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Chapter

Brown Adipose Tissue Energy Metabolism

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Abstract

The brown adipose tissue (BAT) evolved as a specialized thermogenic organ in mammals. Nutrients (i.e., fatty acids and glucose) from the intracellular storage and peripheral tissues are critical to the BAT thermogenic function. The BAT converts the chemical energy stored in nutrients to thermo energy through UCP1-mediated nonshivering thermogenesis (NST). Activated BAT contributes significantly to the whole body energy substrate homeostasis. It is now well-recognized that adult humans possess BAT with functional thermoactivity. Thus, BAT energy metabolism has a significant therapeutic potential in the management of metabolic disorders, such as obesity, insulin resistance, type 2 diabetes, and lipid abnormality in humans.

Keywords: brown adipose tissue, brown adipocyte, metabolism, fatty acid, glucose, metabolic disorders

1. Introduction

Brown adipose tissue (BAT) evolved as a specialized thermogenic organ in the modern eutherian mammals, including *Homo sapiens* [1–7]. The main function of BAT is mediating adaptive thermogenesis or nonshivering thermogenesis (NST), when mammals are challenged in the cold environment. The NST function is a critical adaptation, which helps to maintain the homeothermy in mammals and gives them the evolutionary advantage to survive in the cold habitat, and to thrive from the Artic to the Antarctic region [3, 4]. During the cold challenge, BAT metabolizes nutrients (i.e., fatty acids and glucose) and converts the chemical energy to thermo energy through NST. In addition to its thermogenic function, activated BAT also contributes significantly to whole body energy substrate homeostasis. When activated, BAT presents physiologically significant benefit in fatty acids and glucose homeostasis as well as insulin sensitivity in mammals [8–24]. It is now well-recognized that adult humans possess BAT, which has functional thermoactivity [8–10, 25]. Cold challenge-activated BAT is detected mainly in the supraclavicular, paravertebral, and cervical regions in adult humans [9, 10, 25, 26]. Accumulating evidences indicate that BAT function is inversely associated with age, body mass index (BMI), and diabetic status in adult humans, which indicates that the activation of BAT has potential translational implication in the management of metabolic disorders, such as diabetes and obesity in humans [8–14, 16, 17, 21, 27].

Brown adipocyte, which is endowed with mitochondria, is the most important thermogenic functional unit of BAT [4, 28]. In the mitochondrion of brown

adipocyte, energy generated from nutrients is initially stored as proton gradient membrane potential across the mitochondrial inner membrane, and then converted directly to thermo energy by the uncoupling protein 1 (UCP1)-mediated proton flow [4, 29]. In BAT, brown adipocytes are surrounded by abundant capillaries. The heat generated in the brown adipocyte mitochondria can be quickly distributed by the blood flow to maintain the steady core body temperature in mammals [4]. In addition to classical brown adipocytes, beige/brite adipocytes can be induced from the white adipocytes to conduct thermogenesis upon the cold challenge or catecholamine stimulation [26, 30, 31]. This process is termed as "browning" [26, 30, 31]. Thermogenesis in beige/brite adipocytes can also contribute to mammals' body temperature maintenance and whole body metabolism [26, 30, 31]. Beige/brite adipose tissue generates heat through both UCP1-independent thermogenesis, including calcium cycling in and out of endoplasmic reticulum, futile cycle between creatine and phosphocreatine, and UCP1-dependent thermogenesis in mitochondria [26, 30–33]. The energy metabolism is essential for the optimal UCP1-mediated mitochondrial thermogenic function of brown and beige/brite adipocytes [4, 34, 35]. In this chapter, we discuss the importance of energy metabolism in maintaining brown adipocyte thermogenic function and the proceeding of targeting metabolic disorder through BAT activation in human studies.

2. Fatty acid metabolism in brown adipocytes

Thermogenic brown adipocyte possesses a high capacity for fatty acid β-oxidation that has been reported in both rodent and humans [4, 12, 14, 36]. Fatty acids serve as the activator for UCP1, which is a fatty acid/H^+ symporter directly mediating proton flow and thermogenesis [37]. Fatty acids also serve as the main energy substrate mediating the uncoupling and thermogenic function in brown adipocytes [2, 36–41]. In addition, fatty acids can enhance brown adipocyte thermogenic capacity through the nuclear receptor peroxisome proliferator-activated receptors (PPARs), which are the master transcription regulators for the expression of genes involved in lipid metabolism, oxidative phosphorylation, and the key thermogenic protein UCP1 in brown adipocytes [42, 43].

Intracellular fatty acids are stored in the format of triglyceride in the heterogeneous multilocular lipid droplets in the brown adipocyte [4]. Upon the cold challenge, triglycerides stored in the brown adipocyte lipid droplet are lipolysed. Triglyceride lipolysis is a sequential process that involves different enzymes, resulting in the liberation of glycerol and fatty acids for heat production [44]. The lipid droplet is composed of triglycerides and cholesterol esters, which are surrounded by a monolayer of phospholipids [44]. Important proteins with regulatory and enzymatic functions, including perilipin and CGI58, coexist on the phospholipid monolayer to regulate the lipid trafficking and other functions of the lipid droplet [44–47]. Perilipin stabilizes the lipid droplet and prevents it from lipolysis under basal condition. Upon cold challenge, β-adrenergic stimulation leads to the activation of G-protein-coupled receptor (GPCR) and adenylate cyclase, which subsequently increases the cAMP level in brown adipocyte [48]. cAMP then activates protein kinase A (PKA), which phosphorylates perilipin at its serine residues [49–52]. The phosphorylated perilipin releases CGI-58, an adipose triglyceride lipase (ATGL)-activating protein. CGI-58, subsequently, binds and actives ATGL. Activated ATGL hydrolyzes triglycerides and generates free fatty acids and diglycerides [50, 53–55]. Upon the cold challenge, PKA also phosphorylates serine residues on another key lipolysis enzyme hormone sensitive lipase (HSL) [56]. Although HSL is capable of hydrolyzing triglycerides, diglycerides, monoglycerides,

and a broad array of other lipid substrate, it is the rate-limiting enzyme for hydrolyzing the diglycerides *in vivo* [56]. The phosphorylated HSL catalyzes the diglycerides and generates free fatty acids and monoglycerides [56]. As the last step of lipolysis, monoglycerides is hydrolyzed by monoacylglycerol lipase (MGL) to form free fatty acids and glycerol [57].

Given the importance of fatty acid in brown adipocyte thermogenic function, it is reasonable to predict that the deficiency of the key lipolysis enzymes and fatty acid transportation proteins in brown adipocytes can lead to a defected BAT thermogenic function. Human and rodent *in vivo* studies using both pharmacological and genetic approaches to manipulate triglyceride lipolysis processes were reported [36, 39, 58–60]. In the initial studies, nicotinic acid (NiAc) was used to inhibit intracellular triglyceride lipolysis through acting on metabolite-sensing Gi-protein-coupled GPR109A and subsequent PKA activation [36, 58, 61]. A study in rats suggested the brown adipocyte intracellular lipid lipolysis played a major role in thermogenesis in rodents [36]. It showed that the intracellular triglyceride lipolysis contributes to 84% of thermogenesis during an acute cold challenge (10°C, 2–6 hours) and 74% of thermogenesis during a chronic cold challenge (10°C, 21 days) [36]. The importance of the brown adipocyte intracellular triglyceride-derived fatty acids was also reported in a human study [58]. In this study, administrating intracellular triglyceride lipolysis inhibitor NiAc significantly blunted BAT oxidative metabolism in cold-challenged young healthy humans (average 30 years of age with an average BMI of 24.5 kg/m^2). During a 3-hour cold challenge at 10°C, NiAc administration suppressed BAT intracellular triglyceride lipolysis by about 50% and BAT oxidative metabolism by 70%, despite of the increased blood flow in the BAT [58].

One caveat from both the aforementioned human and rodent *in vivo* studies is that NiAc-mediated lipolysis inhibition took effect in both brown and white adipocytes in addition to other tissues, which makes it hard to delineate the contribution of lipolysis from each cell type. An *in vitro* study nicely confirmed the importance of intracellular lipolysis in brown adipocytes [2]. In cultured primary mouse brown adipocytes, the inhibition of both of the key lipolysis enzymes adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) led to a 97% decrease in isopropanol-induced brown adipocyte respiration, indicating the imperative requirement of intracellular lipolysis in activated brown adipocytes [2]. These studies suggested that fatty acids liberated from the intracellular triglyceride storage serve as a critical fuel resource and contribute significantly to BAT thermogenesis in brown adipocytes upon activation.

Further studies in genetically manipulated mice showed similar results. The ATGL-knockout mouse presented defective triglyceride lipolysis function with increased BAT weight (8.5-fold), enlarged lipid droplet (20-fold), and decreased BAT explant lipid hydrolysis activity (−85%) [38]. The impaired triglyceride lipolysis activity in the *ATGL-knockout* mouse led to a defective thermogenic function. Upon a 5-hour 4°C challenge, mouse body temperature dropped to a critical low point at around 25°C [38]. Another study using mice with CGI-58 deficiency in both white and brown adipocytes also showed decreased BAT thermogenic function. The *adipose-CGI-58-knockout* mice were cold-sensitive, but only under fasted state [60]. The *HSL-null* mouse adipose tissue also presented defected triglyceride lipolysis function. Upon catecholamine-stimulation, *HSL-null* adipose tissue explants exhibited significantly reduced fatty acid and almost blunted glycerol release into the culture medium, parallel with diglycerides accumulation in both white and brown adipose tissue [56, 62]. However, it seems that HSL-mediated lipolysis function is not as critical as ATGL-mediated lipolysis function in brown adipocytes. An in vitro study showed that isopropanol-induced UCP1 activity was

largely dependent on ATGL function (80%) compared to HSL function (35%) in cultured primary brown adipocytes, and the combined inhibition of both ATGL and HSL functions led to an almost complete block of UCP1-mediated thermogenic function (97%) [2].

These studies highlight the cardinal role of intracellular triglyceride liberation in the brown adipocyte thermogenic function, but raise the question if the brown adipocyte intracellular lipolysis is essential for BAT to maintain the adaptive thermogenesis *in vivo*. Interestingly, recent studies using *UCP1-Cre*-mediated knockout of either ATGL or CGI-58 gene in mice showed an unexpected insignificant impact of brown adipocyte intracellular lipolysis and suggested that the circulating fatty acids mobilized from peripheral tissues may play more important roles in BAT thermogenesis in mice [59, 60]. These studies demonstrated that the loss of either ATGL or CGI58-mediated triglycerides lipolysis in brown adipocytes did not compromise the cold challenge-induced thermogenic response in mice [59, 60]. The phenotypic discrepancy between the systemic and BAT-specific *ATGL* and *CGI-58* knockout mice brings very important insights to *in vivo* BAT fatty acid metabolism, and indicates that the brown adipocyte intracellular lipolysis is not the only energy resource for *in vivo* adaptive thermogenesis during cold challenge. Although it is not clear about the exact partition of intracellular and circulating fatty acids contribution to the BAT thermogenic function, it is reasonable to speculate that the core body temperature maintenance is vital for mammals to maintain their optimal function during the cold challenge, when the intracellular lipolysis function is impaired or insufficient, circulating energy substrates from other metabolic tissues, that is, white adipose tissue and liver, are mobilized to provide energy substrates for BAT-mediated adaptive thermogenesis to maintain the adequate core body temperature and ensure the optimal functionality of mammals.

Long-chain fatty acids (LCFAs) are the most abundant format of fatty acid energy substrate in mammals [63–66]. The liberated LCFAs from intracellular lipid droplet are facilitated and transported to mitochondrion and nucleus by fatty acid-binding proteins (FABPs) to conduct their functions [67]. Of the six FABP isoforms, FABP4 (also termed adipocyte p2) and FABP5 are the major FABP isoforms in brown adipocytes [68–74]. Mice mutated in both *FABP4* and *FABP5* gene developed severe hypothermia during fasting after an acute cold challenge (1–3 hours), indicating that fatty acids transportation plays an indispensable role in BAT thermogenic function [74].

The LCFAs-mediated mitochondrial oxidation and UCP-1 activation function require sequential carnitine acyltransferases in order to translocate the LCFA into mitochondrial matrix. Carnitine palmitoyltransferase 1 (CPT1), located on the mitochondrial outer membrane, is the rate-limiting enzyme that mediates LCFAs inward translocation into mitochondrial matrix [63–66]. CPT1 exists in tissue-specific isoforms, and CPT1b is the major isoform expressed in brown adipocytes [75–77]. Mouse embryos-carried homozygous knockout of *Cpt1b* gene were lethal before embryonic day 9.5–11.5 and a normal percentage of $CPT1b^{+/-}$ mice was born from the $CPT1b^{+/-}$ and wild type breeding pairs [78]. However, more than 50% of the $CPT1b^{+/-}$ pups were lost before weaning [78]. The detailed experiment showed that ~7% $CPT1b^{+/-}$ mice developed fatal hypothermia following a 3-hour cold challenge (4°C) and ~52% $CPT1b^{+/-}$ mice developed fatal hypothermia following a 6-hour cold challenge (4°C), indicating the critical contribution of CPT1b-mediated LCFAs transportation and thermogenesis during the cold challenge in infant/young mice [78]. Carnitine palmitoyltransferase 2 (CPT2) is located on mitochondrial inner membrane to further medicate LCFAs translocation into mitochondrial matrix. In line with the study using the *CPT1b*-deficient mice, an *adipose-specific CPT2 knockout* mice also presented hypothermic phenotype after 3 hours cold

challenge (4°C) and decreased LCFAs oxidation in isolated MEF cells [39, 79]. These studies indicate that fatty acid transportation is critical for BAT thermogenic function during the cold challenge.

The intramitochondrial LCFAs contribute to the thermogenesis through UCP-1 activation and β-oxidation. A detailed study confirmed that mice with impaired fatty acid β-oxidation are cold-intolerant [80]. The acyl-CoA dehydrogenases, which catalyze the initial steps of fatty acid β-oxidation, are composed of a group of enzymes, including very long-chain acyl CoA dehydrogenase (VLCAD), long-chain acyl CoA dehydrogenase (LCAD), and short-chain acyl CoA dehydrogenase (SCAD) [81, 82]. A detailed experiment showed that fetal hypothermia was presented in all of the homozygous knockout mice ($VLCAD^{-/-}$, $LCAD^{-/-}$, and $SCAD^{-/-}$) after 1-hour 4°C challenge [80]. Although the mice with single heterozygous of VLCAD, LCAD, and SCAD genes were cold tolerate; more than 30% of mice with double heterozygous $VLCAD^{+/-}//LCAD^{+/-}$ *or* $LCAD^{+/-}//SCAD^{+/-}$ and triple heterozygous $VLCAD^{+/-}//LCAD^{+/-}//SCAD^{+/-}$ combinations developed hypothermia upon the cold challenge [80], indicating the essential role of the LCFAs-mediated thermogenic function in mitochondria.

In summary, these studies highlight the importance of the brown adipocyte lipolysis and the liberated intracellular fatty acid transportation and oxidation during thermogenesis. In addition, these studies indicate that brown adipocytes not only use the intracellular lipid storage, but also the circulating energy substrate to maintain the critical thermogenic function for the organismal survival and optimal function in mammals [2, 12, 38, 44, 56, 59, 60, 80]. In modern days, BAT's ability to metabolize fatty acids mobilized from other peripheral storages, including white adipose tissue and liver, makes it a good potential therapeutic target in humans. Studies have shown that BAT mediates significant plasma lipid clearance during the cold challenge in rodent and humans under both physiological and pathological conditions [83–85].

One study showed that the activated BAT is involved in the basal and post-prandial triglyceride metabolism in rodents [83]. In this study, compared to mice kept at 22°C, mice kept at 4°C had significantly lower triglyceride-rich lipoproteins (TRLs)-triglyceride concentration [83]. The study also showed that the activated BAT was involved in the post-prandial triglyceride metabolic process, evidenced by an oral ^{3}H-triolein tolerance test. Under cold challenge condition, the BAT ^{3}H-triolein uptake was significantly higher than that of either liver or skeletal muscle, suggesting the significant triglyceride/triglyceride-rich lipoprotein metabolism in activated BAT. For the triglyceride clearance, circulating triglycerides rose to a peak at 2 hours postprandial and declined subsequently in mice kept at 22°C. In contrast, the triglyceride level reminded persistently low in mice kept at 4°C, suggesting that the BAT possesses a high postprandial triglyceride clearance function [83]. Most interestingly, the cold challenge increased the uptake of radio-labeled lipoprotein in BAT and reduced the uptake in liver [83]. The cold challenge-induced lipid clearance shift suggests that BAT can be targeted for lipid metabolism *in vivo*. In a pathological setting, the genetically manipulated $Apoa5^{-/-}$ mice with severe hyperlipidemia were studied. A 4–24 hours 4°C challenge corrected $Apoa5^{-/-}$ mouse plasma lipid concentration to the values comparable to wild-type mice, indicating the significant impact of BAT on whole body lipid metabolism in the rodent under the pathological condition [83, 86, 87]. Other studies also showed that the BAT preferentially uptook plasma triglycerides through peripheral tissue lipolysis, and the selective fatty acids uptake from triglyceride-rich lipoprotein ameliorated hyperlipidemia in rodents [84, 85].

Although the studies in rodent strongly support the importance of BAT in lipid clearance, the significance of BAT in human lipid metabolism is still unclear. The contribution of activated BAT in human body was studied using a dietary radio-labeled LCFAs tracer 14(R,S)-[^{18}F]-fluoro-6-thia-heptadecanoic acid (^{18}FTHA) [88].

This study showed that a 4-hour mild cold-challenge at 18°C significantly increased dietary fatty acids distribution in BAT in humans [88]. However, given the relative small volume of BAT tissue, the dietary fatty acids clearance contribution from the BAT is less significant compared to other organs including heart, liver, white adipose tissue, or skeletal muscles, and only contributed to ~0.3% of total body dietary fatty acids clearance upon the mild cold challenge [88]. Similarly, another study using fatty acid tracer ^{18}F-fluoro-thiaheptadecanoic acid (^{18}FTHA) showed that a 3-hour cold challenge at 18°C led to four times higher radio-labeled tracer uptake and ~80% metabolic rate increase in the BAT, contributing <1% of total fatty acids clearance rate in human subjects [12]. Nevertheless, these experiments suggest that BAT not only exists, but also is functionally active and can contribute to systemic metabolism in humans. One possible reason for the relatively low BAT contribution in fatty acids metabolism in humans could be due to the short cold challenge time and mild cold challenge conditions. It is possible that during the acute cold challenge, intracellular lipid lipolysis serves as the main energy resource so that it ameliorates the clearance of circulating TRLs or fatty acids.

The circulating TRLs or fatty acids are transported into brown adipocyte by a group of proteins, including lipoprotein lipase (LPL) and fatty acid transport proteins [83, 89–93]. LPL is a multifunctional protein produced by many tissues, including the adipose tissue [94]. LPL serves as a rate-limiting enzyme mediating extracellular lipolysis [94]. It hydrolyzes triglycerides into lipid-rich proteins into fatty acids and monoacylglycerol. It also mediates the cellular uptake of triglyceride and other lipids [94]. Studies have shown that cold challenge or catecholamine-stimulation induce the expression and activity of LPL in brown adipocytes through a cAMP-mediated mechanism [89–91]. After activation, LPL is released from the brown adipocyte, transferred to the capillary endothelium lumen, and serves as the anchor between the endothelium cell surface and the TRLs [95–97]. Next, the LCFAs liberated from LPL channel into brown adipocyte for thermogenic function [98, 99]. A study indicated that the local LPL activity is required for TRLs uptake into the BAT [83]. In this study, it is shown that either LPL-specific inhibitor tetrahydrolipstatin pretreatment or releasing LPL from endothelium by heparin significantly blocked the uptake of TRL and fatty acids in BAT [83].

The liberated LCFAs in circulation can be transported into cells and activated by both transmembrane fatty acid transporter proteins (FATPs) and scavenger receptor CD36 [92, 93]. FATPs are composed by a family of six proteins mediating circulating LCFAs uptake and distribution in cells [83, 92, 93, 100]. Among the six isoforms, FATP1 (SLC27A1) is the major isoform in brown and white adipose tissue [83, 92, 93, 100]. Studies showed that postprandial lipid uptake is highly dependent on the adipose tissue FATP1 [101]. The FATP1-knockout mice have decreased lipid uptake in adipose tissue and a compensatory lipid redistribution in liver and heart, where FATP1-mediated lipid uptake function is not required under normal conditions [101]. The cold challenge can induce FATP1 expression in BAT [100]. In line with this, the isolated FATP1-null brown adipocytes showed significantly less fatty acids uptake upon catecholamine stimulation [100]. *In vivo* studies showed that in cold challenged FATP1-knockout mice, BAT triglyceride storage was decreased and circulating serum free fatty acids was increased, indicating the importance of FATP1-mediated fatty acid uptake in BAT [100]. The LCFAs uptake can also be mediated by the transmembrane class B scavenger receptor CD36 [83, 102, 103]. Upon the cold challenge, CD36 is significantly upregulated in adipocytes [83, 102]. Other studies using *CD36*$^{-/-}$ mice clearly indicated the importance of CD36-mediated LCFAs transportation during thermogenesis. Around 60% of *Cd36*$^{-/-}$ mice died during a 24-hour cold challenge, which is paralleled with drastically increased plasma free fatty acids concentration [83, 102]. A recent

study indicated that CD36-medicated coenzyme Q (CoQ) uptake is required for the BAT mitochondrial thermogenic function [104].

In summary, fatty acid is indispensable for the brown adipocyte thermogenic function. Fatty acid mediates brown adipocyte thermogenesis through mitochondrial β-oxidation, UCP1-activation, and fatty acid-mediated thermogenic gene regulation. Both the intracellular fatty acids from brown adipocyte lipolysis and the liberated fatty acids from peripheral tissues play essential roles mediating the critical BAT adaptive thermogenic function to main the adequate core body temperature in mammals. In modern days, activating BAT thermogenic function to increase fatty acids uptake and utilization may offer new therapeutics to treat human metabolic disorders.

3. Glucose metabolism in BAT

The radio-labeled glucose analog ^{18}F-fluorodeoxyglucose (^{18}F-FDG), in combination with positron emission tomography (PET) and computed tomography (CT), provides a reliable method for the *in vivo* tissue glucose uptake study [105, 106]. Based on studies using this method, it is now well-recognized that BAT in both rodents and humans possesses a significant glucose uptake capacity upon the cold challenge [4, 9–12, 25, 26, 107–112].

Glucose uptake is regulated in brown adipocytes. *In vivo* studies showed that cold challenge significantly enhanced insulin sensitivity and subsequent glucose uptake in the BAT [9, 10, 25, 26, 107, 112, 113]. Interestingly, other studies showed that starved rats with low insulin level also had increased BAT glucose uptake during the cold challenge, indicating that the enhanced glucose uptake is not completely insulin-dependent [114]. Additional studies also indicated that the enhanced glucose uptake in brown adipocytes can be mediated by different pathways in addition to insulin stimulation, including β-adrenergic receptor activation, AMP-activated protein kinase (AMPK) activation, and mTOR activation [115–117].

The importance of glucose metabolism in BAT is a long-standing question. Glucose transporters, which facilitate glucose across the cell plasma membrane is the first rate-limiting step of the glucose metabolism [34, 118, 119]. Intracellular glucose is subsequently phosphorylated to glucose-6-phosphate (G6P) by the enzyme hexokinase (HK). Glucose-6-phosphate serves as the substrate into different pathways, including glycolysis, glycogen synthesis, and the pentose phosphate pathway (PPP) [34, 118, 119]. Glycolysis breaks down glucose to pyruvate to generate small amount of ATP and NADH [34, 118, 119]. The pyruvate is then transported into mitochondria for oxidation and energy production. Under hypoxia condition, pyruvate is disposed in the form of lactate [34, 118, 119].

Early study indicates that glucose and its metabolites contribute to the brown adipocyte thermogenesis by showing that catecholamine-induced glucose uptake was decreased when mitochondrial β-oxidation was inhibited in brown adipocytes [120]. Other studies in brown adipocyte glucose transportation also indicate the importance of the glucose in brown adipocyte metabolism [6, 112, 121, 122]. Both glucose transporter 1 (Glut1) and glucose transporter 4 (Glut4) are abundantly expressed in brown adipocytes and the insulin sensitive Glut4 is the major isoform [112, 122]. The *in vitro* study showed that knock-down of Glut1 and/or Glut4 gene in cultured brown adipocytes impaired the catecholamine-induced cell oxygen consumption by 30–50% [6, 121]. Other studies indicate the importance of glycolysis in brown adipocyte thermogenesis [115, 123, 124]. An *in vitro* study showed that the knockdown of two glycolysis enzymes, HK2 or pyruvate kinase M (PKM), decreased glucose uptake and catecholamine-induced cell oxygen consumption by 67% or

34% respectively, in brown adipocytes [6]. These studies suggested the importance of glucose metabolism in brown adipocyte function. However, another detailed study indicated that glucose oxidation does not play a major role in brown adipocyte metabolism and thermogenesis, by showing that the rate of $^{14}CO_2$ formation from the ^{14}C glucose was relatively small compared with the maximum rate of oxygen consumption in activated brown adipocytes [125]. Studies using radio-labeled glucose also suggested that glucose uptake was only sufficient to fuel maximally ~15% of the thermogenic capacity in activated rodent brown adipocytes, suggesting that the significantly upregulated glucose uptake in activated brown adipocytes is disassociated with the relative low glucose-mediated thermogenic capacity [123, 125, 126]. A more recent study indicates that the brown adipocyte energy production from glucose depends on the state of the cell: glucose and fatty acid contribute equally to brown adipocyte oxygen consumption under the basal condition; upon catecholamine-activation, oxygen consumption is mainly fueled by fatty acids [6].

The dissociation between high glucose uptake and low glucose-mediated thermogenesis in activated BAT raises an important question: what is the function of the intracellular glucose in brown adipocytes? The importance of glucose in the *de novo* lipogenesis in brown adipocytes has been reported [127–129]. Studies indicate that glucose uptake is an independent process of thermogenesis in both cold-challenged and catecholamine-activated BAT, which further supports that glucose uptake might play other role as energy substrate in activated brown adipocytes [116, 117, 130–132]. One detailed *in vitro* study using rat brown adipocytes showed that norepinephrine significantly enhanced glucose oxidation by sevenfold, while it also inhibited lipogenesis at the same time. On the other hand, insulin stimulation increased the lipogenesis by sevenfold in brown adipocytes whereas glucose oxidation remained very low. Most interestingly, the addition of insulin to the norepinephrine only potentiated the enhanced glucose oxidation, but do not enhance the lipogenesis [115]. On the other hand, another study showed that brown adipocytes converted a greater proportion of metabolized glucose into lactate and pyruvate, but only a smaller proportion into fatty acids through insulin-mediated pathway [125]. These studies suggest that glucose metabolism is involved in two different states in brown adipocytes, the thermogenic state and nonthermogenic state. It is plausible that during the thermogenic state, glucose contributes to both the energy production and lipogenesis; and during the nonthermogenic state, glucose contributes mainly to the lipogenesis and energy storage process in brown adipocytes, which explains the disassociation of glucose uptake and glucose metabolism in brown adipocytes. In addition to glucose, other energy substrates, including glycerol and amino acid (glutamate), might also contribute to BAT metabolism and thermogenesis in human and rodent brown adipocytes. However, the relative contribution and partition of these energy substrates are unclear [132–135].

4. Energy storage in BAT

The cold challenge not only enhances catabolic processes mediating energy substrate metabolism and heat generation, but also induces anabolic processes mediating fatty acid synthesis and lipogenesis, as well as glycogenesis [36, 111, 136–139].

Glucose can be stored as glycogen in brown adipocytes [36, 139]. Studies showed that glycogen synthases (GStot) and uridine diphosphate glucose pyrophosphorylase (Udgp), which mediates glycogenesis, were upregulated upon the cold challenge in BAT [36, 139]. Interestingly, glycogen hydrolysis enzyme, glycogen phosphorylase (Pygl), was also upregulated after cold challenge [36]. Although cold-challenge upregulates both glycogen synthesis and degradation, it is reported

that the stored glycogen is used up shortly after the cold challenge [139]. Indicating that glycogen is not an efficient format for energy storage and a sustainable energy resource in brown adipocytes.

The glucose uptaken by brown adipocyte can also be converted to fatty acid through the *de novo* lipogenesis. Carbohydrate response element-binding protein (ChREBP) is the major transcription factor mediating fatty acid synthesis in adipocytes. There are two isoforms of ChREBP, ChREBPα and ChREBPβ. ChREBPα is abundantly expressed in BAT from rodents and humans [137, 140, 141]. *De novo* lipogenesis involves a series of enzymes mediating the sequential reactions converting glucose-derived citrate into fatty acids, including ATP-citrate lyase (ACLY), acetyl-CoA carboxylases 1 (ACC1), fatty acid synthase (FASN), and stearoyl-CoA desaturase-1 (SCD1). ChREBPα, coordinates with another transcription factor Max-like protein (MLX), directly binds to the carbohydrate response elements (ChoRE) and upregulates these *de novo* lipogenic genes expressions. ChREBPα can also increase the expression of ChREBPβ to further activate the *de novo* lipogenesis enzymes [142, 143]. It has been reported that lipogenic genes and AKT2-ChREBP pathways are upregulated to optimize fuel storage and thermogenesis upon cold stimulation in BAT [136]. In accordance with these studies, *ChREBP*$^{-/-}$ mice presented less BAT weight [142], and adipose-specific *ChREBP-knockout* mice had decreased carbohydrate-induced lipogenesis in BAT [144]. These studies indicate that ChREBP plays important roles in brown adipocyte's *de novo* lipogenesis and energy storage.

Sterol regulatory element-binding protein-1 (SREBP-1) is another transcription factor mediating the *de novo* lipogenesis [145]. Of the three different SREBP isoforms, SREBP1c is more abundant and SREBP1a is less abundant in the adipose tissue [137, 145, 146]. *In vitro* study showed that SREBP1c is sufficient to regulate lipogenic enzymes in cultured adipocytes [147]. In the adipose-specific *ap2-SREBP1c* transgenic mice, lipogenic enzyme Acc1, FASN and Scd1 expression as well as fatty acids synthesis rate were significantly upregulated in brown adipocytes [148]. In line with this study, *ap2-SREBP1a* transgenic mice also developed adipose tissue hypertrophy in accordance with an increased lipogenic enzyme profile and enhance *de novo* lipogenesis in the BAT [148]. However, some *in vivo* studies indicated that SREBP1c's role in the *de novo* lipogenesis in adipocytes is dispensable, as evidenced by *SREBP1-knockout* mice have normal lipogenic enzymes gene expression profile and normal lipid storage in their adipose tissue [149, 150]. These studies suggest that SREBP-1 is involved in the brown adipocyte lipogenesis and triglyceride storage when the excessive energy resources are available; however, SREBP-1's function can be compensated by other factors when it is absent.

In summary, these studies suggest that in activated brown adipocytes, glycogenesis and lipogenesis are upregulated to store/restore energy substrates, which is parallel with energy substrate metabolism and thermogenesis. These coordinated anabolic and catabolic processes are important to maintain the brown adipocyte energy homeostasis.

5. The proceeding of targeting BAT in human metabolic disorders

The understating of BAT energy homeostasis and the discovery of the functional BAT in humans lead to significant interests in targeting BAT for metabolic disorders, for example, obesity, insulin resistance, type 2 diabetes, and lipid profile abnormality. The ability of BAT metabolizing fatty acid and glucose from the intracellular storage, the peripheral tissues liberation, and teh dietary nutrition absorption makes it a good potential therapeutic target for combating metabolic disorders in humans. In addition to the BAT, beige/brite fat, which is coexisted in white adipose tissue,

can be recruited and activated (browning) in respond to cold challenge or pharmacological stimulation and serves as a target for metabolic disorders [151–154].

It has been reported that the BAT ^{18}F-FDG uptake in humans correlates inversely with aging, adiposity, diabetic status, and BMI, indicating that the manipulation of BAT function is a possible approach for combating metabolic disorders [8–14, 16, 21–24, 155, 156]. The studies in mouse models and humans provide evidences for the metabolic benefit of BAT. Mice with genetic ablation of BAT and the *UCP1-knockout* mice under thermoneutrality, both developed obesity [18–20, 85]. In addition, BAT activation reduced hypercholesterolemia and protected mice from atherosclerosis development and liver steatosis [18–20, 85]. It is well-recognized that BAT can be activated upon seasonal temperature changes or short time period (several hours) mild cold challenge (16-19°C) in humans [7, 9, 10, 12, 21, 22, 24–27, 157]. However, the significance of BAT's contribution in human metabolism has not been clearly elucidated. Human studies indicated that BAT activity is inversely correlated with body fat deposition, suggests BAT can serve as a target for obesity [9, 10, 16, 21–24]. Studies also showed that fasting glucose was lower in the human subjects with higher BAT prevalence and the BAT activity were blunted in subjects with obesity [11, 16, 155]. More interestingly, in the same patients with multiple PET scans, BAT was more detectable when fasting glucose in the subjects were lower [16]. Other studies showed that a mild cold challenge significantly increased whole body glucose disposal, glucose oxidation, insulin sensitivity, and whole body energy expenditure in human subjects [13, 17, 158, 159]. Additional study showed that moderate cold challenge (18.06°C) significantly improved the peripheral glucose uptake and insulin sensitivity by 20%, but did not impact the pancreatic insulin secretion [17]. These studies strongly indicate the therapeutic potentials of targeting BAT for glucose metabolism in humans, but leave the question of the relative contribution of activated BAT in whole body glucose metabolism to be answered.

Cold challenge can also enhance BAT lipid metabolism. Studies showed that cold challenge led to significantly enhanced lipid mobilization, increased plasma fatty acid levels, as well as upregulated genes for lipid metabolism in human BAT [12, 58, 156, 160]. It is reported that circulating fatty acids were uptaken by BAT in cold-challenged humans by using the ^{18}FTHA tracing method [88]. In addition, it has been shown that cold-activated BAT significantly contributed to whole body fatty acid utilization in healthy humans [17]. The fatty acid uptake is significantly lower in overweight human subjects compare to healthy humans [156]. Importantly, BAT activation upon mild cold challenge significantly increased systemic lipid metabolism, whole body lipolysis, triglyceride-fatty acid cycling, and fatty acid oxidation in overweight/obese subjects [14]. These studies suggest that BAT contributes to whole body lipid metabolism and homeostasis in healthy humans as well as in humans with metabolic disorders, suggesting that BAT can serve as a target for lipid abnormality in humans.

The significance of BAT/beige adipose tissue in human whole body metabolism has been studied and remarkable progress has been made in recent years. The acknowledgment that BAT can be activated and subsequently contributes to the human whole body energy expenditure is encouraging. Although the capacity and relative significance of BAT's contribution to whole body energy substrate metabolism has not been elucidated, it should be noticed that majority of the studies in humans were conducted for relative short time period with mild cold challenge conditions. Given, humans usually live under thermoneutrality, we can assume that their BAT functions are repressed under this condition. In addition, the prevalence of BAT in humans varies significantly, which depends on individual's life style, physical activity, and health conditions, which makes it harder to evaluate the contribution of BAT in whole body metabolism [7, 9–12, 21, 22, 24–27, 88, 156, 157].

Hence, a sustainable chronic mild cold challenge strategy aiming to recruit more BAT/beige adipose tissue and enhance their oxidative capacity might provide more significant therapeutic potential in humans over time, especially the human subjects with metabolic disorders. Future studies should also delineate the relative contribution from glucose and fatty acid in human BAT under physiological and pathological conditions; as well as compare the glucose and fatty acid partitioning in different tissues and organs, including skeletal muscle, heart, liver and BAT. These details will guide us to establish better strategies targeting metabolic disorders through BAT activation in the future.

6. Conclusion

The energy metabolism plays critical role in maintaining BAT thermogenic function in mammals. Through the energy metabolism, the chemical energy stored in nutrients (i.e., fatty acids and glucose) can be converted to thermoenergy and dissipated as heat in the BAT. Activated brown adipocytes not only contribute to the intracellular substrate homeostasis, but also contribute significantly to the whole body energy metabolism. BAT with functional thermoactivity is present in adult humans. Thus, BAT activation has remarkable therapeutic implications in human metabolic disorder management.

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Conflict of interest

The author has no conflict of interest to declare.

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