revived interest in targeting BAT in adults to drive increases in energy expenditure with subsequent decreases in adiposity. To target BAT energetics for the treatment of obesity requires BAT activation that can occur during thermoneutrality.

Obesity treatment – beiging of WAT; a brite tool to counter obesity

As previously discussed, increases in adiposity decrease BAT stores, ultimately leading to the inhibition of cold-induced increases in energy expenditure. Therefore, therapies aimed to reduce adiposity by non-shivering thermogenesis will likely not produce significant results. Rather, mechanisms that intend to use BAT for obesity therapy should first be efficient at reestablishing BAT mass lost in obesity and, second, activate newly established BAT at thermoneutrality. As such, an anti-obesity treatment should not solely activate but also increase the amount of BAT. This process naturally occurs in the body via differentiation (recruitment) from “inducible” brite/beige progenitor cells located in WAT. Cold can naturally induce the beiging of WAT (For review, see [33]); however, beiging/browning can also be induced by systemically administered agents (Table 1). Hence, the beiging phenomenon has received much attention as a possible mechanism to alter WAT metabolism toward enhanced energy utilization, which subsequently counters obesity.

Table 1: Drug molecules/natural products that activate browning phenomenon in vitro and in vivo.

Agent	Mechanism of induction of browning of WAT	Model organism (rodents/humans/cell lines)
Thiazolidinediones	SIRT-1-dependent deacetylation of PPAR γ [34]	Mouse
	Stabilization of PPAR γ [35]	HIB-1B cells
	Kruppel-like factor (KLF11) [36]	
Fenofibrate	PPAR α activation [37]	Mouse

Capsaicin	– SIRT-1 activation, SIRT-1-dependent PPAR γ and PRDM-16 deacetylation and their interaction [38] – Increase in brown fat-specific genes in vitro [39]	Mouse 3T3-L1 preadipocytes
Berberine	AMPK and PGC-1 α -dependent mechanism [40]	Mouse
Resveratrol	AMPK activation [41]	Mouse
Retinoic acid	p38 MAPK signaling [42]	Mouse
Menthol	UCP-1-dependent thermogenesis [43]	Human adipocytes
Green/black tea	AMPK activation [44]	Mouse
Melatonin	UCP-1- and PGC-1 α -dependent mechanism [45]	Zucker diabetic fatty rats
Ginsenoside	PPAR γ induction [46]	3T3-L1 adipocytes
Irisin	p38 MAPK and ERK pathways [47]	Mouse
Green tea	AMPK, induction of UCP-1 and IGF binding protein 1 [44]	Mouse
Curcumin	Stimulation of norepinephrine- β 3-adrenergic receptor [48], [49]	Mouse
Butein	Prdm4 induction [50]	Mouse
β -adrenergic receptor stimulation by catecholamines	mTORC1-dependent mechanism [51]	Mouse
Artepillin C	Increased UCP-1 and PRDM-16 [52]	Mouse
Bitter melon seed oil (BMSO)	Mitochondrial uncoupling [53]	Mouse
Omega 3 fatty acid	Increase UCP-1 and fatty acid oxidation [54], [55]	Mouse

Beiging/browning of WAT favors energy expenditure by triggering thermogenesis, which suppresses diet-induced weight gain [56], [57], [58]. Increased beiging/browning of WAT also enhances the efficiency of brown fat activity. Further analysis of molecular mechanisms underscoring the induction of beiging/browning of WAT led to the identification of adipogenic factors and their stabilization and interaction with proteins, which serve as catalysts for the browning of WAT [34], [35], [59]. Harnessing brown fat thermogenesis has opened new strategies to counteract obesity [60], [61], [62]. Previous research also indicates an inverse relation between body mass and BAT activation and suggests that recruitment of beige or brown cells in WAT increases energy expenditure to antagonize obesity [63]. Table 2 characterizes studies that demonstrate WAT depots capable of being induced toward beiging/browning, markers and transcription factors known to be involved in the process and activators that can promote the progression.

Table 2: Location of beige-able WAT, markers of beiging and activators of browning of WAT.

Table I	Beige-able fat (fat that exhibits browning phenomenon)
Location in mice	Subcutaneous, inguinal and visceral [64]
Location in humans	Supraclavicular [64], perirenal, visceral and subcutaneous depots [65]
Markers	Cd137 [66], Shox2 [67], Cited 1 [64], Tmem26 [66], Tbx1 [66], [68], BMP8b [37], [69], [70], UCP-1 [71], [72], SIRT-1-dependent mechanisms [34], [38]
Transcription factors	C/ebp β , PRDM-16 [34], [73], [74], PGC-1 α [75], [76], [77], PPAR α [37], PPAR γ [34], [38]
Activators	Thiazolidinediones [34], [78], [79], [80], natriuretic peptides [57], [81], [82], [83], capsaicin [38], [39], [84], [85], irisin [47], [86], [87], [88], FGF21 [84], [89], green tea [44], [90], resveratrol [41], [84], quercetin [91], berberine [40], bile acids [84], [92], retinoic acid [84], [93], melatonin [45], ginsenoside [46], β -aminoisobutyric acid [94], amino acid restriction [84], menthol [43], [95], caffeine [44], curcumin [48], [49], butein [50]
Mechanism that promotes browning of WAT	Inhibition of notch signaling [56], inhibition of myostatin [96], BDNF signaling [58], inhibition of Sam68 [97]

Thermoneutrality and beiging of WAT

The amount of energy used for thermogenesis is critically regulated by environmental temperature [98], [99]. As the environmental temperature increases, the energy required to maintain body temperature decreases [100]. Thermoneutrality is a critical temperature zone of the body in which no extra heat is required to maintain body temperature [100]. Most of the studies describing the beiging of WAT have been conducted at temperatures below thermoneutrality. At thermoneutrality, minimal energy is required to maintain body temperature. Maintaining mice at temperatures below thermoneutrality (around 30 °C) presents a mild cold stress to mice, which stimulates an increase in energy expenditure needed to defend body temperature against the cold environmental temperature. Mitochondrial uncoupling protein 1 (UCP-1) is a key regulator of adaptive thermogenesis that generates heat by uncoupling oxidative phosphorylation ATP generation during thermogenesis. Several studies have demonstrated UCP-1 upregulation in white fat as a mechanism for browning of WAT [37], [38], [84], [101]. Lack of UCP-1 ablates adaptive thermogenesis, but the effect is temperature-dependent. For example, UCP-1^{-/-} mice are resistant to diet-induced obesity when maintained below thermoneutrality [102]. This suggests that UCP-1-dependent thermogenesis is not fundamental when mice were maintained at ambient temperature (22 °C). Therefore, below thermoneutrality, UCP-1^{-/-} mice are forced to use alternate mechanisms for thermogenesis. However, ablation of cold stress by rearing at 30 °C makes UCP-1^{-/-} mice prone to obesity, which suggest a role for UCP-1 that at thermoneutrality, which when expressed, leads to increases in energy expenditure and protection against obesity in UCP1^{+/+} mice. Although these data have implications toward humans, there are significant differences in thermoneutrality and resting metabolic rate between humans and mice [103], [104]. Further, mice can easily regulate heat loss via the tail [105]. Therefore, care should be exercised in interpreting data from mice and extrapolating the rodent research to humans, as clothing and external environmental temperature facilitate thermoneutrality in humans. Despite these differences, recent research aims to develop human strategies to counter obesity by enhancement of thermogenesis and energy expenditure through WAT browning.

Mechanisms involved in the browning of WAT

Brown adipocytes arise from the lineage of myogenic factor 5 (Myf5)-expressing cells while white adipocytes arise from a cell lineage lacking Myf5. However, there is a subpopulation of WAT derived from the Myf5-positive cell lineage. These adipocytes exhibit the potential of beiging (or brite, defined as brown in white) and such beige-able WAT has enhanced metabolism due to enhanced expression BAT-specific genes of thermogenesis. However, the functional implication of Myf5-positive and Myf5-negative cells lineages in WAT remains unclear. Molecular signaling mediated by adipocyte progenitors significantly differ among the various depots of adipocytes, yet cellular metabolic sensor, sirtuin-1 (SIRT-1), peroxisome proliferator-activated receptor gamma (PPAR γ) and positive regulatory domain-containing protein 16 (PRDM-16) are commonly recognized as beiging factors [106].

Beiging/browning of WAT involves the expression and activation of the brown fat-specific genes in white adipocytes [67], [73], [107], [108]. For example, bone morphogenetic proteins (BMP) regulate thermogenesis and fatty acid oxidation [109], [110], [111]. A distinct form, bone morphogenetic protein 8b (BMP8b), is specifically demonstrated to facilitate energy dissipation by thermogenesis [112]. Another important protein that regulates brown fat thermogenesis is UCP-1 [113]. UCP-1, localized on the inner mitochondrial membrane, short-circuits the mitochondrial proton gradient to promote thermogenesis via oxidation of fatty acids. Hence, as discussed earlier, mice lacking UCP-1 are prone to obesity at thermoneutrality [114], [115]. Conversely, enhancement of BMP8b and UCP-1 in the subcutaneous adipose depot is associated with browning of WAT and decreased adiposity [43], [44]. Another important factor that governs beiging and BAT thermogenesis is the activation of β -adrenergic receptors [78]. Fibroblast growth factor 21 (FGF21) and bone morphogenetic protein 9 (BMP9) are also identified as important metabolic regulators involved in the browning of WAT [116].

The development and function of the classical beige adipocyte is primarily governed by PRDM-16, a transcriptional co-regulator that controls the production of the brown adipocyte gene [35], [73]. Posttranslational modification, such as deacetylation, of PRDM-16 by SIRT-1 (a cellular energy sensor [34], [38]) has been shown to be involved in the browning of WAT [38]. SIRT-1 deacetylation of PPAR γ [34], [38], [117] leading to the stabilization of the PRDM-16/PPAR γ protein complex [38], which is also demonstrated to fundamentally contribute to the browning of WAT. As such, research focuses on SIRT-1 as a potential strategy to induce browning of WAT. This is further supported by the findings that ablation of PRDM-16 in mice presents metabolic dysfunctions and ablates the thermogenic program of beige fat cells. PRDM-16 ablation also drives subcutaneous adipocytes

to alter inherent characteristics to resemble those of the visceral depot [59], by enrichment of Willms tumor-1 (Wt1). Taken together, this emphasizes the importance of PRDM-16 in the browning of WAT.

SIRT-1, a central player that regulates the browning program in WAT, is a sensor of cellular metabolism and energy utilization. SIRT-1 is phosphorylated and activated by cellular protein kinases including Ca^{2+} /calmodulin-dependent protein kinase kinase β (CaMKK β [118]) and 5'-adenosine monophosphate-activated protein kinase (AMPK [119], [120], [121]). Intracellular Ca^{2+} -dependent CaMKII-AMPK signaling plays a role in metabolism and fatty acid oxidation, and CaMKII is an upstream regulator of AMPK [122]. Thus, crosstalk signaling between intracellular Ca^{2+} -activated CaMKII-AMPK signaling and SIRT-1 are essential for the regulation of browning of WAT [38]. Overall, the browning phenomenon has been recognized in specific depots of WAT based on the expression of these several specific thermogenic markers that regulate beige transcription. Figure 1 is a diagram of the proposed mechanisms that regulate the induction of WAT browning.

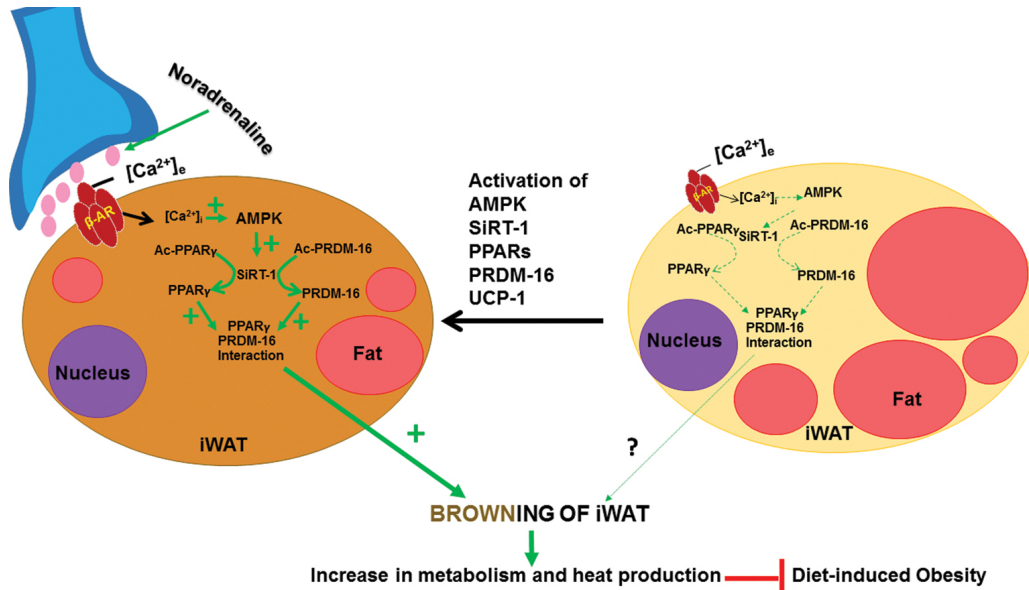


Figure 1: Model describing possible mechanisms involved in the browning of WAT.

Left. Noradrenaline release at the sympathetic innervations, which activates β -adrenergic receptors. The resultant Ca^{2+} influx enhances AMPK-dependent SIRT-1 activation. SIRT-1 deacetylates PPAR γ and PRDM-16. This causes a PPAR γ -PRDM-16 interaction leading to the browning of inguinal WAT, and counters diet-induced obesity. Right. High-fat diet (HFD) feeding TRPV1-dependent noradrenaline- β -adrenergic receptor signaling. This decreases AMPK-SIRT-1-dependent deacetylation of PPAR γ and PRDM-16. Browning of inguinal WAT does not occur and HFD promotes obesity.

Pharmacological approaches to BAT induction

Pharmacological approaches used to induce browning of WAT include the use of specific agonists of PPARs [34], [37], [38], SIRT-1 [34], [38], β 3-adrenergic receptor stimulation [51], thyroid hormone, irisin and FGF21 [84], [89] induction. In addition, activation of the transcription factor peroxisome proliferator-activated receptor gamma coactivator 1 α (PGC-1 α) enhances mitochondrial biogenesis and increases burning of fat by the upregulation of UCP-1 expression in WAT [43], [123]. Several natural products that also enhance metabolism and thermogenesis thus have the potential to trigger the induction of browning in WAT. Specific examples include specific amino acid restrictions, 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 (Refer to Table 2 for specific mechanisms). Metabolic signaling mechanisms from the muscle and liver, such as irisin and FGF21, are also recognized as activators of BAT and beige/brite adipocytes in humans [124]. The classes of molecules, synthetic and from natural origin, and the mechanisms by which the molecules stimulate browning of WAT are summarized in Table 1.

Targeting WAT depots for beiging

The identification of genes that are metabolically important in the regulation of thermogenesis in WAT creates numerous potential targets that can be favorably altered to enhance the beiging of WAT. This will allow WAT to acquire the functional phenotype of BAT and subsequently enhance WAT metabolic activity toward an increased ability to burn the stored fat into heat energy. A recent discovery that suggests the transformation of sWAT from energy-storing to energy-dissipating tissue has geared up novel strategies to target WAT for obesity management [37].

A viable strategy to enhance thermogenesis and energy expenditure in humans is by stimulating the browning of WAT by UCP-1 upregulation. There are numerous natural and synthetic drug molecules presented in Table 2 that are promising therapies to promote browning of WAT and mediate weight loss in humans. Table 3 summarizes the clinically relevant targets evaluated in humans for BAT activation and enhancement of browning of WAT to counter obesity and metabolic diseases in humans.

Table 3: Clinically relevant targets for human BAT activation.

Molecules/targets	Mechanism and effect
Capsinoids (non-pungent analogs of capsaicin)/capsaicinoids (analogs of capsaicin)	–Capsinoids increased BAT density [125] –A combination of capsaicinoids, epigallocatechin gallate, piperin and L-carnitine – Increased satiety and stimulated thermogenesis [126] –Repetitive stimulation by cold and capsinoids increased energy expenditure, BAT activity and a concomitant decrease in body fat mass [22] –Capsinoid increased fat oxidation and facilitated abdominal fat loss [127]
β -adrenergic stimulation	– Induced thermogenic program but did not improve insulin-dependent glucose uptake [128] – Isoprenaline increased β-adrenergic stimulation, increased metabolic activity but did not activate BAT [129] – Chronic epinephrine reduced body fat content but did not increase BAT activity [130]
Vagus nerve stimulation	Increased energy expenditure and BAT activation [131]
PPAR α	Binds to PPAR-responsive element, promotes PGC-1 α and enhances UCP-1 transcription [132]
PPAR γ	Promotes mitochondrial fatty acid oxidation [133]
BMP8B	Thermogenic activation of PRDM-16 [132]
	Deacetylation of PRDM-16 [35]
	Beiging of WAT [35], [39], [84]
	Upregulated by capsaicin to enhance browning of WAT [38] and BAT thermogenesis [117]
	AMPK and orexin-mediated signaling [69]
	Regulate energy expenditure in association with AMPK [112]
Irisin	Promotes osteogenesis and browning of WAT [134]
	(Effect is controversial) [135], [136]
Chili pepper extracts/capsinoids and capsaicinoids	Energy expenditure and weight loss [137]
	Suppress fat accumulation via lipid metabolism [138]
	Increase fat oxidation [127]
Capsiate	Increase in UCP-1 expression [139]
Exercise	Whole-body energy homeostasis [140]

Conclusion

The concept of browning of WAT in humans opens the possibility of targeting white fat with drug molecules and modulators that induce and enhance the expression and signaling of genes of thermogenesis in these tissues. The use of orally bioavailable PPAR agonists dual and pan agonists has provided immense benefit to control hyperlipidemia and insulin resistance associated with obesity [141]. However, there are also significant adverse

effects associated with such therapy [142], [143], [144]. Therefore, there is a clear need for selective PPAR agonists with minimal side effects.

Attempts to enhance SIRT-1 and AMPK with natural compounds like resveratrol has been proven to be fruitful in developing browning of WAT [145]. Although several other natural products have been accolated for their ability to counter obesity by triggering browning of WAT (refer to Table 2), further studies are warranted to characterize their effective doses in their pure form for human use.

Another important aspect of targeting WAT for obesity management will be developing adipose tissue target-specific drug delivery systems. Methods such as ligand targeting of liposomes provide new hope for targeting WAT [146], [147], which may significantly decrease undesired adverse effects of drug molecules. The use of magnetic nanoparticles for target site-specific delivery [148], [149], [150], [151] for agents that can trigger browning of WAT is another novel approach. Lastly, recent research has provided new hope for developing topical capsaicin formulation for reducing visceral adipose fat [152].

Taken together, WAT contributes the greatest proportion toward overall adipose tissue mass in the body. Unlike BAT, which decreases in obesity, WAT effortlessly expands with excessive calorie intake, making BAT thermogenesis obsolete for numerous reasons. BAT can effectively burn through lipids, and when consistently activated, can reduced adiposity. However, cold-induced therapy is not a feasible option for the reversal of obesity. Instead, research demonstrates that this same system, burning of lipids through UCP-1, can be targeted at thermoneutrality and use WAT as a fuel source. Beiging of WAT has great potential as a treatment for obesity reversal, but the most effective treatments for targeted, safe and specific adipose tissue delivery still remain to be elucidated.

Author Statement

Research funding: This work was supported by the pilot project funding from the AHA award no. 15BGIA23250030 to BT.

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.

References

- [1] O'Brien PE, Dixon JB. The extent of the problem of obesity. *Am J Surg.* 2002;184:4S–8S.
- [2] Vega GL. Obesity and the metabolic syndrome. *Minerva Endocrinol.* 2004;29:47–54.
- [3] Mlinar B, Marc J. New insights into adipose tissue dysfunction in insulin resistance. *Clin Chem Lab Med.* 2011;49:1925–35.
- [4] Ceska R. Clinical implications of the metabolic syndrome. *Diab Vasc Dis Res.* 2007;4(Suppl 3):S2–4.
- [5] Merklin RJ. Growth and distribution of human fetal brown fat. *Anat Rec.* 1974;178:637–45.
- [6] Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. *Am J Physiol Endocrinol Metab.* 2007;293:E444–52.
- [7] Heaton JM. The distribution of brown adipose tissue in the human. *J Anat.* 1972;112(Pt 1):35–9.
- [8] Cypess AM, Kahn CR. Brown fat as a therapy for obesity and diabetes. *Curr Opin Endocrinol Diabetes Obes.* 2010;17:143–9.
- [9] Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med.* 2009;360:1509–17.
- [10] van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, et al. Cold-activated brown adipose tissue in healthy men. *N Engl J Med.* 2009;360:1500–8.
- [11] Cohade C, Osman M, Pannu HK, Wahl RL. Uptake in supraclavicular area fat ("USA-Fat"): description on 18F-FDG PET/CT. *J Nucl Med.* 2003;44:170–6.
- [12] Yeung HW, Grewal RK, Conen M, Schoder H, Larson SM. Patterns of (18)F-FDG uptake in adipose tissue and muscle: a potential source of false-positives for PET. *J Nucl Med.* 2003;44:1789–96.
- [13] Virtanen KA, Lidell ME, Orava J, Heglin M, Westergren R, Niemi T, et al. Functional brown adipose tissue in healthy adults. *N Engl J Med.* 2009;360:1518–25.
- [14] Klein S, Allison DB, Heymsfield SB, Kelley DE, Leibel RL, Nonas C, et al. Waist circumference and cardiometabolic risk: a consensus statement from shaping America's health: Association for Weight Management and Obesity Prevention; NAASO, the Obesity Society; the American Society for Nutrition; and the American Diabetes Association. *Diabetes Care.* 2007;30:1647–52.
- [15] Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev.* 2004;84:277–359.
- [16] Lidell ME, Betz M, Dahlqvist Leinhard O, Heglin M, Elander L, Slawik M, et al. Evidence for two types of brown adipose tissue in humans. *Nat Med.* 2013;19:631–4.

- [17] Lee P, Greenfield JR, Ho KK, Fulham MJ. A critical appraisal of the prevalence and metabolic significance of brown adipose tissue in adult humans. *Am J Physiol Endocrinol Metab.* 2010;299:E601–6.
- [18] Sacks H, Symonds ME. Anatomical locations of human brown adipose tissue: functional relevance and implications in obesity and type 2 diabetes. *Diabetes.* 2013;62:1783–90.
- [19] Smith RE, Horwitz BA. Brown fat and thermogenesis. *Physiol Rev.* 1969;49:330–425.
- [20] Huttunen P, Hirvonen J, Kinnula V. The occurrence of brown adipose tissue in outdoor workers. *Eur J Appl Physiol Occup Physiol.* 1981;46:339–45.
- [21] Matsushita M, Yoneshiro T, Aita S, Kameya T, Sugie H, Saito M. Impact of brown adipose tissue on body fatness and glucose metabolism in healthy humans. *Int J Obes.* 2014;38:812–7.
- [22] Yoneshiro T, Aita S, Matsushita M, Kayahara T, Kameya T, Kawai Y, et al. Recruited brown adipose tissue as an antiobesity agent in humans. *J Clin Invest.* 2013;123:3404–8.
- [23] van der Lans AA, Hoeks J, Brans B, Vijgen GH, Visser MC, Vosselman M, et al. Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. *J Clin Invest.* 2013;123:3395–403.
- [24] Hanssen MJ, Hoeks J, Brans B, van der Lans AA, Schaart G, van den Driessche JJ, et al. Short-term cold acclimation improves insulin sensitivity in patients with type 2 diabetes mellitus. *Nat Med.* 2015;21:863–5.
- [25] Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, et al. High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes.* 2009;58:1526–31.
- [26] Chondronikola M, Volpi E, Borsheim E, Porter C, Annamalai P, Enerback S, et al. Brown adipose tissue improves whole-body glucose homeostasis and insulin sensitivity in humans. *Diabetes.* 2014;63:4089–99.
- [27] Chen KY, Brychta RJ, Linderman JD, Smith S, Courville A, Dieckmann W, et al. Brown fat activation mediates cold-induced thermogenesis in adult humans in response to a mild decrease in ambient temperature. *J Clin Endocrinol Metab.* 2013;98:E1218–23.
- [28] Blondin DP, Labbe SM, Phoenix S, Guerin B, Turcotte EE, Richard D, et al. Contributions of white and brown adipose tissues and skeletal muscles to acute cold-induced metabolic responses in healthy men. *J Physiol.* 2015;593:701–14.
- [29] Zingaretti MC, Crosta F, Vitali A, Guerrieri M, Frontini A, Cannon B, et al. The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J.* 2009;23:3113–20.
- [30] Vijgen GH, Bouvy ND, Teule GJ, Brans B, Schrauwen P, van Marken Lichtenbelt WD. Brown adipose tissue in morbidly obese subjects. *PLoS One.* 2011;6:e17247.
- [31] Wijers SL, Saris WH, van Marken Lichtenbelt WD. Cold-induced adaptive thermogenesis in lean and obese. *Obesity (Silver Spring).* 2010;18:1092–9.
- [32] Lee P, Smith S, Linderman J, Courville AB, Brychta RJ, Dieckmann W, et al. Temperature-acclimated brown adipose tissue modulates insulin sensitivity in humans. *Diabetes.* 2014;63:3686–98.
- [33] Giralt M, Villarroya F. White, brown, beige/brite: different adipose cells for different functions?. *Endocrinology.* 2013;154:2992–3000.
- [34] Qiang L, Wang L, Kon N, Zhao W, Lee S, Zhang Y, et al. Brown remodeling of white adipose tissue by SirT1-dependent deacetylation of Ppargamma. *Cell.* 2012;150:620–32.
- [35] Ohno H, Shinoda K, Spiegelman BM, Kajimura S. PPARgamma agonists induce a white-to-brown fat conversion through stabilization of PRDM16 protein. *Cell Metab.* 2012;15:395–404.
- [36] Loft A, Forss I, Siersbaek MS, Schmidt SF, Larsen AS, Madsen JG, et al. Browning of human adipocytes requires KLF11 and reprogramming of PPARgamma superenhancers. *Genes Dev.* 2015;29:7–22.
- [37] Rachid TL, Penna-de-Carvalho A, Brighenti I, Aguila MB, Mandarin-de-Lacerda CA, Souza-Mello V. Fenofibrate (PPARalpha agonist) induces beige cell formation in subcutaneous white adipose tissue from diet-induced male obese mice. *Mol Cell Endocrinol.* 2015;402:86–94.
- [38] Baskaran P, Krishnan V, Ren J, Thyagarajan B. Capsaicin induces browning of white adipose tissue and counters obesity by activating TRPV1 channel-dependent mechanisms. *Br J Pharmacol.* 2016;173:2369–89.
- [39] Baboota RK, Singh DP, Sarma SM, Kaur J, Sandhir R, Boparai RK, et al. Capsaicin induces “brite” phenotype in differentiating 3T3-L1 preadipocytes. *PLoS One.* 2014;9:e103093.
- [40] Zhang Z, Zhang H, Li B, Meng X, Wang J, Zhang Y, et al. Berberine activates thermogenesis in white and brown adipose tissue. *Nat Commun.* 2014;5:5493.
- [41] Wang S, Liang X, Yang Q, Fu X, Rogers CJ, Zhu M, et al. Resveratrol induces brown-like adipocyte formation in white fat through activation of AMP-activated protein kinase (AMPK) alpha1. *Int J Obes (Lond).* 2015;39:967–76.
- [42] Wang J, Sun GJ, Ding J, Zhang JX, Cui Y, Li HR, et al. WY14643 combined with all-trans retinoic acid acts via p38 MAPK to induce “browning” of white adipocytes in mice. *Genet Mol Res.* 2015;14:6978–84.
- [43] Rossato M, Granzotto M, Macchi V, Porzionato A, Petrelli L, Calcagno A, et al. Human white adipocytes express the cold receptor TRPM8 which activation induces UCP1 expression, mitochondrial activation and heat production. *Mol Cell Endocrinol.* 2014;383:137–46.
- [44] Yamashita Y, Wang L, Wang L, Tanaka Y, Zhang T, Ashida H. Oolong, black and pu-erh tea suppresses adiposity in mice via activation of AMP-activated protein kinase. *Food Funct.* 2014;5:2420–9.
- [45] Jimenez-Aranda A, Fernandez-Vazquez G, Campos D, Tassi M, Velasco-Perez L, Tan DX, et al. Melatonin induces browning of inguinal white adipose tissue in Zucker diabetic fatty rats. *J Pineal Res.* 2013;55:416–23.
- [46] Mu Q, Fang X, Li X, Zhao D, Mo F, Jiang G, et al. Ginsenoside Rb1 promotes browning through regulation of PPARgamma in 3T3-L1 adipocytes. *Biochem Biophys Res Commun.* 2015;466:530–5.
- [47] Zhang Y, Li R, Meng Y, Li S, Donelan W, Zhao Y, et al. Irisin stimulates browning of white adipocytes through mitogen-activated protein kinase p38 MAP kinase and ERK MAP kinase signaling. *Diabetes.* 2014;63:514–25.
- [48] Wang S, Wang X, Ye Z, Xu C, Zhang M, Ruan B, et al. Curcumin promotes browning of white adipose tissue in a norepinephrine-dependent way. *Biochem Biophys Res Commun.* 2015;466:247–53.
- [49] Zhang X, Tian Y, Zhang H, Kavishwar A, Lynes M, Brownell AL, et al. Curcumin analogues as selective fluorescence imaging probes for brown adipose tissue and monitoring browning. *Sci Rep.* 2015;5:13116.

- [50] Song NJ, Choi S, Rajbhandari P, Chang SH, Kim S, Vergnes L, et al. Prdm4 induction by the small molecule butein promotes white adipose tissue browning. *Nat Chem Biol.* 2016;12:479–81.
- [51] Liu D, Bordicchia M, Zhang C, Fang H, Wei W, Li J, et al. Activation of mTORC1 is essential for beta-adrenergic stimulation of adipose browning. *J Clin Invest.* 2016;126:1704–16.
- [52] Nishikawa S, Aoyama H, Kamiya M, Higuchi J, Kato A, Soga M, et al. Artepillin C, a typical Brazilian propolis-derived component, induces brown-like adipocyte formation in C3H10T1/2 cells, primary inguinal white adipose tissue-derived adipocytes, and mice. *PLoS One.* 2016;11:e0162512.
- [53] Chang YY, Su HM, Chen SH, Hsieh WT, Chyuan JH, Chao PM. Roles of peroxisome proliferator-activated receptor alpha in bitter melon seed oil-corrected lipid disorders and conversion of alpha-eleostearic acid into rumenic acid in C57BL/6 mice. *Nutrients.* 2016;8:805.
- [54] Simopoulos A. The FTO gene, browning of adipose tissue and omega-3 fatty acids. *J Nutrigenet Nutrigenomics.* 2016;9:123–6.
- [55] Simopoulos AP. An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients.* 2016;8:128.
- [56] Bi P, Shan T, Liu W, Yue F, Yang X, Liang XR, et al. Inhibition of Notch signaling promotes browning of white adipose tissue and ameliorates obesity. *Nat Med.* 2014;20:911–8.
- [57] Bordicchia M, Liu D, Amri EZ, Ailhaud G, Dessi-Fulgheri P, Zhang C, et al. Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. *J Clin Invest.* 2012;122:1022–36.
- [58] Cao L, Choi EY, Liu X, Martin A, Wang C, Xu X, et al. White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis. *Cell Metab.* 2011;14:324–38.
- [59] Cohen P, Levy JD, Zhang Y, Frontini A, Kolodin DP, Svensson KJ, et al. Ablation of PRDM16 and beige adipose causes metabolic dysfunction and a subcutaneous to visceral fat switch. *Cell.* 2014;156:304–16.
- [60] Wu J, Cohen P, Spiegelman BM. Adaptive thermogenesis in adipocytes: is beige the new brown?. *Genes Dev.* 2013;27:234–50.
- [61] Vosselman MJ, van Marken Lichtenbelt WD, Schrauwen P. Energy dissipation in brown adipose tissue: from mice to men. *Mol Cell Endocrinol.* 2013;379:43–50.
- [62] Peschechera A, Eckel J. “Browning” of adipose tissue—regulation and therapeutic perspectives. *Arch Physiol Biochem.* 2013;119:151–60.
- [63] Qian S, Huang H, Tang Q. Brown and beige fat: the metabolic function, induction, and therapeutic potential. *Front Med.* 2015;9:162–72.
- [64] Harms M, Seale P. Brown and beige fat: development, function and therapeutic potential. *Nat Med.* 2013;19:1252–63.
- [65] Rockstroh D, Landgraf K, Wagner IV, Gesing J, Tauscher R, Lakowa N, et al. Direct evidence of brown adipocytes in different fat depots in children. *PLoS One.* 2015;e0117841.
- [66] Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell.* 2012;150:366–76.
- [67] Servera M, Lopez N, Serra F, Palou A. Expression of “brown-in-white” adipocyte biomarkers shows gender differences and the influence of early dietary exposure. *Genes Nutr.* 2014;9:372.
- [68] Petrovic N, Walden TB, Shabalina IG, Timmons JA, Cannon B, Nedergaard J. Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. *J Biol Chem.* 2010;285:7153–64.
- [69] Martins L, Seoane-Collazo P, Contreras C, Gonzalez-Garcia I, Martinez-Sanchez N, Gonzalez F, et al. A functional link between AMPK and orexin mediates the effect of BMP8B on energy balance. *Cell Rep.* 2016;16:2231–42.
- [70] Poher AL, Altirriba J, Veyrat-Durebex C, Rohner-Jeanrenaud F. Brown adipose tissue activity as a target for the treatment of obesity/insulin resistance. *Front Physiol.* 2015;6:4.
- [71] Valero-Munoz M, Li S, Wilson RM, Hulsmans M, Aprahamian T, Fuster JJ, et al. Heart failure with preserved ejection fraction induces Beiging in adipose tissue. *Circ Heart Fail.* 2016;e0027249.
- [72] Srinivasa S, Wong K, Fitch KV, Wei J, Petrow E, Cypess AM, et al. Effects of lifestyle modification and metformin on irisin and FGF21 among HIV-infected subjects with the metabolic syndrome. *Clin Endocrinol (Oxf).* 2015;82:678–85.
- [73] Lo KA, Sun L. Turning WAT into BAT: a review on regulators controlling the browning of white adipocytes. *Biosci Rep.* 2013;pii:e0006533. DOI:10.1042/BSR20130046.
- [74] Park JW, Jung KH, Lee JH, Quach CH, Moon SH, Cho YS, et al. 18F-FDG PET/CT monitoring of beta3 agonist-stimulated brown adipocyte recruitment in white adipose tissue. *J Nucl Med.* 2015;56:153–8.
- [75] Stanford KI, Middelbeek RJ, Goodyear LJ. Exercise effects on white adipose tissue: Beiging and metabolic adaptations. *Diabetes.* 2015;64:2361–8.
- [76] Wu MV, Bikopoulos G, Hung S, Ceddia RB. Thermogenic capacity is antagonistically regulated in classical brown and white subcutaneous fat depots by high fat diet and endurance training in rats: impact on whole-body energy expenditure. *J Biol Chem.* 2014;289:34129–40.
- [77] Fisher FM, Kleiner S, Douris N, Fox EC, Mepani RJ, Verdeguer F, et al. FGF21 regulates PGC-1alpha and browning of white adipose tissues in adaptive thermogenesis. *Genes Dev.* 2012;26:271–81.
- [78] Keipert S, Jastroch M. Brite/beige fat and UCP1 – is it thermogenesis?. *Biochim Biophys Acta.* 2014;1837:1075–82.
- [79] Coelho MS, de Lima CL, Royer C, Silva JB, Oliveira FC, Christ CG, et al. GQ-16, a TZD-derived partial PPARgamma agonist, induces the expression of thermogenesis-related genes in brown fat and visceral white fat and decreases visceral adiposity in obese and hyperglycemic mice. *PLoS One.* 2016;11:e0154310.
- [80] Zadeegan FG, Ghaedi K, Kalantar SM, Peymani M, Hashemi MS, Baharvand H, et al. Cardiac differentiation of mouse embryonic stem cells is influenced by a PPAR gamma/PGC-1alpha-FNDC5 pathway during the stage of cardiac precursor cell formation. *Eur J Cell Biol.* 2015;94:257–66.
- [81] Cereijo R, Villarroja J, Villarroja F. Non-sympathetic control of brown adipose tissue. *Int J Obes Suppl.* 2015;5(Suppl 1):S40–4.
- [82] Neinast MD, Frank AP, Zechner JF, Li Q, Vishvanath L, Palmer BF, et al. Activation of natriuretic peptides and the sympathetic nervous system following Roux-en-Y gastric bypass is associated with gonadal adipose tissues browning. *Mol Metab.* 2015;4:427–36.
- [83] Leiss V, Illison J, Domes K, Hofmann F, Lukowski R. Expression of cGMP-dependent protein kinase type I in mature white adipocytes. *Biochem Biophys Res Commun.* 2014;452:151–6.

- [84] Bargut TC, Souza-Mello V, Aguila MB, Mandarim-de-Lacerda CA. Browning of white adipose tissue: lessons from experimental models. *Horm Mol Biol Clin Investig* 2017. DOI:10.1515/hmbci-2016-0051.
- [85] Azhar Y, Parmar A, Miller CN, Samuels JS, Rayalam S. Phytochemicals as novel agents for the induction of browning in white adipose tissue. *Nutr Metab (Lond)*. 2016;13:89.
- [86] Jeremic N, Chaturvedi P, Tyagi SC. Browning of white fat: novel insight into factors, mechanisms, and therapeutics. *J Cell Physiol*. 2017;232:61–8.
- [87] Irving BA, Still CD, Argyropoulos G. Does IRISIN have a BRITE future as a therapeutic agent in humans?. *Curr Obes Rep*. 2014;3:235–41.
- [88] Elsen M, Raschke S, Eckel J. Browning of white fat: does irisin play a role in humans?. *J Endocrinol*. 2014;222:R25–38.
- [89] Schlessinger K, Li W, Tan Y, Liu F, Souza SC, Tozzo E, et al. Gene expression in WAT from healthy humans and monkeys correlates with FGF21-induced browning of WAT in mice. *Obesity (Silver Spring)*. 2015;23:1818–29.
- [90] Sae-Tan S, Rogers CJ, Lambert JD. Decaffeinated green tea and voluntary exercise induce gene changes related to beige adipocyte formation in high fat-fed obese mice. *J Funct Foods*. 2015;14:210–4.
- [91] Arias N, Pico C, Teresa Macarulla M, Oliver P, Miranda J, Palou A, et al. A combination of resveratrol and quercetin induces browning in white adipose tissue of rats fed an obesogenic diet. *Obesity (Silver Spring)*. 2017;25:111–21.
- [92] Fang S, Suh JM, Reilly SM, Yu E, Osborn O, Lackey D, et al. Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. *Nat Med*. 2015;21:159–65.
- [93] Tourniaire F, Musinovic H, Gouranton E, Astier J, Marcotrichino J, Arreguin A, et al. All-trans retinoic acid induces oxidative phosphorylation and mitochondria biogenesis in adipocytes. *J Lipid Res*. 2015;56:1100–9.
- [94] Roberts LD, Bostrom P, O'Sullivan JF, Schinzel RT, Lewis GD, Dejam A, et al. Beta-aminoisobutyric acid induces browning of white fat and hepatic beta-oxidation and is inversely correlated with cardiometabolic risk factors. *Cell Metab*. 2014;19:96–108.
- [95] Sakellariou P, Valente A, Carrillo AE, Metsios GS, Nadolnik L, Jamurtas AZ, et al. Chronic l-menthol-induced browning of white adipose tissue hypothesis: a putative therapeutic regime for combating obesity and improving metabolic health. *Med Hypotheses*. 2016;93:21–6.
- [96] Shan T, Liang X, Bi P, Kuang S. Myostatin knockout drives browning of white adipose tissue through activating the AMPK-PGC1alpha-Fndc5 pathway in muscle. *FASEB J*. 2013;27:1981–9.
- [97] Zhou J, Cheng M, Boriboun C, Ardehali MM, Jiang C, Liu Q, et al. Inhibition of Sam68 triggers adipose tissue browning. *J Endocrinol*. 2015;225:181–9.
- [98] Abreu-Vieira G, Xiao C, Gavrilova O, Reitman ML. Integration of body temperature into the analysis of energy expenditure in the mouse. *Mol Metab*. 2015;4:461–70.
- [99] Hoevenaars FP, Bekkenkamp-Grovenstein M, Janssen RJ, Heil SC, Bunschoten A, Hoek-van den Hil EF, et al. Thermoneutrality results in prominent diet-induced body weight differences in C57BL/6 mice, not paralleled by diet-induced metabolic differences. *Mol Nutr Food Res*. 2014;58:799–807.
- [100] Cui X, Nguyen NL, Zarebidaki E, Cao Q, Li F, Zha L, et al. Thermoneutrality decreases thermogenic program and promotes adiposity in high-fat diet-fed mice. *Physiol Rep*. 2016;pii: e127994. DOI:10.14814/phy2.12799.
- [101] Joffin N, Jaubert AM, Bamba J, Barouki R, Noirez P, Forest C. Acute induction of uncoupling protein 1 by citrulline in cultured explants of white adipose tissue from lean and high-fat-diet-fed rats. *Adipocyte*. 2015;4:129–34.
- [102] Feldmann HM, Golozoubova V, Cannon B, Nedergaard J. UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab*. 2009;9:203–9.
- [103] Kingma B, Frijns A, van Marken Lichtenbelt W. The thermoneutral zone: implications for metabolic studies. *Front Biosci (Elite Ed)*. 2012;4:1975–85.
- [104] Nieman DC, Trone GA, Austin MD. A new handheld device for measuring resting metabolic rate and oxygen consumption. *J Am Diet Assoc*. 2003;103:588–92.
- [105] Serrat MA, King D, Lovejoy CO. Temperature regulates limb length in homeotherms by directly modulating cartilage growth. *Proc Natl Acad Sci U S A*. 2008;105:19348–53.
- [106] Chi J, Cohen P. The multifaceted roles of PRDM16: adipose biology and beyond. *Trends Endocrinol Metab*. 2016;27:11–23.
- [107] Rosell M, Kaforou M, Frontini A, Okolo A, Chan YW, Nikolopoulou E, et al. Brown and white adipose tissues: intrinsic differences in gene expression and response to cold exposure in mice. *Am J Physiol Endocrinol Metab*. 2014;306:E945–64.
- [108] Lu X, Ji Y, Zhang L, Zhang Y, Zhang S, An Y, et al. Resistance to obesity by repression of VEGF gene expression through induction of brown-like adipocyte differentiation. *Endocrinology*. 2012;153:3123–32.
- [109] Futamura M, Goto S, Kimura R, Kimoto I, Miyake M, Ito K, et al. Differential effects of topically applied formalin and aromatic compounds on neurogenic-mediated microvascular leakage in rat skin. *Toxicology*. 2009;255:100–6.
- [110] Tramontana M, Giuliani S, Valenti C, Cialdai C, Lazzeri M, Turini D, et al. Excitatory and inhibitory urinary bladder reflexes induced by stimulation of cervicovaginal capsaicin-sensitive sensory fibers in rats. *Naunyn Schmiedeberg's Arch Pharmacol*. 2009;379:107–14.
- [111] Molliver DC, Immke DC, Fierro L, Pare M, Rice FL, McCleskey EW. ASIC3, an acid-sensing ion channel, is expressed in metaboreceptive sensory neurons. *Mol Pain*. 2005;1:35.
- [112] Whittle A, Carobbio S, Martins L, Slawik M, Hondares E, Vazquez MJ, et al. BMP8B increases brown adipose tissue thermogenesis through both central and peripheral actions. *Cell*. 2012;149:871–85.
- [113] Hesselink MK, Mensink M, Schrauwen P. Human uncoupling protein-3 and obesity: an update. *Obes Res*. 2003;11:1429–43.
- [114] Denjean F, Lachuer J, Geloën A, Cohen-Adad F, Moulin C, Barre H, et al. Differential regulation of uncoupling protein-1, -2 and -3 gene expression by sympathetic innervation in brown adipose tissue of thermoneutral or cold-exposed rats. *FEBS Lett*. 1999;444:181–5.
- [115] Melnyk A, Himms-Hagen J. Temperature-dependent feeding: lack of role for leptin and defect in brown adipose tissue-ablated obese mice. *Am J Physiol*. 1998;274:R1131–5 Pt 2.
- [116] Kim S, Choe S, Lee DK. BMP-9 enhances fibroblast growth factor 21 expression and suppresses obesity. *Biochim Biophys Acta*. 2016;1862:1237–46.
- [117] Baskaran P, Krishnan V, Fettel K, Gao P, Zhu Z, Ren J, et al. TRPV1 activation counters diet-induced obesity through sirtuin-1 activation and PRDM-16 deacetylation in brown adipose tissue. *Int J Obes (Lond)*. 2017;41:739–49.

- [118] Iwabu M, Yamauchi T, Okada-Iwabu M, Sato K, Nakagawa T, Funata M, et al. Adiponectin and AdipoR1 regulate PGC-1 α and mitochondria by Ca²⁺ and AMPK/SIRT1. *Nature*. 2010;464:1313–9.
- [119] Passariello CL, Zini M, Nassi PA, Pignatti C, Stefanelli C. Upregulation of SIRT1 deacetylase in phenylephrine-treated cardiomyoblasts. *Biochem Biophys Res Commun*. 2011;407:512–6.
- [120] Lau AW, Liu P, Inuzuka H, Gao D. SIRT1 phosphorylation by AMP-activated protein kinase regulates p53 acetylation. *Am J Cancer Res*. 2014;4:245–55.
- [121] Peng Y, Rideout DA, Rakita SS, Gower WR, You M, Murr MM. Does LKB1 mediate activation of hepatic AMP-protein kinase (AMPK) and sirtuin1 (SIRT1) after Roux-en-Y gastric bypass in obese rats? *J Gastrointest Surg*. 2010;14:221–8.
- [122] Raney MA, Turcotte LP. Evidence for the involvement of CaMKII and AMPK in Ca²⁺-dependent signaling pathways regulating FA uptake and oxidation in contracting rodent muscle. *J Appl Physiol* (1985). 2008;104:1366–73.
- [123] Singh SP, Schragenheim J, Cao J, Falck JR, Abraham NG, Bellner L. PGC-1 α regulates HO-1 expression, mitochondrial dynamics and biogenesis: Role of epoxyeicosatrienoic acid. *Prostaglandins Other Lipid Mediat*. 2016;125:8–18.
- [124] Cereijo R, Giralt M, Villarroya F. Thermogenic brown and beige/brite adipogenesis in humans. *Ann Med*. 2015;47:169–77.
- [125] Nirengi S, Homma T, Inoue N, Sato H, Yoneshiro T, Matsushita M, et al. Assessment of human brown adipose tissue density during daily ingestion of thermogenic capsinoids using near-infrared time-resolved spectroscopy. *J Biomed Opt*. 2016;21:091305.
- [126] Rondanelli M, Opizzi A, Perna S, Faliva M, Solerte SB, Fioravanti M, et al. Acute effect on satiety, resting energy expenditure, respiratory quotient, glucagon-like peptide-1, free fatty acids, and glycerol following consumption of a combination of bioactive food ingredients in overweight subjects. *J Am Coll Nutr*. 2013;32:41–9.
- [127] Snitker S, Fujishima Y, Shen H, Ott S, Pi-Sunyer X, Furuhashi Y, et al. Effects of novel capsinoid treatment on fatness and energy metabolism in humans: possible pharmacogenetic implications. *Am J Clin Nutr*. 2009;89:45–50.
- [128] Mossenbock K, Vegiopoulos A, Rose AJ, Sijmonsma TP, Herzig S, Schafmeier T. Browning of white adipose tissue uncouples glucose uptake from insulin signaling. *PLoS One*. 2014;e1104289.
- [129] Vosselman MJ, van der Lans AA, Brans B, Wiers R, van Baak MA, Schrauwen P, et al. Systemic beta-adrenergic stimulation of thermogenesis is not accompanied by brown adipose tissue activity in humans. *Diabetes*. 2012;61:3106–13.
- [130] Carey AL, Pajtak R, Formosa MF, Van Every B, Bertovic DA, Anderson MJ, et al. Chronic ephedrine administration decreases brown adipose tissue activity in a randomised controlled human trial: implications for obesity. *Diabetologia*. 2015;58:1045–54.
- [131] Vijgen GH, Bouvy ND, Leenen L, Rijkers K, Cornips E, Majoie M, et al. Vagus nerve stimulation increases energy expenditure: relation to brown adipose tissue activity. *PLoS One*. 2013;e772218.
- [132] Hondares E, Rosell M, Diaz-Delfin J, Olmos Y, Monsalve M, Iglesias R, et al. Peroxisome proliferator-activated receptor alpha (PPAR α) induces PPARgamma coactivator 1alpha (PGC-1alpha) gene expression and contributes to thermogenic activation of brown fat: involvement of PRDM16. *J Biol Chem*. 2011;286:43112–22.
- [133] Wang L, Teng R, Di L, Rogers H, Wu H, Kopp JB, et al. PPAR α and Sirt1 mediate erythropoietin action in increasing metabolic activity and browning of white adipocytes to protect against obesity and metabolic disorders. *Diabetes*. 2013;62:4122–31.
- [134] Zhang Y, Xie C, Wang H, Foss RM, Clare M, George EV, et al. Irisin exerts dual effects on browning and adipogenesis of human white adipocytes. *Am J Physiol Endocrinol Metab*. 2016;311:E530–41.
- [135] Raschke S, Elsen M, Gassenhuber H, Sommerfeld M, Schwahn U, Brockmann B, et al. Evidence against a beneficial effect of irisin in humans. *PLoS One*. 2013;e736808.
- [136] Elbelt U, Hofmann T, Stengel A. Irisin: what promise does it hold? *Curr Opin Clin Nutr Metab Care*. 2013;16:541–7.
- [137] Cannon NP, Lambalot EL, Vaughan RA. The effects of capsaicin and capsaicinoid analogs on metabolic molecular targets in highly energetic tissues and cell types. *Biofactors*. 2016;42:229–46.
- [138] Hong Q, Xia C, Xiangying H, Quan Y. Capsinoids suppress fat accumulation via lipid metabolism. *Mol Med Rep*. 2015;11:1669–74.
- [139] Masuda Y, Haramizu S, Oki K, Ohnuki K, Watanabe T, Yazawa S, et al. Upregulation of uncoupling proteins by oral administration of capsiate, a nonpungent capsaicin analog. *J Appl Physiol* (1985). 2003;95:2408–15.
- [140] Sepa-Kishi DM, Ceddia RB. Exercise-mediated effects on white and brown adipose tissue plasticity and metabolism. *Exerc Sport Sci Rev*. 2016;44:37–44.
- [141] Harrington WW, Britt SC, Wilson CJ, Milliken ON, Binz CJ, Lobe CD, et al. The effect of PPAR α , PPAR δ , PPAR γ , and PPAR γ agonists on body weight, body mass, and serum lipid profiles in diet-induced obese AKR/J mice. *PPAR Res*. 2007;2007:97125.
- [142] Kung J, Henry RR. Thiazolidinedione safety. *Expert Opin Drug Saf*. 2012;11:565–79.
- [143] Home P. Safety of PPAR agonists. *Diabetes Care*. 2011;34(Suppl 2):S215–9.
- [144] Abbas A, Blandon J, Rude J, Elfar A, Mukherjee D. PPAR- γ agonist in treatment of diabetes: cardiovascular safety considerations. *Cardiovasc Hematol Agents Med Chem*. 2012;10:124–34.
- [145] Zou T, Chen D, Yang Q, Wang B, Zhu MJ, Nathanielsz PW, et al. Resveratrol supplementation of high-fat diet-fed pregnant mice promotes brown and beige adipocyte development and prevents obesity in male offspring. *J Physiol*. 2017;595:1547–62.
- [146] Bu L, Gao M, Qu S, Liu D. Intraperitoneal injection of clodronate liposomes eliminates visceral adipose macrophages and blocks high-fat diet-induced weight gain and development of insulin resistance. *AAPS J*. 2013;15:1001–11.
- [147] Feng B, Jiao P, Nie Y, Kim T, Jun D, van Rooijen N, et al. Clodronate liposomes improve metabolic profile and reduce visceral adipose macrophage content in diet-induced obese mice. *PLoS One*. 2011;e243586.
- [148] Baskaran M, Baskaran P, Arulsamy N, Thyagarajan B. Preparation and evaluation of PLGA-coated capsaicin magnetic nanoparticles. *Pharm Res*. 2017;34:1255–63. DOI:10.1007/s11095-017-2142-2.
- [149] Rocca A, Moscato S, Ronca F, Nitti S, Mattoli V, Giorgi M, et al. Pilot in vivo investigation of cerium oxide nanoparticles as a novel anti-obesity pharmaceutical formulation. *Nanomedicine*. 2015;11:1725–34.
- [150] Sharifi S, Daghighi S, Motazacker MM, Badlou B, Sanjabi B, Akbarkhanzadeh A, et al. Superparamagnetic iron oxide nanoparticles alter expression of obesity and T2D-associated risk genes in human adipocytes. *Sci Rep*. 2013;3:2173.
- [151] Kim D, Park JH, Kweon DJ, Han GD. Bioavailability of nanoemulsified conjugated linoleic acid for an antiobesity effect. *Int J Nanomedicine*. 2013;8:451–9.

- [152] Lee GR, Shin MK, Yoon DJ, Kim AR, Yu R, Park NH, et al. Topical application of capsaicin reduces visceral adipose fat by affecting adipokine levels in high-fat diet-induced obese mice. *Obesity (Silver Spring)*. 2013;21:115–22.