Oil does more than light the lamp: the multifaceted role of lipids in thermogenic fat

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Summary

Brown and beige adipocytes, or thermogenic fat, were initially thought to be merely a thermogenic organ. However, emerging evidence suggests its multifaceted roles in the regulation of systemic glucose and lipid homeostasis. One of the important functions of thermogenic fat is as a "metabolic-sink" for glucose, fatty acids, and amino acids, which profoundly impacts metabolite clearance and oxidation. Importantly, lipids are not only the predominant fuel source used for thermogenesis, but also essential molecules for development, cellular signaling, and structural components. Here we review the multifaceted role of lipids in thermogenic adipocytes.

Introduction

The development of obesity is strongly related to the development of type 2 diabetes, cardiovascular disease, and several cancers. Obesity occurs from chronic energy imbalance where caloric intake exceeds caloric expenditure. The majority of excess energy is safely sequestered as triglycerides (TAG) in lipid droplets of adipose tissues. However, adipocytes have a finite storage capacity, which once full, redirect excess lipids for storage toward other tissues resulting in lipotoxicity (Lee et al., 1994; Unger et al., 2010). Several strategies to regulate energy balance and energy storage exist. One promising strategy to remove excess energy storage is to increase energy expenditure through the activation of thermogenic fat (Sidossis and Kajimura, 2015).

Thermogenic fat - brown adipocytes and the recruitable form, *a.k.a.,* beige adipocytes - are the predominant tissue that is responsible for non-shivering thermogenesis. Non-shivering thermogenesis occurs by dissipating energy in the form of heat through mitochondrial uncoupling proton 1 (UCP1) and UCP1-independent pathways (Chouchani and Kajimura,

2019). When activated by external stimuli (e.g., cold exposure), thermogenic fat acts as a metabolic-sink for the uptake and disposal of glucose, lipids, and branched-chain amino acids (BCAA). Thus, enhancing the thermogenic activity and/or the development would effectively remove excess energy from the body.

Whereas the abundance of brown adipose tissue (BAT) is highest in newborns (Lean et al., 1986), compelling evidence demonstrates that adult humans possess both brown and beige adipocytes (Finlin et al., 2018; Lidell et al., 2013; Shinoda et al., 2015; Wu et al., 2012). Importantly, a recent epidemiological study demonstrates that the prevalence of thermogenic fat, as assessed by ^{18}F-fluorodeoxyglucose positron emission tomography combined with computed tomography (FDG-PET/CT), was significantly associated with a lower incidence of type 2 diabetes, dyslipidemia, coronary artery disease, cerebrovascular disease, congestive heart failure, and hypertension (Becher et al., 2021). In thermogenic fat, lipids are more than a fuel source: they have essential roles in triggering thermogenesis, cellular signaling, and organelle formation. Here, we discuss the current understandings of the multifaceted roles of lipids in thermogenic adipocytes.

Role of lipids in brown/beige fat thermogenesis

The best-characterized thermogenic mechanism in thermogenic fat is mitochondrial proton uncoupling through UCP1 (Cannon and Nedergaard, 2004). In general, the mitochondrial electron transport system exports protons to generate an energetic gradient across the inner mitochondrial membrane (IMM) to generate a thermodynamically favorable free energy (ΔG) for the synthesis of ATP by the ATP synthase (Neufer, 2018). However, UCP1, located on the IMM transmembrane, can hijack the energetic gradient across the IMM to convert the energy potential generated from electron transport chain proton pumping away from ATP generation and toward heat production (Smith et al., 1966). The process of uncoupling mitochondrial proton pumping generates large energy demand to maintain the electrical gradient of the mitochondrial membrane. To meet this high energetic demand, the mitochondrial electron transport system is highly activated, and macronutrient catabolism is increased to generate the reducing equivalents (e.g., NADH and $FADH_2$) to meet the demand of membrane depolarization caused by UCP1 transport of protons into the mitochondrial matrix. At the molecular level, long-chain fatty acids (FAs) are required for UCP1 activity. Long-chain FAs directly bind to UCP1 protein, which overcome the inhibitory effect of purine nucleotides to UCP1, leading to activation of proton conductance in the mitochondrial inner membrane (Jezek et al., 1994; Urbankova et al., 2003; Winkler and Klingenberg, 1994). Long-chain FAs are negatively charged at a carboxyl end, and thus, the direct binding to UCP1 appears to trigger the H^+ flux into the matrix like a fatty-acid anion/H^+ symporter (Fedorenko et al., 2012).

Recent studies also highlight the role of UCP1-independent thermogenesis. This involves ATP-dependent futile cycling, such as Ca^{2+} cycling and creatine cycling (Ikeda et al., 2017; Kazak et al., 2015; Kazak et al., 2019; Tajima et al., 2020). In contrast to UCP1, substrate futile cycling requires active ATP synthesis, and thus, also requires active fuel oxidation in the mitochondria. The underlying mechanisms of substrate preference of fatty acids or other substrates for UCP1-independent thermogenesis remain unclear.

Essential fuels for thermogenesis:

Active uptake of glucose and fatty acids into thermogenic fat is a common distinguishing feature and is used as an effective method to characterize and quantify BAT depot volume in humans, as demonstrated by the studies using ^{18}F-FDG-PET/CT (Cypess et al., 2009; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009) and ^{18}F-fluoro-thiaheptadecanoic acid (18FTHA) PET-CT (Ouellet et al., 2012). While the glucose analog, ^{18}F-FDG, has been the predominant molecule utilized to characterize and quantify thermogenic fat, it is important to recognize glucose is not the only molecule that is sequestered upon cold stimulation in thermogenic fat. As highlighted in this review, lipid metabolism is essential for the thermogenic response in BAT, and thus, the utilization of lipid analog ^{18}FTHA, should be considered as an essential maker of BAT activity in humans. Together, glucose, lipid, and amino acid imaging can provide a comprehensive evaluation of the sequestering ability of thermogenic fat and in turn provide important insight into the role of thermogenic fat on systemic metabolism.

In response to cold stimuli, norepinephrine released from the sympathetic nerve terminals and subsequent activation of the β-adrenergic receptor (β-AR) induces a signaling cascade that stimulates lipolysis in adipocytes, which stimulates supplies free fatty acids to support the increased demand for electron transport chain reducing equivalents. The current dogma surrounding human BAT activation through β-AR signaling is that the signal transduction occurs through the β3-AR. Work conducted by Cypess et al. (2015), demonstrates that a 200 mg dose of mirabegron, a β3-AR specific agonist, augments human BAT activity as measured through ^{18}F-FDG PET/CT. While follow up studies conducted by O'Mara et al. (2020) demonstrate that chronic treatment of 100 mg of mirabegron increased BAT activity and resting energy expenditure, while improving insulin sensitivity, without changes in body mass. On the other hand, a recent study by Blondin et al. (2020) reported that human BAT was activated by β2-AR stimulation. In differentiated human brown adipocytes, the mRNA expression of the β2- AR is the most highly expressed isoform and β2-AR mRNA expression is augmented when stimulated with norepinephrine. Furthermore, differentiated human primary adipocytes generate an increase in oxygen consumption when stimulated with formoterol, a selective β2-AR agonist, while the effect was blunted in the presence of β2-AR antagonist, ICI-118,551. While these results suggest that human thermogenic fat can be activated through β2-AR, continued research is needed to validate the significance of β2-AR relative to β3-AR in activation of human BAT.

The source of FAs for UCP1 uncoupling was initially thought to derive from *de novo* lipolysis within brown adipocytes, *i.e.,* norepinephrine acts on β3-AR in brown adipocytes, leading to increased FAs synthesis for beta-oxidation. However, BAT-specific defects in lipolysis, such as genetic deletion of adipose triglyceride lipase (ATGL) or the ATGL-activating protein comparative gene identification-58 (CGI-58) by *Ucp1*-Cre, did not impair BAT thermogenesis *in vivo.* In contrast, deletion of ATGL or CGI-58 in both brown and white fat, by the use of *adiponectin*-Cre, significantly attenuates BAT thermogenesis (Schreiber et al., 2017; Shin et al., 2017). These data suggest that FAs released from WAT, rather than *de novo* lipolysis in BAT, are the primary fuel source for thermogenesis (Fig. 1). FFA transported to brown adipose tissue are taken up by plasma membrane fatty acid

transport proteins such as CD36 or Fatty acid transport protein 1 (FATP1) (Bartelt et al., 2011; Wu et al., 2006). In mice, a loss of FATP1 is sufficient to inhibit fatty acid uptake into BAT and impair the thermogenic response to cold exposure, suggesting that active FA transport into BAT, rather than *de novo* lipolysis, is required for BAT thermogenesis (Wu et al., 2006). Besides lipolysis in WAT, FFA can be processed in the liver to acylcarnitine, which significantly contributes to optimal thermogenesis in BAT (Simcox et al., 2017).

In addition to fatty acids, glucose and BCAA oxidation are essential for optimal thermogenesis. Panic et al. (2020) demonstrate that a lack of pyruvate entry into brown adipose tissue mitochondria is required for a robust thermogenic response to a cold challenge in mice. Likewise, Yoneshiro et al. (2019) demonstrate that thermogenic fat acts as a major metabolic-sink for BCAA and that active BCAA oxidation is required for optimal thermogenesis. Together, these studies suggest that one fuel alone is not sufficient for a robust thermogenic response without compensational adaptation, while each fuel source is regulated to work in concert to supply necessary reducing equivalents to meet the energetic demand to produce heat.

The role of lipids as signaling entities (lipokines)

Lipids are a diverse class of molecules and are more than fuel for cellular energy. For example, specific lipids are signaling molecules known as lipokines that mediate inter-organ or inter-cellular communication (Cao et al., 2008). The diverse structures of lipids allow for a potentially wide range of the currently uncharacterized function of lipokines. Lipid structure varies by the lipid properties, such as the lipid class, fatty acid chain length, and degrees of unsaturation. The diversity of these features allows for a wide range of lipids to be lipokines that act on specific receptors (Fig.2).

G-Protein Coupled Receptors:

G-protein coupled receptors (GPRs) can be stimulated by certain FFAs, where different species of FFA are ligands for different receptors (Vinolo et al., 2012). Short-chain fatty acids (SCFA) are lipid molecules less than 6 carbons long, and they are a ligand for GPR43 (Free fatty acid receptor 2, FFAR2). In brown adipocytes, GPR43 expression is markedly enhanced in differentiated cells. Stimulation of GPR43 in brown adipocytes increased expression of UCP1 and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), whereas knockdown of GPR43 attenuated UCP1 expression and mitochondrial biogenesis in differentiated brown adipocytes (Hu et al., 2016). However, whole-body knockout of GPR43 in mice demonstrates discrepancies when compared to cell culture data. Mice with a whole-body knockout of GPR43 have lower body weight than controls when fed a high-fat diet. GPR43 whole-body knockout mice also have less lipid accumulation in the BAT and greater core body temperature after 20 weeks of high-fat diet feeding. However, GPR43 whole-body knockout mice consume more food and absorb less energy than control mice, suggesting that GPR43 signaling in the gastrointestinal tract is critical for calorie absorption (Bjursell et al., 2011). Future studies should examine mice with brown adipose tissue-specific knockout of GPR43 to determine the role of brown adipose tissue GPR43 signaling in core body temperature.

Medium-chain fatty acids (MCFA) are lipid molecules between 6–12 carbons long, and they activate GPR84. Tissue-specific expression of GPR84 is highest in skeletal muscle, but BAT and inguinal WAT both have high levels of GPR84 protein expression (Montgomery et al., 2019). GPR84 has been predominantly studied for its role in immune cell response (Recio et al., 2018). In mice, a whole-body knockout of GPR84 does have a minor reduction in glucose tolerance, but only when mice were fed an MCFA enriched diet. Body mass and energy expenditure are not affected by GPR84 loss (Montgomery et al., 2019).

The unsaturation or number of double bonds also change the shape of the lipid, and polyunsaturated omega-3 fatty acids stimulate GPR120 (FFAR4). In humans with obesity, GPR120 expression in adipose tissue is greater when compared to lean controls. A deleterious mutation of *GPR120* (p.R270H) in humans was determined to be a risk factor for obesity in European populations (Ichimura et al., 2012). Also, GPR120 expression is elevated during brown adipocyte differentiation (Schilperoort et al., 2018) and in response to cold stimulation (Quesada-Lopez et al., 2016). A knockout of GPR120 in mice was sufficient to increase body weight gain with high-fat diet feeding and attenuate energy expenditure, glucose tolerance, and insulin tolerance (Ichimura et al., 2012). When fed a standard chow diet, mice with an absence of GPR120 have a mild impairment in glucose tolerance that is accompanied by a significant increase in circulating insulin, suggesting that GPR120 knockout mice are insulin resistant (Oh et al., 2010). GPR120 knockout mice additionally display mild cold intolerance that is accompanied by attenuated levels of UCP1 expression in inguinal WAT (Quesada-Lopez et al., 2016), while a knockout of GPR120 in brown adipocytes was sufficient to reduce *Ucp1* expression in differentiated brown adipocytes (Schilperoort et al., 2018). Conversely, activation of GPR120 in white adipocytes with docosahexaenoic acid (DHA) potently increased glucose uptake and augmented GLUT4 translocation (Oh et al., 2010). Pharmacological activation of GPR120 with CpdA, a selective agonist, did improve glucose and insulin tolerance from a high-fat diet in wild-type mice without stimulating differences in body weight (Oh et al., 2014). A separate GPR120 agonist, TUG-891, also increases BAT activity, while treatment of TUG-891 to brown adipocytes did not increase *Ucp1* expression compared to vehicle-treated cells. Mice treated with GPR120 agonist, GW9508, had augmented UCP1 protein expression in inguinal WAT (i.e., beige adipocytes) and greater oxygen consumption under basal conditions and when stimulated with CL316,243, a β-adrenergic agonist, when compared to untreated controls (Quesada-Lopez et al., 2016). Combined these studies suggest that treatment with a GPR120 agonist may improve glucose tolerance mediated, at least in part, through activation of thermogenic adipocytes.

Palmitic acid hydroxystearic acids (PAHSAs) are considered endogenous ligands for GPR120 in adipose (Yore et al., 2014) and GPR40 in pancreatic β cells (Syed et al., 2018). Acute administration of 5-PAHSA or 9-PAHSA was sufficient to lower basal glucose levels and improve glucose tolerance in mice on a high-fat diet (Yore et al., 2014). In cultured white adipocytes, treatment with 9-PAHSA induced a thermogenic transcriptional profile with upregulation of UCP1, PGC-1α, C/EBPβ, and PRDM16. In wild-type and ob/ob mice chronically treated with 9-PAHSA, white adipose tissue UCP1 and PGC-1α protein expression were upregulated in a GPR120-dependent manner (Wang et al., 2018). Chronic treatment of 5- and 9-PAHSA or 9-PAHSA in chow or high fat diet-fed mice did not alter

body weight gain but did improve glucose and insulin tolerance, which are thought to be in part mediated through GPR40 signaling in pancreatic β cells (Syed et al., 2018). While chronic PAHSA treatment significantly improves glucose and insulin tolerance, which is observed with augmentation in insulin secretion mediated through GPR40 signaling, the role of PAHSAs in thermogenic adipose is an exciting area requiring more characterization.

Thermogenic lipokines:

Certain lipokines are shown to activate thermogenic fat via upregulating UCP1 expression or in a UCP1-independent fashion. Such lipids include N-acyl amino acids, 12-hydroxyeicosapentaenoic acid (12-HEPE), and 12,13-dihydroxy-9z-octadecenoic acid (12,13-diHOME).

Amino acids conjugated to fatty acids are known as N-acyl amino acids. These modified fatty acids have unusual signaling activity with metabolic consequences. A biosynthetic pathway for N-acyl amino acids is through the peptidase M20 domain containing 1 (PM20D1) protein, which has enzymatic activities to both synthesize and hydrolyze N-acyl amino acids. PM20D1 is enriched in UCP1-positive adipocytes, and mRNA levels are increased in brown adipose tissue with cold exposure (Long et al., 2016). Overexpression of PM20D1 increased energy expenditure, reduced fat mass, reduced food intake, and improved glucose tolerance in mice fed a high-fat diet. Additionally, studies with supplemented N-acyl amides mirrored the findings of PMD20D1 overexpression. Notably, N-acyl amino acids can be natural uncouplers that induce mitochondrial uncoupling respiration independent of UCP1 (Long et al., 2016). This action appears to be through mitochondrial ATP/ADP symporters, ANT1 and ANT2 (SLC25A4 and SLC25A5), although the mechanism of the action needs further investigation. In mice with a whole-body knockout of *Pm20d1*, energy expenditure and diet-induced body weight gain were not different from control mice. However, loss of *Pm20d1* was sufficient to exacerbate glucose and insulin tolerance. Of note, *Pm20d1* knockout mice did not have lower levels of N-acyl amino acids. Instead, several lipid species were upregulated compared to control, which corresponded with *Pm20d1* knockout mice having an improved thermogenic response to acute cold stress that might be attributed to an elevation in *N*-oleoyl-glutamine (C18:1-Gln) (Long et al., 2018).

Oxylipins are a group of bioactive lipids which are synthesized from polyunsaturated omega-3 or omega-6 fatty acids (Barquissau et al., 2017). 12-HEPE is an oxylipin that is produced by 12-lipoxygenase (*Alox12*, 12-LOX) from the precursor fatty acid eicosapentaenoic acid (C20:5) (Hamberg, 1980). Cold exposure in humans and mice increased the abundance of circulating 12-LOX generated oxylipins (Kulterer et al., 2020; Leiria et al., 2019). While in mice multiple 12-LOX generated lipids were upregulated in cold, the genetic reduction of BAT only ablated cold-induced upregulation of 12-HEPE. Mice with *Alox12*-deletion in UCP1-positive cells have exacerbated tolerance to cold compared to controls. In brown adipocytes absent of *Alox12*, glucose uptake and glycolytic metabolism was impaired compared to controls. Consistent with impaired cold tolerance and glucose metabolism, *Alox12*-deletion in brown adipocytes resulted in drastically reduced maximal mitochondrial oxygen consumption. Daily treatment with 12(*S*)-HEPE for two

weeks in mice was unable to alter body weight. However, 12(S)-HEPE treated mice had improved glucose and insulin tolerance compared to vehicle treated mice. Consistent with improved glucose and insulin tolerance, glucose uptake was greater in BAT and skeletal muscle of mice treated with 12(S)-HEPE (Leiria et al., 2019). Together these data support the role of 12-HEPE as a cold-induced thermogenic lipid which has positive outcomes on glucose uptake in skeletal muscle.

Another example of thermogenic lipokine is 12,13 diHOME that is released from brown adipose or white adipose tissue. This lipokine is induced by cold exposure and a downstream fatty acid metabolite of linoleic acid (C18:2). Plasma levels of 12,13 diHOME are elevated with cold exposure in humans and positively correlates with BAT activity. In mice, acute injection of 12,13 diHOME augments body temperature in response to a cold challenge and increases the uptake of fatty acids into BAT. The increase in fatty acid uptake to BAT was mediated through an increase in fatty acid transporters translocation. A chronic treatment of 12,13 diHOME was not sufficient to reduce body weight or ameliorate glucose tolerance, but did improve oral lipid tolerance in mice fed a high-fat diet. Additionally, chronic treatment did not increase BAT UCP1 mRNA expression, but did increase lipoprotein lipase (LPL) expression in BAT (Lynes et al., 2017). 12,13 diHOME as a fatty acid uptake stimulating endocrine molecule has also been observed in skeletal muscle (Stanford et al., 2018), suggesting that many 12,13 diHOME endocrine actions are yet to be characterized.

The role of lipids in membrane structure and function

Phospholipids, sphingolipids, and cholesterol are integral components of cellular membranes. Classically, they provide organizational barriers and structural support for membrane-bound proteins. However, membrane lipids are highly compartmentalized among cellular organelles, and their regulation is critical to cellular function (Funai et al., 2020). The composition of membrane lipids has been demonstrated to alter the structure, function (Laganowsky et al., 2014), and efficiency (Verkerke et al., 2019) of membrane-bound proteins. Further, phospholipids are responsive to environmental stressors such as exercise and inactivity (Heden et al., 2019). Membrane phospholipids in BAT mitochondria are also responsive to environmental cues, and BAT phospholipids increase in response to cold, while re-acclimation to room temperature will bring phospholipid levels back to pre-cold exposure (Ricquier et al., 1978; Strunecka et al., 1981). The metabolic phenotypes of mouse models with altered lipid species are summarized in Table. 1.

Phosphatidylcholine (PC) is the most abundant phospholipid in mammalian cells (van der Veen et al., 2017). It is primarily synthesized through the Kennedy pathway, which produces new phospholipid molecules (DeLong et al., 1999). Additionally, PC can be formed by the methylation of phosphatidylethanolamine (PE) by the enzyme PE methyltransferase (PEMT) (Ridgway and Vance, 1987) or through the conversion of lyso-PC to PC by acyltransferases (Wang and Tontonoz, 2019). Phosphatidylcholine abundance in BAT is responsive to environmental stimuli such as cold exposure (Ricquier et al., 1978) and exercise (May et al., 2017). Whole-body deletion of PEMT increases energy expenditure and protects mice from diet-induced obesity (Jacobs et al., 2010). However, mice with whole-body deletion of PEMT are cold intolerant (Gao et al., 2015) and have PC species-specific changes (Johnson

et al., 2020). PEMT expression increases during differentiation of brown adipocytes, and a knockdown of PEMT corresponds to a reduced thermogenic program, including the lower abundance of UCP1. However, BAT-specific knockout of PEMT does not result in a lower abundance of UCP1, cold intolerance, or changes in the species abundance of PC. In mice with a whole-body knockout of PEMT, alternative splicing of UCP1 occurs in a non-cell autonomous action, which generates a non-functional UCP1 protein (Johnson et al., 2020).

Cardiolipin is a unique class of phospholipids. Unlike other phospholipids, cardiolipin has two phosphatidic acid groups, which are connected through a glycerol backbone. Thus, creating a lipid with four acyl chains and a cone shape (Ikon and Ryan, 2017). Cardiolipin synthase generates nascent cardiolipin from cytidine diphosphate diacylglycerol (CDP-DAG) (Houtkooper et al., 2006). The acyl chains of the nascent cardiolipin are remodeled in a process catalyzed by taffazin to form mature cardiolipin molecules, which are predominately tetralinoleic (Xu et al., 2006). Cardiolipin is highly concentrated in the inner mitochondrial membrane where it is thought to be critical for the structure and function of many IMM proteins (Houtkooper and Vaz, 2008). The isolation of UCP1 has demonstrated that cardiolipin does bind to UCP1 (Lee et al., 2015). Additionally, biochemical studies utilizing reconstituted UCP1 demonstrate that the addition of cardiolipin alleviates the inhibition of UCP1 proton transport by purine nucleotide-binding (Klingenberg, 2009). In brown adipocytes, cardiolipin synthase gain of function was accompanied by an increase in UCP1 mRNA abundance as well as an increase in NE-induced oxygen consumption. Congruently, a reduction in cardiolipin synthase reduced NE-induced oxygen consumption in brown adipocytes (Sustarsic et al., 2018). However, reducing cardiolipin synthase did not reduce maximal respiration rates in brown adipocytes. In mice, fat-specific knockout of cardiolipin synthase impaired the ability of thermogenic adipose to respond to cold stimulus and these KO mice were cold intolerant. Interestingly, fat-specific loss of cardiolipin synthase did not result in augmented weight gain in mice fed a high-fat diet. Rather, the KO mice had attenuated weight gain with a high-fat diet feeding compared to controls. Loss of adipose cardiolipin synthase did cause metabolic inflexibility, and mice without adipose cardiolipin synthase were insulin intolerant (Sustarsic et al., 2018). Similar to mice with fat-specific cardiolipin synthase knockout, mice with whole-body taffazin knockdown are cold intolerant, although UCP1 protein levels were unchanged in mice with taffazin-knockdown (Johnson et al., 2020). These results may suggest a differential role between nascent cardiolipin and mature cardiolipin in mitochondrial function for thermogenesis that is not clearly characterized.

Ceramides, a lipid class of sphingolipids, are low in abundance compared to phospholipids, but have significant biological activity (Summers et al., 2019). Ceramide abundance increases with obesity, and inhibiting ceramide production has been demonstrated to improve metabolic health (Chaurasia et al., 2019). In human white adipose tissue, ceramide synthase 6 (CerS6) positively correlates with body mass index (BMI) and insulin resistance. A whole-body knockout of *CerS6* was sufficient to protect mice from diet-induced obesity, increase energy expenditure levels, and protect glucose and insulin tolerance from effects of HFD feeding. The BAT-specific knockout of *CerS*6 was sufficient to increase energy expenditure levels and blunt exacerbated glucose tolerance with high-fat diet feeding (Turpin et al., 2014). Inhibiting ceramide synthesis through BAT-specific deletion of serine

palmitoyltransferase subunit 2 (Sptlc2) also increased energy expenditure, reduced weight gain, and protected mice from diet-induced impairments in glucose and insulin intolerance. Conversely, increases in ceramide accumulation mediated through BAT-specific loss of acid ceramidase 1 (*Asah1*) lowered energy expenditure, increased diet-induced weight gain, and exacerbated glucose tolerance (Chaurasia et al., 2020). Together, these studies suggest that the regulation of ceramide abundance is critical to thermogenic function and systemic lipid and glucose homeostasis.

Perspectives: The best ability is the availability

Lipids serve thermogenic fat in many ways, from serving as structural support and their utilization as signaling entities to their most well-characterized role as a fuel source. While highlighted in this review is that lipids are essential for thermogenic fat, glucose and BCAA metabolism are also crucial for an optimal thermogenic response. Which raises the key question: how do cells determine the optimal fuels? The glucose-fatty acid cycle, or the Randle Cycle, is the biological process by which the optimal fuel selection of glucose to fatty acid oxidation is most commonly viewed and best understood (Randle et al., 1963). In the glucose-fatty acid cycle, as the fatty acid metabolism increases metabolite flux into the TCA cycle, there is an accumulation of acetyl-CoA, which inhibits pyruvate dehydrogenase complex (Hue and Taegtmeyer, 2009). Additionally, the availability of TCA intermediates induces a cascade, which results in transport of citrate out of the mitochondrial matrix and into the cytosol, where it has inhibitory effects on glycolysis (Garland et al., 1963). Thus, allowing for the influx of fatty acids into brown/beige adipocytes to predominate as an essential fuel source for thermogenesis.

An essential aspect of the glucose-fatty acid cycle is the importance of compartmentalization of metabolites between the cytosol and the mitochondrial matrix. As more metabolites are characterized as essential for optimal thermogenic response, it will be critical to developing a deeper understanding of fuel selection mechanisms. In this regard, accumulating evidence indicates the importance of the solute carrier family 25 (SLC25) family, a mitochondria-localized transporter family of proteins (Palmieri, 2014). The majority of proteins in the SLC25 family localize to the inner mitochondrial membrane and regulate mitochondrial matrix ion and metabolite flux. Included in the SLC25 family are UCP1–3 (SLC25A7–9), nicotinamide adenine dinucleotide transporter (SLC25A51), mitochondrial BCAA carrier (SLC25A44, MBC), and the carnitine-acylcarnitine translocase (SLC25A20, CACT). As an example, SLC25A44 is recently characterized as the MBC that is required for mitochondrial BCAA uptake and oxidation in BAT (Yoneshiro et al. (2019). The MBC determines the intracellular BCAA fate, *i.e.,* mitochondria BCAA catabolism vs. anabolic reactions, such as protein synthesis. Thus, the utilization of inner mitochondrial membrane gatekeepers to study the relationship of subcellular metabolite fate may help us understanding the regulation of optimal fuel selection and metabolic flexibility.

Acknowledgements

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Verkerke and Kajimura present a Review about the multifaceted role of lipids in thermogenic (brown and beige) adipocytes. They discuss lipids as a fuel source used for thermogenesis, and as essential molecules for development, cellular signaling, and structural components
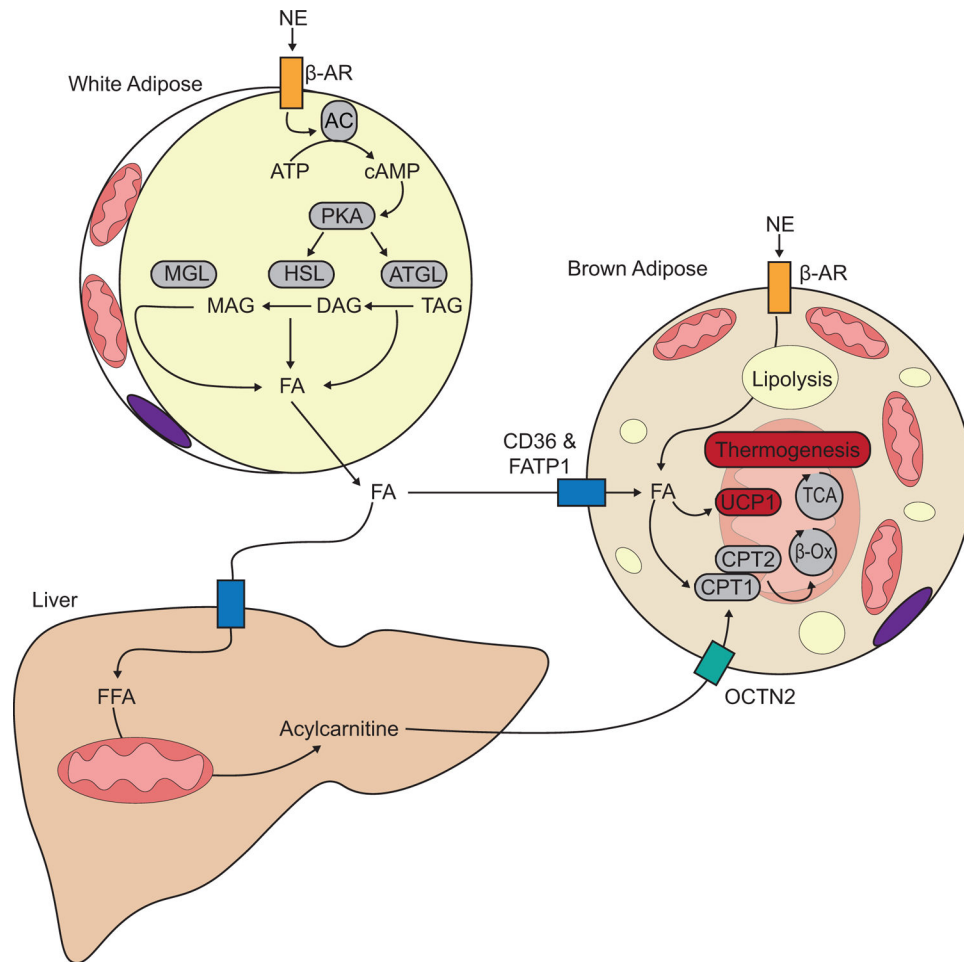
Figure 1. Role of lipids in thermogenesis.
Cold-induced norepinephrine (NE) activation of β- adrenergic receptor (β-AR) induces a lipolytic signaling cascade with activates adenylate cyclase (AC) to produce cyclic-AMP (cAMP), which activates protein kinase A (PKA). PKA activates lipase activity and adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) and monoglyceride lipase breakdown triacylglyceride (TAG), diacylglyceride (DAG), monoacylglyceride (MAG) into fatty acids (FAs) and glycerol. FAs are taken up by brown/beige adipocytes and transported into the mitochondria through carnitine palmitoyltransferase 1 and 2 (CPT1, CPT2), where they undergo β-oxidation and feed into the tricarboxylic acid (TCA) cycle to support energy requirements of thermogenesis.
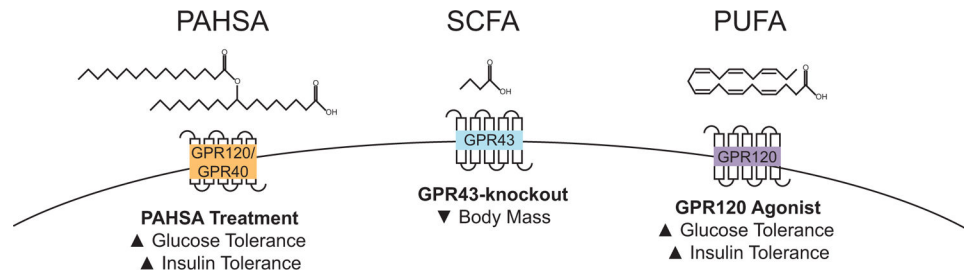
Figure 2. Lipid activated G protein-coupled receptors and mouse phenotypes.
Short-chain fatty acids (SCFA) stimulate GPR43. Polyunsaturated fatty acids (PUFA) activate GPR120. PAHSAs activate adipose GPR120 and pancreatic β cell GPR40.
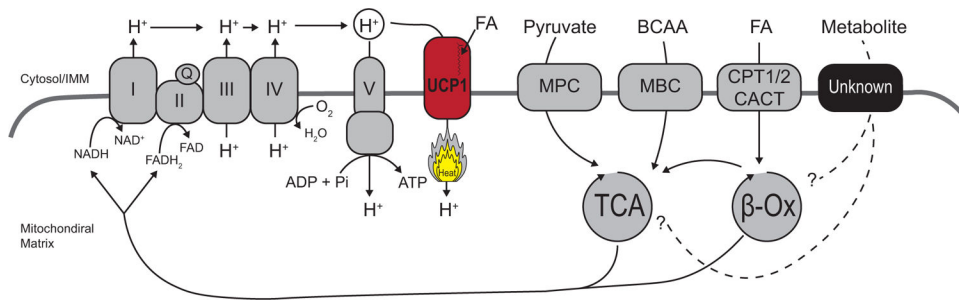
Figure 3. Fuel selection during thermogenesis.

Glucose, fatty acids (FA), and branched-chain amino acids (BCAA) are necessary for optimal thermogenesis in brown and beige fat. A better understanding of metabolite gating into the mitochondrial matrix is needed to develop a comprehensive model of fuel selection. Genetic or pharmacological regulation of inner mitochondrial membrane (IMM) transport proteins allows for the control of the fate of metabolites from cytosolic actions and that of mitochondrial metabolism action.

Table 1.

Summary of lipid modifying models and their metabolic outcomes.

Lipid	Model	Metabolic Response	Source
Cardiolipin	*Cls1* knockout	▼Body Mass ▼ Insulin Tolerance ▼Cold Tolerance	Sustaric et al., *Cell Metab* 2018
	Taz knockdown	▼Body Mass ▼Cold Tolerance	Johnson et al., *Mol Metab* 2019
Phosphatidylcholine	*Pemt* knockout	▲Energy Expenditure ▼Body Mass ▼Fat Mass ▼Cold Tolerance	Gao et al., *J Lipid Res* 2015 Johnson et al., *Mol Metab* 2019
Ceramide	*Cers6* knockout	▲Energy Expenditure ▲Glucose Tolerance	Turpin et al., *Cell Metab* 2014
	Sptlc2 knockout	▲Energy Expenditure ▼Body Weight Gain ▼Fat Mass ▲Glucose Tolerance	Chaurasia et al., *Mol Metab* 2020
	Asah1 knockout	▼ Energy Expenditure ▲ Body Weight Gain ▲ Fat Mass ▼Glucose Tolerance	Chaurasia et al., *Mol Metab* 2020
12,13-DiHOME	Acute Injection	▲Cold Tolerance ▲Fatty acid uptake	Lynes et al., *Nat Med* 2017
12-HEPE	*Alox12* knockout	▼Cold Tolerance ▼Glucose Uptake	Osório Leiria *et al.*, *Cell Metab* 2019
	12(S)HEPE Treatment	▲Glucose Tolerance ▲Insulin Tolerance	Osório Leiria *et al.*, *Cell Metab* 2019
PAHSA	5-PAHSA or 9-PAHSA Acute Treatment	▼Basal Glucose ▲Glucose Tolerance	Yore et al., *Cell* 2014
	5- and 9-PAHSA or 9-PAHSA Chronic Treatment	▲Glucose Tolerance ▲Insulin Tolerance	Syed et al., *Cell Metab* 2018
N-Acyl amino acids	*PM20D1* overexpression (AAV)	▲Energy Expenditure ▼Body Weight Gain ▼Fat Mass ▼Food Intake ▲Glucose Tolerance	Long et al., *Cell* 2016
	C18:1-Leu, C18:1-Phe, or C20:4-Gly Supplementation	▲Energy Expenditure ▼Body Weight Gain ▼Fat Mass ▼Food Intake ▲Glucose Tolerance	Long et al., *Cell* 2016
	Pm20d1 whole-body knockout	▼ Glucose Tolerance ▼ Insulin Tolerance ▲ Cold Tolerance	Long et al., PNAS 2018