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PATHOGENESIS OF TYPE 2 DIABETES AND INSULIN RESISTANCE (M-E PATTI, SECTION EDITOR)

Emerging Role of AMPK in Brown and Beige Adipose Tissue (BAT): Implications for Obesity, Insulin Resistance, and Type 2 Diabetes

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Abstract

Purpose of Review The global prevalence of type 2 diabetes (T2D) is escalating at alarming rates, demanding the development of additional classes of therapeutics to further reduce the burden of disease. Recent studies have indicated that increasing the metabolic activity of brown and beige adipose tissue may represent a novel means to reduce circulating glucose and lipids in people with T2D. The AMP-activated protein kinase (AMPK) is a cellular energy sensor that has recently been demonstrated to be important in potentially regulating the metabolic activity of brown and beige adipose tissue. The goal of this review is to summarize recent work describing the role of AMPK in brown and beige adipose tissue, focusing on its role in adipogenesis and non-shivering thermogenesis.

Recent Findings Ablation of AMPK in mouse adipocytes results in cold intolerance, a reduction in non-shivering thermogenesis in brown adipose tissue (BAT), and the development of non-alcoholic fatty liver disease (NAFLD) and insulin resistance; effects associated with a defect in mitochondrial specific autophagy (mitophagy) within BAT. The effects of a β3-adrenergic agonist on the induction of BAT thermogenesis and the browning of white adipose tissue (WAT) are also blunted in mice lacking adipose tissue AMPK. A specific AMPK activator, A-769662, also results in the activation of BAT and the browning of WAT, effects which may involve demethylation of the PR domain containing 16 (Prdm16) promoter region, which is important for BAT development.

Summary AMPK plays an important role in the development and maintenance of brown and beige adipose tissue. Adipose tissue AMPK is reduced in people with insulin resistance, consistent with findings that mice lacking adipocyte AMPK develop greater NAFLD and insulin resistance. These data suggest that pharmacologically targeting adipose tissue AMPK may represent a promising strategy to enhance energy expenditure and reduce circulating glucose and lipids, which may be effective for the treatment of NAFLD and T2D.

Keywords AMPK · Adipose tissue · Adipogenesis · Lipolysis · Non-shivering thermogenesis

Introduction

The global prevalence of type 2 diabetes (T2D) is escalating at alarming rates due to increased obesity, physical inactivity, and an aging population [1]; trends that are only expected to worsen given the tenfold increase in childhood obesity observed over the last four decades [2]. In addition to inducing a marked physical and financial cost on individuals and society, T2D also dramatically increases the risk of death from cardiovascular disease and certain cancers. While tremendous progress over the last decade has been made in developing a new arsenal of therapeutics aimed at restoring euglycemia in individuals with T2D, the development of additional classes of therapeutics are needed to further help reduce the burden of disease.

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One potentially important therapeutic target for the treatment of T2D is the AMP-activated protein kinase (AMPK). AMPK is a highly conserved and ubiquitously expressed protein composed of catalytic α (α1, α2), and regulatory β (β1, β2) and γ (γ1, γ2, γ3) subunits [3]. The activity of AMPK is increased in response to alterations in cellular adenine nucleotides. Specifically, a reduction in ATP and elevations in AMP and ADP, which compete for binding to specific residues within the γ isoform, leads to enhanced activating phosphorylation of AMPK at Thr172 within the α catalytic subunit by the upstream kinase LKB1 [4]. In addition to changes in adenine nucleotides, in some tissues, calcium through the activation of calcium/calmodulin-dependent protein kinase kinase 2 (CAMKK2) can also increase AMPK Thr172 phosphorylation. AMPK also responds to changes in carbohydrate availability, where glycogen suppresses AMPK activity by binding the β-isoform and low glucose increases AMPK through aldolase and fructose-1,6-biphosphate [5–7]. These effects may be further potentiated by the autophagy protein unc-51 like autophagy activating kinase 1 (ULK1), which is switched on in response to low levels of nutrients and promotes allosteric activation of AMPK through the phosphorylation of the β1 subunit (Ser108) [8]. Collectively, these mechanisms for AMPK activation allow cells to respond to numerous metabolic perturbations; matching energy demand with production.

Once activated, AMPK modulates metabolism through the phosphorylation of key metabolic proteins and transcription factors that promote energy-producing pathways (catabolism) while concurrently suppressing energy-storing pathways (anabolism). In skeletal muscle and liver, AMPK activation promotes glucose and fatty acid uptake, enhances mitochondrial function and fatty acid oxidation, and suppresses lipid and cholesterol synthesis; all of which may be beneficial in people with T2D. Consistent with this concept, skeletal muscle AMPK activity is increased by endurance exercise [9] and this is important for stimulating glucose uptake [10]. Therapeutics that lower blood glucose such as metformin and the sodium glucose cotransporter 2 inhibitor (SGLT2i) canagliflozin increase the activity of AMPK in the liver by suppressing mitochondrial function [11, 12]. With metformin, this activation of liver AMPK and subsequent phosphorylation of acetyl-CoA carboxylase—a critical enzyme regulating fatty acid synthesis—is important for improving insulin sensitivity, but is not required for the acute effects of metformin on hepatic gluconeogenesis [13] which is instead dependent on alterations in redox status [14]. Numerous other natural products that reduce blood glucose such as berberine, quercetin, and resveratrol also activate AMPK by affecting mitochondrial function, but their effects in humans may be limited due to low bioavailability [15]. Since alterations in mitochondrial function would be expected to alter the function of numerous enzymes, it is likely that some of the effects of these natural products and therapeutics (e.g., metformin, canagliflozin) on glucose control are independent of AMPK.

Salsalate, a prodrug of salicylate that lowers blood glucose in people with insulin resistance or T2D [16, 17] also increases AMPK activity in the liver [18], but, in contrast to other glucose lowering agents, does so through direct interactions with the allosteric binding site (Ser108) within the β1 isoform [19]. Given the distinct mechanism of AMPK activation compared to metformin, there are additive effects on glucose control when salsalate and metformin are delivered in combination [20]; however, some of these effects are independent of liver AMPK since salsalate also induces mitochondrial uncoupling [18]. To avoid potential off-target effects, highly specific agonists that increase AMPK activity in skeletal muscle, without affecting mitochondrial function, have recently been developed and found to lower blood glucose in mouse models, non-human primates and patients with T2D [21–23]. However, in contrast to activating skeletal muscle AMPK, agonists that primarily increase AMPK activity in the liver (by targeting the AMPK β1 isoform) have little effect on blood glucose in mouse models and non-human primates, but are effective for treating non-alcoholic fatty liver disease (NAFLD) and lowering LDL-cholesterol [24]. Collectively, these data indicate the therapeutic potential of targeting both skeletal muscle and liver AMPK for treating T2D and common comorbidities such as NAFLD and cardiovascular disease.

In addition to targeting skeletal muscle and liver, a potentially emerging treatment for obesity and T2D involves enhancing the metabolic capacity of brown adipose tissue (BAT) [25, 26]. In contrast to classical white adipose tissue (WAT), which is primarily noted for being a storage depot and endocrine organ, BAT can dissipate large amounts of chemical energy in the form of heat to maintain thermal homeostasis. To add to classical BAT and WAT, beige (or brite) adipocytes are brown-like adipocytes found within WAT that can have a high thermogenic capacity. In contrast to WAT, both brown and beige adipocytes have an abundance of mitochondria that, unlike other tissues such as liver or skeletal muscle, are enriched in mitochondrial uncoupling protein 1 (UCP1). UCP1 dissipates the electrochemical proton gradient through a proton leak in the inner mitochondrial membrane, resulting in the uncoupling of oxidative phosphorylation. In addition to mitochondrial UCP1, beige adipocytes also possess UCP1-independent pathways such as creatine and calcium futile cycling [27, 28]. Collectively, this futile cycling increases flux through the β-oxidation pathway, the tricarboxylic acid cycle, the electron transport chain, and the free fatty acid (FFA) re-esterification pathway to enhance energy expenditure—a process referred to as non-shivering thermogenesis.

The capacity of brown and beige adipocytes to increase non-shivering thermogenesis is highly dependent on a thermogenic program that is predominately regulated by the sympathetic nervous system (SNS), which stimulates the local release of norepinephrine in response to reductions in temperature. Norepinephrine then activates the β3-adrenergic receptor,

increasing cAMP and activating the cAMP-dependent protein kinase (PKA), which results in the following: (1) the liberation of free fatty acids from triglycerides, diglycerides, and monoglycerides through a process termed lipolysis, and (2) the promotion of transcription factors which increase genes regulating mitochondrial biogenesis. In addition to the activation of PKA, cold and norepinephrine also increase the activity of AMPK, which is more highly expressed in BAT than any other tissue [29]. Interestingly, this activation of AMPK by β-adrenergic agonists and cold is dependent on the induction of lipolysis [30] but does not require UCP1 [31]. AMPK is also activated in cultured human brown adipocytes in response to norepinephrine [32•]. Given the critical role for AMPK in regulating glucose uptake, fatty acid metabolism, and mitochondrial function, AMPK may play a potentially important role in regulating the thermogenic program which allows for brown and beige adipocytes to take up more glucose and burn more lipid through non-shivering thermogenesis.

Despite the potentially important therapeutic contribution of BAT to both glucose and lipid homeostasis, people with T2D have an impaired ability to increase glucose uptake in BAT (as measured using positron emission tomography (PET) imaging of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake) in response to cold exposure [33, 34]. And although no studies have examined AMPK activity in the BAT of humans with T2D, it is known that AMPK is reduced in the WAT of individuals with marked obesity and insulin resistance compared with participants that are weight-matched and insulin sensitive [35]. Furthermore, it has been shown that reductions in body mass or improvements in insulin sensitivity after weight loss induced by a low-calorie diet or Roux-en-Y gastric bypass surgery are associated with increases in adipose tissue AMPK α Thr172 phosphorylation [36–38]. Taken together, these data highlight the potential role of AMPK in brown and beige adipose tissue metabolism and encourage research to be undertaken to understand its role in systemic metabolism so that novel activators may be formulated for the prevention and treatment of T2D. The goal of this review is to summarize recent work describing the role of AMPK in brown and beige adipose tissue, focusing on its role in adipogenesis and non-shivering thermogenesis.

The Role of AMPK in Brown Adipocyte Development

Adipocyte development is complex and there is a growing body of evidence showing that developmental origins of brown, beige, and white adipocytes are multifarious [25]. Briefly, multipotent progenitor cell populations' commitment to brown preadipocytes is regulated by the transcription factor early B cell factor 2 (EBF2), with subsequent differentiation into brown adipocytes through interaction with the transcriptional co-regulator PR domain containing 16 (PRDM16). This then increases expression of the thermogenic program including peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1α) and UCP1 [26]. Therefore, it can be assumed that there is an increase in brown and beige adipocyte development when these markers are increased in an orchestrated manner in either cells or tissue.

Most studies alluding to AMPK's role in brown adipogenesis focus on differentiation. One of the first studies demonstrating this relationship were those of Qi et al., who found that the increased energy expenditure and protection from high-fat diet observed in *Cidea*$^{-/-}$ mice was associated with enhanced AMPK expression and activity in BAT [39]. Subsequent studies established that repressing the expression of Cidea—a protein that regulates ubiquitin-dependent proteasome degradation of the AMPK β1 subunit—could enhance AMPK expression and BAT differentiation. More recently, a high-throughput screen using a combination of knockdown and pharmacological inhibitors identified AMPK as a kinase that promoted the formation of UCP1-abundant brown adipocytes, with a specific role for AMPK α1, β1, β2, γ1, and γ3 [40]. Consistent with these observations, genetic ablation of AMPK α1 (gene *Prkaa1*) reduces progenitor density in BAT and suppresses brown adipogenesis, instead favoring fibrogenesis [41]. Furthermore, *Prkaa1* deletion in uncommitted stem cells resulted in a profound reduction in the formation of brown adipocytes [42••]. It is important to note that these results are not generalizable to white adipocytes, as there are often opposing effects of AMPK on brown and white adipocyte development [42••, 43, 44]. Collectively, these results indicate that AMPK has an important role in the development of brown adipocytes, but the key pathways mediating these effects require further study.

AMPK exerts effects on nearly all branches of metabolism through phosphorylation of key downstream substrates. A critical regulator of cell growth, proliferation, and metabolism is the mammalian target of rapamycin complex (mTOR); AMPK inhibits this pathway through phosphorylation of the key regulatory proteins tuberous sclerosis complex 2 (TSC2) and regulatory-associated protein of mTOR (Raptor) [45]. Consistent with a role for AMPK in brown fat differentiation via the inhibition of Raptor, an early study indicated that AMPK inhibition of mTOR is important for the differentiation of brown but not white adipocytes [44]. Moreover, mice lacking adipocyte Raptor have more beige adipose tissue, increased energy expenditure, and are protected from developing diet-induced obesity [46]. However, more recent studies have shown a critical role for mTOR in regulating BAT glucose uptake and glycolysis, suggesting that, while mTOR inhibition is important for differentiation, sustained inhibition might impair the normal function of BAT in adult mice [47–49]. This suggests that directly inhibiting mTOR to enhance BAT metabolic activity is unlikely to be therapeutically viable.

AMPK may also control BAT differentiation through DNA methylation. DNA methylation is a dynamically regulated process that plays a large role in cellular differentiation via transcriptional regulation, and methylation and hydroxymethylation marks have been shown to be responsive to cellular metabolic status and enzyme co-factors such as the TCA cycle-intermediate α-ketoglutarate (α-KG) [50]. Recently, Yang and colleagues observed that neonatal *Prkaa1*$^{-/-}$ mice had lower BAT mass, dorsal interscapular temperature, Ucp1 expression, and density and organization of mitochondria [42••], paralleling increased DNA methylation and reduced hydroxymethylation in the Prdm16 promoter of neonatal *Prkaa1*$^{-/-}$ mice. Further experiments unearthed a novel mechanism whereby AMPK increases the activity of the TCA cycle enzyme isocitrate dehydrogenase 2 (IDH2), which yields α-KG, and the eventual demethylation of the Prdm16 promoter region. Thus, AMPK-induced increases in IDH2 activity leads to demethylation of Prdm16, promoting the differentiation of brown adipocytes. How AMPK increases IDH2 activity remains incompletely understood.

The differentiation of brown and beige adipose tissue may also be controlled by systemic factors beyond the SNS. For example, the insulin sensitizing adipokine apelin activates AMPK and PRDM16 to enhance brown adipocytes differentiation [51]. Circulating microRNAs (miRNAs) may also modulate brown adipose differentiation [52]. Specifically, miRNA-455 can suppress the translation of hypoxia-inducible factor 1-alpha inhibitor, which in turn reduces the hydroxylation of AMPK in brown adipocytes [53]; this led to increased activating phosphorylation of AMPKα (Thr172) and subsequent upregulation of PGC1α, UCP1, and mitochondrial biogenesis. A remarkably similar phenotype has also been observed following the genetic deletion of another negative regulator of AMPK, folliculin [54]. AMPK activation can also be achieved using the natural products resveratrol, cryptotanshinone, and medicarpin, all of which have been shown to promote the commitment of pluripotent stem cells or stromal vascular cells to brown adipocytes [55–57]. In vivo, the treatment of neonate mice born from obese parents with the AMPK activators metformin or AICAR also increases brown adipocyte progenitor cells and BAT weight [42••]. Collectively, this suggests that therapies aimed at activating AMPK in progenitor cells may have a noteworthy role in promoting the development of brown adipocytes.

The Role of AMPK in Maintaining Mitochondrial Health in BAT

Until recently, most studies investigating the role of AMPK in adipose tissue have utilized cultured cells or mice lacking single subunits of AMPK that retain some AMPK activity. With technology now allowing for the complete ablation of AMPK in adipose tissue, two major reports have demonstrated that AMPK is essential for brown and beige adipose function. Adipocyte-specific deletion of either AMPK α1α2 (AMPKα-AKO) or the inducible deletion of AMPK β1β2 (iβ1β2AKO, hereafter AMPKβ-AKO) isoforms greatly reduced AMPK activity in both white and brown adipose tissue of adult mice [32•, 58•]. Although phenotypically normal when fed chow diet and housed at standard room temperature (22–23 °C), the adipose-specific AMPK-null mice had a profound defect in thermogenesis, with rapid drops in core body temperatures as compared with controls when housed in the cold (4 °C). This defect in thermogenesis was not attributed to defective WAT lipolysis, as there were no differences in circulating free fatty acids [32•]. Rather, both cold exposure and acute injection with the β3-adrenergic receptor agonist CL-316243 yielded subnormal increments in oxygen consumption (VO_2) and BAT temperature responses, likely related to profound defects in BAT mitochondrial morphology characterized by large swollen mitochondria and disrupted cristae. Moreover, mitochondria from AMPKβ-AKO mice had impaired fatty acid oxidation, independent of acetyl CoA carboxylase activity. Thus, AMPK is essential for maintaining BAT mitochondrial function.

Multiple mechanisms may contribute to AMPK's regulation of mitochondrial function. A healthy network of mitochondria requires an efficient remodeling system to balance production (biogenesis) and degradation (selective mitochondrial autophagy, known as mitophagy). AMPK not only regulates biogenesis through communication with the transcriptional co-activator PGC1α and transcription factor EB (TFEB), but also controls fission (the dividing of mitochondria from their network) through mitochondrial fission factor (MFF), and mitophagy through ULK1 [59]. Interestingly, BAT mitochondrial number was not altered in AMPKβ-AKO mice nor was there an alteration in MFF phosphorylation [32•]. Instead, reductions in mitochondrial quality in AMPKβ-AKO mice were associated with reductions in ULK1 phosphorylation and other markers of mitophagy (p62, LC3B), suggesting that a reduction in the clearance of old/damaged mitochondria was contributing to the defect in cold-induced thermogenesis [32•]. These data suggest that AMPK regulation of mitophagy is essential for maintaining BAT mitochondrial function.

AMPK Activation Is Important for the Browning of WAT

The presence of UCP1-positive adipocytes within WAT has been recognized for many decades, but it is only within the last decade, through the use of sophisticated lineage analysis tools, that these beige/brite adipocytes have been identified as cells that are closely related to human BAT [60–62]. In addition, recent findings have indicated that beige/brite cells can undergo UCP1-independent thermogenesis, potentially due to both calcium

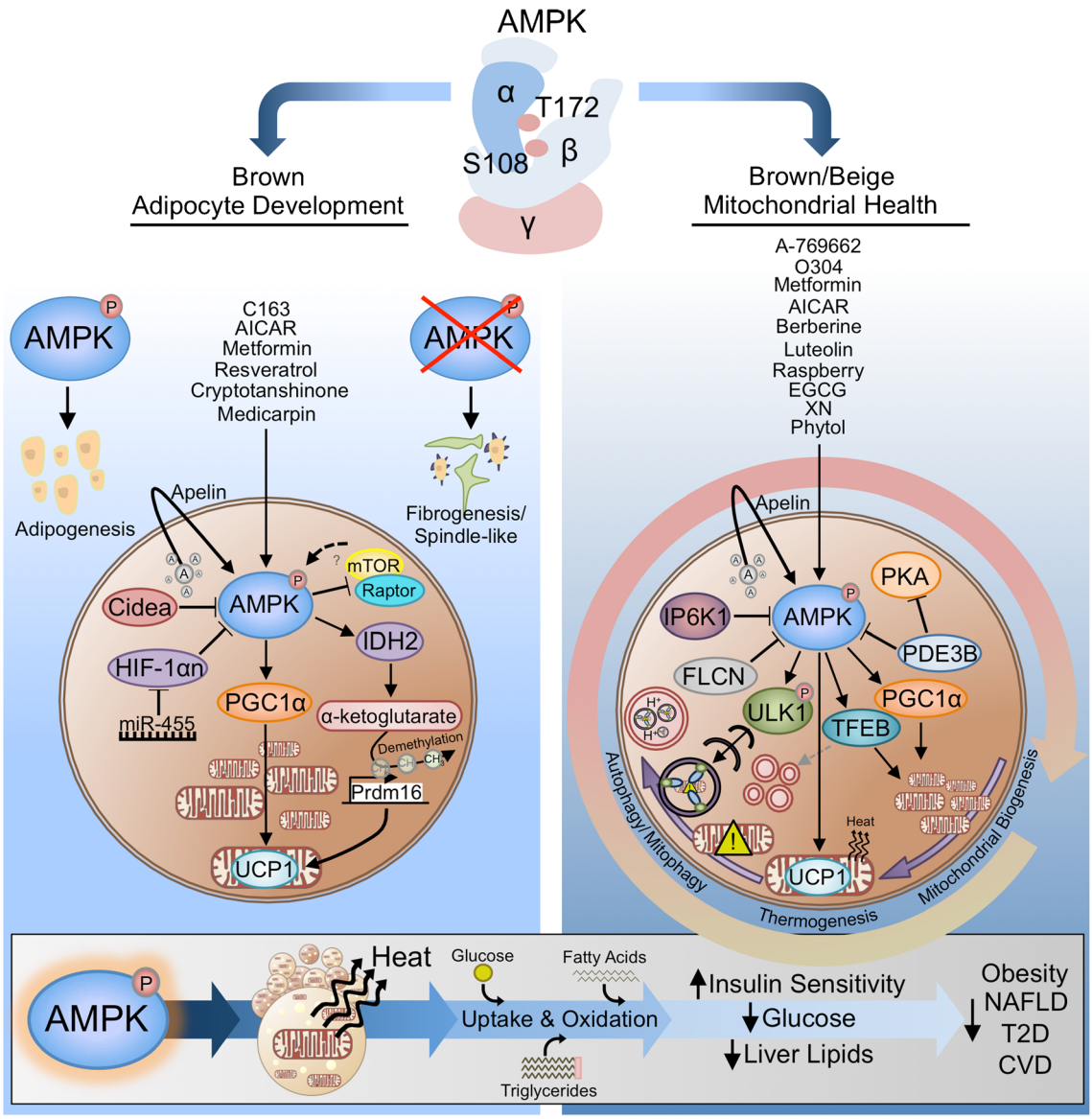

Fig. 1 AMP-activated protein kinase (AMPK)'s emerging role in brown and beige adipose tissue. Left panel: In the development of brown adipocytes, natural and pharmacological activators can activate AMPK to produce more adipocytes expressing uncoupling protein 1 (UCP1). Positive (*solid arrows*) and negative (*solid lines with bars across end*) regulators of AMPK affect the activity of AMPK within brown adipocytes. The presence and activity of AMPK leads to the formation of functional brown adipocytes, while the absence leads to undifferentiated, spindle-like cells, or adipocytes with an abundance of fibrogenesis. Right panel: In the promotion of mitochondrial health in brown/beige adipocytes, natural and pharmacological activators can increase the activation of AMPK to promote non-shivering thermogenesis. AMPK activity is regulated by multiple substrates in brown/beige adipose tissue, and when activated, AMPK promotes mitochondrial health by balancing the degradation of old/dysfunctional mitochondria via autophagy/mitophagy—phosphorylating unc-51 like autophagy activating kinase 1 (ULK1)—and promoting biogenesis through the stimulation of transcription factor EB (TFEB) and PGC1α. In turn, this results in an abundance of mitochondria that can generate heat. To summarize, activating AMPK in brown and beige adipocytes can increase non-shivering thermogenesis; promote the uptake and oxidation of glucose, fatty acids, and triglycerides; improve insulin sensitivity, lower glucose levels and liver lipids; and potentially reduce the risks associated with obesity, non-alcoholic fatty liver disease (NAFLD), type 2 diabetes (T2D), and cardiovascular diseases (CVD) (figure was made using Microsoft PowerPoint for Mac)

and creatine cycling [27, 28]. Although the importance of these cells for regulating non-shivering thermogenesis in humans has yet to be firmly established, the concept of converting white to beige/brite fat in order to increase energy expenditure beyond that achievable through classical BAT is of potentially significant therapeutic importance [63].

The browning of WAT can be achieved through pharmacological activation of AMPK using the specific AMPK β1 agonist A-769662 [58•]. Increases in WAT thermogenesis have also been observed in mice treated with the AMPK activator O304; however, this effect was not attributed to increases in UCP1-positive cells but instead was associated with

increased markers of lipolysis, mitochondrial content, and fatty acid futile cycling [23]. Consistent with these observations, numerous other studies have found that indirectly increasing AMPK activity in WAT also results in browning of white adipose tissue and increases in energy expenditure [15, 54, 64–73]. However, it should be noted that an absolute requirement for adipose tissue AMPK in mediating these phenotypes has not been investigated in most studies. For example, while FGF21 increases AMPK activity in cultured adipocytes [74, 75], the beneficial effects of this compound are not mediated through adipose tissue AMPK [76]. Nevertheless, experimental reduction in adipose tissue AMPK, achieved via germline deletion in AMPK $\beta1^{-/-}$ or adipose-specific reductions in both AMPK α and β subunits, leads to reduced WAT PGC1α and mitochondrial markers, both in the basal state and in response to β-adrenergic receptor agonists such as norepinephrine or CL-316243 [32•, 58•, 77]. Future studies investigating the mechanisms by which AMPK promotes the browning of WAT and whether concomitant increases in energy expenditure are independent of UCP1 are now required.

Adipose Tissue AMPK, Obesity, Nonalcoholic Fatty Liver Disease, and Type 2 Diabetes

Humans with obesity and T2D have reductions in cold-stimulated glucose uptake into BAT and reductions in adipose tissue AMPK [33, 35]. However, in humans, it is difficult to establish whether these reductions in adipose tissue AMPK may be a consequence or a cause of either insulin resistance, T2D, or impairments in BAT function. As noted above, studies using adipose tissue-specific AMPK-KO mice have begun to address these important questions [32•, 78]. Although phenotypically normal when fed chow, AMPK adipose specific-null mice develop slightly greater obesity than control littermates after a high-fat diet challenge. Importantly, despite the relatively modest effect on adiposity, mice lacking adipose tissue AMPK develop greater liver steatosis, inflammation, and insulin resistance than wildtype littermates. These data suggest that reductions in adipose tissue AMPK may be a cause, and not just a consequence of insulin resistance, and that pharmacologically targeting adipose tissue AMPK may be effective for reducing liver lipids and glucose. However, future studies in mice with constitutively active AMPK mutations or treated with adipose-targeted AMPK activating therapeutics will be required to further test this hypothesis.

Conclusions and Perspectives

Since the discovery of functional BAT in humans roughly a decade ago, there has been renewed interest in the therapeutic potential of targeting this tissue for the treatment of metabolic disorders including obesity, T2D, and NAFLD. There is now clear and compelling evidence that AMPK plays a vital role in regulating the development of BAT, maintenance of BAT mitochondrial function, and browning of WAT (Fig. 1).

However, many important questions remain to be answered. Firstly, since AMPK is reduced in WAT in humans with obesity and insulin resistance, it will be important to know whether AMPK activity is also altered in BAT. Secondly, although in vitro and in vivo findings are promising, it is still unclear whether the effects of AMPK activation—resulting in the induction of BAT and browning of WAT—will have a positive therapeutic effect in humans living at thermoneutrality. Moreover, there are still many mechanisms to be investigated regarding the role of AMPK in the browning of WAT. An exciting avenue is to identify whether AMPK is involved in UCP1-independent non-shivering thermogenesis pathways such as the creatine and calcium shuttles. Lastly, AMPK integrates a multitude of inputs and can act on many different substrates. Determining which enzymes are the predominant effectors of AMPK-mediated non-shivering thermogenesis will advise the development of future adipose tissue-specific activators so that maximal effectiveness can be achieved with minimal side effects. Answering these questions will help establish whether targeting adipose tissue AMPK may be effective for treating obesity, NAFLD, and T2D.

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Compliance with Ethical Standards

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Informed Consent This article does not contain any experiments with human or animal subjects.

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