REVIEW

NEW INSIGHTS INTO BROWN ADIPOSE TISSUE AS A PHARMACOLOGICAL TARGET IN OBESITY

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Abstract

The adipose tissue is composed of at least two types of adipose cells – unilocular adipocytes in the white adipose tissue (WAT) and multilocular in brown adipose tissue (BAT). Accumulation of WAT in physiological depots leads to obesity and its known metabolic and cardiovascular consequences. Recently, it has been proved that BAT is metabolically active not only in new-borns but also in adult humans. Imaging and molecular studies revealed two varieties of BAT: constitutive (cBAT) and cells from WAT depots that could be activated – beige adipocytes forming the inducible BAT (iBAT). Activation of BAT thermogenesis is the main controller of the balance between energy intake (EI) and expenditure (EE). Non-pharmacological strategies based on reducing EI or increasing EE has been proved inefficient in the treatment of obesity and its related diseases because many subjects show poor adherence. Therefore, scientific interest for BAT is revived in order to develop new therapeutic strategies targeting adipose tissue metabolism alterations. In this paper we review recent data regarding the potential benefit of dietary factors and pharmacological agents in BAT activation and WAT browning as a strategy to treat obesity.

Rezumat

Țesutul adipos este considerat un mare organ compus din cel puțin două tipuri de celule adipoase – adipocitele unilocculare din țesutul adipos alb (WAT) și multilocculare în țesutul adipos brun (BAT). Acumularea WAT în depozitele fiziologice conduce la obezitate cu consecințele metabolice și cardiovasculare cunoscute. S-a dovedit recent că BAT poate fi activ metabolic și la subiecții umani adulți, nu doar la nou-născuți. Studiile imagistice au arătat că există două varietăți de BAT: constitutiv și celule din depozitele WAT ce pot fi activate – adipocite bej, care formează BAT inductibil. Activarea termogenezei în BAT este reglatorul principal al balanței între aportul (EI) și consumul de energie (EE). Strategiile non-farmacologice bazate pe reducerea EI sau creșterea EE s-au dovedit ineficiente pentru tratamentul obezității și bolilor asociate deoarece mulți dintre subiecți manifestă o aderență redusă. De aceea, interesul științific pentru BAT este resuscitat cu scopul de a dezvolta noi strategii terapeutice care țintesc activarea acestuia. În acest articol sunt revizuite date recente referitoare la potențiala eficiență a unor factori de dietă și agenți farmacologici în tratamentul obezității prin activarea BAT sau "brunificarea" WAT.

Keywords: obesity, therapeutic target, BAT activation, WAT browning

Introduction

Obesity and its related diseases represent a significant health problem with major social and economic consequences. Weight gain and metabolic syndrome not only decrease dramatically the quality of life, but considerably increase the costs for medical care [19]. At present, fat is considered a huge adipose organ composed of at least two types of adipose cells – uni-locular adipocytes for the white adipose tissue (WAT) and multilocular in brown adipose tissue (BAT). Accumulation of WAT in physiological depots leads to obesity. An imbalance of adipocytes secretion and the crosstalk between adipocytes and non-adipocytic elements from the adipose depots had distinguished the role of various types of cells in obesity. Recently, it has been proved that BAT is metabolically active not only in new-borns but also in adult humans. Activation of BAT thermogenesis is the main controller of the balance between EI and EE. UCP-1 (uncoupling protein-1), which is uniquely present in BAT, provides it with the ability to transform in heat the stores of fatty acids. Therefore, scientific interest for BAT is revived in order to develop new therapeutic strategies targeting adipose tissue metabolism alterations. We review research data regarding the plasticity of the adipose tissue and recent advances on the potential

benefit of some dietary factors and pharmacological agents acting as BAT activators or inducers of WAT browning as a strategy to treat obesity and its related diseases.

BAT in humans

The adipose organ is composed by two types of adipose depots mainly divided into adipose lobules of unilocular adipose tissue or WAT composed of unilocular cells and BAT formed by multilocular adipocytes. Both variety of cells are sustained by the non-adipocytic fraction vascularized, innervated and infiltrated with immune cells and preadipocytes [12, 20]. Recent studies reveal the presence of a third type of adipocytes with features of both WAT and BAT named "brite" (from brown-in-white) cells [21].

BAT so named because of its yellow–brown colour *in vivo*, is formed by cells distinguishable morphologically from WAT adipocytes by cytoplasmic multiple droplets of stored lipids while WAT cells contains a single large droplet. WAT is recognized as the site of fat storage, while BAT acts as a heat-generating tissue.

The dogma that BAT is not present in adult humans as it is in rodents because it dramatically reduces or disappear after infancy, is now contradicted by the discovery of human BAT using [18]FDG PET/CT (positron emission tomography with 2-deoxy-2-fluorine-18-fluoro-D-glucose/computed tomography). In adult humans, this method considered the "gold standard" to identify BAT [11] localized the BAT depots (named constitutive or canonical BAT) in a symmetrical cervical position and around the clavicles. The magnitude of [18]FDG uptake by BAT was reported to increase with the exposure to low temperature, to be higher in women than men, and to decrease with age and body fat mass [34, 65]. It is presumed that at least 30% of old adults and probably close to 100% of young adults possess BAT [46, 55].

Two types of BAT are present: the classical (cBAT) that are fully developed at birth and then reduced to remain only in the regions mentioned above and the inducible or recruitable BAT (rBAT) composed by a different type of isolated brown multilocular cells, "beige" or "brite" (because they are intricate into the WAT depots), resident between white cells mainly from the subcutaneous or visceral depots [42, 50] which cannot be detected by PET/CT.

BAT metabolism and physiology

Since the 1970s, BAT has been recognized as the main site of non-shivering thermogenesis in mammals, and also as the site of the so-called "diet-induced thermogenesis" [21]. Brown adipocytes harbour numerous mitochondria containing UCP-1, a 32 kDa mitochondrial membrane protein uniquely present in BAT, able to actively oxidize metabolic substrates and to uncouple ATP production from oxidative phosphorylation for the purpose of dissipating chemical energy as heat [43].

In response to cold or overfeeding-triggered activation of the sympathetic nervous system, the thermogenic activity of BAT is induced *via* distinct cellular processes. To generate heat for thermogenesis, BAT first uses its stored lipids as substrate. The early phase is controlled by norepinephrine released from the sympathetic nerves activating the release of free fatty acids from triglyceride droplets. Some of the fatty acids activate UCP-1. The remaining fatty acids are imported into the mitochondria and combusted, releasing energy as heat, due to UCP-1 action [22].

A lot of studies reconsider the importance of BAT as a metabolically active part of systemic energy homeostasis and thermoregulation as it responds to environmental stimuli such as cold (as it results in the most important controller of non-shivering thermogenesis) [5] and also to the increase of caloric intake "cafeteria diet" by a biologic process defined as "adaptative thermogenesis" or "diet induced thermogenesis" [6].

Any meal is followed by two types of thermogenesis - one necessary related to the direct handling of the food and the second, "diet induced thermogenesis", facultative, an extra-thermogenic component that reduces the amount of energy stored as fat in WAT [36] and consequently prevent obesity. Probably what matters in weight gain is each person thermogenic response to a meal. This may be considered one of the most attractive concepts concerning BAT as a therapeutic target in obesity.

BAT activation and WAT browning

BAT is now reconsidered as an alternative way to fight against obesity, targeting the consumption side of the balance food intake-energy expenditure. As morbid obesity is associated with low BAT activity [56], and more BAT promotes a metabolic health, improving glucose metabolism [35, 41], a huge scientific interest rises through enhancing BAT activity. Two possibilities are currently emphasized: stimulating BAT activation or WAT to BAT remodelling (browning of the white fat), defined as BAT induction.

BAT activation
Low temperatures activates the sympathetic nerves that innervate BAT and norepinephrine (NE) is released which stimulates brown fat cells [36].

The study of Ouellet *et al.* responds to another huge challenge demonstrating that BAT depots in human adults are metabolically active and it contributes to cold induced non-shivering thermogenesis [39]. This activation was associated with an increase in total EE. Wide interindividual differences in detectable BAT volume of activity (from 31 - 329 mL) suggest that unknown factors may modulate BAT volume and thermogenic capacity, in addition to age, sex, BMI and diabetes [39, 45].

Several hormones like catecholamines hyperrepressed in pheochromocytoma and thyroid hormones enhance the activity and the expansion of BAT by an adrenergic stimulation through β3-receptor, the sympathetic nervous system (SNS) being the most important regulator of BAT function [7]. Thyroid hormones are necessary for full BAT activation and development, the effect of hyperthyroidism of human BAT being emphasized by recent study using FDG PET/CT and calorimetry [28]. The thyroid hormones are mainly secreted as T4 which is converted to the more active T3 by deiodinase 2 (DIO2). DIO2 is induced in BAT by 6 days acclimation and its level increases upon 10x, representing a characteristic feature of activated BAT cells. Loss of DIO2 negatively affects the capacity of BAT thermogenesis [16, 30, 32].

Despite the excited positive aspects of BAT activation in mice and on metabolic homeostasis in obese subjects, we lack a method that directly measures the total BAT volume and BAT specific thermogenesis; moreover, some data argues against the real benefit of BAT human activation: the real contribution of BAT on resting metabolic rate is quite low 1% - 7% [10] and a fully stimulated BAT provides an energy expenditure of 100 Kcal/day [33]; BAT activation in humans is not easy, and a severe cold exposure may lead to a compensatory increase in appetite and food intake [8, 44]. When cBAT activation is scarce, an increased energy consumption seems to be provided by beige cells or rBAT, compensatory browning occurring in WAT.

BAT induction

A lot of studies focused on the main molecules implicated in BAT or brite differentiation. During embryogenesis cells from cBAT arise from a common progenitor that expresses myogenic transcription factors such as *myf 5* and gives rise both to BAT and skeletal muscle myocytes [49, 51]. The origin of brite cells is very controversial, either from the differentiation of pre-existent white adipocytes or the recruitment of different precursor cells resident in the stromo-vascular fraction [40]. Seale *et al*. demonstrated that cells that expressed *myf* 5 during embryogenesis could develop into BAT or muscle cells but never into cells found in WAT [49].

Available data from animal studies indicate that brite cells can be induced, similar to cBAT, in particular conditions in response to appropriate stimuli (cold, high caloric diet, β3 adrenergic agonists or thiazolidinediones) and that WAT browning cells can confer protection from obesity [52] expressing a high UCP-1 gene concentration and increased energy consumption similar to brown adipocytes. These brite/beige cells express some similar genes as cBAT (for example, peroxisome proliferator–activated receptor gamma coactivator 1 alpha, PGC-1α) but a lot of other genes are different from both BAT and WAT cells [61]. These controversial data suggest two possible origins

of the brite cells: they are arising from the trans-differentiation of existing mature white adipocytes, or from a distinct precursor population [13, 29, 61]. The heterogeneity of the bright cells from different depots (inguinal, perigonadal or interscapular) is an intriguing observation that sustains their different origin [47]. This suggests that the browning process depends of the local *milieu* such as vascularization, innervation (noradrenergic parenchymal nerves), ppO₂ and nutrients [13, 14, 61]. Wu *et al*. suggested that BAT and brite cells show different responsiveness to specific differentiating factors, such as BMP-7 for cBAT and irisin for brite cells induced by the browning of subcutaneous WAT [61]. Irisin (a polypeptide secreted by muscles and increased with exercise) has little effects on BAT isolated from the interscapular depot [4], so irisin could be a selective marker for differentiated beige/brite cells.

Wu *et al*. demonstrated that selective molecular markers, such as UCP-1, are markedly expressed in stimulated brite fat cells rather than classical BAT cells. This could explain why a relative low proportion of humans show PET-positive BAT fat depots unless activated with a brief cold exposure. These observations lead to the conclusion that in adult humans, where hypothermia is less frequent than in rodents, brite adipocytes, rather than cBAT, remain present in the adult stage [61].

First identified as a bone inducer, bone morphogenetic protein-7 (BMP-7), a member of the transforming growth factor β superfamily, is now recognized as a multifunctional cytokine and has been implicated as a potential therapeutic agent for cardiovascular, metabolic and degenerative diseases [53]. Tseng *et al*. emphasized the important role of BMP-7 in performing the full program of BAT function [54]. In brown preadipocytes, BMP-7 induces UCP-1 mRNA expression in the presence of PGC-1α. BMP-7 induces the expression of PRDM-16, a zinc-finger-binding protein, identified as an early regulator determining brown fat fate [48]. In cell cultures, BMP-7 treatment significantly increases the expression of genes involved in mitochondrial biogenesis and function, including PGC-1α responsible also for white to brown adipocytes transdifferentiation [1]. Fibroblast growth factor 21 (FGF21) is a protein secreted predominantly by the liver whose level rises during fasting, ketogenic diet and after aminoacid deprivation. Skeletal muscles and pancreatic β cells are also able to secrete FGF21 after different metabolic signals [17, 60]. Additionally, FGF21 seems to be secreted by BAT in an auto/paracrine manner after cold exposure, in response to adrenergic stimulation [30]. FGF21 increases the expression of thermogenic genes in BAT and regulates the browning of WAT through PGC1α signalling [23]. Very few and controversial data about FGF21 are reported in humans, circulating levels being increased in obese subjects as well as in mice [63]. Further

studies are necessary to understand the metabolic effect of FGF21 at the tissue level in overfeeding conditions.

Pharmacological and dietary approach to BAT activation and BAT induction

BAT activity may be increased by parallel strategies based on the enhancement of adrenergic stimulation. The most promising target might be the β3-adreno-receptor, which is expressed at high levels in BAT, WAT, gastrointestinal tract, prostate and bladder [25]. Signalling through β3-adrenoreceptors is the mechanism of action for some drugs used to treat various diseases but having an effect on fat storage and increasing the energy expenditure.

Mirabegron is a selective β3-adrenoreceptor agonist used for the treatment of overactive bladder [33]. Cypess *et al*. demonstrated that it acutely stimulates human BAT thermogenesis enhancing glucose uptake in both kinds of BAT: cBAT from the cervical and supra-clavicular depots and beige from supraclavicular, abdominal and other site depots [15]. Moreover, it increased the resting metabolic rate by 13%/day in humans. Despite the effect of activating BAT in healthy subjects, administration of mirabegron was accompanied by cardiovascular and muscular side effects mediated through β1 and/or β2-adrenoreceptors [10].

Several studies revealed that chronic treatment with thiazolidinediones, PPARγ synthetic ligands, can also induce the browning process. Animal models and *in vitro* studies demonstrated that PRDM16 is required for the development of TZD-inducible brown adipocytes in many subcutaneous depots [37, 42]. A recent study proved that long-term lobeglitazone treatment is important for ameliorating WAT inflammation by the decrease of M1 to M2 macrophages ratio [27]. Moreover, it is beneficial for WAT remodelling and induces the differentiation of brown and beige adipocytes. However, a similar WAT phenotypic change has not been proved in humans.

Treatment with liraglutide, the first incretin mimetic prescribed for weight loss in obese type 2 diabetic patients, revealed an increase in EE. Experimental studies in mice demonstrated that it stimulates BAT thermogenesis, WAT browning and reduces food intake [3, 59]. Further studies must be performed to explain if the weight loss in humans is also related to increased BAT activity and the imbalance in the incidence of pancreatitis [25] is an incretin mimetic-related side effect.

Sympathetic stimulation is the best-known activator of BAT and is necessary to develop sympathomimetic agents relatively specific for BAT but with less adverse effects on the cardiovascular system.

Over the last decades numerous research studies that dietary compounds can act as critical environmental factors for BAT activation and thermogenesis.

Several bioactive food ingredients: selective phyto-chemicals (capsaicin, menthol, resveratrol, curcumin, green tea, berberine, caffeine, theophylline, catechins, quercetin); dietary fatty acids (fish oil and conjugated linoleic acid); all-trans retinoic acid are efficient in thermogenesis through activation of SNS [38, 60].

Capsaicin, a compound found in hot red chili peppers, and menthol are known to act as agonists for the isoforms of the temperature receptors targeting the central regulation of BAT [60]. Capsaicin triggered BAT induction by promoting the expression of SIRT-1, UCP-1, BMP-8B, PPARγ and PGC1α in white adipocytes [2]. Menthol stimulates thermogenesis through a temperature receptor-TRPM8 expressed in the sympathetic and central nervous system, as well as in BAT cells. In mice, both capsaicin and menthol protect from obesity, the effect being attributed to their direct effect on BAT itself [60]. In humans, their action needs to be further investigated.

Investigating the effect of oral ingestion of capsinoids, found in nonpungent type of red pepper, in relation to BAT activation, Yoneshiro *et al*. proved that their ingestion resulted in a three-fold increase in EE [62]. The mechanisms involved in capsinoids activity on BAT include ADRB3 activation, acting as direct β3-agonists or having indirect effects *via* SNS activation. High doses of capsinoids facilitate cold-induced BAT recruitment and thermogenesis [38].

Research data regarding the effects of resveratrol, proved to have two mechanisms targeting obesity, being important for inguinal WAT browning [57] and also to supress adipogenesis in some depots, for example epididymal WAT [26].

Studies regarding the effects of curcumin in cell cultures and animal models proved that it is more efficient for WAT browning then for activating cBAT. Curcumin induces the expression of genes encoding several brown specific markers (UCP-1, PRDM16, PGC1α) in various cell cultures possible *via* AMP-activated protein kinase (AMPK) activation [31].

If the results regarding the efficiency of many compounds in humans are very scarce, for curcumin Di Pierro *et al*. reported its tolerability and efficacy to increase weight loss and reduce some anthropometric features of 44 overweight subjects treated for 30 days with a bioavailable form of curcumin complex [18].

Studies regarding the effect of berberine, a derivative from the group of benzylisoquinoline alkaloids extracted from various medicinal plants, such as *Berberis vulgaris*, emphasized that it improves glucose and lipid metabolism, inhibits lipogenesis and prevents WAT accumulation in several different rodent models [24, 58]. Recently, it was found that berberine has a role in adaptative thermogenesis both by BAT activation and by white to brown adipose tissue conversion. Zhang *et al*. showed that administration of 5 mg/kg bw of berberine in *db/db* mice increased EE by 20%

because it is able to increase mitochondrial biogenesis and the expression of main thermogenic markers in a depot-dependent manner, inguinal WAT depots being the most responsive [64]. Berberine can lead to mitochondrial biogenesis and trigger thermogenic responses *via* AMPK/PGC1α signalling [24].

Conclusions

A lot of studies showed that even in humans BAT has an anti-obesity role being involved in the reduction of metabolic imbalance. Numerous dietary and pharmacological factors act either through cBAT activation or WAT browning and some of them through both mechanisms. These encourage toward a personalized medicine approach which provides a realistic base for the development of more efficient therapeutic anti-obesity strategies with less side effects.

Conflict of interest

The authors declare no conflict of interest.

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