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Brown Fat Fuel Utilization and Thermogenesis

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Abstract

Brown adipose tissue (BAT) dissipates energy as heat to maintain optimal thermogenesis and to contribute to energy expenditure, in rodents and possibly humans. The energetic processes executed by BAT require a readily available fuel supply, which includes glucose and fatty acids (FAs). FAs become available by cellular uptake, *de novo* lipogenesis, and from multilocular lipid droplets in brown adipocytes. BAT also possesses a great capacity for glucose uptake and metabolism, and an ability to regulate insulin sensitivity. These properties make BAT an appealing target for the treatment of obesity, diabetes and other metabolic disorders. Recent research has revealed a better understanding of the processes of fuel utilization carried out by brown adipocytes, which is the focus of the current review.

Keywords

Brown Adipose Tissue; Energy Expenditure; Uncoupling Protein 1

Significance of Brown Fat

The main function of brown adipose tissue (BAT) is to dissipate energy in the form of heat, a property driven by the presence of the mitochondrial protein uncoupling protein 1 (UCP1) that uncouples mitochondrial respiration. BAT is also densely innervated by the sympathetic nervous system (SNS), and highly vascularized[1]. The thermogenic capacity of BAT may be important for heat-production in newborns, essential for rodents and hibernating mammals, and possibly helps burn excess dietary energy consumption.

Imaging studies in humans have revealed that adults possess BAT depots around the neck, clavicle and spinal cord[2–8], that are metabolically active and able to take up and utilize glucose and FAs[9,10]. This observation has sparked interest in the possibility that human BAT manipulation might represent a target for obesity management. However, the amount of BAT and its level of activity vary greatly among people, with higher levels observed in younger, leaner people, or by season and cold-exposure[2–10]. Nevertheless, uptake and oxidation of glucose and FAs for heat production makes BAT an attractive target for the treatment of obesity, diabetes and other metabolic disorders (Fig. 1). In fact, two recent studies have demonstrated that cold acclimation in humans, after repeated daily cold exposure, results in an increase of BAT activity with a surge in energy expenditure[11,12].

In addition to classical BAT, brown adipocytes also can be induced to appear in white adipose tissue (WAT), through a process termed `browning'. These recruitable brown

adipocytes[13] (also known as beige[14] or brite[15] adipocytes) can appear after cold-exposure or after treatment with sympathetic mimetics such as the β3-adrenergic receptor agonist CL 316, 243, as well as other agents (for review see 16–18). The induction of browning may be a novel method for increasing whole body energy expenditure and combating obesity.

An important goal in the study of BAT biology is to better understand the mechanisms underlying the uptake and utilization of FAs and glucose in energy expending processes, including the role of fuel switching (Box 1). Enabling brown adipocytes to increase these rates of catabolism may lead to greater energy expenditure in order to combat obesity and diabetes. Here we will discuss recent findings related to fuel utilization and activation of classical and recruitable brown adipocytes, including uptake and utilization of glucose and FAs, as well as what is known about the regulation of these processes.

Glucose utilization by BAT

The use of positron emission tomography - computed tomography (PET-CT) imaging with the tracer fluorodeoxyglyucose (FDG) allows imaging of metabolically active BAT in humans that readily takes up glucose. Experiments in adult humans demonstrated that the rate of cold-activated glucose uptake exceeded that of insulin-stimulated glucose uptake in skeletal muscle. Specifically, glucose uptake after cold exposure was increased by 12-fold in BAT, and was correlated with an increase in whole body energy expenditure, while insulin stimulated glucose uptake in BAT increased by 5-fold[9]. Interestingly, gene expression of the glucose transporter GLUT4 was higher in BAT than white adipose tissue (WAT)[9] in these subjects, and in mice GLUT1 and GLUT4 are more highly expressed after cold exposure in BAT than in other tissues[19], further underscoring the importance of glucose for BAT function. Previous experiments using cold-exposed mice have also shown that many of the genes up-regulated in BAT are involved in glucose uptake and catabolism,[20] and activation of adrenergic signaling by cold exposure resulted in translocation of the glucose transporter GLUT1 and GLUT4 in the plasma membrane of brown adipocytes[21]. In obese, glucose-intolerant mice, cold exposure was able to normalize glucose tolerance and increased both glucose and FA uptake in BAT of lean and obese mice[19,22,23]. This increase in glucose uptake in BAT was greater than in brain, heart, liver, WAT and muscle combined[19,23]. Collectively, these data indicate that BAT may serve as an important glucose sink able to improve insulin sensitivity and glucose uptake after cold exposure[24,25].

BAT can also take up glucose in an insulin-independent matter. Using a class of selective partial agonists of the non-canonical hedgehog signaling pathway, Teperino et al. demonstrated that these compounds cause robust insulin-independent glucose uptake in BAT and skeletal muscle via activation of the Smo-AMPK axis[26], that involves the G protein-coupled receptor (GPCR or GPR) of the hedgehog pathway Smoothened (Smo), and AMP-activated protein kinase (AMPK), indicating that energy sensing by AMPK in BAT may regulate fuel utilization. Another hormone that might regulate BAT glucose uptake and thermogenesis is thyroid hormone. Thyroid hormone is converted from the low-activity form thyroxine (T4) to the active form 3, 3', 5-triiodothryonine (T3) by the enzymes type 1 and type 2 deiodinases (DIO1 and DIO2). BAT is a site of high expression of DIO2 and DIO2 knock-out mice have defects in both lipolysis, lipogenesis and adaptive thermogenesis, despite increased levels of UCP1[27,28]. Additionally, DIO2 knock-out mice are insulin resistant and susceptible to diet-induced obesity (DIO), perhaps a result of defects in BAT energy expenditure[29]. Together, these findings indicate there may be some insulin-independent pathways which can regulate BAT glucose uptake.

BAT mass and glucose disposal

Given the ability of BAT to take up glucose, one hypothesis is that increasing BAT mass in an individual may increase their glucose disposal. Indeed, a recent rodent study utilizing BAT transplantation from donor mice into the visceral cavity of recipient mice in an effort to increase BAT mass, demonstrated improved glucose tolerance, increased insulin sensitivity, lower body weight, and decreased fat mass[30]. Similarly, BAT transplantation from chow-fed donor mice into the visceral cavity of high-fat diet (HFD) recipient mice resulted in complete reversal of HFD-induced insulin resistance[30]. Likewise, in a separate study transplantation of BAT into the interscapular region was able to reverse diet-induced obesity and improve insulin sensitivity[31]. In another study, subcutaneous transplantation of embryonic BAT was also able to restore euglycemia in streotozotocin (STZ)-treated type 1 diabetic mice[32].

It is well established that administration of CL 316, 243 via subcutaneous miniosmotic pumps leads to an increase in BAT mass and increased basal and insulin-stimulated whole-body glucose disposal, without affecting body weight in non-obese rats[33], an effect mostly mediated by WAT and BAT, and not muscle. Collectively these data suggest that manipulation of BAT mass might be a powerful way of increasing glucose disposal.

FAs as Fuel for BAT

FAs fulfill a wide variety of roles in physiology (reviewed in[34] and[35]), including providing structural support in cell membranes, affecting the activities of certain transcription factors and activating GPCRs. After a meal, FAs and glucose are stored in the adipose as triglycerides (TG), and when energy is depleted, TGs are degraded to release FAs via the process of lipolysis. Dietary nutrients are also stored in liver, muscle and heart, but these are relatively short-lived as compared to fuel stored in adipocytes. Most cells are able to take up FAs secreted from adipose upon metabolic demand[36]. Upon internalization, FAs are esterified, to become available for β –oxidation, or reassembly for storage as inert TG via lipogenesis. Thus, the balance between adipose FAs and TGs is tightly regulated by the biochemical processes of lipolysis and lipogenesis. In the BAT of cold-exposed rodents, genes involved in glucose metabolism, lipogenesis, and uptake and catabolism of FAs are up-regulated as part of cold adaptation[20], and fatty acids are utilized for UCP1 activation (Box 2). It is currently unclear how brown adipocytes regulate *de novo* lipogenesis vs. FA uptake, but it has been reported that BAT undergoes more lipogenesis than WAT[37].

The high level of vascularization of BAT may also play a key role in fuel utilization. Indeed, PPARγ in the endothelium is able to regulate lipids, and may integrate vascular and metabolic responses[38]. Additionally, vascular endothelial growth factor B (VEGF-B) is able to control endothelial uptake of FA in heart and skeletal muscle, although the role for VEGF-B in the vasculature of BAT remains to be explored[39]. A newly identified transcription factor, TLE3, may act as a white and brown adipocyte switch, as it is able to interfere with PRDM16's interaction with PPARγ, in order to reduce fatty acid oxidation and thermogenesis[40]. Deletion of TLE3 has the opposite effect and is able to increase thermogenesis in subcutaneous adipose tissue[40].

Lipolysis and Lipogenesis in BAT

Lipolysis requires lipases including adipose triglyceride lipase (ATGL; which hydrolyzes TG to diacylglycerols and NEFAs), hormone sensitive lipase (HSL; which can hydrolyze a variety of acylesters including TG, diacylglycerols and monoacylglycerols), and monoglyceride lipase (MGL; which cleaves monoacylglycerols to NEFAs)[36] (Fig. 1). These processes occur intracellularly, whereas lipoprotein lipase (LPL) acts in extracellular/

vascular lipolysis (see below). Mice with a whole-body deletion of ATGL have lower energy expenditure, including reduced insulin-stimulated glucose disposal in BAT, but overall the mice are protected from DIO due to increased cardiac and liver glucose clearance, despite increased lipid content in these tissues[41]. However, these mice are sick and their condition closely mimics neutral lipid storage disease with myopathy[42]. Adipose-specific deletion of ATGL converts BAT to a WAT-like tissue, with impaired thermogenesis and lower expression of UCP1[43]. This study also clarified a mechanism for ATGL action, which involves AMPK-mediated phosphorylation to activate TG hydrolase activity[43]. Together, these studies indicate that ATGL's role in lipolysis is required to maintain a brown-like phenotype.

LPL is capable of chylomicron- and VLDL-TG lipolysis as well as cellular uptake of TG and other lipids[44], including the uptake of lipids into BAT[19]. LPL is highly expressed and secreted by adipose tissues[45]. Cold exposure results in a decrease in triglycerides and an increase in `good' HDL-cholesterol[19] in the circulation, although recent evidence indicates that while HDL cholesterol is considered beneficial, it may in fact increase cardiovascular risk[46]. Indeed, UCP1-dependent lipolysis induced by cold-exposure in mice genetically prone to atherosclerosis further promotes plaque growth and instability[47]. However, paradoxically the BAT-like perivascular adipose tissue is actually beneficial in preventing atherosclerosis[48]. Mice with a lack of perivascular adipose tissue, due to smooth muscle cell deletion of PPARγ, do not exhibit the inhibition of atherosclerosis by cold-exposure as was observed in wild-type mice, due to impaired lipid clearance in the mice lacking perivascular adipose[48].

Through a mechanism requiring the scavenger receptor cluster of differentiation 36 (CD36) and LPL, BAT is capable of taking up TG, and the overall rate of FA uptake by BAT during cold exposure is greater than that of skeletal muscle and requires LPL action[19]. Higher activity of LPL results in greater adiposity and insulin resistance[49]. Conversely, loss of LPL selectively from adipose tissue and not skeletal muscle resulted in an increase in lipogenesis in BAT and WAT. Despite previous findings showing that lipogenesis is important for BAT function, removal of LPL to prevent its lipolysis and promote *de novo* lipogenesis, did not activate BAT or induce browning of WAT in response to high fat diet or ADRB3 stimulation[50]. Adipocyte-specific deletion of LPL leads to an increase of de novo lipogenesis products, such as palmitoleate (C16n1–7)[50], however these mice are not protected from metabolic disease and do not undergo adipose tissue browning, thus increasing lipogenesis alone is not sufficient to induce browning.

LPL is transported across capillary endothelial cells by the GPI-anchored glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 (GPIHBP1; reviewed in[51,52], which can bind LPL and chylomicrons[53]. The movement of LPL and GPIHBP1 across endothelial cell membranes is bidirectional[51], and GPIHBP1 knockout mice display mislocalized LPL (including in brown adipose tissue)[54], decreased adipose tissue TG and defective lipolysis[55].

FAs consisting of 16 carbon atoms or less are synthesized by fatty acid synthase (FAS), but elongation of these FAs to very-long-chain-fatty acids (VLCFAs) is done via the elongation activities of very long-chain fatty acid enzymes (ELOVL). ELOVL3 is highly expressed in BAT after cold exposure[56], and mice lacking ELOVL3 are only able to survive the cold by shivering, as they lack the FA elongation activity needed to supply fuel for BAT thermogenesis, although the mice are able to eventually adapt and restore their elongation abilities in BAT[57]. Paradoxically, Elovl3−/− mice are resistant to DIO due to increased energy expenditure, not reduction of food intake. These mice also display reduced hepatic lipogenesis and triglyceride formation[58]. Conversely, adipose-specific ablation of FAS

resulted in increased energy expenditure and browning of subcutaneous WAT[59]. The pattern of FA composition is different in BAT versus WAT and between lean and obese mice[60], and the profile can be altered by transplantation of BAT from obese mice to lean mice, with the graft adopting the FA composition of the host[60]. Interestingly, loss of adipose LPL also changes the FA composition of both BAT and WAT, with a tendency for an increase in products of *de novo* lipogenesis and an accompanying increase in expression of genes for lipogenesis, as well as a decrease in polyunsaturated fatty acids (PUFAs)[61]. These findings are consistent with the role of LPL in releasing PUFAs from triglyceride-rich lipoproteins, which cannot be synthesized by the cells. Notably, one of the up-regulated FAs, palmitoleate (C16:1n-7), has been shown to have insulin-sensitizing and anti-inflammatory effects, thereby acting as a `lipokine', allowing adipose tissue to communicate with other tissues regarding metabolic status[6062]. Palmitoleate was identified in the serum of fatty acid binding proteins (FABP) −/− mice, and is released from adipocytes in response to physiological stimuli in order to communicate with liver and skeletal muscle[62]. Therefore, not only is the oxidation and storage of lipids influenced by BAT, but activation of BAT could also have beneficial effects on whole body lipid composition, independent of weight loss.

De novo lipogenesis in adipose tissue, producing palmitoleate, is thought to be beneficial, whereas excessive lipogenesis in liver is linked with metabolic disease[63]. In brown adipocytes, PPARα and carbohydrate response element binding protein (ChREBP), which has also been linked to liver lipogenesis, are part of a feedback loop involved in BAT lipogenesis[64]. Recently, PPARδ has also been implicated in liver *de novo* lipogenesis, producing a serum lipid (phosphatidylcholiune 18:0/18:1) which communicates with skeletal muscle to increase FA utilization, and in mice is regulated by diurnal changes in the activity of hepatic PPARδ[65]. Whether this new lipokine can communicate with fat is currently unknown.

Transporters and sensors

Sympathetic activation of BAT results in recruitment of enzymes needed for uptake, mobilization and oxidation of lipids, as well as turning on a thermogenic gene expression profile; these effects are synergistically enhanced by thyroid hormone[66]. Therefore, given the unwanted side effects of sympathomimetics in humans[67], identifying other pathways which can mimic these effects specifically in BAT may increase energy expenditure more safely. For instance, targeting FA sensing and transport into BAT may be an appealing option, along with a safer level of BAT activation.

FAs are essential for proper function and energy expenditure of brown adipocytes. FA uptake in adipocytes is mainly regulated by the fatty acid translocase CD36, as well as certain isoforms of fatty acid transport proteins (FATPs) such as FATP1 and FATP4 (reviewed in[34]), which bring FAs across the cell membrane (Fig. 2). Once inside the cell, FAs may either be stored as TG in lipid droplets or transported into the mitochondria for oxidation. CD36 variants have been associated with BMI in humans[68], and loss of CD36 function renders mice unable to survive the cold due to lack of availability of fats for combustion in brown fat thermogenesis[19]. Similarly, lack of the FATP member FATP1 leaves mice unable to defend body temperature due to lack of FA transport into brown adipocytes for thermogenesis[69]. CD36 may also act as a FA sensor as well as a transporter, which has been shown in taste buds of mice, where CD36 and GPR120 act together[70] in fat sensing.

GPCRs are widely regarded as the fat sensors of the cell. As a family, these seven-transmembrane receptors are found on various cell types and are activated by various ligands, including FAs. In adipose tissues, FA sensing GPR41, 43 and 120 have been identified, and are known to play roles in leptin secretion, lipolysis, adipogenesis and

glucose uptake via GLUT4 translocation (reviewed in[35]). This latter example provides a hint at the potential cross-talk between FA and glucose uptake in the adipocyte. However, much of this work has been done in white, and not brown, adipose tissues. Determining how FAs are sensed and transported in brown adipocytes will go a long way in elucidating the mechanisms of fuel utilization in BAT.

FABPs in adipocytes

FABPs are important for FA trafficking and act as lipid chaperones to transport lipids to certain cell compartments or outside of the cell. Brown adipose tissue expresses a cohort of FABPs that are also expressed in WAT or muscle, including FABP3 (or heart type; also expressed in muscle and other tissues) and FABP4 (or adipocyte type; also called aP2) (reviewed in[71]). After cold exposure all ten known FABP isoforms were assessed in BAT of rats, and it was found that FABPs 3–5 were all expressed in BAT of room-temperature and cold-exposed animals, with FABP4 being the most abundant and FABP3 having the highest (10-fold) induction by cold exposure[72]. FABP3 knock-out mice display reduced fatty acid uptake (reviewed in[71]) and FABP3 expression in BAT is increased in UCP1 knock-out mice and with diet induced obesity, both correlating with an increased demand for adaptive thermogenesis[73]., While FABP4 has been regarded as a marker for mature adipocytes, it has recently been identified as a marker for adipocyte progenitors in WAT and BAT, which reside in the stem cell niche and express known progenitor markers[74]. Importantly, this finding indicates that in addition to aP2-Cre expression in mature adipocytes, this driver also targets adipocyte progenitor cells, making it a useful genetic tool for investigation of adipogenesis.

FA oxidation in mitochondria

FAs are released in cells in part by the action of acyl-coA thioesterases (Acots) which hydrolyze fatty acyl-CoAs. Recently, Acot11 (also known as thioesterase superfamily 1, or Them1), which is highly expressed in BAT, has been shown to decrease energy expenditure and promote conservation of calories. In particular, mice lacking Them1 were protected against DIO and had increased FA oxidation in BAT, as well as improved whole-body glucose homeostasis[75]. Carnitine palmityl-transferase1 (CPT1) is the rate limiting enzyme in the carnitine shuttle (Fig. 2), which transports FAs into the mitochondria for oxidation. Mice lacking the muscle-form of CPT1 (mCPT1; which along with the liver form are expressed in BAT) die during cold exposure due to their inability to undergo thermogenesis, consistent with the expression of CPT1 in BAT and muscle[76]. In Sprague Dawley rats, BAT has the highest CPT1 activity and palmitate oxidation rate of the tissues examined[77]. In addition to FA coordination in the adipose tissue itself, lipolysis in WAT which releases FAs into circulation, as well as FA flux in the liver, are also important[78] but not necessary[79] for thermogenesis in rodents, as long as a sufficient supply of dietary nutrients is sustained. Fasted animals rely heavily/essentially on flux from WAT and liver to BAT and muscle for thermogenesis. Therefore, targeting FA oxidation in BAT mitochondria by increasing fuel availability or flux through mitochondrial pathways is another appealing method for increasing BAT energy expenditure.

FA activation of PPARs; lipid droplet proteins in BAT

In addition to FA oxidation in the mitochondria, FAs may also act as signaling molecules (reviewed in[80]), including the ability to act as ligands for the family of nuclear receptors, peroxisome proliferator-activated receptors (PPARs), which includes PPAR α, δ and γ. In response to adrenergic signaling activation, cyclic adenosine monophosphate (cAMP) activates protein kinase A (PKA) which in turn activates the co-activator cAMP response element binding protein (CREB), which increases transcription of genes such as peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) and UCP1. PKA also activates p38

MAPK signaling, which is known to regulate UCP1 and PGC1α expression[81]. The genes for UCP1 and PGC1α contain binding sites for PPARs, and together these genes are able to regulate FA oxidation and lipid storage. In parallel to this pathway, PKA also activates lipolysis via ATGL and HSL (summarized in[82]). PPAR γ activation in mice and humans initiates TG uptake into adipose tissue via LPL[83] without any significant changes in glucose or insulin. Treatment with the PPAR γ agonist rosiglitazone increases *de novo* lipogenesis in WAT without increasing glucose uptake, but it is unknown if the same effects would be observed in BAT[84]. Overall, PPAR γ interaction with FAs in BAT may provide an important link between fuel availability and energy expending processes.

Recently, it has been demonstrated that adrenergic activation of thermogenic genes in BAT requires lipolysis, and that increased levels of FAs activating PPAR α and δ were sufficient to increase FA oxidation[82]. Ligands for PPAR α and δ are created at the lipid droplet surface within minutes of stimulation[82]. The lipid droplet is an important storage component for TG in adipose tissues, shielding the other cell organelles from the potential lipotoxicity of FA, and providing a surface area that is able to be acted upon by water-soluble lipases in the cytosol. The lipid droplet also stores cholesterol esters, preventing cholesterol lipotoxicity. The surface of the lipid droplet is comprised of phospholipids and proteins (Fig. 2), which help regulate the process of lipolysis (reviewed in[85]), and many of which are now known to play a role in the phenotype of brown adipocytes, especially given that brown adipocytes contain more lipid droplet surface area due to their multilocularity. Lipid droplet proteins include the Plin family (such as Plin1, or perilipin), the Cide family (such as CIDEC or Fsp27), and several others, with the majority being more highly expressed in BAT than WAT, indicating an important role for lipid droplet access and utilization in brown adipocytes[86]. Nearly all the lipid-droplet proteins are up-regulated in cold-exposed subcutaneous white fat, which is known to readily undergo browning, but this was not seen in mesenteric white fat[86], which does not regularly undergo browning. Lipid droplet remodeling may represent a mechanism for the proposed process of transdifferentiation, whereby white adipocytes can directly transform into UCP1-positive brown adipocytes[86].

Cell death-inducing DNA fragmentation factor-α-like effector A (CIDEA) is a lipid droplet protein and an inhibitor of lipolysis that is also highly expressed in the mitochondria of BAT. CIDEA can directly interact with UCP1 and suppress its activity[87]. Mice lacking CIDEA exhibit higher energy expenditure, increased lipolysis in BAT, and are resistant to HFD-induced obesity[87]. Along these lines, CIDEA is down-regulated (mRNA and protein) after cold-exposure through a mechanism involving adrenergic signaling[88]. CIDEA is also expressed around lipid droplets in BAT, and its expression and promoter activity can be repressed by receptor-interacting protein 140 (RIP140) and induced by PGC1α[89]. A more recently described BAT lipid droplet protein in the CIDEA family is CIDEC or Fat-specific protein 27 (Fsp27). During adipogenesis, levels of Fsp27 are greatly increased, and it appears to act opposite of CIDEA by promoting lipid accumulation through interaction with perilipin[90], another well-described lipid droplet protein. Fsp27 is also positively correlated with obesity, and is upregulated in adipose and liver of *ob/ob* mice[91]. The Fsp27−/− mice have reduced adiposity and increased browning of WAT, with improved insulin sensitivity that may be attributed to both of these changes. Interestingly, Fsp27−/− mouse embryonic fibroblasts (MEFs) obtained from these mice also showed an increased likelihood to differentiate to brown adipocytes[91].

While deletion of Fsp27 leads to browning of WAT, overexpression of perilipin leads to the same effect[92], revealing a complex regulatory network of lipid droplet-associated proteins. Normally, perilipin acts to suppress lipolysis in the absence of PKA stimulation, and perilipin transgenic mice are resistant to a HFD[93], through a mechanism now known to involve browning of WAT, increased energy expenditure, and a reduction of Fsp27

expression in WAT[92]. Whether lipid droplet protein function differs between WAT and BAT remains to be determined, but it is likely that lipid droplet proteins in BAT play a role in regulating fuel availability and may be a target for increasing BAT energy expenditure.

Signaling Pathways Regulating BAT Fuel Utilization

In order for BAT to be effective at burning extra calories, it needs a readily available fuel supply, as well as activation of its energy-expending capacity for β-oxidation and thermogenesis. The classic method of activating BAT energy expenditure is via the SNS, and although this pathway and its synergistic activation of BAT with the thyroid hormone system are well characterized, recently several novel factors and signaling pathways (such as fibroblast growth factor 21 (FGF21), cardiac naturietic peptides, and bone morphogenetic proteins (BMPs)) have been described which can affect fuel availability, activation of energy expenditure in BAT, or both. These factors and pathways have been nicely reviewed elsewhere[17,94].

Given the importance of the SNS in innervating and activating BAT, it is no surprise that adrenergic signaling controls hydrolysis of TG and the regulation of the enzymatic machinery for lipolysis and fuel utilization[81]. Recently, the β1- isoform of the adrenergic receptor has been shown to be instrumental for this purpose[95], although the β3- isoform is the highest expressed in BAT and WAT of mice, with lesser expression in WAT, BAT and intestine of humans[96,97].

In humans, few factors or treatments aside from cold-exposure have been shown to directly activate BAT. Indeed, it has recently been shown that ephedrine activates the SNS without activating BAT[67], although others have reported that ephedrine, when used at a higher dosage, activates BAT in lean but not obese subjects[98]. Overall, adrenergic agonists used in humans represent several challenges, necessitating the development of sympathomimetics with fewer adverse side effects.

In mice, more factors have been identified which activate BAT. For example, BMP7 is able to activate mitochondrial activity of brown adipocytes in culture by a mechanism that involves SMAD and p38 signaling, and upregulation of FA transporters CPT1 and CD36[99]. In mice, systemic BMP7 treatment also acts to lower RER and increase energy expenditure[99]. FGF21 can directly increase PGC1α protein levels and thereby inducing the expression of thermogenic genes in BAT and WAT in an autocrine/paracrine manner[100,101]. Accordingly, FGF21 deficient mice display an impaired response to chronic cold challenges[101].

Concluding remarks and future perspectives

Given that obesity results from an energy imbalance leading to caloric overload, WAT must appropriately respond by storing excess energy in lipid droplets. The ability of adipose tissue to undertake this role, or the `adipose expandability' of a given person[102], may be a factor predisposing to the metabolic disturbances with obesity, which include ectopic lipid deposition in tissues such as skeletal muscle and liver, and leading to lipotoxicity. If agents can 1) specifically increase SNS activation of lipolysis in WAT to provide FA for thermogenesis and β -oxidation in BAT, 2) maximally activate the available BAT or 3) increase BAT mass, then perhaps the energy imbalance can be righted by increasing energy expenditure. As summarized in this review, given the ability of BAT to take up FA and glucose, it could help ameliorate the excess circulating levels of these fuels, thereby preventing adverse physiological effects.

As described above, while we are beginning to understand the responsiveness of activated BAT to affect insulin and glucose homeostasis as well as sensing and taking up lipids, a better understanding of these processes may provide novel opportunities for the development of therapies for obesity and metabolic disease. Additionally, the ability to target adipose thermogenesis specifically, without adverse effects in other tissues, either by developing specific adrenergic agonists acting only on adipose tissues or by utilizing other chemical uncouplers to mimic the actions of UCP1, would be important steps in increasing whole body energy expenditure. As described in Box 3, we do not yet understand the relative importance of glucose vs FAs as fuel for BAT, how WAT lipolysis regulates BAT fuel supply, or what role the SNS plays in regulating fuel availability to BAT. We also do not yet fully understand the complement of circulating factors coming from adipose and non-adipose tissues that may drive BAT energetics. Finally, the lipid droplet proteins making stored fuel available for catabolism are not fully identified or understood yet, and may provide additional clues for how to increase lipolysis and FA oxidation. Overall, the energy expending capacity of BAT is great, and targeting BAT catabolic processes is an important potential tool for reducing adiposity.

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BOX1: Fuel Switching

The exact metabolic controls and consequences for fuel switching from carbohydrate to lipid or vice versa are currently poorly understood. Measurement of respiratory exchange ratio (RER) is one indication of fuel switching, where RER is calculated as the ratio of VCO2/VO2, and RER closer to 1 indicates greater carbohydrate utilization and closer to 0.7 is greater fat utilization. One hope is that switching from glucose to greater lipid oxidation would result in increased energy expenditure and could be utilized as an obesity treatment. However, it is incorrect to believe that increasing substrate into the mitochondria alone would necessarily increase energy expenditure (reviewed in [103]). The ability of FAs to enter the mitochondria via the carnitine shuttle is essential for their availability to be oxidized, however they will not be utilized until the cell requires the energy. Metabolism of lipids requires the presence of ADP for this purpose. Additionally, NADH and $FADH_2$ feed the electron transport chain as products from both glucose *or* lipid metabolism, and therefore switching fuel source would not necessarily increase energy expenditure overall[103]. Therefore, in obesity where there is an overabundance of FAs as fuel from the diet and WAT lipolysis, the oxidation of these fats would need to be stimulated in addition to transportation of the fuel to the mitochondria, in order to raise energy expenditure. Chemical uncouplers which mimic the actions of activated UCP1 may be one way to achieve this[103], in addition to activation of UCP1 in existing brown fat depots, enabling more fuel oxidation for thermogenesis.

Fuel switching in humans occurs on a daily basis, with greater carbohydrate usage after eating, and greater utilization of lipids during fasting or sleep. During these changes, lipid flux into and out of the adipose tissue needs to be tightly regulated. The ability to make this switch, termed `metabolic flexibility', can be measured by RER and has been shown to be blunted in subjects with a family history of type 2 diabetes, which may be a factor predisposing to insulin resistance[104].

One factor that may regulate fuel switching in BAT is lipocalin prostaglandin D synthase (L-PGDS), which is capable of both synthesizing D-series prostaglandins as well as carrying lipophilic molecules[105]. L-PGDS KO mice exposed to cold had lower basal metabolic rates, despite the same thermogenic capacity. The KO mice also displayed lower lipid utilization and increased carbohydrate utilization and improved glucose tolerance on a high-fat diet, indicating that this synthase is important for fuel preference[105]. The flexibility of BAT in terms of fuel switching requires further investigation, but given the ability of BAT to utilize glucose and fatty acids as fuel, the tissue holds great promise for increasing metabolic flexibility.

BOX 2: Fatty Acid-Dependent and Independent Regulation of UCP1 Activity and Thermogenesis

UCP1 is a mitochondrial proton channel that is able to catalyze a proton leak, leading to uncoupling of oxidative phosphorylation from ATP production, and instead producing heat as a by-product. UCP1-activity is thought to involve activation by FAs[106,107], as well as inhibition by purine nucleotides, such as ATP or GDP[108]. Specifically, LCFAs released by lipolysis of BAT lipid droplets upon adrenergic stimulation, activate UCP1-mediated thermogenesis[107]. These mechanisms were largely unknown, but several hypotheses exist including: 1) allosteric binding of LCFAs to an H+ or OH-uniporter channel, 2) LCFAs binding to the pore of UCP1 and regulating its function, or 3) UCP1 as an LCFA anion carrier bringing them outside the mitochondria to bind protons, or 4) FAs induce a conformational change in UCP1 (summarized in[109]). A recent approach used patch-clamp to measure the UCP1 currents in the native inner mitochondrial membrane of BAT, and found that UCP1 has no constitutive activity (likely due to purine inhibition) until it is activated by LCFA generated within the inner mitochondrial membrane, which bind to the cytosolic side of UCP1[106]. There is also a difference in H+ transport by UCP1 depending on the pKa of the LCFA that is activating it, indicating that the LCFA anions directly bind and carry H+ as they are transported by UCP1, and that this LCFA activation overcomes the purine inhibition. Fuel substrate and uncoupling state regulate the state of BAT mitochondria, which involves pyruvate/malate coupling with GDP, while oleate and succinate promote uncoupling[108]. Related to the ability of fuel status to influence UCP1 activity, is the concept of diet-induced thermogenesis[110], or the increased energy expenditure conferred by intake of certain diets, which is lost after UCP1 ablation in mice housed at thermoneutrality[111]. In humans, however, a 24hr period of overfeeding was not found to activate BAT [112]. While the concept remains controversial [113], numerous studies report findings of diet-induced thermogenesis [95].

BOX 3: Outstanding Questions

Outstanding questions regarding the control of energy expenditure and fuel utilization in BAT

- What are the relative contribution and importance of glucose versus FAs as a fuel source for the energy expending processes of BAT, and is there a master `switch' that regulates changes in fuel supply in BAT?
- What role does the SNS play in the regulation of fuel supply, sensing or uptake in BAT? Do other cell types in BAT provide noradrenergic stimulation?
- How does BAT regulate de novo lipogenesis versus beta-oxidation?
- What physiological or pathophysiological states induce secretion of factors from non-adipose tissues (skeletal muscle, cardiac muscle, liver, gut, and other locations) that may regulate adipose tissue thermogenesis and fuel utilization?
- What is the complete milieu of lipid droplet-associated proteins and how do they act together or independently to regulate availability of stored lipid and overall cellular energetics?

Highlights

1. BAT is a very energy expending tissue, undergoing high levels of thermogenesis and β-oxidation.
2. Brown adipose tissue utilizes both glucose and fatty acids as fuel.
3. The regulation of these fuels occurs via availability, sensing, uptake and utilization.
4. All of the above may be potential targets for increasing BAT activation and combating obesity.

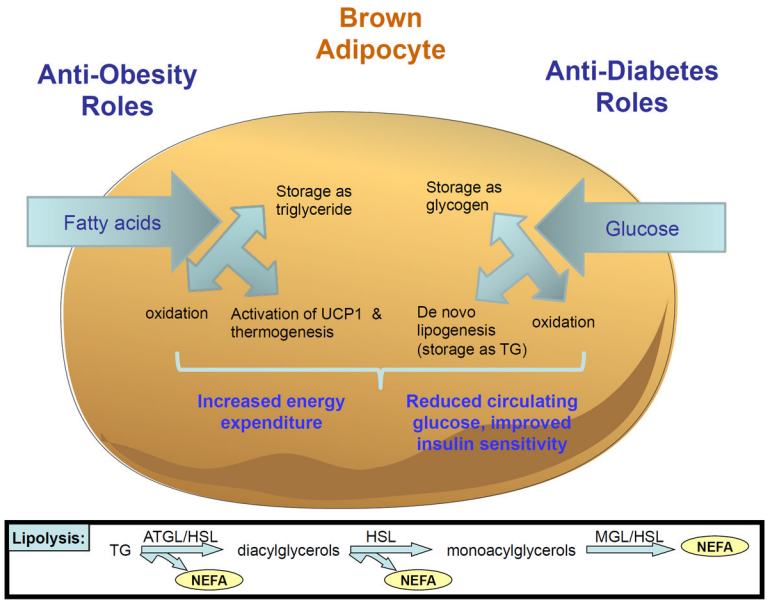

Figure 1. Glucose and FAs in BAT Combat Diabetes and Obesity

BAT holds great promise for combating metabolic diseases such as obesity and diabetes, in part through its ability to take up and oxidize (or store) FAs and glucose. By targeting glucose and fatty acid as fuels, BAT may be able to mitigate the weight gain caused by high sugar and high fat diets. Fatty acids may be stored as TG, oxidized, or utilized to activate thermogenesis via UCP1 (Box 2). Glucose may be oxidized, stored as glycogen, or it may undergo de novo lipogenesis to provide TG for storage. Pathways involved in lipolysis are presented in the lower box. (NEFA = non-esterified fatty acids; ATGL = adipose triglyceride lipase; HSL = hormone sensitive lipase; MGL = monoglyceride lipase)

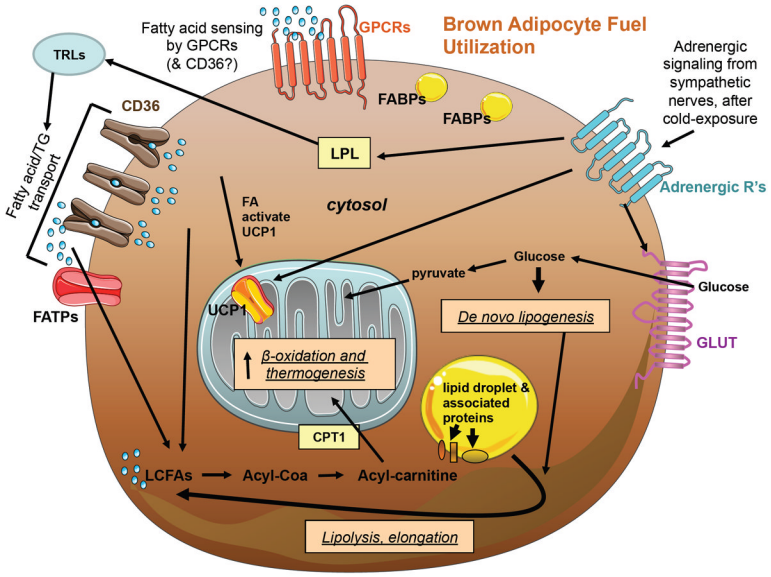

Figure 2. Fuel Utilization in Brown Adipocytes

The schematic shows a summary of FA sensing, uptake and oxidation pathways, as well as glucose uptake and downstream metabolism pathways in a typical brown adipocyte. Shown are glucose uptake by GLUT transporters, including GLUT translocation stimulated by adrenergic signaling, and the fate of glucose in *de novo* lipogenesis, storage as glycogen, or conversion to pyruvate and mitochondrial oxidation. FAs are sensed by GPCRs and possibly also CD36, and are taken up by CD36 and FATPs. LPL produced and secreted by adipocytes after adrenergic stimulation is also able to break down triglyceride-rich lipoproteins (TRLs), providing additional lipid fuel for uptake. FAs activate mitochondrial UCP1, as does activation from the sympathetic nervous system and adrenergic signaling, enabling energy expenditure via thermogenesis. FAs also become available from the lipolysis of lipid droplets (which is partially under regulation by lipid droplet-associated proteins). FAs may be elongated and/or converted to acyl-carnitine for transport into the mitochondria via the carnitine shuttle, where they become fuel for β-oxidation. FABPs also contribute to intracellular FA handling.