Review

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Brown adipocyte glucose metabolism: a heated subject

Mohammed K Hankir[1,2,*] & Martin Klingenspor[3,4,**]

Abstract

The energy expending and glucose sink properties of brown adipose tissue (BAT) make it an attractive target for new obesity and diabetes treatments. Despite decades of research, only recently have mechanistic studies started to provide a more complete and consistent picture of how activated brown adipocytes handle glucose. Here, we discuss the importance of intracellular glycolysis, lactate production, lipogenesis, lipolysis, and beta-oxidation for BAT thermogenesis in response to natural (temperature) and artificial (pharmacological and optogenetic) forms of sympathetic nervous system stimulation. It is now clear that together, these metabolic processes in series and in parallel flexibly power ATP-dependent and independent futile cycles in brown adipocytes to impact on whole-body thermal, energy, and glucose balance.

Keywords brown adipose tissue thermogenesis; fatty acid metabolism; glucose metabolism; positron emission tomography; uncoupling protein 1
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See the Glossary for abbreviations used in this article.

Lessons from the brain

Human functional neuroimaging was first introduced in the early 1980s on the basis that metabolically active brain regions can be visualized by ^{18}flurodeoxyglucose positron emission tomography (^{18}F-FDG PET) [1]. What ensued was a heated and lengthy debate about precisely how and where glucose is handled by brain cells to power neuronal activity, with glycolytic and oxidative processes at presynaptic and postsynaptic sites as well as in astrocytes being tabled [2–5]. Only recently, however, have optogenetic and pharmacological small-animal ^{18}F-FDG PET imaging studies unequivocally confirmed neuronal [6] and glial [7] contributions to the brain ^{18}F-FDG PET signal, respectively. Despite these issues, there is at least unanimous agreement that during brain activity, glucose is metabolized to produce ATP required for the function of active transporters and ion pumps essential for synaptic transmission [8].

^{18}F-FDG PET imaging of brown adipose tissue

Human functional imaging of thermogenic brown adipose tissue (BAT) was first introduced in the late 2000s again using ^{18}F-FDG PET [9–11]. It followed from careful retrospective analysis of clinical ^{18}F-FDG PET imaging data that pointed to the existence of metabolically active BAT mainly in the supraclavicular area of a small proportion of adults [12–14] (Fig 1), and that increased in prevalence during the winter months [15]. Through the use of ^{18}F-fluoroheptadonic acid (^{18}F-FTHA) [16] and ^{11}C-acetate PET imaging [17], respectively, human BAT was subsequently shown to consume large amounts of circulating fatty acids and to be highly oxidative during temperature-induced thermogenesis [18]. Prior to these seminal studies, it had generally been thought that BAT only exists in the interscapular region in human infants [19]. Coupled with the knowledge from animal work that increasing energy expenditure by stimulating BAT thermogenesis promotes a negative whole-body energy balance [19], nothing short of a biomedical revolution was heralded.

BAT glucose uptake and thermogenesis do not go hand in hand

While in the last decade, it has become clear that BAT can potentially be harnessed to independently manage hyperglycemia [20] and hyperlipidemia [21] as well as obesity [22,23] in humans, a new but somewhat familiar controversy was gaining traction in the background: Can ^{18}F-FDG PET imaging really be considered a reliable technique to measure BAT thermogenic activity? The demonstration that BAT ^{18}F-FDG uptake is directly proportional to the degree of non-shivering thermogenesis measured by indirect calorimetry first suggested that this could be the case [18,22–24]. However, several findings arose which challenged this notion. For example, like the small molecule drug Mirabegron which activates beta-3 adrenergic receptors in brown adipocytes to stimulate ^{18}F-FDG uptake [23], insulin does the same through the insulin receptor

1 Department of Experimental Surgery, University Hospital Wuerzburg, Wuerzburg, Germany
2 German Research Foundation Collaborative Research Center in Obesity Mechanisms 1052, University of Leipzig, Leipzig, Germany
3 Chair of Molecular Nutritional Medicine, TUM School of Life Sciences Weihenstephan, Technical University of Munich, Freising, Germany
4 EKFZ - Else Kröner-Fresenius Center for Nutritional Medicine, Technical University of Munich, Freising, Germany
*Corresponding author. Tel: +49 9312 01 31728; E-mail: hankir_m@klinik.uni-wuerzburg.de
**Corresponding author. Tel: +49 8161 71 2386; E-mail: mk@tum.de

Glossary

^{123}/^{125}I-BMIPP	[^{123}/^{125}I]-b-Methyl-p-iodophenyl-pentadecanoic acid
^{18}F-FDG PET	^{18}Flurodeoxyglucose positron emission tomography
^{18}F-FTHA	^{18}F-Fluroheptadonic acid
ACL	ATP citrate lyase
AGPAT2	1-Acylglycerol-3-phosphate O-acyltransferase 2
ARC	Arcuate nucleus
ATGL	Adipose triglyceride lipase
BAT	Brown adipose tissue
CCK	Cholecystokinin
CPT1	Carnitine palmitoyltransferase 1
DGAT2	Diacylglycerol acyltransferase 2
EPAC1	Exchange protein activated by cyclic AMP 1
ErbB3/4	Epidermal growth factor receptor 3/4
FASN	Fatty acid synthase
FGF21	Fibroblast growth factor 21
G6PDX	Glucose-6-phosphate dehydrogenase, X-linked
GD1	Glycerol-3-phosphate dehydrogenase 1
GLUT1/4	Glucose transporter 1/4
GPAT3	Glycerol-3-phosphat-O-acyltransferase 3
GYS1/2	Glycogen synthase 1/2
HK2	Hexokinase 2
HSL	Hormone-sensitive lipase
IMM	Inner mitochondrial membrane
LDH	Lactate dehydrogenase
MCC	Malonyl coenzyme A carboxylase
MCT	Monocarboxylate transporter 1
MPC1/MPC	Mitochondrial pyruvate carriers 1/2
mTORC2	Mammalian target of rapamycin complex 2
NRG4	Neuregulin 4
NTS	Nucleus tractus solitarius
PCK1	Phosphoenolpyruvate carboxykinase 1
PGD	6-Phosphogluconate dehydrogenase
PKA	Protein kinase
PKM	Pyruvate kinase M
POMC	Pro-opiomelanocortin
RYR2	Ryanodine receptor 2
SERCA2b	Sarco/ER Ca^{2+}- ATPase 2b
SLC25A1	Mitochondrial citrate transporter
SPECT	Single-photon emission computed tomography
TCA	Tricarboxylic acid
TGR5	Takeda G-protein receptor 5
TRPV1	Transient receptor potential vanilloid receptor 1
UCP1/2	Uncoupling protein 1/2
WAT	White adipose tissue

[25]. Consequently, insulin-resistant individuals accumulate less ^{18}F-FDG in BAT but show normal non-shivering thermogenesis as well as BAT fatty acid uptake and oxidative metabolism [26].

More definitive data eventually came again from small-animal ^{18}F-FDG PET imaging studies performed on mice that lack uncoupling protein 1 (UCP1) [27–29]. This inner mitochondrial membrane (IMM) protein is most highly expressed in brown adipocytes and is essential for both forms of non-shivering thermogenesis, i.e., temperature-induced [30] and diet-induced [31]. It was found that stimulation of sympathetic nerves innervating BAT by acute cold exposure results in BAT ^{18}F-FDG uptake in female UCP1 knockout mice as it does in wild-type mice [27]. In line with this, in subsequent studies, BAT ^{18}F-FDG uptake in response to a beta-3 adrenergic receptor agonist in UCP1 knockout mice was fully retained despite defective BAT thermogenesis upon the same pharmacological treatment [28,29]. Furthermore, independent experiments on isolated brown adipocytes lacking UCP1 have all corroborated the *in vivo* imaging results [28,32,33]. These findings clearly show that BAT glucose uptake can be dissociated from UCP1-mediated thermogenesis.

However, evidence in support of the contrary does exist. Acute noradrenaline treatment of UCP1 knockout mice did not further promote BAT 2-[^{3}H]deoxyglucose uptake compared to vehicle treatment [34]. Similarly, unlike for female UCP1 knockout mice, cold exposure of male UCP1 knockout mice did not stimulate BAT ^{18}F-FDG uptake [27]. Optogenetic stimulation of BAT following siRNA-mediated knockdown of *Ucp1* in mice further failed to regulate glycemia [35]. Because this intervention resulted in markedly lower blood glucose levels in mice with normal BAT, it was inferred that brown adipocyte glucose utilization is secondary to UCP1-mediated thermogenesis [35]. However, this conclusion was not supported by any direct measurements of glucose uptake by BAT. The discrepancies between the *in vivo* studies with beta-3 adrenergic receptor agonist and noradrenaline treatments may be due to the different housing temperatures of mice prior to scanning [28,29,34]. It will be of interest to determine the causes underlying the gender differences in UCP1 knockout mice in terms of temperature-induced BAT glucose uptake, *i.e.,* whether they are at the level of sympathetic nerve or brown adipocyte responsivity, although the latter is not supported by data from male and female UCP1 knockout mice treated with a beta-3 adrenergic receptor agonist [29].

The retained glucose uptake by brown adipocytes upon adrenergic stimulation in the absence of UCP1-mediated thermogenesis can be explained by the fact that the two processes occur in parallel rather than in series. This differs from the situation in hippocampal neurons for instance where electrical stimulation leads to GLUT4 trafficking to the presynaptic membrane, where it promotes glucose uptake secondary to increases in the intracellular AMP:ATP ratio sensed by AMP kinase [36]. Instead, upon beta-3 adrenergic receptor activation of brown adipocytes and the subsequent rise in intracellular cyclic AMP concentrations, the EPAC1 signaling arm causes GLUT1 trafficking to the plasma membrane and glucose uptake, while simultaneously the PKA signaling arm causes UCP1-mediated thermogenesis through fatty acids released upon lipolysis [37–39]. Further, despite the fact that UCP1-mediated thermogenesis in brown adipocytes decreases ATP production by dissipating the proton gradient across the IMM [40], this does not appreciably influence AMP kinase activity [32]. Thus, unlike neurons, activated brown adipocytes do not take up glucose as a result of increased AMP kinase activity in response to energy demands placed on the cell.

Insight into BAT function guides technique and drug development

In light of the false negatives and positives that occur with ^{18}F-FDG PET imaging described above and the exposure it causes to ionizing radiation, other techniques are now being considered to visualize human BAT thermogenic activity, such as infrared thermal [41] and near-infrared optoacoustic [42] imaging, that both have potential to supersede the current gold standard. The studies highlighted above also raise a fundamental question concerning BAT fuel utilization: If so much glucose is taken up by activated brown adipocytes during thermogenesis to the point of visualization with ^{18}F-FDG PET, what exactly happens to it? The answer has strong implications for the design of new strategies to treat hyperglycemia aimed at taking advantage of the glucose sink property of BAT. Elegant work

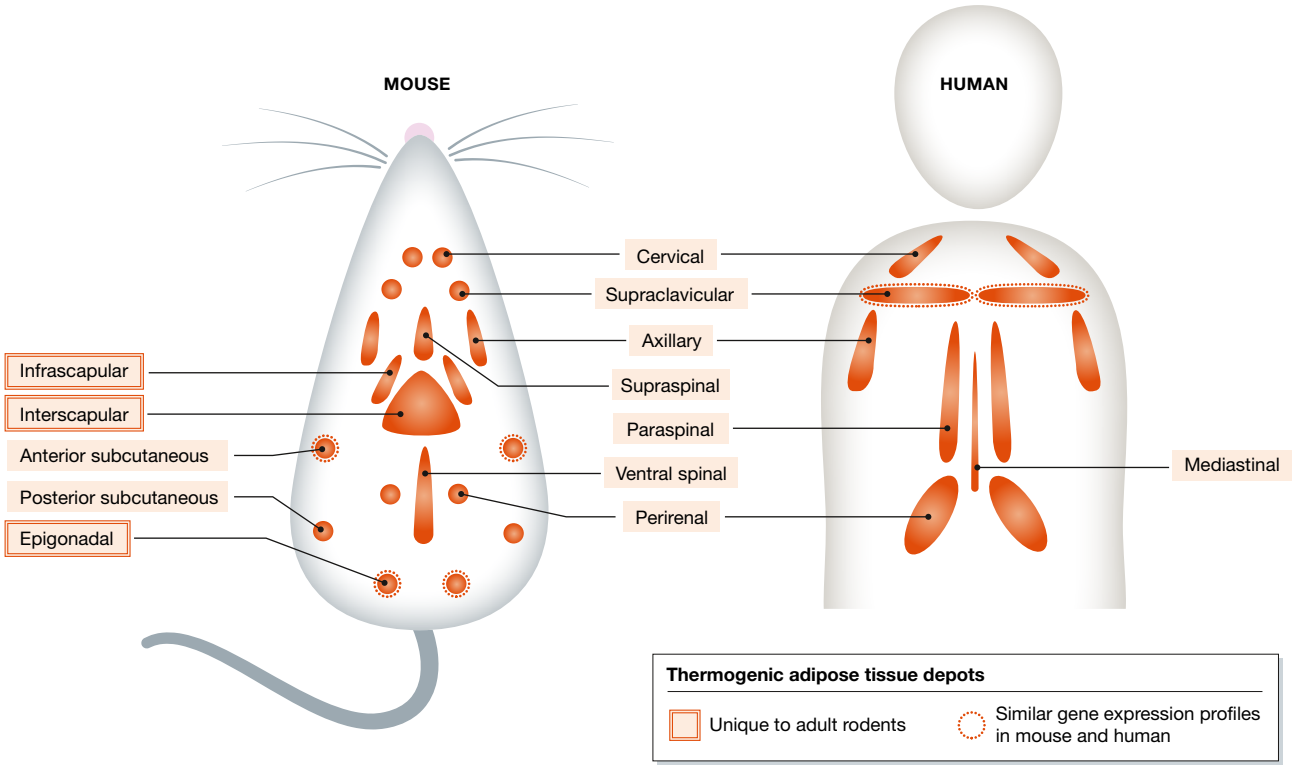

Figure 1. The distribution of thermogenic adipose tissue depots in mice and humans.

Molecular imaging techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT) have allowed for the identification of various thermogenic adipose tissue depots in animals and humans. This is principally because these depots consume large amounts of glucose and fatty acids. Cervical, supraclavicular, axillary, and spinal depots are shared between species, whereas scapular and gonadal depots are unique to adult rodents. Gene expression profiling has revealed that the molecular signature of interscapular brown adipose tissue (BAT) of mice is unique, whereas that of the browned anterior subcutaneous and epigonadal adipose tissue depots of mice are more similar to the supraclavicular BAT depot of humans.

performed decades ago in which rats were administered either ^{14}C-glucose [43] or tritiated water [44] first revealed that a substantial degree of lipogenesis takes place in BAT following acclimation to cold. These findings have now been extended by those from more recent cell-based, animal and human studies that have finally provided us with a more detailed and complete picture of glucose metabolism in brown adipocytes (Fig 2) and that will be discussed below.

The significance of glucose uptake and glycolysis for BAT thermogenesis

Early estimates on the contribution of glucose as an energy source for BAT thermogenesis were in the range of 2–16% [45–47]. They were based on glucose and oxygen consumption rates of isolated rat brown adipocytes upon acute adrenergic stimulation [46,47] or on arteriovenous differences in blood glucose and oxygen across BAT of anesthetized cold-acclimated rats [45]. To specifically address the requirement of BAT glucose uptake and subsequent glycolysis for thermogenesis, loss-of-function experiments were performed on cultured immortalized mouse brown adipocytes [33]. The increase in oxygen consumption by these cells upon acute adrenergic stimulation was prevented by siRNA-mediated knockdown of glucose transporters *Glut1* and *Glut4*. Similar results were obtained with siRNA-mediated knockdown of hexokinase 2 (*Hk2*) and pyruvate kinase M (*Pkm*), the first and last enzymes in glycolysis, respectively. This is consistent with infrared thermal imaging data from differentiated human brown adipocytes showing that the GLUT4 inhibitor indinavir dose-dependently reduces thermogenesis [48], and with the acute cold intolerance of mice genetically engineered to have defective glucose uptake and glycolysis in brown adipocytes through inactivation of the serine/threonine kinase mTORC2, a downstream target of EPAC1 [39]. Remarkably, adeno-associated virus-mediated overexpression of *Hk2* in the BAT of these mice restored its glucose uptake and glycolytic capacity as well as their cold tolerance [39]. Therefore, while quantitatively speaking glucose may only modestly contribute to fueling BAT thermogenesis compared to fatty acids [33,45–47,49], it is nevertheless essential. Furthermore, ^{18}F-FDG uptake increases in BAT when fatty acid uptake and oxidation are diminished in UCP2-deficient mice [50], suggesting that when necessary, glucose can make a larger contribution to fueling BAT thermogenesis in the short term. This is in line with findings from differentiated T37i cells (a brown adipocyte cell line) acutely treated with a beta-3 adrenergic receptor agonist, in which glucose oxidation doubled when fatty acid oxidation was pharmacologically inhibited [49].

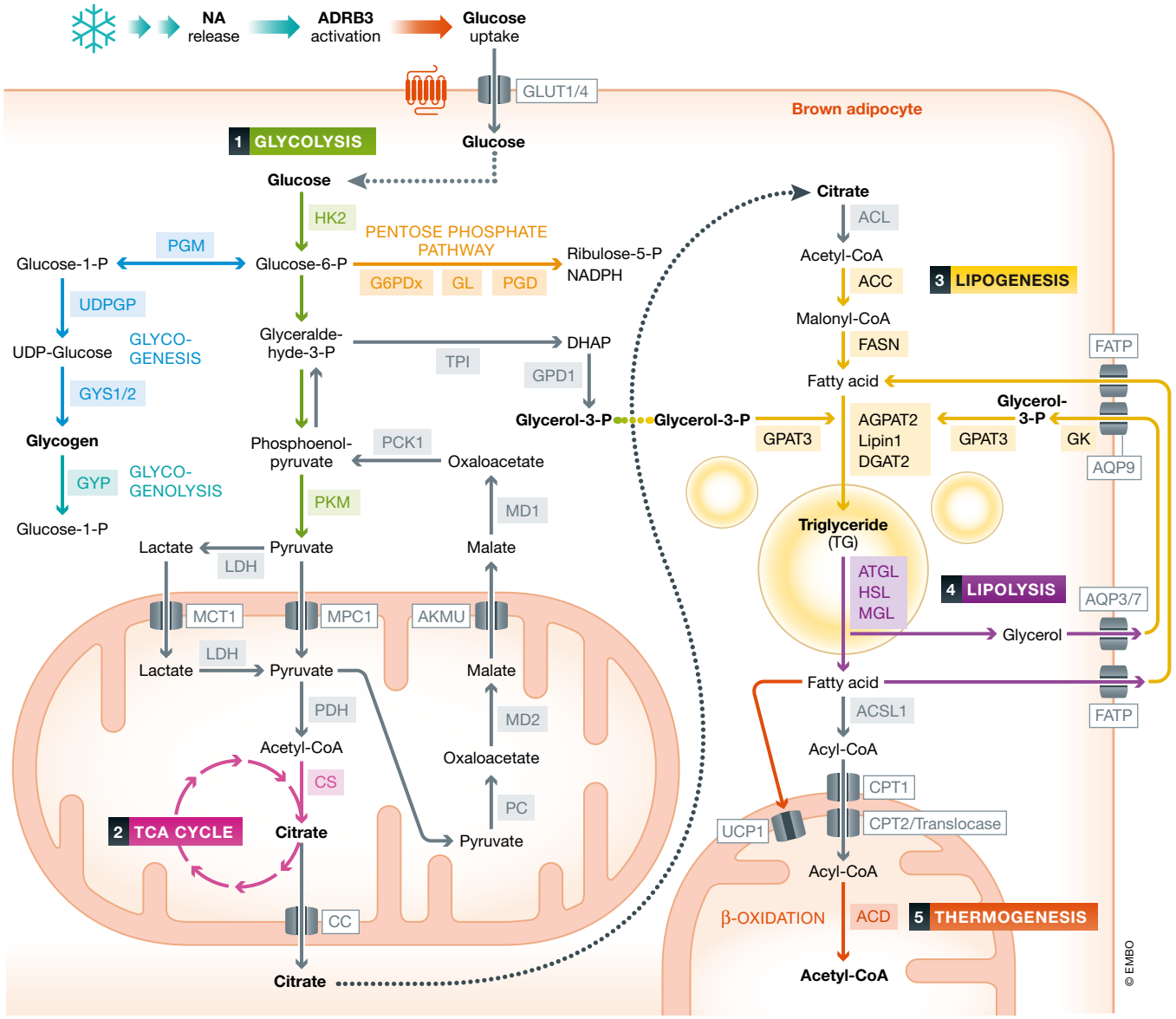

Figure 2. The metabolic fate of glucose in brown adipocytes during thermogenesis.

Upon beta-3 adrenergic receptor activation following noradrenaline release by sympathetic nerve endings in response to cold, glucose is taken up by brown adipocytes via glucose transporters 1 and 4 (GLUT1/4). Glucose then undergoes glycolysis to generate dihydroxyacetone phosphate (DHAP), pyruvate, and lactate in the cytosol. Simultaneously, glucose-6-phosphate (glucose-6-P) feeds into the pentose phosphate pathway to generate ribulose-5-P and NADPH which is used for lipogenesis and also into glycogen synthesis and breakdown pathways. Pyruvate and/or lactate are next transported into the mitochondria via monocarboxylate transporter 1 (MCT1) and for pyruvate, the pyruvate carrier 1 (PC1), while DHAP is converted into glycerol-3-P by glycerol-3-phosphate dehydrogenase 1 (GPD1). Once inside the mitochondria, pyruvate is converted into acetyl-CoA by pyruvate dehydrogenase (PDH). If lactate is indeed transported into mitochondria, it would be converted by LDH back into pyruvate. Acetyl-CoA then undergoes partial breakdown in the TCA cycle into citrate by citrate synthase (CS) which is then exported by the citrate carrier (CC) into the cytosol. Citrate then feeds into a lipogenic pathway after being converted back into acetyl-CoA by ATP citrate lyase (ACL). Acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), glycerol-3-phosphat-O-acyltransferase 3 (GPAT3), AGPAT2, lipin 1, and diacylglycerol O-acyltransferase 2 (DGAT2) then contribute to the generation of triglycerides (TG) from fatty acids and glycerol-3-P. These then rapidly undergo lipolysis in cytosolic lipid droplets through the action of adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase (MGL). The liberated fatty acids either activate UCP1 to generate heat or are next converted into acyl-CoA by long chain acyl-CoA synthase 1 (ACSL1) and then transported into the mitochondria via the sequential action of carnitine palmitoyltransferase 1 (CPT1), translocase, and CPT2. Once inside the mitochondria, acyl-CoA undergoes β-oxidation by acyl-CoA deyhyrogeneses (ACD) to generate the necessary proton gradient across the inner mitochondrial membrane. The liberated glycerol from lipolysis is phosphorylated by glycerol kinase (GK) into glycerol-3-P to facilitate fatty acid re-esterification. Similarly, pyruvate carboxylase (PC) generates oxaloacetate (OA) from pyruvate, which is then converted into malate (MA) by malate dehydrogenase 2 (MD2). MA is exported outside of the mitochondria into the cytosol by the alpha-ketoglutarate malate uniporter (AKMU) where it is reconverted back into OA by MD1. Finally, phosphoenolpyruvate carboxykinase 1 (PCK1) generates phosphoenolpyruvate which feeds G3P synthesis required for fatty acid re-esterification. Fatty acids and glycerol released from lipolysis may enter an extracellular loop via fatty acid transport protein1 (FATP) and aquaporin 3/7/9 (AQP 3/7/9) as part of the fatty acid recycling pathway. Abbreviations: HK2, hexokinase 2; PGM, phosphoglucomutase; G1P, glucose-1-phosphate; G6PDx, glucose-6-phosphate dehydrogenase X-type; GL, gluconolactolase GYP, glycogen phosphorylase; PGD, 6-phosphogluconate dehydrogenase; UDP, uracil-diphosphate; UDPGP, UDP glucose pyrophosphorylase; PK, pyruvate kinase; and TPI, triosephosphate isomerase. Some reactions have been omitted for clarity.

Offshoots of glycolysis

Glucose-6-phosphate generated at the first step of glycolysis can also proceed to the biosynthetic pentose phosphate pathway (PPP). A transcriptome analysis revealed that mRNA levels of *G6pdx*, *Pgl,* and *Pgd* whose protein products produce ribulose-5-phosphate from glucose-6-phosphate as part of the PPP (Fig 2) increased in the BAT of mice upon chronic cold exposure [51]. The significance of this is unclear, but the extra NADPH produced from the PPP may be used for lipogenesis (see below). Recruitment of the PPP may also provide the nucleotides and amino acids for cold-induced cell proliferation and differentiation in BAT and the dramatic changes in gene expression and protein translation that take place in activated brown adipocytes to boost thermogenic capacity. The glycogen production rate is also enhanced in BAT during cold acclimation reflected by increased *Gys1/2* mRNA expressions [51]. This provides another avenue for the deposition of excess glucose in brown adipocytes. Complementing these findings, glycogen phosphorylase mRNA expression also increases in BAT of rats upon acute and chronic cold exposure, which generates an on-demand intracellular source of glucose from glycogen [52].

The different routes taken by pyruvate in brown adipocytes during thermogenesis

Fatty acid and glycerol production

Clues about what happens to the pyruvate generated during glycolysis in activated brown adipocytes have been obtained from another more comprehensive transcriptome study comparing BAT samples from mice chronically housed at thermoneutrality (30°C) with those at standard room (22°C) and extreme cold (4°C) temperatures [53]. An astonishing degree of differential gene expression was reported between thermoneutrality and room temperature that did not generally change much further in extreme cold. In particular, a wide array of lipogenic gene products was upregulated [53]. Of interest here was that mRNA expression of the mitochondrial pyruvate carriers 1 and 2 (*Mpc1* and *Mpc2*) and the mitochondrial citrate transporter (*Slc25a1*) increased. These two sets of transcripts translate into proteins that provide gateways for the entrance of cytosolic pyruvate into mitochondria and for the exit of citrate generated during the TCA cycle back out, respectively (Fig 2). It is in the cytosolic compartment that ATP citrate lyase (ACL) then generates acetyl coenzyme A which feeds into lipogenesis through the catalytic activities of malonyl coenzyme A carboxylase (MCC) and fatty acid synthase (FASN) (Fig 2). Importantly, mRNA and protein expression of these three enzymes markedly increase from thermoneutrality to room/extreme cold temperatures [53].

The transcription of genes involved in glycerol metabolism also increases in BAT of mice when the temperature drops for prolonged time periods, such as glycerol kinase and *Agpat2* [53]. These two enzymes produce glycerol-3-phosphate from glycerol and phosphatidic acid from lysophosphatidic acid, respectively (Fig 2). Interestingly, analysis of the mitochondrial proteome in BAT of mice chronically exposed to cold revealed a two- to threefold increase in glycerol kinase and AGPAT2 protein expressions [54]. Considering that AGPAT2 is an ER-localized enzyme, this may be due to the establishment of ER–mitochondrial contacts during thermogenesis [55]. In line with the findings in mice, an increase in *Gpd1*, *Pck1,* and *Gpat3* mRNA expression in BAT of rats occurs upon acute and chronic cold exposure [52]. These three enzymes catalyze the production of glycerol-3-phosphate from dihydroxyacetonephosphate generated during glycolysis, phosphoenolpyruvate from oxaloacetate generated from mitochondrial pyruvate carboxylase, and lysophosphatidic acid from glycerol-3-phosphate, respectively (Fig 2). Correspondingly, AGPAT enzymatic activity is approximately five-fold higher in BAT of rats following cold acclimation [56]. The mRNA expression of *Lipin1* in BAT of mice also increases upon acute cold exposure [57], the protein product of which produces diacylglycerol from phosphatidic acid [57]. Importantly, the significance of glycerol-metabolizing enzymes for thermogenesis was demonstrated by the acute cold intolerance of mice lacking lipin 1 specifically in WAT and BAT [57].

Like glycerol kinase, the aforementioned glycerol-metabolizing enzymes could also be important in brown adipocytes for fatty acid re-esterification as part of a dynamic steady state with lipolysis. This so-called fatty acid recycling has long been known to be a UCP1-independent ATP-consuming form of thermogenesis in white adipocytes [58]. In these cells, GPD and PCK1 play a dominant role in glycerol-3-phosphate production due to the absence of appreciable amounts of glycerol kinase [58,59]. The increased oxygen consumption by epididymal white adipose tissue (WAT) but not BAT explants from UCP1 knockout mice chronically treated with a beta-3 adrenergic receptor agonist suggests that fatty acid recycling does not play a significant thermogenic role in BAT in response to this particular pharmacological stimulus [60]. Rather, the regulation of *Ucp1* transcription by glycerol kinase by as yet unknown mechanisms in brown adipocytes may contribute to regulating whole-body thermal and energy balance [61].

Beige adipocyte thermogenesis can compensate for diminished brown adipocyte thermogenesis

Sanchez-Gurmaches *et al* [53] further went on to show that while signaling of the serine/threonine kinase Akt2 in brown adipocytes is required for the global changes in gene expression in response to cold acclimation, it is dispensable for acute cold tolerance. This is most likely because the inguinal WAT of mice lacking Akt2 specifically in BAT underwent compensatory browning, which involves the formation of brite/beige adipocytes. These thermogenic cells intersperse in WAT and have a unique gene expression profile [62]. They can arise either through chronic activation of beta-1 adrenergic receptors in dedicated precursor cells in response to cold exposure or by transdifferentiation of existing white adipocytes due to chronic pharmacological activation of beta-3 adrenergic receptors [63]. Besides UCP1-mediated thermogenesis [64], inguinal beige adipocytes can also generate heat upon alpha-1 and beta-3 adrenergic receptor activation from calcium cycling into and out of the endoplasmic reticulum (ER) through the ER Ca^{2+}-ATPase SERCA2b and the ryanodine receptor RYR2, respectively [65]. As calcium cycling is fueled almost entirely by ATP generated from glycolysis, inguinal beige adipose tissue functions as another glucose sink that favorably regulates glycemia [65]. Furthermore, upon chronic cold exposure, inguinal beige adipocytes initiate another UCP1-independent, ATP-dependent thermogenic futile cycle between creatine and phosphocreatine in the mitochondrial intermembrane space [66]. Creatine kinase-mediated phosphocreatine production from creatine also operates in epididymal beige adipocytes following chronic beta-3 adrenergic receptor agonist treatment [67]. Notably, fatty acid recycling upon chronic

cold exposure and beta-3 adrenergic receptor agonist treatment in inguinal [68] and epididymal [60] beige adipocytes, respectively, may intersect with creatine and phosphocreatine cycling to accept the high energy phosphate from phosphocreatine for the required ATP. Thus, adipocytes from distinct adipose tissue depots can exhibit numerous forms of thermogenic futile cycling depending on the environmental, pharmacological, and genetic conditions found.

Lactate production

Another direction of pyruvate metabolism in cells particularly under anaerobic conditions is toward lactate production through the catalytic activity of lactate dehydrogenase (LDH) (Fig 2). In the brain, this also takes place when oxygen levels are normal, which is referred to as aerobic glycolysis [3]. The mismatch between an excess of glucose but normal oxygen consumption in activated brown adipocytes [25,45–47] is indicative that these cells utilize aerobic glycolysis that achieves expedience at the expense of energetic efficiency. Indeed, early studies revealed that rat brown adipocytes release lactate upon acute adrenergic stimulation [69] and that intact BAT does the same in cold-acclimated rats [45].

Lactate metabolism was recently shown to occur in response to optogenetic stimulation of BAT thermogenesis in mice [35], which more realistically mimics cold exposure than pharmacological approaches. One-hour photostimulation of BAT sympathetic nerve endings expressing the blue light-activated photoreceptor channelrhodopsin 2 not only raised BAT noradrenaline concentrations and temperature measured by telemetry but also precipitously decreased blood glucose levels. This was prevented by local siRNA-mediated knockdown of *Glut1* in BAT and inhibition of glycolysis with 2-deoxyglucose. Similar results were obtained from local pharmacological inhibition of LDH in BAT and siRNA-mediated knockdown of monocarboxylate transporter 1 (*Mct1*), which transports both lactate and pyruvate from the cytosol into mitochondria (Fig 2). These remarkable findings confirm the importance of glucose uptake and glycolysis for BAT thermogenesis in the acute setting. They also provide new causal evidence that intracellular lactate metabolism significantly contributes to this process. However, despite the recent discovery that in most peripheral tissues (including WAT), lactate is not merely a metabolic end-product but actively feeds into the TCA cycle [70], it remains to be shown whether this is also the case in BAT. If LDH can be found in brown adipocyte mitochondria as part of a lactate oxidation complex characterized in neurons [71], this uncertainty might be resolved. Also, it is unclear whether the improved glycemia from optogenetic stimulation of BAT results from the release of a glucoregulatory endocrine factor such as FGF21 from brown adipocytes [72]. This pleiotropic peptide is as effective in clearing blood glucose in mice as optogenetic BAT stimulation, through enhancing glucose uptake by WAT and liver [73]. Regardless of the mechanism, the fact that optogenetic stimulation of BAT can be achieved non-invasively due to the skin-penetrating properties of blue light [35] opens up new therapeutic potential.

Lipogenesis is quickly followed by lipolysis in brown adipocytes during thermogenesis

Up to here, we have discussed the generation of triglyceride components from glucose (i.e., fatty acids and glycerol metabolites) in brown adipocytes during thermogenesis. To specifically address the mechanism of triglyceride formation in activated BAT, differentiated/immortalized mouse brown adipocytes were acutely treated with a selective beta-3 adrenergic receptor agonist following siRNA-mediated knockdown of diacylglycerol acyltransferases 1 or 2 (*Dgat1/2*) [74]. These ER-localized enzymes catalyze the addition of a final fatty acid to diacylglycerol which is the rate-limiting reaction in triglyceride formation (Fig 2). It was found that the incorporation of ^{14}C-glucose-derived carbons into fatty acid and glycerol moieties of triglycerides into specialized cytosolic lipid droplet pools occurred entirely via the action of DGAT2. Furthermore, these *de novo* generated triglycerides in wild-type cells rapidly underwent lipolysis such that the released ^{14}C-labeled free fatty acids return back to the mitochondria to be oxidized into $^{14}CO_2$. Accordingly, this was fully prevented by pharmacological inhibition of ACL, ATGL, and CPT1. Induction of lipogenesis and fatty acid oxidation also occurs in mouse BAT under cold conditions [75]. Such simultaneous catabolism of glucose and fatty acids in brown adipocytes goes against the fundamental tenets of the Randle cycle, which stipulates that each occurs individually by inhibiting the other. It may be that in this cell type, however, there is a unique partitioning of inhibitory glucose and fatty acid metabolites away from their protein targets such as malonyl-CoA from CPT1 [75]. Alternatively/additionally, post-translational modifications may render these proteins resistant to inhibitory allosteric modulation [75], such as that of acetyl-CoA on pyruvate dehydrogenase. Nevertheless, the *in vitro* findings provide perhaps the most in-depth and detailed account of the metabolic fate of glucose in brown adipocytes. Future studies implementing hyperpolarized ^{13}C spectroscopy with ^{13}C-labeled glucose, as is typically applied to study brain energetics, can provide a more global view of metabolites generated from glucose in BAT in the *in vivo* setting as well as in isolated brown adipocytes [76]. This can build upon the results from a previous hyperpolarized ^{13}C spectroscopy study that only revealed changes in the distribution of endogenous ^{13}C-labeled species in rat BAT upon acute cold exposure and in isolated brown adipocytes upon acute adrenergic stimulation, without tracking the fate of glucose [76]. Interestingly, untargeted metabolomics of differentiated T37i cells acutely treated with a beta-3 adrenergic receptor agonist revealed that ^{13}C-labeled glucose feeds into the PPP, glycolysis, and TCA cycle but only into the glycerol moiety of triglycerides [49]. The reason for a lack of fatty acid synthesis from glucose —as has been amply described for activated BAT/primary brown adipocytes [43–47,74]—is unclear but might be an issue inherent to T37i cells.

The importance of lipolysis for BAT thermogenesis

The above-mentioned study by Irshad *et al* [74] revealed that lipolysis is required for the full oxidation of glucose in brown adipocytes but did not address its role in UCP1-mediated thermogenesis. Initial pharmacological experiments performed on cultured differentiated mouse brown and brite/beige adipocytes with ATGL and hormone-sensitive lipase (HSL) inhibitors suggested that lipolysis is mandatory for thermogenesis [37]. This was supported by subsequent *in vivo* findings in rats [52] and humans [77] treated with nicotinic acid, a GPR109a agonist that opposes beta-3 adrenergic receptor signaling and thus inhibits lipolysis and severely impairs

temperature-induced thermogenesis. However, nicotinic acid also markedly reduced circulating glucose and fatty acid uptake by BAT in these studies [52,77] so it remains unclear to what extent its effect on intracellular lipolysis impacts on thermogenesis.

These observations generally supported the model that free fatty acids released by lipolysis upon stimulation of sympathetic nerves innervating BAT act as activators of UCP1. This was in fact postulated early on, based on the observations that removal of intracellular fatty acids by activation of mitochondrial beta-oxidation inhibited uncoupled respiration of brown adipocytes [78], and nanomolar concentrations of fatty acids could override the inhibitory action of purine nucleotides on UCP1 [79]. The latter was confirmed more recently by detailed patch-clamp electrophysiological studies of brown fat IMM preparations [80]. The patch-clamp electrophysiological approach also revealed that UCP1 acts as a thermogenic symporter that transports protons bound to fatty acids from the intermembrane space into the mitochondrial matrix [80]. This insight was gained using fatty acid analogues in the external solution (representing the intermembrane space) with varying dissociation constants. The large UCP1-mediated proton conductance across the IMM was only recorded for fatty acid analogues that bind to protons at physiological pH [80].

The unexpected finding that genetically interfering with BAT ATGL function does not affect cold sensitivity in mice [81,82] suggests that while in the normal genetic landscape, fatty acids derived from intracellular lipolysis act as UCP1 activators and provide a major source of energy for BAT thermogenesis, the fatty acid sources can be redundant. Indeed, it was proposed from the studies of Shin *et al* [81] and Scheiber *et al* [82] that circulating fatty acids derived from WAT lipolysis can fully compensate for defective BAT lipolysis [81,82]. This is because fasted mice with defective lipolysis in both WAT and BAT, as opposed to in BAT alone, are in fact cold-intolerant [81,82]. Interestingly, the provision of food to these mice can preserve body temperature upon acute [81,82] and chronic [82] exposure to cold, whereas for mice with defective glycolysis in WAT and BAT, this is not the case—at least in the acute setting [39]. Together, these results suggest that liproprotein lipase on capillaries near brown adipocytes acting on circulating triglyceride-rich lipoproteins can provide exogenous fatty acids from ingested food [83,84]. They further attest that intracellular glucose but not fatty acid metabolism is indispensable for rapid thermogenesis in brown adipocytes. Notably, when fatty acids cannot be obtained from lipid droplets within the brown adipocyte, increased glucose uptake occurs, which improves glycemic control [80]. Here, we have another example of the therapeutic potential of increasing glucose metabolism in BAT through blocking intracellular lipolysis.

The higher production of the electron donating reducing equivalents NADH and $FADH_2$ from exogenous fatty acids compared with glucose in brown adipocytes may be more important for acclimation to cold [50]. This is consistent with the finding that exogenous fatty acids seem to feed into a separate and larger lipid droplet pool than that replenished by glucose in brown adipocytes, which is not as readily regulated by acute beta-3 adrenergic receptor activation [74]. Accordingly, mice with reduced BAT fatty acid uptake due to a lack of fatty acid transport protein 1 (FATP1) [85] or cluster of differentiation 36 (CD36) [86] in brown adipocytes initially handle the cold when given access to food but eventually succumb to it after prolonged exposure [85]. The avid uptake of circulating fatty acids by activated BAT has recently been underscored by a [123/125I]-b-methyl-p-iodophenyl-pentadecanoic acid (123/125I-BMIPP) single-photon emission computed tomography (SPECT) imaging study in mice, which led to the identification of several new *bona fide* BAT depots with a similar distribution pattern to that in man [87] (Fig 1).

Mitochondrial beta-oxidation: a final common pathway?

Intracellular fatty acids are activated for mitochondrial entrance by acyl-CoA synthases (ACS) and are then converted into acylcarnitine by CPT1 for transport across the IMM by translocase. CPT2 then regenerates acyl-CoA from acylcarnitine in the mitochondrial matrix in preparation for beta-oxidation (Fig 2). While long-chain ACS 1 (ACSL1) [88], CPT1b [89], CPT2 [90], and various acyl-CoA dehydrogenases [91,92], and thus beta-oxidation, have been shown to be essential for mice to mount a proper acute thermogenic response to cold, the role of fatty acids derived from glucose remains unclear. One way this can be addressed explicitly is by deleting ACL or DGAT2 from BAT. Interestingly, this has already been achieved in principle by crossing adiponectin-Cre mice with floxed *Acl* counterparts [39,81,82], but the BAT and cold tolerance of their offspring were not assessed in this particular study [93]. Lipogenesis from exogenous acetate may, however, compensate for that from exogenous glucose in this model [93]. This is because isolated inguinal and epididymal white adipocytes lacking ACL were found to incorporate considerably more ^{13}C acetate into acetyl-CoA and malonyl-CoA than wild-type cells [93].

From a therapeutic perspective, the negative role of mitochondrial acyl-CoA thioesterases (ACOT) in regulating BAT thermogenesis deserves consideration. These enzymes generate acetyl-CoA and free fatty acids from acyl-CoA in the cytosol and the mitochondrial matrix. Consequently, blocking ACOT action would be expected to have the dual effect of increasing cytosolic acyl-CoA availability for mitochondrial import and for beta-oxidation by preventing its breakdown in the mitochondrial matrix. Consistent with this notion, genetic inactivation of ACOT11 was first shown to protect mice from developing obesity and insulin resistance from a high-fat diet through increased energy expenditure [94]. This was associated with enhanced fatty acid oxidation in brown adipocytes [94]. Subsequently, genetic inactivation of ACOT13 in mice was found to improve cold tolerance [95] and to increase energy expenditure [95,96]. Interestingly, this seems to be through enhanced oxidation of fatty acids released by intracellular lipolysis in brown adipocytes [96]. Thus, compounds that inhibit ACOT11/13 might be useful drugs to treat obesity and associated insulin resistance by promoting BAT thermogenesis.

BAT crosstalk with other tissues

In this Review, we have focused on how intracellular glucose metabolism in brown adipocytes regulates systemic homeostasis. Analogous to the previously long-held view that WAT is simply an energy storage organ, it is becoming increasingly evident that BAT does not function in isolation just to dissipate chemical energy as heat. Instead, BAT has been shown to interact extensively with other tissues through neural and endocrine pathways in both health and disease

(Fig 3). This crosstalk involves some of the intracellular catabolic and anabolic processes mentioned here. For instance, the predisposition to developing obesity from chronic overnutrition may have its origins in aberrant liver to BAT communication [97]. This involves increased hepatic glucokinase activity which, via vagal afferents to the hindbrain nucleus tractus solitarius (NTS), decreases sympathetic regulation of BAT thermogenesis (Fig 3) [97]. Additionally, the impaired thermoregulation that occurs with aging associates with decreased acylcarnitine release from the liver [98]. In healthy young mice, these lipid species are generated by the action of CPT1/2 in hepatocytes from free fatty acids derived from WAT lipolysis upon acute cold exposure. They are then channeled to BAT where they are ultimately metabolized in the TCA cycle to promote thermogenesis (Fig 3) [98].

In the reverse direction, BAT communicates with the liver to prevent excessive lipid accumulation in hepatocytes and insulin resistance in the face of a high-fat diet [99]. This is through the constitutive release of the extracellular domain of the transmembrane protein neuregulin 4 (NRG4) following proteolytic cleavage at Ser53. The circulating NRG4 fragment is then thought to bind to epidermal growth factor receptor 3 and 4 (ErbB3/4) in hepatocytes to limit the expression of lipogenic genes such as *Fasn* (Fig 3) [99]. Interestingly, while mRNA expression of *Nrg4* in BAT increases upon both acute and chronic cold exposure, it is not involved in the acute maintenance of body temperature [99]. This underscores the fact that BAT can regulate different physiological functions independently from thermogenesis.

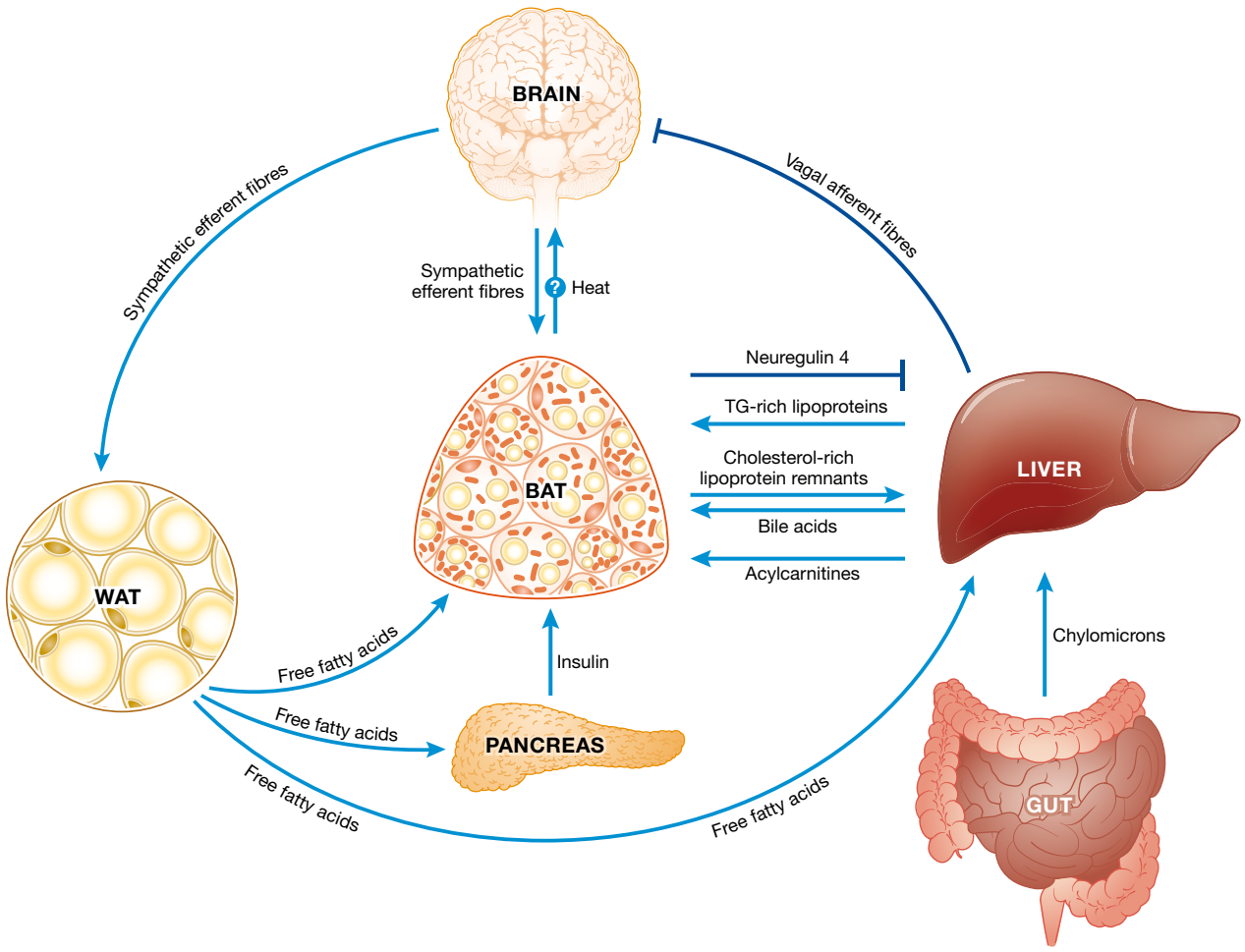

Figure 3. BAT crosstalk with other organs in health and disease.

Here we provide examples of crosstalk between BAT and other organs in health and disease states. During a high-fat meal, cholesterol in the form of chylomicrons is delivered first to the liver and then in the form of triglyceride-rich lipoproteins to BAT. Cholesterol-rich lipoprotein remnants are then released from the action of lipoprotein lipase and are in turn delivered via the bloodstream back to the liver where hepatocytes convert cholesterol into bile acids. These dietary cholesterol-derived circulating bile acids then return to BAT to promote thermogenesis. There may also be a line of communication from BAT to the brain during feeding due to the heat-sensing properties of hypothalamic pro-opiomelanocortin neurons. During chronic consumption of a high-carbohydrate diet, increased glucose kinase activity in the liver results in the activation of vagal afferents which inhibit sympathetic efferents to BAT resulting in decreased thermogenesis in those susceptible to weight gain. Similarly, during aging decreased acylcarnitine released from the liver produced by free fatty acids released from WAT lipolysis results in diminished BAT thermogenesis and cold intolerance. BAT itself releases neuregulin 4 to protect against fatty liver and diabetes from chronic consumption of a high-fat diet. Fatty acids released by WAT lipolysis also promote insulin release from the pancreas which then causes glucose, fatty acid and triglyceride-rich lipoprotein uptake by BAT to sustain thermogenesis.

Box A: In need of answers

(i) To what extent do nutrients and metabolites other than glucose and fatty acids fuel BAT thermogenesis?
(ii) What is the overall metabolic fate of glucose in brown adipocytes during thermogenesis? Is there a difference with beige adipocytes? Is there a species difference?
(iii) Is BAT glucose and fatty acid uptake and metabolism differentially required for acute and chronic tolerance to cold? Is there a difference in BAT fuel utilization for diet-induced thermogenesis?
(iv) Can chronic activation of BAT reduce circulating blood glucose in hyperglycemic humans by acting as a glucose sink as it does in rodents?
(v) Can the full benefits of BAT glucose uptake on glycemia be obtained without invoking BAT thermogenesis?

The fatty acids released by WAT lipolysis upon acute cold exposure or beta-3 adrenergic receptor agonist treatment also stimulates insulin release from pancreatic beta-cells [100]. Remarkably, this was shown to be required for the normal uptake of glucose, fatty acids and triglyceride-rich lipoproteins by activated BAT in mice and, consequently, thermogenesis. As such, mice lacking ATGL in BAT do not release insulin in response to acute cold exposure or beta-3 adrenergic receptor agonist treatment and mice treated with an insulin receptor antagonist or lacking insulin receptors in brown adipocytes are cold intolerant [100].

Communication also exists between the gut and BAT especially during diet-induced thermogenesis. An elaborate example of this involves the delivery of dietary cholesterol via the liver to BAT (Fig 3) [101]. Upon acute cold exposure or beta-3 adrenergic receptor agonist treatment, LPL in BAT capillaries causes the breakdown of triglycerides in triglyceride-rich lipoproteins and the uptake of the fatty acids by brown adipocytes. Consequently, cholesterol-rich lipoprotein remnants are released from BAT into the circulation. These are then directed to the liver where the cholesterol is converted into bile acids in hepatocytes via the alternative pathway through the action of CYP7B1-genetic inhibition or promotion of which decreases and increases BAT thermogenesis, respectively [101]. Interestingly, gut microbiota may add an additional layer to this crosstalk as they dramatically change in diversity upon acute cold exposure to regulate hepatic *Cyb7b1* mRNA expression, circulating bile acid species and BAT thermogenesis [102]. The clinical potential of bile acids is supported by the finding that acute administration of chenodeoxycholate to human subjects increases whole-body energy expenditure associated with increased BAT ^{18}FDG uptake [103]. This was proposed to be mediated by the Takeda G-protein receptor (TGR5) based on pharmacological experiments on cultured differentiated human brown adipocytes [103].

Lastly, the finding that anorexigenic hypothalamic arcuate nucleus (ARC) pro-opiomelanocortin (POMC) neurons are heat-sensitive [104] opens up the possibility of a BAT-brain axis in feeding control. This is consistent with the thermostatic regulation of feeding by BAT which was originally proposed to act as a negative feedback pathway during diet-induced thermogenesis [105]. Indeed, CRISPR-Cas-9-mediated deletion of the heat-activated transient receptor potential vanilloid 1 receptor (TRPV1) in ARC POMC neurons prevents the reduced food intake caused by exercise in mice, which itself robustly increases core body as well as ARC temperatures [104].

Conclusions and future directions

The use of ^{18}F-FDG PET imaging has led to tremendous strides in the translational fields of experimental neuroscience and metabolism not only as a functional technique, but also in motivating research into the energetics of neurons and brown adipocytes, respectively. Despite this, questions about why glucose takes such an inefficient route from mitochondria to lipid droplets and back in brown adipocytes during thermogenesis still remain. One obvious explanation could be that in itself, this contributes to heat production [106]. Another could be that fatty acid recycling, as part of this route, may contribute to the sensitivity of metabolic control [107]. Alternatively, it could serve as a backup mechanism to maintain BAT triglycerides stores. Indeed, during chronic caloric restriction, BAT glucose uptake and metabolism increases markedly even under unstimulated conditions [108].

Much work has been performed on how BAT is fueled during temperature-induced thermogenesis. It is less clear how BAT is fueled during diet-induced thermogenesis. Studies originally suggested contributions from circulating glucose [109,110]. This could provide the ATP from glycolysis required for creatine and phosphocreatine cycling in brown adipocytes that has recently been shown in mice to be an essential component of diet-induced thermogenesis and to prevent weight gain on a high-fat diet [111]. Additionally, circulating fatty acid uptake by BAT was shown to be directly proportional to BAT thermogenesis in response to a mixed carbohydrate-rich meal in humans [112]. Interestingly, despite initially being thought to drive diet-induced thermogenesis [113], BAT sympathetic nerve function does not seem to be involved in both rodents [114,115] and humans [112]. In this context, WAT sympathetic nerve function and the formation of beige adipocytes may play a more dominant role than that of BAT through insulin receptor signaling in ARC neurons [116]. Another important key point is that species differences may exist concerning BAT fuel utilization and intracellular mechanisms of thermogenesis. This is accentuated by the fact that human brown adipocytes have a gene expression profile closer to mouse beige adipocytes than classical (interscapular) brown adipocytes [62,117–119]. Indeed, unlike rodent BAT, human BAT consumes a high amount of glucose at thermoneutrality as recently determined by microdialysis [118]. This technique also revealed that human BAT uptake of the amino acid glutamate increases upon acute cold exposure which may fuel thermogenesis by feeding into the TCA cycle through the action of glutamate dehydrogenase [118]. In line with this, the concentration of glutamate markedly increases in rodent BAT upon acute and chronic cold exposure as does the enzymatic activity of glutamate dehydrogenase [119,120]. Overall, the explosion of knowledge in brown adipocyte biology has generated new hope for the development of novel treatments for the devastating consequences of metabolic disease.

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Author contributions

MKH produced the original draft of the manuscript which MK substantially revised.

Conflict of interest

The authors declare that they have no conflict of interest.

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