Brown adipose tissue (BAT) is well known to burn calories through uncoupled respiration, producing heat to maintain body temperature. This ‘calorie wasting’ feature makes BAT a special tissue, which can function as an ‘energy sink’ in mammals. While a combination of high energy intake and low energy expenditure is the leading cause of overweight and obesity in modern society, activating a safe ‘energy sink’ has been proposed as a promising obesity treatment strategy. Metabolically, lipids and glucose have been viewed as the major energy substrates in BAT, while succinate, lactate, branched-chain amino acids, and other metabolites can also serve as energy substrates for thermogenesis. Since the cataplerotic and anaplerotic reactions of these metabolites interconnect with each other, BAT relies on its dynamic, flexible, and complex metabolism to support its special function. In this review, we summarize how BAT orchestrates the metabolic utilization of various nutrients to support thermogenesis and contributes to whole-body metabolic homeostasis.

Introduction

Brown adipose tissue (BAT) was first reported in the 1950s [1]. In humans, thermogenic BAT used to be thought to only exist in babies and disappear in adults [2,3]. In 2009, three independent groups first detected thermogenic adipose tissue in human adults through positron emission tomography (PET) imaging with 18F-fluorodeoxyglucose (18F-FDG) [4–6]. Since then, BAT studies have been blooming over the last 12 years (2009–2020). Because BAT can burn a significant amount of energy through uncoupling respiration to

Abbreviations

18F-FDG, 18F-fluorodeoxyglucose; 18F-THA, 18F-fluo-thiaheptadecanoic acid; ACC, acetyl-CoA carboxylase; AcCoA, acetyl-CoA; ACLY, ATP-citrate lyase; ACS2, acetyl-CoA synthetase 2; ALDH1A1, aldehyde dehydrogenase 1 family member A1; ALT, alanine aminotransferase; ATGL, adipose triglycerol lipase; BAT, brown adipose tissue; BCAAs, branched-chain amino acids; BCKDH, branched-chain alpha-keto acid dehydrogenase; CGI-58, Comparative Gene Identification-58; CK, creatine kinase; CPT1/2, carnitine acyltransferase ½; CrAT, carnitine acetyltransferase; CRT, creatine transporter; CS, citrate synthase; CTP, citrate transporter protein; DGAT, diglycerol acyltransferase; DHAP, dihydroxyacetone phosphate; DNL, de novo lipogenesis; FASN, fatty acid synthase; G1P, glucose 1-phosphate; G3P, glycerol 3-phosphate; G6P, glucose 6-phosphate; GATM, glycine amidinotransferase; GLUD, glutamate dehydrogenase; GLUT1/4, glucose transporters 1/4; GPDH, glycerol 3-phosphate dehydrogenase; GYS 1/2, glycogen synthase 1/2; HK2, hexokinase 2; HSL, hormone-sensitive lipase; ISO, isoproterenol; LDH, lactate dehydrogenase; MCT1/4, monocarboxylate transporter 1/4; MGL, monoacylglycerol lipase; mmBCFAs, monomethyl branched-chain fatty acids; MPC 1/2, mitochondrial pyruvate carrier 1/2; NiAc, nicotinic acid; OAA, oxaloacetate; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PEP, phosphoenolpyruvate; PET, positron emission tomography; PGM, phosphoglucomutase; PKM, pyruvate kinase muscle isozyme; PYGL, glycogen phosphorylase, liver form; ROS, reactive oxygen species; SREBP1c, sterol regulatory element-binding protein 1c; sWAT, subcutaneous white adipose tissue; TCA cycle, tricarboxylic acid cycle; TG, triglycerol; UCP1, uncoupling protein 1; UGP, uridine diphosphate-glucose pyrophosphorylase; WAT, white adipose tissue; aKG, α-ketoglutarate; β3-AR, β3-adrenergic receptor.
generate heat, enhancing thermogenesis in BAT has been proposed as a promising strategy to treat adult obesity in modern society [7–9].

Brown adipocytes are the major calorie-consuming cells in BAT, which are enriched with mitochondria and express a high level of uncoupling protein 1 (UCP1) [10]. In comparison, the classic white adipose tissue (WAT) comprises white adipocytes, which only have a few mitochondria. Morphologically, brown adipocytes have multiple small lipid droplets, while most white adipocytes have one large lipid droplet per cell. While activating brown adipocytes, cold exposure also turns white adipocytes into brown-like adipocytes known as beige/brite adipocytes. Beige/brite adipocyte-enriched adipose tissue is also known as beige adipose tissue, such as the subcutaneous WAT (sWAT) in mice. Of note, many studies suggest that the 18F-FDG-PET-detected thermogenic adipose tissue in human adults may be more like the beige adipose tissue in mice [11]. A large portion of BAT studies focuses on inducing beige adipocytes in WAT and identifying preadipocytes with the potential to differentiate into the thermogenic beige/brite adipocytes. The relation between brown, beige, and white adipose has been extensively discussed in previous reviews [12–15]. For simplicity, we will primarily use BAT to refer to the thermogenic adipose tissue in both mice and humans in this review.

Uncoupled respiration is a unique metabolic feature of brown adipocytes and BAT. In most cells, catastrophic reactions produce a proton gradient across the mitochondrial inner membrane, which powers ATP synthase to generate ATP. In comparison, mitochondrial proton gradient can be released without ATP production in brown adipocytes. The best-characterized uncoupling mechanism is mediated by UCP1, which functions as a molecular basis for the protonophoric activity through the mitochondrial inner membrane [16]. There are also UCP1-independent uncoupling mechanisms that contribute to the thermogenesis in brown adipocytes, as beautifully summarized in a recent review [17].

As an ‘energy sink’, BAT utilizes a broad source of nutrients. There are three main nutrients, carbohydrates, lipids (fats), and proteins, in mice and humans. Glucose is the most common carbohydrate, and 18F-FDG-PET shows active glucose metabolism in human BAT. While adipose tissue is also known as fat-storing tissue, it is not surprising that fats (lipids) serve as an energy source in BAT. BAT utilizes both intracellular and extracellular lipids as the fuel for thermogenesis. More interestingly, BAT can simultaneously carry out fatty acid β-oxidation and de novo fatty acids synthesis, which can antagonize each other in other metabolic tissues [18]. As the major energy source for BAT, the role of glucose and lipid metabolism in thermogenesis has been extensively studied and summarized in previous reviews on energy metabolism in BAT [19–22].

Most BAT metabolism studies, especially those in vivo, primarily rely on several classic approaches to measure metabolic alterations. First, metabolic imaging with radioactive tracers is routinely used to visualize the active uptake of glucose and fatty acids in BAT. Second, metabolomics is widely used to directly profile the level of metabolites in BAT upon activation. Third, the mRNA and protein expression level of metabolic genes is commonly used to analyze the metabolic enzymes and pathways. More recently, the application of stable isotope tracing in metabolic research, especially in vivo studies, has extended our understanding of the intracellular glucose and lipid metabolism in adipose tissue [23]. Additionally, the stable isotope tracing approach has also contributed to the identification of other fuels for BAT thermogenesis. In this review, we focus our discussion on the source and metabolic fate of glucose, lipids, and other fuels in BAT thermogenesis (Fig. 1).

Metabolic imaging

Thermogenic adipocytes were first detected in adult humans through 18F-FDG-PET in 2009, as these special adipocytes can uptake a large amount of glucose [4–8]. 18F-FDG-PET is a powerful imaging tool to visualize the tissues with a high glucose update rate, as 18F-FDG is a radioactive nonmetabolizable analog of glucose. Upon uptake, 18F-FDG is catalyzed by hexokinase (HK) into 18F-FDG-6-phosphate, which accumulates the 18F radioactive signal within the cells in live animals. As a noninvasive imaging tool, 18F-FDG-PET is best known for its clinical application in oncology, since most tumors have a high glucose uptake rate [24]. Taking advantage of the available 18F-FDG-PET images from 52 487 cancer patients, a recent study reanalyzed those images to show that higher BAT activity promotes cardiometabolic health [25].

Since 2009, 18F-FDG-PET has been extensively used to study glucose metabolism in the thermogenic adipose tissues. 18F-FDG-PET revealed that cold exposure activates glucose uptake in BAT of healthy human subjects [5,6,26,27]; obese patients have less BAT activity than their lean counterparts upon acute cold exposure [4–6,28]; patients with type 2 diabetes show smaller volumes of 18F-FDG-positive BAT and lower glucose uptake per volume of BAT relative to
young healthy control subjects [29]; and insulin enhances glucose uptake in human BAT [30]. Recently, a large study cohort of 134,529 18F-FDG-PET from 52,487 patients confirmed most of the findings in those earlier smaller human studies. Human BAT is significantly and independently associated with reduced odds of type 2 diabetes mellitus, dyslipidemia, coronary artery disease, cerebrovascular disease, congestive heart failure, and hypertension [25]. Most importantly, the protective effects are more pronounced in individuals with obesity or overweight, which suggests BAT has a great potential to play a role in alleviating the deleterious obesity-related metabolic disorders.

As a noninvasive tool, PET imaging is not limited to studies of glucose metabolism. In addition to the glucose tracer (18F-FDG), 18F-fluorothiaheptadecanoic acid (18FTHA) is used to measure fatty acid uptake, and 11C-acetate tracer, to measure the mitochondrial oxidative metabolism in the thermogenic adipose tissues [26,29]. Similar to the enhanced glucose uptake, cold exposure also enhances fatty acid uptake and oxidation in BAT of healthy men [31–33].
It is worth noting that cold-induced fatty acid uptake and oxidation are not defective in type 2 diabetes, although $^{18}$F-FDG-PET indicates reduced glucose uptake in these patients [29]. These imaging studies also show that a carbohydrate-rich meal triggers human BAT thermogenesis to the same extent as cold stress, and activated BAT uses both glucose and lipoprotein lipase-derived fatty acids as energy substrates [34].

In addition to tracing the nutrients through PET imaging, $^{15}$O$_2$ and $^{15}$O-H$_2$O are used to directly assay the rates of oxygen consumption and blood flow in the cold-activated human BAT [34]. Measurement of oxygen consumption provides direct evidence for the underlying mitochondrial respiratory chain reactions irrespective of the choice of substrate utilization [34]. The oxygen consumption of supraclavicular BAT is similar upon cold exposure and food consumption [35]. More recently, PET/magnetic resonance imaging is also developed as a feasible alternative to $^{18}$F-FDG-PET/CT for BAT quantification [36,37].

In addition to applications in clinical studies, PET imaging is used to study cold-activated BAT in rodent models as well [38]. Both acute and chronic cold exposures increase glucose and fatty acid uptake, as well as oxidative metabolism, in rat and mouse BAT [38–40]. More recently, the new $^{[123/125]}$-iodophenyl-pentadecanoic acid with single-photon emission computed tomography/computed tomography imaging approach displays the human-like BAT and beige depots in mice, and a large number of novel fat pads in mice [41]. Additionally, the infrared camera is also used to examine BAT activity based on its thermal imaging. BAT, infrared imaging is still widely used as a quick and easy tool in rodent studies [43,44].

**Glucose metabolism**

The active glucose metabolism in BAT can be routinely imaged by $^{18}$F-FDG-PET. It is worth noting that $^{18}$F-FDG-PET is originally developed to detect the enhanced glucose uptake and retention in tumors, as most cancer cells rely on active glycolysis to support their uncontrolled proliferation [24]. As brown adipocytes do not proliferate like cancer cells, BAT is most likely to have distinct cellular metabolism from that in tumors. In BAT, glucose is directly metabolized to produce energy through both cytosolic glycolysis and mitochondrial oxidation. Additionally, glucose also serves as an important carbon source for glycerol and acetyl-CoA (ACC) production, which are essential for lipogenesis in BAT.

**Glucose uptake and cytosolic glycolysis**

Glucose transporters (GLUTs) mediate cellular glucose uptake. There are four GLUTs in mammals, and BAT expresses GLUT1 and GLUT4. Upon uptake, glucose is phosphorylated by hexokinase 2 (HK2) into glucose 6-phosphate (G6P), which keeps glucose within the brown adipocytes. G6P can be further metabolized by several glycolytic enzymes, including pyruvate kinase M (PKM) at the final step [45].

Human BAT expresses a high level of GLUT1 and GLUT4 relative to WAT from the same human subject, which is consistent with the enhanced $^{18}$F-FDG-PET signals in BAT [30]. Cold exposure significantly increases GLUT1 and GLUT4 expression in BAT of mice [46,47], and β3-adrenergic receptor (β3-AR) agonist also triggers glycolysis in BAT and sWAT [48]. In brown adipocytes, knockout of GLUT1 and GLUT4 impairs isoproterenol (ISO)-stimulated glycolytic flux and oxygen consumption [49]. In addition to GLUT1 and GLUT4, knockout of HK2 or PKM also decreases ISO-stimulated glycolytic flux and oxygen consumption; and interestingly, HK2 knockout exhibits a more severe effect than PKM knockout [49]. Together with findings from $^{18}$F-FDG-PET imaging, these studies indicate that activated glucose uptake and glycolysis are a feature of the thermogenic adipocytes in BAT.

**Mitochondrial pyruvate oxidation**

Pyruvate, the end product of glucose-dependent glycolysis, can enter mitochondria for further oxidation, and mitochondrial pyruvate oxidation starts with mitochondrial pyruvate uptake. Mitochondrial pyruvate carrier (MPC), a heterodimer encoded by two genes (Mpc1 and Mpc2), connects the cytosolic glycolysis and mitochondrial oxidation [50,51]. Within mitochondria, pyruvate is metabolized by pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC). The catabolic PDH reaction catalyzes the conversion of pyruvate to ACC, which subsequently enters the tricarboxylic acid (TCA) cycle to be completely oxidized into CO$_2$ [52]. The anaplerotic PC catalyzes the conversion of pyruvate into oxaloacetate, which can be incorporated with ACC to form citrate [53].

In mice, cold exposure significantly induces the expression of Mpc1/2 in BAT [54], and conditional deletion of Mpc1 in brown adipocytes leads to impaired cold adaptation [55]. In addition to MPC, PDH activity in BAT is higher than that in WAT, muscle, and heart [56]. Furthermore, insulin activates PDH in BAT, which is repressed by fasting and high-
Glucose tracing has advanced our understanding of glucose and \( ^{14}\text{C}\)glucose are fully metabolizable. In contrast to the classical \( ^{14}\text{C}\)glucose-based radioactive tracing, mass spectrometry can separate and quantify all the individual metabolic intermediates derived from the \( ^{1-13}\text{C}\)glucose nonradioactive tracer in mice and humans [68,69]. The in vivo and in vitro application of \( ^{1-13}\text{C}\)glucose tracing has advanced our understanding of intracellular glucose metabolism in brown adipocytes and BAT [70]. In vitro \( ^{1-13}\text{C}\)glucose tracing-based metabolomics showed that \( ^{3}\text{AR}\) stimulation enhances PDH flux and TCA cycle activity in differentiated T37i brown adipocytes [66]. Consistently, \( ^{1-13}\text{C}\)glucose tracing and metabolic flux analysis revealed that \( ^{3}\text{AR}\) agonist significantly enhances the flux of mitochondrial pyruvate uptake in differentiated primary brown adipocytes [54]. More importantly, in vivo \( ^{1-13}\text{C}\)glucose tracing directly showed that BAT has the most active glucose-dependent oxidation, as compared to other metabolic tissues such as WAT, liver, and muscle. Furthermore, chronic cold exposure significantly increases glucose-dependent oxidation without altering glycolysis in BAT [54]. However, acute cold exposure (4 °C for 3 h) does not alter the enrichment of glycolytic and TCA cycle intermediates from the \( ^{1-13}\text{C}\)glucose tracer in BAT [70]. These differences upon acute versus chronic cold exposure suggest that mitochondrial pyruvate/glucose oxidation is dynamically regulated in BAT in response to cold stimulation.

**Glycogen**

Glucose can be stored as glycogen in BAT. Glycogen level is fivefold higher, and glycogen synthase (GYS) activity is increased in lipid droplet-absent BAT [71]. Uridine diphosphate-glucose pyrophosphorylase (UGP) is a key enzyme in glycogenesis, and glycogen phosphorylase (PYGL) is a key enzyme in glycogenolysis. Both UGP and PYGL are increased after chronic cold exposure for 21 days at 10 °C in rats, indicating higher activity of the glycogen cycle [39]. Whereas increased \( \text{Gys1/2} \) mRNA expression during 2/4-day cold exposure at 4 °C in mice indicated enhanced glycogen production, their protein level expression was found to be unchanged [59]. These findings suggest that both glycogenesis and glycogenolysis are activated upon cold exposure.

**Glucose-dependent acetyl-CoA production**

Glucose is also an important source of ACC, which provides building blocks for de novo fatty acid synthesis in most tissues, including BAT [72]. Within mitochondria, glucose/pyruvate-derived ACC is converted by citrate synthase into citrate, which can be used in two major metabolic pathways. One pathway is the TCA cycle, with two carbons burned into CO\(_2\) in each cycle, as discussed in the previous section. In the other pathway, citrate exits mitochondria into the cytosol through citrate transporter protein (CTP), which is encoded by gene \( \text{Slc25a1} \) [73]. Cytosolic citrate is then converted by ATP-citrate lyase (ACLY) back into ACC, which serves as the major carbon source for de novo fatty acid synthesis [74–76]. CTP and ACLY are the key players connecting glucose and fatty acid metabolism in all lipogenic tissues, including the liver and adipose tissues.

In humans, cold exposure increases the expression of ACLY specifically in BAT but not in WAT [77]. In mice, cold exposure induces the expression of ACLY in BAT in a time-dependent manner, and CTP expression in BAT is lower in mice when housed in a warm environment [47]. ACLY expression is regulated by the mechanistic target of rapamycin complex 1, which was reported as a cold-activated metabolic sensor in BAT [78]. Besides the gene expression profiling in cold-activated BAT, early radioactive \( ^{14}\text{C}\)glucose tracing provided direct evidence for glucose-dependent de novo
fatty acid synthesis in BAT [72]. More recently, [U-13C]glucose tracing showed that cold exposure induces palmitate synthesis from glucose-derived ACC in BAT, despite that the enrichment of palmitate is only a small fraction of that of TCA cycle intermediates [54]. This suggests that glucose may not be a major carbon source for lipogenic ACC in BAT. Thus, additional studies are required to further quantify ACC production from various sources.

Glucose-dependent glycerol production

While generating lipogenic ACC, glucose is also a major source for glycerol 3-phosphate (G3P), which provides the glycerol backbone for triglycerol (TG) synthesis in brown adipocytes [79-81]. G3P can be synthesized from dihydroxyacetone phosphate, a glycolytic intermediate. The induction of glucose-derived G3P was also observed in white adipocytes treated by peroxisome proliferator-activated receptors g and a agonists, which are known to promote the beiging of white adipocytes in culture [60]. PDK4 can redirect glucose from oxidation toward G3P production for TG synthesis, and PDK4 expression is markedly induced in wild-type mice in response to β3-AR agonist treatment [60]. In differentiated T37i brown adipocytes cultured with [U-13C]glucose, β3-AR stimulation increases the m + 3 enrichment in intracellular TG, suggesting that the glucose carbons are converted into TG in the form of G3P [66]. More importantly, in vivo [U-13C]glucose tracing directly showed that the m + 3 enrichment of G3P is also significantly induced in BAT of mice upon chronic cold exposure [54].

In addition to [U-13C]glucose tracing, in vivo incorporation of [1-13C]glucose shows that the incorporation rate of glucose carbon into glyceride-glycerol is 10-fold higher in cold-exposed BAT relative to that in BAT at 25 °C [80]. Additionally, glyceroneogenesis, a metabolic pathway that synthesizes G3P from precursors other than glucose, is also significantly induced in cold-exposed BAT [80]. More interestingly, the incorporation in vivo of [1-13C]glucose into glyceride-glycerol is much higher than that into glyceride-FA in BAT [79,80]. These data indicate that glucose may contribute to TG synthesis in BAT primarily through G3P, not through the citrate–ACC–fatty acid route.

Lipid metabolism

Lipids are commonly viewed as the primary fuel for uncoupling respiration in BAT, although the thermogenic BAT in adult humans is featured with a high rate of glucose uptake as shown by 18F-FDG-PET. Upon activation, BAT can utilize both intracellular TG through lipolysis and extracellular lipids through fatty acid uptake. Fatty acids not only serve as fuels, but also act as an activator of thermogenesis in BAT. Most interestingly, cold exposure simultaneously activates fatty acid β-oxidation and fatty acid synthesis in BAT, although these metabolic pathways antagonize each other in most tissues.

Lipolysis

Cold exposure increases whole-body lipolysis in humans [77], and microdialysis assay showed that cold exposure increases glycerol release by human BAT [82]. In rodent models, a decrease in relative lipid content is seen in activated BAT in rats by histology [83,84]. Several studies also showed that nicotinic acid (NiAc, an inhibitor of intracellular TG lipolysis) blocks cold-induced fatty acid uptake and glucose uptake in mice [39], and oral NiAc administration abolishes cold-induced BAT thermogenesis in humans [85]. These studies suggest that lipolysis plays a major role in BAT thermogenesis.

The lipolysis of intracellular TG occurs in three steps, which are sequentially catalyzed by adipose TG lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase. ATGL catalyzes the hydrolysis of TG to diacylglycerol (DAG), and ATGL is the rate-limiting step of the lipolysis of intracellular TG [86]. Both HSL and ATGL are required for β3-AR-mediated induction of oxidative genes in brown adipocytes [87].

The role of ATGL in thermogenesis has been extensively studied in whole-body ATGL-deficient and several tissue-specific ATGL-deficient mouse models [88–91]. First, whole-body ATGL-deficient mice have defects in cold adaptation, suggesting ATGL-mediated lipolysis is essential for thermogenesis [88]. Surprisingly, a follow-up study from the same group showed that the BAT of whole-body ATGL-deficient mice has normal mitochondrial function, and BAT-specific ATGL-deficient mice can maintain body temperature upon acute cold exposure [90]. Surprisingly, whereas both whole-body ATGL deficiency and BAT-specific ATGL deficiency induce BAT hypertrophy and whitening, these ATGL-deficient BATs still have normal oxygen consumption rate, similar to those of their control littersmates [90]. In comparison with the BAT-specific ATGL-deficient mice, adipose-specific ATGL-deficient mice have defects in thermogenesis upon cold adaptation [89–91]. Altogether, these studies of ATGL-deficient mouse models suggest that ATGL in
WAT, but not in BAT, is essential to provide fatty acids for thermogenesis.

Moreover, other mouse model studies also indicate that lipolysis in BAT is not essential for thermogenesis [92]. BAT lipolysis-independent thermogenesis is observed in mice lacking BAT Comparative Gene Identification-58 (CGI-58), a lipolysis activator essential for stimulated LD lipolysis, since BAT-CGI-58-KO mice are not cold-sensitive upon acute cold exposure with food access [93]. In comparison, mice lacking CGI-58 in both BAT and WAT are cold-sensitive, suggesting that BAT lipolysis may not be essential for cold-induced thermogenesis in mice [77,93]. Consistent with findings from the BAT-CGI-58-KO mouse model, a recent study showed that mice lacking lipid droplets in brown adipocytes can still maintain thermogenic activity during cold exposure, although lipid droplets are absent in brown adipocytes of mice lacking both acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) and DGAT2 [71]. Taken together, despite that cold exposure increases whole-body lipolysis, it is not fully clear whether and how local lipolysis in BAT contributes to thermogenesis. As several mouse models challenge the essentiality of BAT lipolysis in thermogenesis, it will be interesting to see more evidence from related clinical studies in the future.

**Fatty acid uptake and β-oxidation**

Although the essentiality of intracellular TG utilization in BAT has been challenged, extracellular fatty acids are widely accepted as metabolic fuels for BAT thermogenesis [20]. 18FTHA-PET/CT imaging clearly showed enhanced fatty acid update in activated brown/beige adipocytes [26,29]. As discussed in the previous section on lipolysis, ATGL in WAT is essential for providing fatty acids for BAT thermogenesis, which reflects the importance of fatty acid uptake in BAT [89–91]. In contrast to the small lipid droplets in brown/beige adipocytes, the big lipid droplets in white adipocytes are the major lipid storage site in mammals. Upon cold exposure, lipolysis in WAT releases a large amount of fatty acids into circulation, which provide fuels for BAT thermogenesis. BAT activation is associated with altered circulating concentrations of distinct fatty acids in humans [31], and BAT activity controls vascular lipoprotein homeostasis by inducing a metabolic program that boosts TG-rich lipoprotein turnover and channels lipids into BAT in mice [94].

Within the thermogenic adipocytes, both extracellular (from fatty acid uptake) and intracellular (from TG lipolysis) fatty acids can be completely metabolized through mitochondrial β-oxidation. Metabolomics studies showed that cold exposure activates fatty acid β-oxidation in rat BAT [95]. Mitochondrial long-chain fatty acid β-oxidation requires successive carnitine acyltransferases (CPTs) to translocate acyl-coenzyme As (acyl-CoAs) from the cytoplasm into the mitochondrial matrix [96]. The initial and rate-setting enzyme, CPT1, generates acylcarnitines that can traverse the mitochondrial membranes via specific transporters. Once inside the mitochondrial matrix, CPT2 generates acyl-CoAs from acylcarnitines to initiate β-oxidation of long-chain fatty acids to ACC. Cold exposure increases mRNA expression of Cpt1/2 in BAT of mice [46,47]. Mice with adipose-specific CPT2 deletion are hypothermic after an acute cold exposure, which suggests fatty acid oxidation is required for cold-induced thermogenesis in BAT [97]. More interestingly, liver-derived acylcarnitines can also act as an energy source for BAT thermogenesis, and carnitine supplementation protects mice from age-induced cold sensitivity [98].

**De novo fatty acid synthesis and lipogenesis**

In addition to consuming lipids through fatty acid β-oxidation and lipolysis, BAT also actively produces fatty acids and TG through de novo fatty acid synthesis and de novo lipogenesis (DNL), respectively. De novo fatty acid synthesis and fatty acid β-oxidation can antagonize each other in most tissues. Biochemically, simultaneous activation of de novo fatty acid synthesis and fatty acid β-oxidation is a futile cycle, concurrent with the energy wasting through heat production. Since BAT is specialized for heat production, it is not surprising that both de novo fatty acid synthesis and fatty acid β-oxidation are often activated simultaneously in BAT upon cold exposure [99–101].

De novo fatty acid synthesis is catalyzed by three enzymes, ACLY, ACC carboxylase (ACC), and fatty acid synthase (FASN). As discussed in the earlier section, ACLY produces cytosolic ACC, the building block for de novo fatty acid synthesis. Cytosolic ACC can be further converted into malonyl-CoA by ACC, and malonyl-CoA is the direct substrate for FASN to produce fatty acids [102]. In humans, cold exposure increases the expression of ACLY in BAT, but not in WAT [77]. In mice, cold exposure increases the expression of Acc1 and Acc1/2 in BAT in a time-dependent manner [47].

In addition to de novo fatty acid synthesis, DNL and TG/glycerol dynamics are also increased in both BAT and WAT upon chronic adrenergic activation [81,103]. The DGAT1/2 catalyzes the last dedicated step of TG synthesis. DGAT2, not DGAT1, is highly
expressed in BAT, and β3-AR agonist enhances DGAT2-mediated TG synthesis in primary brown adipocytes [65]. Robust incorporation of [3H]-H2O into BAT lipids directly indicates enhanced DNL in BAT upon cold exposure in mice [47]. Several transcriptional factors in lipid metabolism are also regulated by cold exposure. CCAAT/enhancer-binding protein expression increases in BAT upon cold exposure [46]. A recent study showed that carbohydrate response element-binding protein regulates the simultaneous induction of DNL and fatty acid oxidation in brown adipocytes [104], and cold temperature causes circadian rhythms of sterol regulatory element-binding protein 1c to activate DNL in BAT [105].

As the thermogenic tissue, BAT has its unique lipid metabolism, with simultaneous activation of lipogenesis and fatty acid β-oxidation. This special metabolic feature enables BAT to act as a metabolic sink, but the regulatory mechanisms underlying this lipid futile cycle are not fully clear. Additional studies are required to further explore lipid metabolism in BAT, which may provide more targets for stimulating heat production and burning extra fat to treat obesity.

**Beyond glucose and lipids**

Carbohydrates, lipids (fats), and proteins are the three main nutrients in mammals. While glucose (the most common carbohydrate) and lipids have been viewed as the major energy substrates in BAT, other metabolites can also participate in thermogenesis. Here, we further discuss how BAT utilizes succinate, lactate, branched-chain amino acids (BCAAs), and other metabolites to support its thermogenesis.

**Succinate**

Succinate is one of the TCA cycle intermediates. Metabolomics profiling showed that succinate is a selectively accumulated metabolite in BAT upon both acute and chronic cold exposure [70]. Stable isotope tracing showed that cultured brown adipocytes can uptake and oxidize extracellular succinate. More importantly, succinate administration can stimulate thermogenesis in brown adipocytes and BAT of mice, via succinate dehydrogenase-mediated reactive oxygen species (ROS) production [70]. Other studies also showed that physiological ROS initiates thermogenesis in brown and beige adipose tissues [106,107]. In addition to ROS production, succinate is also the substrate for mitochondrial protein succinylation, and the succinylation of UCP1 plays an important role in BAT energy metabolism [108].

**Lactate**

Lactate has been primarily known as a metabolic waste product, especially in the cancer metabolism field [109]. Known as the famous Warburg effect, tumor cells use a large amount of glucose through glycolysis, and secret out most of the carbons from glucose in the form of lactate [110]. Similar to tumors, BAT can release a substantial amount of glucose carbons in the form of lactate and pyruvate [84]. Surprisingly, recent in vivo stable isotope tracing studies revealed that certain lung cancer cells can uptake and oxidize lactate [68,69]. More interestingly, similar approaches showed that lactate is also an important metabolic fuel in several mammalian tissues, including BAT [111–113]. Like glucose, lactate-derived carbon can be used as a precursor for fatty acid synthesis in brown adipocytes [72]. Lactate is also an inducer of UCP1 expression in adipocytes [114,115].

Monocarboxylic transporters (MCTs) mediate lactate transportation across the cell membrane. MCT4 predominantly mediates lactate secretion, while MCT1 predominantly mediates lactate uptake [69]. MCT1 expression is higher in BAT and sWAT upon cold exposure, and MCT1 and MCT4 expressions increase during adipocyte differentiation [116]. MCT1 is also expressed in cold-induced beige adipocytes, and its expression positively correlates with UCP1 expression [117]. Furthermore, lactate induces Ucp1 expression in differentiated white adipocytes, which can be blocked by MCT inhibitor or MCT4 knockdown. More importantly, direct injection of MCT1 inhibitor into BAT impairs BAT thermogenesis [118]. Interestingly, MCT1 was also reported to be expressed on the mitochondrial membrane, and mitochondrial MCT1 can transport lactate into mitochondria for oxidation [119].

**Branched-chain amino acids**

Branched-chain amino acids (valine, leucine, and isoleucine) are essential amino acids in mammals. A recent study documented that, upon cold exposure, BAT actively utilizes BCAAs in mitochondria for thermogenesis and promotes systemic BCAA clearance in mice and humans [120]. 18F-Flucilovine PET-CT imaging directly showed enhanced BCAA uptake in cold-activated BAT, and [U-13C]leucine tracing directly showed the oxidation of BCAAs in cultured human brown adipocytes. More importantly, systemic BCAA clearance is impaired in mice with BAT-specific defect in BCAA metabolism, suggesting that BAT is a major BCAA-consuming tissue in mice. Furthermore, Solute Carrier Family 25 Member 44 is identified as the
mitochondrial transporter for BCAA catabolism in BAT [120].

In addition to producing energy for thermogenesis, BCAAs also provide building blocks for de novo fatty acid synthesis in BAT and sWAT. Whereas glucose-derived ACC is used to generate straight-chain fatty acids (C16:0 and C18:0), BCAA-derived propionyl-CoA is used to generate monomethyl branched-chain fatty acids (mmBCFAs, odd-chain FAs C15:0 and C17:0) in adipocytes [121]. Relative to other tissues, BAT exhibits the highest BCAA catabolic and mmBCFA synthesis fluxes, and mmBCFAs are significantly decreased in obese mice [121]. Additionally, BCAAs contribute to the synthesis of both odd-chain and even-chain fatty acids in differentiated 3T3-L1 adipocytes [122]. Stable isotope tracing showed that, in differentiated 3T3-L1 adipocytes, BCAAs contribute to as much as 30% of the lipogenic ACC pool, which is comparable to the amount of glucose-derived ACC [123].

Other metabolites in BAT thermogenesis
Besides BCAAs, glutamate is another amino acid utilized by BAT. One recent study of microdialysis showed that cold exposure increases glutamate uptake only by BAT, but not by WAT, in human subjects [82]. Different from BCAAs and glutamate, alanine inhibits glucose utilization in rat BAT, which may preserve glucose wastage in postprandial situations [124]. Additionally, a recent single-nucleus RNA-sequencing analysis of mouse and human adipocytes showed that acetate metabolism in a subpopulation of adipocytes regulates thermogenesis [125]. Higher temperature induces a rare subpopulation of adipocytes that express a high level of Cytochrome P450 Family 2 Subfamily E Member 1 and Aldh1a1 (encoding aldehyde dehydrogenase 1 family member A1, ALDH1A1), and ALDH1A1 regulates brown adipocyte's thermogenic capacity through acetate.

Creatine is another special metabolite playing an essential role in BAT thermogenesis [126]. Creatine kinase (CK) catalyzes the conversion of creatine and ATP to create phosphocreatine, which serves as an energy reservoir in various tissues. CK is powerfully induced by thermogenic stimuli in both mouse and human adipocytes [127]. In BAT, creatine and phosphocreatine form a futile cycle, which exists in both UCP1-positive and UCP1-negative beige adipocytes [128]. CK activity increases upon cold exposure, and the creatine futile cycle serves as a thermogenic pathway in beige fat. Fat-specific knockout of the creatine transporter or creatine synthesis enzyme glycine amidinotransferase impairs thermogenesis and induces obesity [129,130]. However, a recent study showed that creatine supplementation does not active BAT in adult vegetarians [131,132]. Additional studies are required to further investigate the role of these less common fuels in BAT thermogenesis.

Perspectives
The intracellular glucose and lipid metabolism are tightly connected in BAT. Brown adipocytes are specialized to generate heat through uncoupled respiration, which relies on its unique metabolic program. First, in most cells, mitochondrial oxidation and ATP production are tightly coordinated; by contrast, while mitochondrial oxidation is highly active in brown adipocytes, ATP production is compromised by proton gradient uncoupling. Second, brown adipocytes can simultaneously activate fatty acid β-oxidation and fatty acid synthesis, which antagonize each other in most cells. Third, brown adipocytes can handle a high level of ROS, which promotes its thermogenesis, whereas ROS impairs mitochondrial function in most cells. Most importantly, BAT utilizes a wide range of metabolites to support thermogenesis, exhibiting extreme flexibility for fuel selection.

As a thermogenic tissue, BAT is specialized to ‘waste’ glucose, lipids, and other nutrients through heat production. This wasting feature of BAT gains a great deal of interest recently, as activating metabolic sink has the potentials in reversing obesity to cure metabolic syndrome in modern society. A large portion of BAT studies has focused on the development of brown and beige adipocytes, as many new markers in preadipocytes are identified to promote brown and beige adipocyte differentiation in mammals. In the meantime, many studies have also focused on which metabolites act as energy sources during thermogenesis, and how these metabolites are regulated and metabolized.

At the molecular level, extracellular glucose, lipids/fats, amino acids, and other metabolites can all serve as fuels for thermogenesis in brown adipocytes. Many studies showed that cold exposure increases the utilization of certain kinds of metabolites in BAT. As these extracellular fuels share intracellular metabolic intermediates, it is hard to define the net contribution of each particular fuel. For example, extracellular lactate can be utilized by mammalian tissues [111–113], but the net contribution of lactate as the fuel has also been challenged by other stable isotope tracing studies [133]. In the meantime, glucose-derived carbons can be converted to glycerol and fatty acids, before being fully
oxidized to CO₂. β-oxidation-derived ACC can also be reused for fatty acid synthesis. It is worth noting that, these metabolic interactions are sensitive to the environmental nutrient’s availability. To better understand the thermogenic metabolism, further quantitative analysis is required to define how various stimulations selectively regulate the fuel utilization in brown adipocytes, especially in the in vivo settings.

At the cellular level, as metabolism plays a fundamental role, metabolic reactions often reserve a large capacity in brown adipocytes. One possibility is that a small fraction of metabolic enzymes can fully maintain their function. Our unpublished data showed that knockdown of metabolic enzyme did not impair its metabolic flux in cells, although there was a dramatic decrease in the protein expression level. These data suggest that the protein expression of enzymes may not always reflect their metabolic activities. Another possibility is that the interconnected network can also preserve central carbon metabolism through compensatory routes, especially in the genetic-deficient animal models [134,135]. Thus, in addition to measuring the expression level of selected metabolic enzymes, it is very important to systemically and directly assay the metabolic fluxes in brown adipocytes, upon pharmacological stimulations or after the genetic modification.

At the tissue level, metabolism in BAT is tightly connected with other tissues. Upon cold exposure, lipolysis in WAT provides a large amount of fatty acids as the fuel for BAT thermogenesis [88–91]. Similar to WAT, BAT also functions as an endocrine tissue, and BAT-derived secretory factors (batokines) can regulate glucose and lipid metabolism in the liver [136–138]. While BAT is the most thermogenic tissue per weight, muscle also produces heat, and the large muscle mass significantly contributes to whole-body heat production [139,140]. Additionally, the muscle may also secret metabolites such as succinate to regulate BAT thermogenesis [70]. Since BAT metabolism is constantly connected with other metabolic tissues, it is essential to simultaneously monitor multiple tissues to understand the metabolic crosstalk involved in the control of whole-body thermogenesis.

Conflicts of interest
The authors declare no conflict of interest.

Author contributions
All authors listed have made a direct and intellectual contribution to the work.

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Z. Wang et al.

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