REVIEW

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AMP-activated protein kinase and its multifaceted regulation of hepatic metabolism

Morgan D. Fullerton

Purpose of review
The current review summarizes recent advancements in our mechanistic and physiological understanding of the energy sensing AMP-activated protein kinase (AMPK) and its regulation of select aspects of hepatic metabolism.

Recent findings
A highly conserved serine/threonine kinase, AMPK governs a multitude of cellular process to activate catabolic and inhibit anabolic pathways. Recent work has provided clarity as to the importance and contribution of the AMPK signaling cascade to various aspects of cellular metabolism, including lipid homeostasis, hepatic glucose production, mitochondrial metabolism, and autophagy.

Summary
With more than 60 confirmed substrates, the physiological significance of AMPK signaling has been difficult to ascertain. The generation of targeted knock-in mutations on key AMPK substrates has begun to shed light on this complex system. Future studies are needed to further decipher the complexity, significance, and potential therapeutic targeting of hepatic AMPK signaling.

Keywords
AMP-activated protein kinase, autophagy, metabolism, metformin, obesity, type 2 diabetes

INTRODUCTION

The liver is an extremely versatile metabolic organ, able to buffer nutritional, hormonal, and neuronal oscillations to accommodate fluctuations in energy demand, and thus plays a central role in regulating whole body metabolism. Anatomically positioned to receive portal delivery of newly absorbed nutrients, oral therapeutics, as well as secreted pancreatic and gut derived hormones, the liver has intricately evolved to interpret endogenous and exogenous signals. During metabolic stress/dysfunction (stemming from environmental or genetic origins), many aspects of hepatic metabolism are dysregulated, which has drawn considerable therapeutic interest toward potential targets capable of re-establishing healthy hepatic regulation. The AMP-activated protein kinase (AMPK) is a master regulator of energy homeostasis and exerts control over numerous metabolic pathways [1]. As a potential target for the treatment of numerous metabolic diseases such as obesity, type 2 diabetes, cardiovascular disease, fatty liver disease, and hepatocellular carcinoma, this review aims to highlight recent advancements in our understanding of various aspects of hepatic AMPK signaling (Fig. 1).

AMP-ACTIVATED PROTEIN KINASE; ACTIVATION AND DIRECT ACTIVATORS

A serine/threonine kinase that is conserved in essentially all eukaryotes, AMPK is activated in response to energy deficit (low ATP and reciprocally high AMP and ADP). AMPK is ubiquitously expressed across cell types, and exists as a heterotrimer that comprises catalytic (α1 and α2) and regulatory (β1, β2 and γ1, γ2, γ3) subunits, all of which are encoded by separate genes, with multiple isoforms and splice variants. In the rodent liver, the AMPK heterotrimer is composed of both α1 and α2 equally, β1 and

Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada

Correspondence to Morgan D. Fullerton, PhD, Department of Biochemistry, Microbiology and Immunology, University of Ottawa, 451 Smyth Rd. Ottawa, ON K1H 8M5, Canada. Tel: +1 613 562 5800 x8310; fax: +1 613 562 5452; e-mail: morgan.fullerton@uottawa.ca

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KEY POINTS

- AMPK is a ubiquitously expressed, highly conserved Ser/Thr kinase that is able to restore energy balance by activating catabolic and inhibiting anabolic pathways.
- AMPK lies at the nexus of many interconnected signaling pathways and regulates lipid metabolism via inhibiting phosphorylation of ACC, HMGCR, SREBPs, and ChREBP.
- During energy deficiency, AMPK activation induces macroautophagy via direct phosphorylation of ULK1 and inhibition of mTORC1 via tubular sclerosis 2 and regulatory-associated protein of mTOR, which leads to lysosomal degradation of hepatic lipid droplets (lipophagy) for β-oxidation.
- Normal AMPK function is critical for mitochondrial homeostasis via the regulation of mitochondrial biogenesis, morphology and clearance, via nutrient-induced or targeted autophagy (mitophagy).
- Fifteen years after the discovery that the antidiabetic drug metformin activates hepatic AMPK, it has become clear that only a fraction of the beneficial effects of this therapy are AMPK-dependent.

γ1 components [2–4]. However, human hepatocytes express exclusively α1, β2, γ1/2 [4,5]. True to its reputation as a sensor of cellular energy, during energy deprivation, (i.e., fasting, exercise, ischemia, etc.), AMP and ADP bind adenine nucleotide binding pockets on the γ subunit. This causes allosteric activation and phosphorylation of the α-subunit via the upstream activating kinase, tumor suppressor liver kinase B1 (LKB-1). In addition, this also acts to protect against dephosphorylation by protein phosphatases (mainly by protein phosphatase 2A; PP2A). Alternatively, AMPK can be activated in response to increases in intracellular Ca^{2+} by Ca^{2+}/calmodulin-dependent kinase kinase β (CaMKKβ) and by the transforming growth factor-β-activating kinase 1 (TAK1). The activation and intrinsic functioning of AMPK in response to its various activators has recently been reviewed [6,7].

Given that AMPK activation is so intricately linked to energy status, it is unsurprising that most (but not all) documented compounds and therapeutics that have the capacity to activate AMPK do so indirectly, via inhibition of mitochondrial bioenergetics and/or altered cellular energy charge. In addition, AMPK is also modulated in response to various endocrine cues, which have recently been reviewed [8]. The thioprenidione class of molecules developed by Abbott Laboratories yielded the first direct activator of AMPK, A-769662, which specifically targets the β1 subunit (via interactions involving Ser108) [9,10], and does not promote LKB-1-mediated phosphorylation, but rather acts to inhibit dephosphorylation. We recently reported that salicylate, the base component and unacetylated form of aspirin, can also directly activate AMPK via binding on the β1 subunit, which was shown to increase hepatic β-oxidation and may explain some of the beneficial metabolic effects of salicylate-based therapies [11]. To date, only a select number of activators have been identified that are both direct and specific, including C-13 (α-specific) [12] and the Merck compound 991 (β-specific), the latter of which has recently been described in concert with novel structural analysis of the AMPK complex, and hints at the potential for an endogenous ligand and activator of AMPK [13▪▪].

LIPID METABOLISM

The first documented role for AMPK (although not known to be the kinase responsible at the time) was the acute inhibition of fatty acid (FA) metabolism via phosphorylation of acetyl-CoA carboxylase (ACC) (ACC1 at Ser 79 and ACC2 at Ser212 in rodents and Ser221 in humans) [14,15] and cholesterol synthesis via phosphorylation of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) (Ser872 in rodents and Ser871 in humans) [16,17]. This is thought to act in concert to shut off FA and cholesterol synthesis and relieve inhibition on carnitine palmitoyltransferase 1, resulting in increased β-oxidation, leading to the conservation and subsequent generation of cellular ATP.

Although it had been known for some time that AMPK regulates ACC activity, the physiological significance of this acute signaling event remained unexplored. Moreover, an overwhelming number of studies had measured the phosphorylation of ACC (by phospho-specific antibodies) as a surrogate readout for AMPK activation. We sought to address this by generating mice that harbor Ser-Ala knock-in (KI) mutations at ACC1 Ser79 and ACC2 Ser212 [18▪▪]. Mice with KI mutations to each gene (ACC1 KI and ACC2 KI) were crossed together to create ACC double knock-in (DKI) mice. In spite of the subtlety of the point mutations, ACC DKI mice on a regular chow diet had higher ACC activity, malonyl-CoA and FA synthesis, along with a diminished capacity for hepatic FA oxidation. This was associated with higher levels of hepatic diacylglycerol and TAG, induction of protein kinase Cε (PKCε), more fibrosis and liver damage compared with wild-type controls *in vivo*, which ultimately inhibited insulin signaling [18▪▪]. The importance of AMPK-directed phosphorylation of ACC was also demonstrated by the

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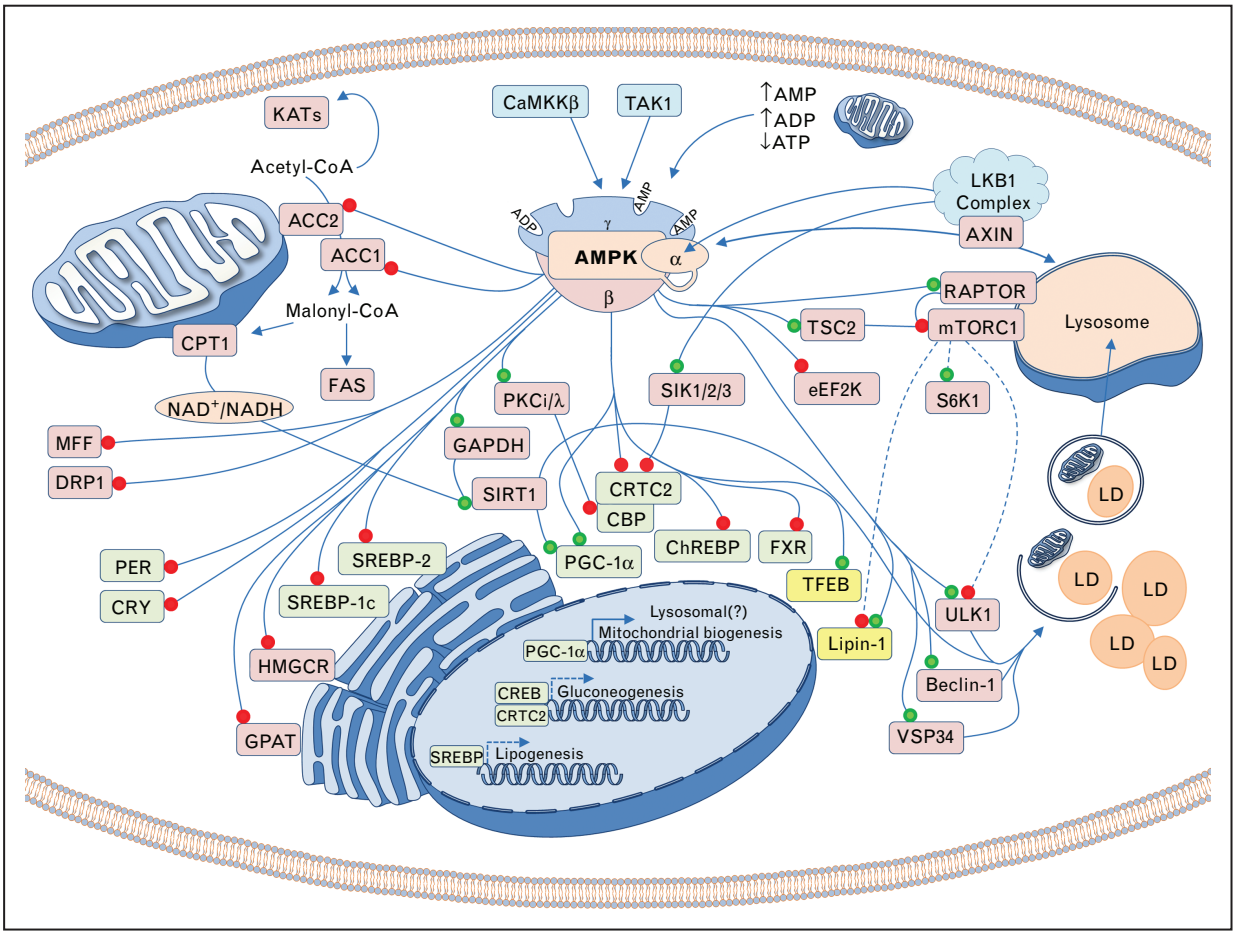

FIGURE 1. Schematic representation of a large majority of known hepatic AMPK targets. Green circles represent activating and red circles represent suppressive phosphorylation. The AMPK heterotrimer is focal, with enzyme targets involved in metabolic pathways shown in light red, transcription factors and coactivators shown in light green and potential, but unsubstantiated interactions shown in yellow. ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; CaMKKβ, Calmodulin modulated kinase kinase β; CBP, CREB binding protein; ChREBP, carbohydrate response element binding protein; CPT1, carnitine palmitoyltransferase 1; CREB, cAMP response element-binding protein; CRTC2, CREB-regulated transcription coactivator 2; CRY, cryptochrome proteins; DRP1, dynamin-related protein 1; eEF2K, eukaryotic elongation factor 2 kinase; FAS, fatty acid synthase; FXR, farnesoid X receptor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GPAT, glycerol-3 phosphate acyltransferase; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; KATs, lysine acetyltransferase; LD, lipid droplet; LKB1, liver kinase B1; MFF, mitochondrial fission factor; mTORC1, mammalian target of rapamycin complex 1; PER, period proteins; PGC-1α, peroxisome proliferator-activated receptor gamma co-activator 1-α; PKC, protein kinase C; RAPTOR, regulatory-associated protein of mTOR; S6K1, S6 kinase1; SIK, salt-inducible kinase; SIRT1, sirtuin-1; SREBP, sterol regulatory element binding protein; TAK1, transforming growth factor-β-activating kinase; TFEB, transcription factor EB; TSC2, tubular sclerosis 2; ULK1, unc-51 like kinase.

interaction of the serine/threonine phosphatase phosphatase 4 (PP4), a member of the PP2A family of protein phosphatases, with ACC in the liver [19]. PP4-mediated dephosphorylation of ACC caused lipid accumulation in hepatocytes and was associated with fatty liver in db/db mice [19].

Whereas chronic ACC activation leads to increased lipogenesis and diminished β-oxidation [18▪▪], it was hypothesized that the opposite would hold true in a newly generated liver-specific ACC1 and ACC2 double knockout model [20▪▪]. However, hepatic deletions of both ACC isoforms unexpectedly lead to an increase in liver TAG, because of reductions in FA oxidation and stimulation of glycolysis. The complete deletion of ACC activity caused a dramatic shift in the acetylation status of numerous metabolic proteins [20▪▪], a finding consistent with the inhibition of ACC in yeast [21,22]. These studies link both ACC activity and potentially its regulation by AMPK in the maintenance of cellular acetyl-CoA and the acetylation of both histone and nonhistone proteins.

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The ablation of AMPK-mediated ACC phosphorylation has also provided insight regarding the potential functional redundancy of the two ACC isoforms. The dogma surrounding ACC has maintained that ACC1 is localized in the cytosol to control lipogenesis, whereas ACC2 is localized to the mitochondrial outer membrane to control FA uptake via carnitine palmitoyltransferase 1 [23]. Furthermore, conflicting evidence has also arisen from the complete genetic knockout models of these genes individually. One model of liver-specific ACC1-deficiency supported redundancy between isoforms because of an upregulation of ACC2 [24], whereas another demonstrated segregation of malonyl-CoA pools [25], which left the question of the extent to which ACC1 and ACC2 might overlap functionally unanswered. In mice harboring single KI mutations to the AMPK-targeted phosphorylation sites (either ACC1 KI or ACC2 KI), hepatic malonyl-CoA, FA synthesis, and FA oxidation was unperturbed compared with wild-type littermate controls [18▪▪]. This strongly suggests an overlapping role for malonyl-CoA derived from liver ACC1 or ACC2, independent of cellular localization.

The activation of hepatic AMPK has also been shown to modulate the activity of sterol regulatory element binding proteins (SREBP-1c and 2; encoded by separate genes that regulate FA and cholesterol synthesis, respectively [26,27]), which are important lipogenic transcription factors regulated by nutritional status. Through direct phosphorylation, AMPK inhibits SREBP-1c at Ser372 and SREBP-2 on an unidentified site, such that acute AMPK-mediated phosphorylation inhibits proteolytic cleavage and nuclear translocation of SREBP isoforms, preventing autoregulation and downstream transcription of FA and cholesterol-related genes [28]. This ultimately diminished lipid synthesis both *in vitro* and in obese and insulin resistant mice *in vivo* [28].

An alternative pathway in the many layers of SREBP regulation involves the highly conserved nutrient sensing kinase mammalian target of rapamycin complex 1 (mTORC1) and its interactions with the phosphatase, lipin-1. In the fed state (when AMPK activity is low), mTORC1 phosphorylates and inhibits the nuclear entry of lipin-1, which promotes SREBP activation; however, upon mTORC1 inhibition, SREBP-mediated lipogenesis is lowered and diet-induced hepatic steatosis is improved [29]. AMPK can inhibit mTORC1 via phosphorylation of both tubular sclerosis 2 [30] and the regulatory-associated protein of mTOR (raptor) [31]. Therefore, in addition to direct phosphorylation and inhibition of SREBP by AMPK, it is possible that AMPK-mediated inhibition of mTORC1 and potentially even lipin-1 directly [32] may contribute to the regulation of SREBP-induced lipid synthesis.

The number of pathways in which AMPK is implicated to regulate lipid metabolism continues to grow, and a number of AMPK activators may have therapeutic benefits extending from obesity and fatty liver, to the progression of atherosclerosis [11,12,13▪▪,28,33]. As the first knock-in model to an AMPK substrate, the ACC DKI mice have yielded interesting results (see metformin section below), as the beneficial effects of high-intensity interval training on hepatic lipid metabolism and insulin sensitivity were independent AMPK-mediated ACC regulation [34▪▪]. Moving forward knock-in models will be necessary for important transcriptional regulators such as SREBP [28], carbohydrate response element binding protein [35] and farnesoid X receptor [36▪], as well as HMGCR, whose phosphorylation was discovered more than 40 years ago [17].

AUTOPHAGY

Autophagy, the process by which intracellular constituents (lipids, proteins, and organelles) are degraded in the lysosome, was originally discovered through studies conducted in the liver. In recent years, AMPK has emerged as an important regulator of macroautophagy (herein referred to as autophagy), which has been reviewed in depth [37,38]. The degradation of lipids via the sequestration of lipid droplets by autophagosomes for lysosomal delivery and processing has been termed lipophagy [39–41], which has been demonstrated to be independent of classical lipolysis [42,43]. mTORC1 is a major negative regulator of autophagy via inhibition of unc-51-like kinase (ULK1/2) at Ser757 [44]. Therefore, AMPK can trigger autophagy via a double-pronged mechanism involving the inhibition of mTORC1 signaling and the direct (activating) phosphorylation of ULK1 at several sites [45,46]. AMPK has also been demonstrated to phosphorylate beclin-1 (Ser91 and Ser94) and VSP34 (Thr 163 and Thr165) [47], and interestingly ULK1 is able to phosphorylate and inhibit AMPK [48,49], potentially as part of a negative feedback loop. Additionally, it was recently shown that AMPK directly phosphorylates glyceraldehyde 3-phosphate dehydrogenase to promote nuclear translocation and activation of sirtuin 1 (SIRT1) deacetylation to stimulate autophagy [50▪▪]. Finally, although classically activated by energy changes, hepatic TAK1 activated AMPK to induce autophagy, regulate FA oxidation and inhibit tumorigenesis, a pathway that may be altered in obesity and fatty liver [51▪].

The transcriptional control over lysosomal homeostasis and autophagy is mainly mediated by

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transcription factor EB (TFEB), which is retained in the cytosol during nutrient sufficiency via mTORC1-mediated phosphorylation [52]. However, when nutrients are scarce, mTORC1 inhibition induces TFEB nuclear translocation and the activation of lysosomal biogenesis. Although a direct link between TFEB and AMPK has not yet been uncovered, recent evidence demonstrates that the upstream activating kinase LKB1, in complex with the scaffolding protein axin, is trafficked to the lysosomal membrane during nutrient deficiency and this is followed by the recruitment and activation of AMPK [53▪▪,54]. Given the lysosomal-localization of activated mTORC1 [55], the observation that AMPK is also recruited and activated at the lysosomal membrane may present a plausible mechanism by which signaling occurs between these two important nutrient-sensing metabolic kinases.

MITOCHONDRIAL HOMEOSTASIS

The maintenance of mitochondrial homeostasis and function are critical for cell survival. To this end, AMPK can dictate the coordination of mitochondrial biogenesis, changes in morphology and lysosomal clearance [56,57]. The peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) is an important regulator that stimulates transcriptional activation of multiple transcription factors involved in mitochondrial proliferation. AMPK activates PGC-1α via direct phosphorylation [58,59], and since AMPK activation causes a change in NAD^+/NADH, this activates SIRT1, which deacetylates PGC-1α to promote its nuclear localization [60]. As described above, AMPK can induce macroautophagy via activation of ULK1 and inhibition of mTORC1, which would result in the clearance and degradation of defective and/or senescent mitochondria. As the process of mitophagy is characterized by the involvement of cargo-recognizing proteins, the level of involvement of hepatic AMPK toward 'mitophagy' 'per se', remains unclear. However, through direct interactions with ULK1, AMPK may affect macroautophagy and mitophagy at a common upstream point, since ablation of AMPK-mediated phosphorylation of ULK1 results in hepatic mitochondrial accumulation with reduced function [45,46].

AMPK may also influence mitochondrial morphology through modulation of dynamin-related protein (DRP1) at Ser637 [a protein kinase A (PKA) phosphorylation site], although direct interaction has not been confirmed nor demonstrated in hepatocytes [61,62]. In a recent proteomic screen of hepatocytes from control and AMPKα1α2 liver-specific knockouts in the presence of absence of AMPK activation, mitochondrial fission factor (MFF) was also shown to be an AMPK substrate, which interacts with DRP1 [63▪▪]. Since mitophagy is preceded by the induction of mitochondrial fission in mammalian cells, AMPK-mediated phosphorylation of mitochondrial fission factor may signal the induction of mitochondrial fragmentation prior to mitophagy, but this requires further investigation.

Therefore, AMPK lies at the nexus of multiple pathways that stand to regulate the biogenesis and lysosomal degradation of hepatocyte organelles. This serves to eliminate defective machinery; regulate the turnover of healthy organelles (such as mitochondria), which may help to regulate mitochondria content; and increase the availability of cellular energy stores (lipid droplet degradation).

HEPATIC GLUCOSE METABOLISM

The level of circulating blood glucose is tightly controlled via intricate multiorgan cross-talk. In the wake of the current epidemic of obesity, insulin resistance and type 2 diabetes, understanding the molecular regulation of these pathways is paramount. Early studies using the nonspecific AMPK activator, 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR), suggested an inhibitory role for AMPK on hepatic glucose production [64,65]. This notion was strengthened by the discovery that the antidiabetic therapy metformin activated hepatic AMPK [66], which fueled speculation that AMPK plays a critical role in the normal regulation of gluconeogenesis and may mediate metformin's glucose-lowering effects.

In the postprandial state, inhibition of gluconeogenesis occurs mainly via the phosphorylation and exclusion of key transcriptional activators from the nucleus, leading to the suppression of gluconeogenic gene expression. In-vivo evidence for AMPK's involvement in hepatic glucose production stems mainly from the observed hyperglycemia in whole body AMPKα2-deficient [67] and liver-specific LKB1-deficient mice [68], as well as a therapeutic lowering of blood glucose in obese mice expressing constitutively active AMPKα2 [69,70]. Interestingly, complete deletion of skeletal muscle AMPK activity results in fasting and ageing-impaired gluconeogenesis, because of dysfunctional autophagy and a lack of substrate availability [71▪]. However, perhaps surprisingly, liver-specific knockout of AMPKα1α2 displayed no change in fasting or fed glycemia [72,73▪▪,74▪] and moreover, specific/direct activation of hepatic AMPK with A-769662 was unable to regulate hepatocyte glucose output [72].

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These data point to the fact that LKB1 (not AMPK) activity is critical for regulating glucose production, and further implicates the related salt-inducible kinases (SIK1/2/3). Recent work has demonstrated that SIK2 is not responsible for the insulin-stimulated suppression of glucose production [73▪▪]. However, through use of a pan SIK inhibitor, it was reasoned that in a nutrient sufficient (fed) state, SIK2 and potentially other SIK isoforms, play an important role in repressing gluconeogenesis [73▪▪]. To this end, SIK3 was recently demonstrated in hepatocytes to be critical for regulating the phosphorylation of cAMP response element-binding protein (CREB)-regulated transcription coactivator 2, causing the suppression of glucose output [75▪]. Conclusions as to the importance of AMPK and LKB1 in mediating control over glucose production were made possible by employing hepatocyte-specific deletions. Future studies using liver-specific (individual and combination) SIK knockout or knock-in mice will undoubtedly tease out the in-vivo mechanisms.

THE CONTINUED SAGA OF METFORMIN AND THE SEARCH FOR ITS MECHANISM(S)

Metformin remains the first line of defense in the treatment of type 2 diabetes; however the precise molecular mechanisms by which metformin conveys its beneficial effects are still being elucidated. Via the inhibition of mitochondrial complex I, metformin alters cellular energy to activate AMPK, which was speculated to be the major mechanism by which metformin lowered glucose production (reviewed recently [76]). However, liver-specific AMPKα1α2 and liver-specific LKB1-deficient mice have demonstrated that metformin limits the required energy for gluconeogenesis, rather than inhibiting transcriptional programs via AMPK or LKB1 [72]. This has recently been countered using transient overexpression and knockdown systems on the basis that earlier studies employed physiologically unachievable concentrations of metformin [77]. However, since the local hepatic concentration of metformin is likely two to three times higher than portal circulation [78,79], the lower dose used to treat liver-specific AMPK/LKB1-null hepatocytes (250 μmol/l) has been shown to be achievable, though it should be considered as the upper end of clinical [72]. The involvement of AMPK with regards to the hepatic mechanisms of metformin might conclusively be demonstrated using the liver-specific null mice at more clinically relevant doses (*in vitro* and more importantly, *in vivo* in the setting of obesity, hyperglycemia and insulin resistance), although evidence suggests this remains AMPK-independent (Viollet B and Foretz M, personal communication).

Several studies have recently described distinct lines of evidence to add to the puzzle that is metformin's mode(s) of action. Biguanides (phenformin and metformin) were shown to limit cAMP production via reductions in ATP, leading to the suppression of glucagon-induced gluconeogenesis [80], although it will be important to determine if more physiological doses of metformin in the setting of obesity act to lower blood glucose via this mechanism. In addition, metformin was shown to promote AMPK phosphorylation of PKCι/λ to lower glucose output [81] and inhibit mitochondrial glycerophosphate dehydrogenase, which reduced mitochondrial gluconeogenic substrates and decreased hepatic gluconeogenesis [82].

Interestingly, intraduodenal metformin delivery (presumably oral delivery as well) activates a GLP-1R/PKA axis that signals via vagal efferent nerves and is completely dependent on duodenal AMPK [83▪▪]. This raises the intriguing possibility that preabsorptive metformin evokes an incretin and neuronal response that culminates with hepatic glucose lowering. However, whether oral administration of metformin induces a duodenal response has yet to be established and will likely be clarified with the use of duodenal-specific AMPK null mice. To that end, it has also been shown that metformin treatment of diet-induced obese mice effectively normalizes the resident gut flora (to a health state), potentially eliciting beneficial metabolic effects for the host [84,85]. Whether or not gut flora dictate efficacy of an intestinal metformin response remains unexplored.

It has become clear that metformin has pleiotropic effects that in addition to glucose lowering, include lipid-lowering and insulin sensitization [86,87]. In the setting of obesity, gluconeogenesis persists through the fasting-feeding transition because of a resistance to the suppressive effects of insulin and contributes to hyperglycemia. In high-fat fed, obese mice, chronic administration of a therapeutic dose of metformin was able to inhibit *de novo* lipogenesis to lower hepatic TAG and diacylglycerol, which was associated with reduced PKCε activation and increased insulin sensitivity, ultimately leading to greater suppression of gluconeogenesis *in vivo* (all independent of any change in weight or adiposity) [18▪▪]. Remarkably, these beneficial chronic effects were completely lost in ACC DKI mice (where AMPK cannot phosphorylate and inhibit ACC). Furthermore, the lipid-lowering and insulin sensitizing effects of metformin could be recapitulated in wild-type, but not ACC DKI hepatocytes made insulin-resistant with palmitate

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treatment [18▪▪]. However, hepatocytes from both genotypes remained sensitive to acute metformin-mediated suppression of cAMP-stimulated glucose production or glucagon-stimulated cAMP, which support previously identified mechanisms of action [72,80]. As dual activation of AMPK has been demonstrated to augment signaling [72,88▪], the synergistic combination of clinically relevant doses of both metformin and salicylate [11] (salsalate, a prodrug of salicylate was used for in-vivo studies) was able to maximally activate AMPK, inhibit lipogenesis and induce a greater protective suppression of hepatic glucose production in obese mice compared with low doses of either drug alone [89▪▪]. Therefore, as potential mechanisms for metformin's effects continue to be discovered, it will be critical to consider therapeutically relevant doses in both in-vitro and in-vivo rodent models, as the use of physiologically unachievable doses cloud interpretation and relevance [90].

CONCLUSION

Understanding the regulation of hepatic metabolism and how these processes become dysfunctional during chronic disease is paramount, especially given the increasing global burden of obesity-related disease. It is clear that hepatic AMPK regulates an ever-expanding repertoire of substrates (a list of 57 existing and novel hepatic substrates was recently identified [63▪▪]), which have diverse functions across more aspects of hepatocyte physiology than was regrettably able to be covered in this review. AMPK has also emerged as a primary readout for countless metabolic studies, and has been credited with mediating the beneficial effects of many genetic and pharmacological interventions. However, as we strive to fully understand and develop therapies that will manipulate aspects of hepatic metabolism, defining cause and effect with respect to AMPK will be important. Open access to specific and direct AMPK activators, the availability of AMPK-deficient mouse models and more importantly the creation of novel targeted Ser/Thr-Ala knock-in mutations of AMPK substrates will discern AMPK-dependent from independent effects. This knowledge will help to reveal the physiological significance of each branch of AMPK signaling and may lead to the use of novel, existing, or combination therapies to combat metabolic disease.

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Conflicts of interest

There are no conflicts of interest.

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- ▪ of special interest
- ▪▪ of outstanding interest

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