



The role of brown and beige adipose tissue in glycaemic control

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ARTICLE INFO

Keywords

Brown adipose tissue
Glucose homeostasis
Lipid homeostasis
Clinical and basic science research
Antiobesity therapies
Batotkines

ABSTRACT

For the past decade, brown adipose tissue (BAT) has been extensively studied as a potential therapy for obesity and metabolic diseases due to its thermogenic and glucose-consuming properties. It is now clear that the function of BAT goes beyond heat production, as it also plays an important endocrine role by secreting the so-called batokines to communicate with other metabolic tissues and regulate systemic energy homeostasis. However, despite numerous studies showing the benefits of BAT in rodents, it is still not clear whether recruitment of BAT can be utilized to treat human patients. Here, we review the advances on understanding the role of BAT in metabolism and its benefits on glucose and lipid homeostasis in both humans and rodents. Moreover, we discuss the latest methodological approaches to assess the contribution of BAT to human metabolism as well as the possibility to target BAT, pharmacologically or by lifestyle adaptations, to treat metabolic disorders.

1. Introduction

At the early discovery stages, brown adipose tissue (BAT) was mainly appreciated for non-shivering thermogenesis, which is essential to defend body temperature from cold in hibernating animals and newborn humans (Cannon and Nedergaard, 2004). As BAT rapidly declines with aging, it was previously thought that it does not play a physiological role in human adults. However, in 2009 it was discovered that adult humans retain functional BAT, which negatively correlates with BMI (Cypess et al., 2009; Saito et al., 2009; van Marken Licht-enbelt et al., 2009; Virtanen et al., 2009). Once active, BAT consumes a great deal of energy substrates and by doing so it contributes to systemic clearance of glucose and lipids from the circulation. Therefore, during the past decade, extensive research has aimed to understand the importance and feasibility of BAT activation as a therapeutic target against obesity and metabolic syndrome.

Classic BAT in humans is a small organ of about 130–170 g (Boon et al., 2017) which is anatomically localized mainly in the supraclavicular area, but also along the spinal cord as a paravertebral depot, and in the mediastinum, particularly in the para-aortic area (van der Lans et al., 2014). In addition, BAT has been found in the infradiaphragmatic depots, particularly in the perirenal area, but this depot is generally smaller than the posterior depots (van der Lans et al., 2014). The distribution pattern of BAT in humans differs to that observed in rodents, where it can be predominantly found in the intrascapular area. BAT is a highly plastic tissue in terms of mass expandability and glucose uptake capacity, and it primarily responds to cold exposure and

sympathetic nervous systems stimulation to prevent hypothermia, hence the term thermogenic BAT (Nedergaard et al., 2007). BAT regulation of systemic glucose and lipids has important therapeutic implications: exposure to cold can reverse glucose intolerance and insulin resistance in mouse models of diet-induced obesity (Bartelt et al., 2011; Stanford et al., 2013). PET-CT studies in humans confirmed high uptake of glucose by BAT (Betz and Enerback, 2015), and found inversed correlation between BAT volume and glucose and HbA1c levels (Lee et al., 2010; Matsushita et al., 2014). Moreover, activation of BAT by cold increases insulin sensitivity in UCP1-positive human subjects, supporting its antidiabetic potential (Chondronikola et al., 2014). BAT also has strong lipid-lowering properties due to the actions of lipoprotein lipase (LPL) and CD36 (Bartelt et al., 2011), which cleave triglyceride-rich lipoproteins from the circulation to enable uptake of free fatty acids by BAT (Khedoe et al., 2015). Activation of BAT by selective $\beta 3$ agonist CL-316,243 protected hyperlipidaemic mice from atherosclerosis due to lowering effect of BAT on triglyceride and cholesterol levels (Berbee et al., 2015), which makes BAT a potent organ in the regulation of systemic glucose and lipid homeostasis.

Beige adipocytes are the inducible form of thermogenic fat cells that, upon stimulation, emerge within the depots of white adipose tissue (WAT), having a distinct localization in comparison to BAT (Petrovic et al., 2010; Wu et al., 2013). The formation of beige cells takes place in a response to a variety of external stimuli, such as chronic cold exposure, long-term treatment with peroxisome proliferator-activated receptor γ (PPAR γ) agonists, cancer cachexia and bariatric surgery,

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caloric restriction and exercise, in a process called beiging or browning (Frontini and Cinti, 2010; Hatori et al., 2012; Ohno et al., 2012; Vegiopoulos et al., 2017). Beige adipocytes are similar to brown adipocytes in that they possess multilocular lipid droplets and the capacity for UCP1-mediated thermogenesis (Kajimura et al., 2015). Even though beige cells intrinsically have lower capacity for thermogenesis in comparison to the classical BAT (Shabalina et al., 2013), they play an equally important role in glucose homeostasis and systemic energy expenditure (Kajimura et al., 2015). Despite the similarity at the cellular level, brown and beige adipocytes arise from different precursor cells. Classical brown adipocytes derive from myogenic factor 5 (Myf5)- and paired-box protein 7 (Pax7)-positive cells in the embryonic mesoderm (Inagaki et al., 2016; Seale et al., 2008), while beige cells seem to originate from multiple different Myf5-negative precursors (Harms and Seale, 2013; Wang and Seale, 2016). Recent experimental evidence show that molecular signatures of thermogenic fat cells in humans resemble rodent beige adipocytes rather than classical brown adipocytes (Sharp et al., 2012; Wu et al., 2012, 2013). However, other reports indicate that the composition of human BAT is more heterogeneous and complex (Cypess et al., 2013; Lidell et al., 2013). However, mitochondria of human and mouse origin seem to have a similar degree of oxidative potential and UCP1 uncoupling capacity (Porter et al., 2016).

In the present review we summarize the recent advances in our understanding of the role of BAT/Beige adipocytes in systemic glucose homeostasis, with emphasis on the signalling pathways involved in glucose clearance by these glucose-consuming cells, at baseline and upon activation. In addition, the role of BAT secreted hormones, named batokines and their role in glucose clearance by peripheral tissues, such as liver, skeletal muscle, pancreas and CNS will be discussed. Finally, we highlight possible pharmacological strategies and lifestyle adaptations in targeting BAT/beige activity to treat glucose intolerance and type 2 diabetes in humans.

2. Signalling pathways that regulate glucose uptake and utilization in brown and beige adipocytes

2.1. Glucose clearance via UCP1-dependent and UCP1-independent thermogenesis

The human body defends its temperature by a) shivering thermogenesis, primarily mediated by skeletal muscle and b) non-shivering thermogenesis, almost entirely fuelled by BAT (Haman and Blondin, 2017; Mineo et al., 2012; Thurlby and Trayhurn, 1980). In addition, BAT contributes to thermogenesis induced after food ingestion, called postprandial thermogenesis, however, this process is not exclusive for BAT (Kozak, 2010). Thermogenesis in BAT is mediated by uncoupling protein 1 or thermogenin (UCP1), which facilitates proton leakage across the inner mitochondrial membrane, leading to heat generation instead of the adenosine triphosphate (ATP) production (Fedorenko et al., 2012). The importance of BAT in thermoregulation was clearly demonstrated by exposing rats to cold (4°C) for 10 days, which resulted in sustained glucose utilization by the BAT in the absence of shivering (Nedergaard and Cannon, 1985). In addition, glucose uptake by the skeletal muscle remained unchanged, suggesting that thermogenesis-induced glucose clearance can be primarily attributed to BAT (Nedergaard and Cannon, 1985). This was also evident from studies that showed impaired BAT thermogenic capacity in mice (Albert et al., 2016) and humans (Lee et al., 2016) upon inhibition of glucose uptake.

Although the importance of mitochondrial UCP1 in BAT thermogenesis is unquestionable (Cannon and Nedergaard, 2004; Golozubova et al., 2001), studies in UCP1-deficient mouse models have revealed the existence of alternative/compensatory mechanisms of thermogenesis which promote glucose clearance. Creatine futile cycling acts as a compensatory mechanism for heat production in the absence of UCP1 by increasing energy expenditure both in BAT and in beige fat

(Kazak et al., 2015). This finding was also translated to human adipocytes *in vitro*, implicating relevance beyond murine genetic models (Kazak et al., 2015). Another pathway that controls UCP1-independent thermogenesis in beige cells was shown to be mediated by the sarco/endoplasmic reticulum Ca^{2+} -ATPase 2b (SERCA2b) and ryanodine receptor 2 (RyR2) (Ikeda et al., 2018; Ukropec et al., 2006). The activation of the SERCA2b pathway increased glycolytic capacity of beige adipocytes and improved glucose tolerance independent of the body weight loss (Ikeda et al., 2017).

2.2. Mechanisms of glucose uptake by brown and beige adipocytes

The uptake of glucose by BAT can be triggered by two major signalling pathways: a) the sympathetic route, induced primarily by cold stimulation and b) insulin signalling (Fig. 1). Sympathetic stimulation is mediated by the release of catecholamines epinephrine and norepinephrine that bind to β -adrenoreceptors widely expressed on brown adipocytes (Cannon and Nedergaard, 2004), thereby increasing the expression and translocation of glucose transporters GLUT1 and GLUT4 to plasma membrane (Townsend and Tseng, 2014). β 3-adrenergic receptor, the most abundant β -receptor in BAT, induces the uptake of glucose primarily via GLUT1 in an insulin-independent manner (Dallner et al., 2006). Adrenergic stimulation of BAT also triggers intracellular catabolic processes in order to provide fuel under conditions of required energy utilization, such as exercise. This occurs via the increase of intracellular cyclic nucleotide cAMP and activation of protein kinase A (PKA), resulting in the activation of lipolysis and glycolysis (Collins, 2011; Jeong et al., 2018) to provide a sufficient amount of substrates to support thermogenesis.

Insulin is a major proadipogenic factor in both BAT and white adipose tissue (Dimitriadis et al., 2011), which also contributes to glucose utilization by BAT. Due to its antilipolytic and anabolic effects (Fain and Rosenberg, 1972), insulin partially antagonizes the actions of sympathetic stimulation on BAT, which is also evident in chronic hyperinsulinemia, which leads to the reduction of expression of adrenergic receptors on the surface of white adipocytes (Rajan et al., 2016). However, catecholamines and insulin seem to have a synergistic effect on glucose utilization by BAT due to activation of distinct pathways. Insulin promotes glucose uptake in brown adipocytes via the phosphoinositide 3-kinase-phosphoinositide-dependent kinase-1-Akt (PI3K-PDK1-Akt) pathway, resulting in translocation of GLUT4 to plasma membrane (Huang and Czech, 2007; Zaid et al., 2008). On the other hand, adrenergic stimulation induces the transcription of glucose transporters mainly via the canonical cAMP pathway, while translocation to the cell surface occurs via a UCP1-independent mechanism which includes the activation of mechanistic target of rapamycin (mTOR) (Olsen et al., 2014) (Fig. 1). The importance of insulin as an endogenous stimulus of glucose uptake by BAT has also been confirmed in humans (Orava et al., 2011).

Studies performed in the mice lacking all β -adrenergic receptors (β -less mice) have shown severely impaired thermogenesis in the interscapular BAT in response to cold, and these mice, therefore, do not survive long-term exposure to low temperatures (4°C) (Bachman et al., 2002). However, exposure of these mice to a milder cold challenge (10°C for 20h) increased thermogenic capacity of subcutaneous adipose tissue, but not of epididymal depots (Ye et al., 2013). These findings suggest the existence of β -adrenergic-independent thermogenic and glucose-consuming pathways in the white fat depots. Recently, a type of highly glycolytic adipocyte was identified to form during prolonged exposure to cold in β -less mice (Chen et al., 2019). This type of adipocytes, called g-beige, is distinct in comparison to conventional beige fat with respect to developmental origin and regulation (Chen et al., 2019). The high glycolytic capacity of the g-beige fat is defined by a transcriptional program controlled by GABP α (Chen et al., 2019).

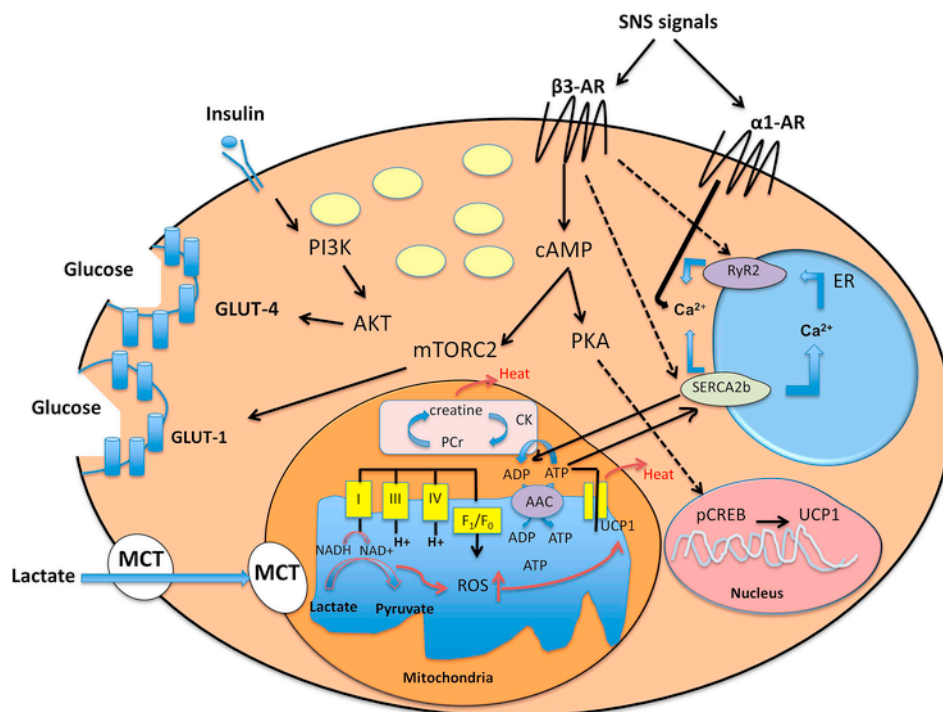


Figure 1. Major signalling pathways involved in glucose uptake and thermogenesis in brown or beige adipocytes. This model describes signalling pathways discussed in the text. Sympathetic stimulation triggers β_3 -AR-cAMP-PKA signalling, which activates transcription factor CREB and leads to increase of UCP1. Synergistic action of β_3 -AR and α_1 -AR induces calcium accumulation in the endoplasmic reticulum (ER), which subsequently leads to increased utilization of glycolysis-derived ATP. Glucose uptake is regulated by either insulin-dependent GLUT-4 or by β_3 -AR-mTORC2-GLUT-1 uptake. In addition, intermediate metabolites of glycolysis, such as lactate, drive thermogenesis when present in high intracellular amounts. Lactate is transported to the mitochondrion via monocarboxylate transporters (MCT). High levels of lactate lead to redox stress and accumulation of reactive oxygen species (ROS), which stimulates UCP1-mediated thermogenesis.

An additional endogenous mechanism which controls BAT and beige fat is realized through intermediate metabolites of glucose metabolism, such as lactate, which has been shown to promote browning of white adipocytes (Carriere et al., 2014). Mice and human white adipocytes express lactate transporters in the cell and in the mitochondrial membrane (Halestrap, 2013). High levels of lactate, which are induced in the white adipocytes by cold exposure and after prolonged exercise, are converted into pyruvate and NADH in mitochondria (thereby altering NADH-to-NAD⁺ ratio), and lead to an increase of UCP1 expression and activity (Carriere et al., 2014) (Fig. 1). The increase in UCP1 is considered to be a protective mechanism against redox stress under high lactate conditions (Jastroch et al., 2010). This mechanism is also consistent with the research showing that mitochondrial reactive oxygen species (ROS) production drives the uncoupling process (Chouchani et al., 2016). In line with this notion, metabolites other than lactate were found to regulate beige fat, such as the ketone body β -hydroxybutyrate, which is a strong inhibitor of browning (Carriere et al., 2014).

2.3. BAT-mediated endocrine signals involved in glycaemic control

BAT transplantation studies in disease models indicated that metabolic benefits exerted by BAT cannot be entirely attributed to its thermogenic function, and suggested that BAT regulates some of its effects on glucose homeostasis via its endocrine actions (Gunawardana and Piston, 2012; Stanford et al., 2013). Brown/beige adipocytes secrete signalling factors, termed batokines that can act in endocrine, paracrine and/or autocrine fashion. A majority of so far identified batokines are small peptides (Villarroya et al., 2017), however, BAT also secretes non-peptide signalling molecules, such as microRNAs and lipids (Thomou et al., 2017; Xiang et al., 2018). Many of BAT-secreted factors act locally to control the remodelling of BAT and beige fat (Klepac et al., 2016), however, BAT also acts on peripheral or-

gans, such as the liver, skeletal muscle, pancreas, bone, the immune system and the CNS, affecting systemic glucose levels in a thermogenesis-dependent and -independent manner. For an extensive review on batokines one can refer to Villarroya et al. (2017) and Scheideler et al. (2017). Here, we will focus on batokines with direct impact on glycaemic control and insulin sensitivity primarily in an endocrine fashion. The major batokines discussed here and their target organs are shown in Fig. 2.

2.3.1. Endocrine BATokines which promote glucose clearance via actions on peripheral tissues

2.3.1.1. Insulin-like growth factor 1 (IGF-1) The role of BAT-secreted IGF-1 in glycaemic control was suggested in a transplantation study, showing that subcutaneous transplantation of BAT corrects hyperglycaemia and insulin resistance in streptozotocin-treated mice, a mouse model of type 1 diabetes (Gunawardana and Piston, 2012). The positive changes in glucose levels were attributed to the increase of plasma IGF-1 levels, possibly secreted by BAT (Gunawardana and Piston, 2012). In addition, glucagon levels were lower in BAT recipients compared to controls, suggesting that in the absence of insulin the role of BAT in glucose homeostasis becomes even more important, as it may act directly on suppression of glucagon (Gunawardana and Piston, 2012).

2.3.1.2. Neuregulin 4 (NRG4) The link in the BAT-liver axis became apparent with the discovery of NRG4, a batokine that belongs to the epidermal growth factor (EGF) family of extracellular ligands and primarily targets the liver (Wang et al., 2014). NRG4 inhibits de-novo lipogenesis in liver by activating ERBB3/4 signalling, thereby protecting against hepatic steatosis and insulin resistance in diet-induced obesity (Wang et al., 2014). In accordance, NRG4 inversely correlates with obesity in humans, and reduced NRG4 in humans is associated with development of non-alcoholic fatty liver disease (NAFLD) and metabolic syndrome (Dai et al., 2015; Wang et al., 2014; Yan et al., 2018).

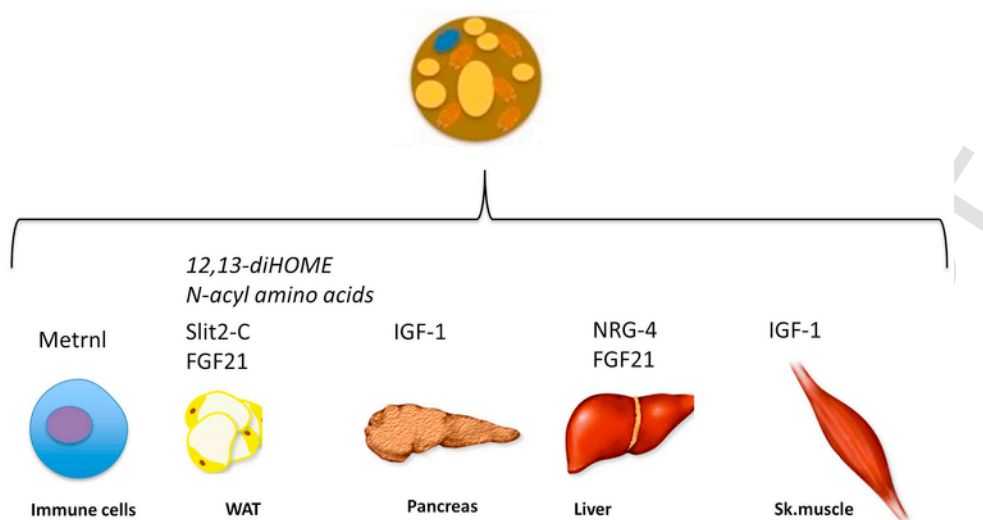


Fig. 2. Batokines with endocrine functions and their target tissues.

2.3.1.3. Slit2 Slit2 is an extracellular matrix glycoprotein of 180 kDa in size. A 50 kDa C-terminal fragment of Slit2 (Slit2-C) was recently discovered as a beige fat-secreted factor which is regulated by PRDM16 (Svensson et al., 2016). Slit2-C, but not Slit2-N, increased energy expenditure and improved glucose tolerance in obese mice via the cAMP/PKA pathway, without inducing changes in body weight (Kang et al., 2017; Svensson et al., 2016). In humans, serum SLIT-2 negatively correlates with fasting glucose, and its levels were found to be decreased in patients with type 2 diabetes (T2D) (Kang et al., 2017). The receptor for SLIT2-C remains unknown.

2.3.1.4. Fibroblast growth factor 21 (FGF21) FGF21 is a hormone mainly secreted by the liver, however, in stimulated conditions, BAT also becomes a major source of circulating FGF21 (Hondares et al., 2011; Markan et al., 2014). FGF21 stimulates thermogenesis in BAT and beige fat both in an autocrine and paracrine fashion (Villarroya et al., 2017), which leads to an increase in energy expenditure and weight loss in mice (Giralt et al., 2015). Moreover, FGF21 reduces hyperglycaemia and hyperlipidaemia in obesity and T2D (Giralt et al., 2015) in an UCP1-dependent manner (Kwon et al., 2015). FGF21 is also secreted in humans exposed to cold and can induce the thermogenic program in human adipocytes *in vitro* (Wu et al., 2013). Moreover, there is evidence that FGF21 activates BAT indirectly by targeting CNS (Douris et al., 2015; Owen et al., 2014), underlining a complex role of FGF21 in metabolism, which includes an interplay between liver, brain and BAT to regulate energy homeostasis.

2.3.1.5. Interleukin-6 (IL-6) Although initially discovered as a proinflammatory cytokine, IL-6 is now also recognized as an insulin-sensitizing factor released by the muscle after exercise (Ikeda et al., 2016). In macrophages, IL-6 promotes the M2 phenotype by augmenting the action of IL-4, which leads to increased insulin sensitivity (Mauer et al., 2014). Lack of IL-6 diminishes the beneficial effects of BAT transplantation, suggesting that this cytokine is an important mediator of the BAT-supported euglycemia (Stanford et al., 2013). Furthermore, IL-6 is required for browning of WAT upon cold exposure, as this process was impaired in IL-6-null mice (Knudsen et al., 2014).

2.3.1.6. Meteorin-like protein (Metrnl) Metrnl is a PGC-1 α -regulated hormone and its expression in BAT increases upon cold stimulation. Elevated levels of circulating Metrnl were shown to increase energy expenditure and improve glucose tolerance in mice. In addition, Metrnl induces the expression of browning genes in WAT as well as the expression of anti-inflammatory cytokines (Rao et al., 2014). Browning seems to be not a direct effect of Metrnl on white adipocytes, but might rather involve the action of alternatively activated macrophages (Rao et al., 2014). It is important to note here, however, that a recent re-

port raises doubt over the notion of macrophage induced-thermogenesis (Fischer et al., 2017).

2.3.1.7. 12,13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME) 12,13-diHOME is a newly identified bioactive lipokine secreted by BAT upon physical exercise, as surgical removal of BAT diminished the increase of circulating 12,13-diHOME (Stanford et al., 2018). In addition, 12,13-diHOME levels were found to be increased in BAT during cold exposure in mice and humans (Lynes et al., 2017). Injections of 12,13-diHOME increased BAT activity and promoted triglycerides uptake by BAT without any effects on glucose uptake or glucose tolerance (Lynes et al., 2017; Stanford et al., 2018). 12,13-diHOME has, at least partially, an autocrine and/or paracrine action on brown or beige adipocytes. However, specific receptors or uptake systems for this lipokine still need to be identified. Due to specific actions on triglycerides, 12,13-diHOME may therapeutically address hypertriglyceridemia in patients with metabolic syndrome and T2D.

2.3.1.8. N-acyl amino acids N-acyl amino acids are lipidated metabolites that directly bind to mitochondria and act as endogenous uncouplers independently of UCP1 (Long et al., 2016). The enzyme which regulates the production of N-acyl amino acids, PM20D1, is enriched in UCP1-positive beige adipocytes in comparison to the UCP1-negative white adipocytes (Long et al., 2016). Due to low levels of circulating N-acyl amino acids, a paracrine-autocrine function of those metabolites is more likely than an endocrine function. Interestingly, authors suggested that N-acyl amino acids might serve as a mechanism to activate thermogenesis in the UCP1-negative adipocytes by the neighbouring UCP1-positive cells (Long et al., 2016). Administration of N-acyl amino acids or PM20D1 in mice increased energy expenditure, beige cell formation, reduced body weight and improved glucose tolerance (Long et al., 2016).

3. BAT regulation of energy homeostasis and therapeutic potential in humans

3.1. The relevance of human BAT in whole-body metabolism

Current therapies to treat obesity and diabetes are not sufficient to stop the worldwide spread of these diseases, which have become an immense burden to modern society (Misra and Khurana, 2008; Swinburn et al., 2011). The approved weight-reducing drugs are inducing only a mild body weight loss, and the most effective treatment, bariatric surgery, includes an extensive surgical procedure not feasible for larger population (Arterburn and Courcoulas, 2014; Melnikova and Wages, 2006). Moreover, significant breakthrough in diabetic therapies has not occurred in years, making metformin the most com-

only prescribed antidiabetic for decades (Rojas and Gomes, 2013). Rational design of drugs to target multiple signalling pathways has shown promise as future approach to treat metabolic disorders (Clemmensen et al., 2019; Finan et al., 2014). However, new therapies are urgently needed, and the discovery of active BAT in human adults has sparked a great interest in exploiting its therapeutic potential in this context (Cypess and Kahn, 2010; Harms and Seale, 2013).

Similarly to the BAT found in rodents, human BAT is also responsive to cold and adrenergic stimulation, and contributes to the whole-body energy expenditure via UCP1-mediated thermogenesis (Chondronikola et al., 2014; Porter et al., 2016). Even mild changes in ambient temperature can recruit BAT in humans, which leads to an increase in insulin sensitivity, decrease in fat mass, and elicits positive changes on glucose metabolism (Chondronikola et al., 2014; Lee et al., 2014; Yoneshiro et al., 2013). In addition to the regulation of glucose, activation of BAT by short-term cold stimulation was shown to increase the expression of genes involved in lipid metabolism and enhance the mobilization of lipids from periphery in overweight and obese men (Chondronikola et al., 2016). We summarized the major findings addressing the role of BAT in systemic homeostasis in Table 1.

Despite cold being the most commonly used stimulus to demonstrate the activity of BAT in human patients, reports are emerging for other physiological regulators. In response to insulin stimulation, the uptake of glucose by BAT was comparable to the uptake of glucose by skeletal muscle in healthy subjects (Orava et al., 2011). Moreover, endogenous signalling molecules nucleoside adenosine and bile acid chenodeoxycholic acid (CDCA) were both shown to increase the metabolic activity of human BAT (Broeders et al., 2015; Lahesmaa et al., 2018), confirming the findings in rodents (Gnad et al., 2014; Teodoro et al., 2014). The differences in physiology of human and rodent BAT were underlined in a study demonstrating the upregulation of UCP1 by acute application of glucocorticoids in lean, healthy volunteers, which is in contrast to the effects observed in murine brown adipocytes (Ramage et al., 2016) and calls for caution when translating the findings from rodent models to clinics.

Although still little is known in regard to the regulation of the human BAT and the molecular mechanisms that govern its biology, these findings demonstrate that BAT plays an important role in both glucose and lipid homeostasis, emphasizing the need to expand our understanding of its physiology in humans.

3.2. BAT/beige diagnostics – imaging techniques and predictive markers

The activity of BAT in human subjects was first demonstrated and is still most commonly measured by positron emission tomography in combination with computed tomography (PET-CT) imaging, which is based on uptake of radioactive tracers, e.g. glucose or fatty acids, into (stimulated) BAT (Cohade et al., 2003; Hany et al., 2002). In addition to the anatomical localization, these studies revealed that BAT in adult humans is a metabolically active by demonstrating considerable uptake of glucose and nonesterified fatty acids in stimulated conditions (Ouellet et al., 2012). Moreover, imaging studies showed that its activity and volume decrease in obesity and diabetes as well as with aging (Betz and Enerback, 2015), which led to the important question whether BAT recruitment and increase in volume might reverse the negative effects of metabolic diseases.

Despite several reports estimating the contribution of BAT to human metabolism, with the most optimistic numbers mounting up to >520 kcal/d in stimulated conditions (Carpentier et al., 2018; Leitner et al., 2017), the extent of contribution of human BAT to energy homeostasis is still not clear. This lies, in part, in shortcomings of the methods that measure its activity. The majority of studies in humans rely on the glucose-based 2-deoxy-2-[fluorine-18]fluoro-D-glucose ([18F]FDG) tracer, which can lead to misinterpretation of its thermogenic and metabolic activity, as fatty acids released by intracellular lipolysis, rather than glucose, represent major fuel for BAT thermogene-

Table 1
Major discoveries from clinical studies that expanded our understanding of impact of BAT on metabolism and glucose homeostasis in humans.

Study	Major findings	Methodology
(Cypess et al., 2009; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009)	Demonstration of metabolically active BAT in human adults and negative correlation between BAT activity and BMI, old age and male sex.	[18F]FDG PET-CT, BAT induction by cold
Lee et al. (2010)	Inverse correlation between BAT and glucose levels. In addition, study suggests much higher prevalence of BAT in human adults than previously assessed, due to more reliable results obtained by repeated PET-CT scans.	[18F]FDG PET-CT at ~21 °C
Yoneshiro et al. (2011)	The study found an association between age-related reduction of BAT and increase of body fat in healthy subjects.	[18F]FDG-PET-CT at 19 °C for 2h
Orava et al. (2011)	Demonstration that human BAT is highly insulin-sensitive tissue.	[18F]FDG PET-CT
Ouellet et al. (2012)	Study reveals substantial uptake of glucose and nonesterified fatty acids by BAT in healthy men upon acute cold exposure, accompanied with increased whole-body energy expenditure.	PET-CT using [11C]acetate, [18F]FDG and [18F]FTHA tracers in cold-exposed subjects with minimal shivering
(Muzik et al., 2012, 2013)	Indication of low contribution of BAT to human metabolism (~20 kcal/day) upon moderate cold exposure.	[18F]FDG PET-CT, dynamic [15O]O ₂ PET imaging, indirect calorimetry
Orava et al. (2013)	Blunted response of BAT upon cold and insulin stimulation in obese subjects.	[18F]FDG PET-CT, indirect calorimetry
Yoneshiro et al. (2013)	Increase of BAT activity and thermogenesis, and reduction of body fat mass upon repeated cold exposure in healthy subjects with low BAT activity.	[18F]FDG PET-CT; 2h/day at 17 °C for 6 weeks
van der Lans et al. (2013)	Increased non-shivering thermogenesis and recruitment of BAT upon repeated cold exposure. Study also shows a shift in subjective experience of patients acclimatized to cold to more comfortable, indicating the feasibility to use cold exposure to recruit BAT in clinics.	[18F]FDG PET-CT 15–16 °C for 10 days
Lee et al. (2014)	Demonstration of human BAT acclimation and plasticity upon exposure to mild cold, accompanied by increased diet-induced thermogenesis and insulin sensitivity.	[18F]FDG PET-CT upon prolonged overnight exposure to 19 °C, indirect calorimetry
Chondronikola et al. (2014)	Demonstration of BAT regulation of systemic glucose: improved glucose metabolism (disposal and oxidation), insulin sensitivity and increased energy expenditure in male, healthy, BAT-positive subjects upon cold exposure.	[18F]FDG PET-CT at temperatures with minimal shivering, indirect calorimetry, assessment of glucose and fatty acids kinetics using stable isotopes, hyperinsulinemic-euglycemic clamp
Cypess et al. (2015)	First demonstration of BAT activation by a β 3 agonist in healthy male subjects; demonstration of BAT responsiveness to pharmacological adrenergic stimulation.	[18F]FDG PET-CT; BAT activation by mirabegron

Table 1 (Continued)

Study	Major findings	Methodology
Chondronikola et al. (2016)	Demonstration of BAT regulation of systemic lipid metabolism in humans: correlation between BAT volume and whole-body lipolysis, triglyceride-free fatty acid cycling and oxidation, and adipose tissue insulin sensitivity in overweight and obese men. Upregulation of genes involved in lipid metabolism in BAT upon cold stimulation.	[18F]FDG PET-CT upon exposure for 5–8 h to 19 °C, assessment of fatty acids kinetics and lipolysis using stable isotopes, hyperinsulinemic-euglycemic clamp, biopsies
u Din et al. (2016)	The study suggests only a minor role of BAT contribution to cold induced thermogenesis, which seems to be rather driven by muscle activity.	PET-CT using [15O]O ₂ , [15O]H ₂ O, and [18F]FTHA upon acute cold exposure, indirect calorimetry
Lee et al. (2016)	Demonstration of circadian rhythmicity of human BAT in healthy individuals, which is dependent of daily glucose fluctuations.	[18F]FDG PET-CT upon cold stimulation, indirect calorimetry, circadian glucose monitoring
Leitner et al. (2017)	Detailed mapping of human BAT distribution and volume in healthy and obese men suggests its higher metabolic capacity than previously estimated.	[18F]FDG PET-CT upon cold exposure
Blondin et al. (2017a)	Study shows the importance of intracellular triglyceride lipolysis to fuel BAT thermogenesis. Upon inhibition of BAT lipolysis, thermogenesis is predominantly supported by muscle action.	[11C]acetate and [18F]FDG PET-CT
Blondin et al. (2017b)	Study shows contribution of human BAT in clearance of dietary fatty acids upon acute cold stimulation. However, its contribution is minor in comparison to other metabolic tissues.	[18F]FTHA PET-CT in cold-exposed subjects
Weir et al. (2018)	Demonstration of BAT metabolic activity in human adults at “warm” temperatures, at which thermogenesis normally does not occur.	[18F]FDG PET-CT, microdialysis, indirect calorimetry

sis, especially under stimulated conditions (Blondin et al., 2017a; Labbe et al., 2015; Ma and Foster, 1986; Paulus et al., 2017). Moreover, the protocols used to stimulate BAT activity and subsequent estimation of the data are poorly standardized (Cypess et al., 2014). More recent reports, using refined detection methods or alternative tracers, suggest that the importance of BAT in whole-body metabolism might be underestimated (Leitner et al., 2017), but also overestimated (u Din et al., 2016) by previous studies. Leitner et al. postulates that human BAT has far greater potential and capacity to increase volume and activity than previously thought, based on calculations and estimations derived from individuals with high BAT activity (Leitner et al., 2017). On the other hand, combining [15O]O₂, [15O]H₂O and 14(R,S)-(18)F-fluoro-6-thia-heptadecanoic acid ([18F]FTHA) tracers, U Din et al. concluded that BAT contribution to the energy expenditure in cold-stimulated conditions is only minor in comparison to skeletal muscle, and adds up to 1% (u Din et al., 2016). Another study, which used [18F]FTHA in combination with PET, showed that the overall contribution of BAT to dietary fatty acids clearance in comparison to the other major metabolic organs, such as liver, skeletal muscle or WAT, is relatively small, amounting to ~0.3% (Blondin et al., 2017b), suggesting only a limited contribution of BAT in lipid metabolism in humans.

Several groups have explored the possibility of using blood biomarkers, such as miRNAs (miRNA92a, which inversely correlates with BAT activity in humans), or circulating lipids (12,13-diHOME and

LysoPC-acyl C16:0, which positively correlate with BAT activity), to measure the recruitment of BAT in humans (Boon et al., 2017; Chen et al., 2016; Lynes et al., 2017). However, the main difficulty in establishing reliable biomarkers lies in the identification of molecules that are selective for BAT (Chen et al., 2016; Nakhuda et al., 2016). Perhaps a combination of several biomarkers could provide a more accurate estimation of BAT activity; the increasing use of screening methods for BAT-secreted factors and metabolites will most certainly help to achieve this goal (Xiang et al., 2018).

Current experimental advancements in the measurement of human BAT activity include (less invasive) infrared thermography, magnetic resonance spectroscopy and MRI (Hu et al., 2013; Lee et al., 2011; Raiko et al., 2015). In addition, microdialysis has recently been developed as a quantitative method to measure BAT substrates utilization and intermediates release (Weir et al., 2018). The study employing this method demonstrated that BAT in adult humans is metabolically active at ambient (~25 °C) temperatures, at which thermogenesis normally does not occur (Weir et al., 2018). The high degree of glycolysis suggests that during “warm” conditions BAT takes up glucose to replenish its triglyceride stores (Weir et al., 2018). This is of interest, as most of the studies on relevance of BAT in humans have been performed in patients exposed to cold, with the prevalent opinion that BAT activity in humans is very low or non-existent under temperatures close to thermoneutrality.

Although the newly developed methods to detect BAT are still of limited use and not yet standardized in a clinical setting, our understanding of BAT significance in humans is rapidly advancing. Due to, in part, conflicting results in regard to contribution of BAT to whole-body metabolism in physiological and pathophysiological conditions its therapeutic potential is, however, still under debate. A combination of different tracers and improvement in methodology to measure BAT activity will help answer these open questions.

3.3. The prospect of pharmacological targeting of BAT in humans

The question whether BAT activation could be used as a long-term therapy for obesity and diabetes in humans is still not clear. The basic idea, to increase energy expenditure by uncoupling mitochondrial respiration is not new. Uncoupler 2,4-Dinitrophenol, which acts on a systemic level, was commonly used as a weight-loss agent in the early 20th century. However, due to toxicity and lethal side-effects such as hyperthermia, its clinical use has since been abandoned (Parascandola, 1974). Tissue-specific uncoupling by targeting BAT mitochondria might represent a more promising strategy to lose weight and the so-called “mild uncoupling” has been proposed as a potential strategy, although this concept is still under discussion and has been reviewed elsewhere (Shabalina and Nedergaard, 2011).

3.3.1. Adrenergic agonists

So far, the efforts to pharmacologically target BAT have mostly been focused on adrenergic system as the main regulator of BAT function. Application of non-selective sympathomimetics such as ephedrine failed to stimulate or had little effect on BAT activity with rather pronounced effects on the cardiovascular system (Carey et al., 2013; Cypess et al., 2012; Vosselman et al., 2012). Targeting β_3 -receptors is considered to be a more promising approach to selectively recruit BAT, due to high expression of these receptors on brown adipocytes (Ursino et al., 2009). However, early β_3 agonists had limitations in regard to bioavailability and selectivity, resulting in cardiovascular effects due to actions on β_1 -receptor (Arch, 2002). A study using a partial, but selective β_3 agonist CL-316,243 showed promising effects on metabolism, leading to increased glucose disposal and fat oxidation, and decreased plasma triglyceride levels (Weyer et al., 1998). Direct activation of BAT by a β_3 agonist was first demonstrated by Cypess et al., in 2015, showing that acute application of mirabegron

increases glucose uptake by BAT and resting metabolic rate in human subjects (Cypess et al., 2015). In addition, mirabegron was shown to induce browning of WAT in obese, insulin-resistant, and in older subjects (Finlin et al., 2018). The retained browning capacity indicates that BAT could be therapeutically exploited in these clinically relevant subjects, despite the reports that BAT volume reduces with age and metabolic diseases (Betz and Enerback, 2015). However, the prospect of long-term activation of BAT by pharmacotherapies directed at adrenergic receptors remains to be evaluated.

3.3.2. Non-adrenergic drugs with direct action on BAT

The identification of a number of paracrine and autocrine factors that modulate BAT activity in the recent years has shifted the focus from adrenergic system to exploring other means of BAT activation in clinics. Aforementioned endogenous ligands adenosine and CDCA target non-adrenergic A_{2A} and TGR5 receptors, but recruit BAT in a similar manner by increasing intracellular cAMP production (Broeders et al., 2015; Lahesmaa et al., 2018). Targeting of an alternative pathway to induce browning in humans, by increasing the activity of cGMP and mTOR pathway, was achieved by treatment with sildenafil, a drug widely used in clinics for treatment of erectile dysfunction (Li et al., 2018a). A 7-day application in overweight patients resulted in reduced adipocyte size in subcutaneous WAT with increased UCP1 and PGC1 α expression (Li et al., 2018a). Importantly, sildenafil was shown to have cardioprotective effects (Kukreja, 2013), which is of great importance for patients that suffer from obesity and metabolic syndrome, that are at increased risk of cardiovascular complications (Grundy, 2004).

3.3.3. Cold-mimetics and nutrients

The most effective mean to increase BAT activity without eliciting negative cardiovascular effects is cold, which drew attention to cold-mimicking substances to target BAT. Sensation of cold is mediated by transient receptor potential (TRP) channels, which leads to stimulation of SNS and BAT activation (Saito and Yoneshiro, 2013). These channels can also be activated by certain food ingredients, such as capsinoids, nonpungent analogues of capsaicin, which were found to increase BAT activity and energy expenditure in "high-BAT" subjects by binding to TRP vanilloid 1 (TRPV1) (Ang et al., 2017; Yoneshiro et al., 2012). Icilin and menthol also belong to the group of cold-mimicking substances, and were shown to induce browning via direct action on human white adipocytes by targeting TRP cation channel subfamily M member 8 (TRPM8) (Rossato et al., 2014). Moreover, icilin activates thermogenesis and reduces body weight in DIO mice through TRPM8-mediated induction of sympathetic tone (Clemmensen et al., 2018), however, clinical data for menthol and icilin are not yet available. Another group of nutrients speculated to exert their action on BAT via cold-sensing receptors are catechins, polyphenols abundantly found in tea (Kurogi et al., 2012), which, synergistically with caffeine, increased BAT density (Nirengi et al., 2016) and BAT-mediated energy expenditure (Yoneshiro et al., 2017). Due to the recent identification of a number of thermogenic nutrients, the idea to employ the so called "functional foods" has emerged with a goal to induce BAT-mediated thermogenesis and whole-body energy expenditure in humans (Nirengi et al., 2016).

3.3.4. Drugs that recruit BAT through central action

In addition to the cold-mimicking substances, liraglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist, was shown to induce BAT and browning through central action, mechanistically targeting hypothalamic AMPK (Beiroa et al., 2014). This indicates that drugs already available on the market for treatment of metabolic diseases possibly exert some of their beneficial effects on metabolism through the action on BAT and beige fat.

4. BAT contribution to glucose uptake in the context of human lifestyle

4.1. Circadian glucose uptake by BAT

In addition to the acute response to cold, BAT appears to consume glucose and produce heat in a circadian manner (Lee et al., 2016). Circadian thermogenesis may have been evolutionarily developed to preserve body temperature and prevent shivering thermogenesis in situations where cold and shortage of food coincide; in these conditions muscle activity could be used for food acquisition and survival rather than to preserve the core temperature. Therefore, BAT-glucose biorhythm may serve to provide BAT with energy substrate independent of nutrient-related hormonal signals and maintain the body temperature under conditions of nutrient scarcity. In accordance, humans with higher BAT mass were found to demonstrate less variation of blood glucose levels throughout the day and thus demonstrate a better glycaemic control.

4.2. Feeding and BAT/beige activation

Humans with higher BAT mass show increased postprandial utilization of carbohydrates after ingestion of a mixed meal high in carbohydrates, and have higher degree of energy expenditure (Vosselman et al., 2013). The evolutionary purpose of postprandial thermogenesis is not understood, however it has been speculated to be linked with the feeling of satiety after a meal. Mechanistically, this could be mediated by secretin, a gut hormone released after a meal which directly activates BAT and thermogenesis in a non-sympathetic manner, promoting satiety in the brain and glucose uptake by BAT (Li et al., 2018b). In combination with circadian thermogenic control and utilization of carbohydrates to sustain BAT thermogenesis, individuals with higher BAT mass may have an advantage in basal as well as postprandial glycaemic control.

Intermittent fasting is gaining in popularity as a natural way of weight control, which also improves glucose homeostasis. Several studies in rodents have shown that alternating days of feeding and fasting, or restricting the food consumption to 8 or less hours during the day (time-restricted feeding), without reducing caloric consumption, leads to improved metabolic health, including better glycaemic control (Longo and Mattson, 2014). Studies in rodents indicate that some of these effects might be mediated by increased activity of BAT and formation of beige fat, which leads to increased energy expenditure and weight loss (Kim et al., 2017; Li et al., 2017). Furthermore, time-restricted feeding was found to increase BAT UCP1 levels in mice (Hatori et al., 2012). Under these conditions, fasting rather than caloric restriction is thought to be the underlying reason of increased browning. In accordance, humans that lose weight by decreasing caloric intake, but without periods of prolonged fasting, did not show increased number of beige adipocytes in the subcutaneous fat depots (Barquissau et al., 2018). The effects of intermittent fasting on browning in humans remain to be evaluated.

4.3. Exercise

Exercise has been shown to induce the formation of beige adipocytes. Transplantation of subcutaneous adipose tissue of mice that underwent exercise improved glucose homeostasis, which did not occur by transplantation of subcutaneous fat from sedentary mice (Stanford et al., 2015). This seems to be mediated mainly through exercise-induced factors secreted by peripheral organs, such as skeletal muscle, adipose tissue and potentially the liver, that induce browning of WAT in an endocrine and/or paracrine manner. Some of these include: a) irisin, a PPAR γ coactivator-1 α (PGC1- α) dependent myokine (Bostrom et al., 2012), which has attracted a lot of attention as a browning agent, although the effects of irisin on browning are still under major

discussion (Perakakis et al., 2017). Numerous other factors that promote browning are also altered during or following exercise, such as interleukin-6 (Ma et al., 2015), B-aminoisobutyric acid (Roberts et al., 2014), meteorin-like (Rao et al., 2014), FGF21 (Giralt et al., 2015), natriuretic peptides (NP's) (Bordbar et al., 2012; Follenius and Brandenberger, 1988) and lactate (Carriere et al., 2014). However, many of these indices have only been studied in rodents, and functional effects on human browning remains to be proven.

5. Closing remarks

Overall, clinical studies that looked into the effects of pharmacological activation of BAT activation were predominantly short-term or acute, and were largely designed to demonstrate the activation of BAT without assessing the effects on whole body-metabolism (Table 1). The potential pitfalls in recruiting BAT as a sustained therapy for metabolic diseases could be desensitization by downregulation of target receptors and downstream signalling as suggested by Larsen et al. (2002). Therefore it is important to consider intracellular targets to induce the thermogenic program in BAT, such as PPAR γ (Loft et al., 2015), which can be targeted by thiazolidinediones, or substances that directly act on mitochondria to promote uncoupling. Another obstacle in increasing energy expenditure might be compensation by overfeeding (Cottle and Carlson, 1954; Ravussin et al., 2014), which might prevent any weight-loss induced by BAT thermogenesis. However, it is important to note that even without the reduction in adiposity, BAT activation might still elicit beneficial effects on metabolic health and glucose homeostasis that are independent of weight-loss. Future studies should reveal whether long-term pharmacological targeting of BAT is feasible to fight obesity and metabolic diseases.

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