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Review Article

AMPK inhibits liver gluconeogenesis: fact or fiction?

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Is there a role for AMPK in the control of hepatic gluconeogenesis and could targeting AMPK in liver be a viable strategy for treating type 2 diabetes? These are frequently asked questions this review tries to answer. After describing properties of AMPK and different small-molecule AMPK activators, we briefly review the various mechanisms for controlling hepatic glucose production, mainly via gluconeogenesis. The different experimental and genetic models that have been used to draw conclusions about the role of AMPK in the control of liver gluconeogenesis are critically discussed. The effects of several anti-diabetic drugs, particularly metformin, on hepatic gluconeogenesis are also considered. We conclude that the main effect of AMPK activation pertinent to the control of hepatic gluconeogenesis is to antagonize glucagon signalling in the short-term and, in the long-term, to improve insulin sensitivity by reducing hepatic lipid content.

Introduction

AMP-activated protein kinase (AMPK) is a highly conserved eukaryotic Ser/Thr protein kinase whose principal role is to sense changes in intracellular adenylate charge. Interest in AMPK as a drug target started with the discovery of its implication in increased glucose uptake induced by muscle contraction during exercise, independent of insulin. This led to the idea that pharmaceutical AMPK activators could be effective in treating insulin resistance in patients with type 2 diabetes (T2D) [1]. It was then found that AMPK might mediate some of the anti-diabetic action of biguanides, such as metformin [2], to inhibit liver gluconeogenesis (GNG). This review discusses the implication of AMPK in the control of GNG and whether AMPK activation in the liver could be beneficial for the management of T2D.

AMPK

Canonical AMPK activation results from an increase in intracellular AMP:ATP ratio, as occurs for example in skeletal muscle during intense exercise. Once activated, AMPK switches on ATP generating processes while simultaneously switching off ATP consuming pathways, in order to restore energy balance [3]. AMPK exists as heterotrimers comprising a catalytic alpha subunit (AMPKα1/α2) associated with regulatory beta (AMPKβ1/β2) and gamma (AMPKγ1/γ2/γ3) subunits. Based on immunoblotting, AMPKα1β2γ1 was shown to be predominant in human hepatocytes, whereas rat and mouse liver predominantly expressed AMPKα2β1γ1 [4]. By mass spectrometry, the major heterotrimer AMPK complex in human liver was AMPKα1β2γ1, but dog and rat livers mainly contained AMPKα1β1γ1 and AMPKα2β1γ1 [5]. AMPKα2 has been shown to have a preferential nuclear localization at least under certain conditions [6] where it might be more important for transcriptional control. AMPKβ1-containing complexes can be selectively activated by small-molecule direct AMPK activators (see below).

Maximal AMPK activation requires phosphorylation of Thr172 located in the activation loop of the AMPK α-subunit by upstream kinases, mainly liver kinase B1 (LKB1) in response to energy deficit [3] and Ca^{2+}/calmodulin-dependent protein kinase kinase 2 (CaMKK2) in response to increased cytosolic Ca^{2+}. The AMPK γ-subunits bind AMP, ADP and ATP (binding ATP is crucial as this is how

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AMPK senses changes in adenine nucleotides), while the AMPK β-subunits possess a carbohydrate-binding module (CBM), possibly to co-localize AMPK with glycogen particles and glycogen synthase [7], an AMPK target in liver [8]. A rise in AMP levels increases AMPK activity via three mechanisms: (i) allosteric stimulation of AMPK activity; (ii) promotion of AMPKα Thr172 phosphorylation; (iii) inhibition of AMPKα Thr172 dephosphorylation by protein phosphatases [9,10]. While intracellular ADP concentrations also increase in response to energy stress, ADP does not allosterically stimulate AMPK activity, although it does mimic effects of AMP on AMPKα Thr172 phosphorylation [11]. Increases in AMP:ATP ratio are now considered the key mechanism for AMPK activation by energy stress *in vivo*, and the allosteric effect of AMP binding to the AMPK γ-subunits probably combines with the effect AMPKα Thr172 phosphorylation to render AMPK exquisitely sensitive to small changes in adenine nucleotides.

AMPK activators

Pharmacological AMPK activators can be subdivided into (i) compounds that act indirectly by inhibiting ATP synthesis [12] or by inhibiting AMP degradation [13,14], thereby increasing the AMP:ATP and ADP:ATP ratios, (ii) pro-drugs that are converted into AMP analogues and (iii) direct activators that bind to sites distinct from the AMPKγ-subunit AMP binding sites [15]. Metformin activates AMPK canonically via mild inhibition of mitochondrial respiratory chain complex I, thus increasing the AMP:ATP ratio [16]. The compound 5-aminoimidazole-4-carboxamide riboside (AICAr) was the first pharmacological AMPK activator. It becomes converted in cells to the AMP analogue 'ZMP' [17], which was subsequently shown to activate AMPK [18]. Interestingly, prior to its discovery as an AMPK activator, AICAr treatment was found to inhibit GNG in rat hepatocytes [19]. A769662 was the first direct AMPK activator [20–22] and is specific for AMPKβ1-containing complexes [23]. Compound 991 also preferentially activates AMPKβ1-containing trimers, at least in cell-free assays [24]. Both A769662 and 991 allosterically increase AMPK activity and inhibit AMPKα Thr172 dephosphorylation. Other AMPKβ1-specific direct activators include PF249, PF06409577 and salicylate [25]. At high doses, 991 also activates AMPKβ2-containing complexes in skeletal muscle associated with increased glucose uptake [26]. A769662 and 991 bind to a site located between the N-lobe of the AMPK α-subunit kinase domain and the AMPK β-subunit CBM [24]. Since this site also binds salicylate [27], the breakdown product of aspirin (acetyl salicylate), it has been called the 'Allosteric Drug and Metabolite' or 'ADaM' binding pocket [28]. Compounds SC4 [29], PF739 [30] and MK8722 [31] are 'pan-β' direct AMPK activators that have recently been developed. Another small-molecule AMPK activator is O304, the first to be used in human clinical trials [32], but its precise mode of action is unclear. Interestingly in cells that lack LKB1, allosteric stimulation of AMPK by A769662 alone can lead to increased phosphorylation of acetyl-CoA carboxylase (ACC), its best known substrate, even in the absence of noticeably increased AMPKα Thr172 phosphorylation [22,33].

AMPK activators can have off-target effects. For example, ZMP can bind to other AMP-regulated enzymes such as fructose-1,6-bisphosphatase (FBPase-1) [34] and AICAr treatment activates glycogen phosphorylase [35], while A769662 inhibits Na^+/K^+-ATPase [36] and increases intracellular Ca^{2+} [37]. Also, we recently showed that compound 991 inhibits mitochondrial pyruvate transport and *sn*-glycerol 3-phosphate (Gro-3-P) dehydrogenase-2 (GPD2) activity independently of AMPK [38].

(Patho)physiological conditions modulating AMPK activity

Physical exercise rapidly leads to AMPK activation in the liver and other tissues [39,40]. The communication between contracting skeletal muscle and liver might involve a myokine/pro-inflammatory cytokine activating AMPK, such as IL-6 [41]. Hepatic AMPK activation during exercise could also be due to mild local hypoxia, since blood flow becomes diverted towards skeletal muscle during contraction [42]. Exercising skeletal muscles convert large amounts of plasma glucose via glycolysis to lactate, that is then shuttled to the liver to be used as substrate for GNG to re-supply muscle and other tissues with glucose (Cori Cycle). Exercise-induced peaks in energy-costly GNG from lactate could cause transient hepatic ATP depletion, with a concomitant rise in AMP leading to AMPK activation [43]. After several hours of starvation, liver AMPK becomes activated [44] and caloric restriction leads to increases in AMPK activity in several tissues [45]. In addition to changes in nucleotides leading to AMPK activation in response to starvation, glucagon transiently activates AMPK in hepatocytes [46] and in intact freeze-clamped liver [47]. The underlying mechanisms involve increased intracellular Ca^{2+} leading to AMPK phosphorylation by CaMKK2 as well as a temporary decrease in energy charge, *i.e.* increased AMP:ATP ratio (for a detailed review, see [48]). Interestingly, glucagon levels in the portal vein are also greatly increased during exercise [49]. The adipokines adiponectin and leptin modulate AMPK activity in tissues.

Leptin, which reduces food intake, decreases hypothalamic AMPK activity [50]. However, leptin activates AMPK in skeletal muscle, increasing fatty acid oxidation [51], while adiponectin activates AMPK in both liver and skeletal muscle [52]. Lastly, ammonia released from (glucogenic) amino acids such as alanine in particular, was shown to activate AMPK in hepatocytes [53].

On the other hand, AMPK and ACC phosphorylation are decreased in several tissues, including liver, of obese humans compared with lean control subjects [54–56]. AMPK and ACC phosphorylation are also reduced in animals on a high-fat diet (HFD) [57] and on a high-fructose diet [58], or in ob/ob [59] and db/db mice [60], or after streptozotocin treatment [61]. The negative correlation between AMPK activity and insulin resistance as well as inflammation reinforces the potential benefits of AMPK activators for treating T2D and obesity.

Hepatic glucose production

The liver takes centre stage in the maintenance of blood glucose during starvation. Depending on glycaemia and hormonal status, the liver releases glucose to be used by tissues having an absolute requirement for the sugar as energy source. Glucose is indeed the main fuel for the brain and renal medulla, and it is the only energy source for erythrocytes and the retina [62]. Hepatic glucose production depends on two major pathways, namely glycogenolysis and GNG, the *de novo* synthesis of glucose from lactate, pyruvate, glycerol and certain amino acids. Both glycogenolysis and GNG produce glucose 6-phosphate (Glc-6-P), which is situated at a metabolic crossroad. Glc-6-P can be hydrolyzed to glucose, which is then released in the bloodstream. Glc-6-P can also enter the glycolytic pathway, the pentose-phosphate pathway and the hexosamine pathway from fructose 6-phosphate (Fru-6-P). It is noteworthy that liver glycogen content in T2D subjects was lower in studies using isotopic tracers [63,64], favouring the development of diabetic ketosis and acidosis. Intriguingly, the same studies also reported lower hepatic glycogen breakdown in T2D patients. Therefore, GNG would be the main contributor to (excessive) hepatic glucose production (HGP) in T2D. Interestingly, lower hepatic glycogen content and glycogenolysis as encountered in T2D were phenocopied by liver-specific knock-out (KO) of AMPK, reducing glycogenic flux and glycaemia during exercise [65].

From the pioneering work of George Cahill and illustrated in many biochemical textbooks, five phases of glucose homeostasis are recognized in humans [66]. In the post-absorptive phase lasting up to ∼16 h, blood glucose is maintained mainly by glycogen breakdown. Glucagon levels peak at ∼4–6 h after a last meal and come down again after insulin release induced by postprandial hyperglycaemia, but only in normal and not in diabetic individuals. During the second phase of early starvation up to 72 h after a last meal, hepatic GNG prevails, primarily from lactate and alanine, and proteins must be hydrolyzed in muscle to produce amino acids for glucose production. Under these conditions, glycolysis is inhibited in the liver and greatly diminished in other tissues because fatty acids and ketone bodies serve as alternate fuels and have a glucose-sparing effect [67,68].

Gluconeogenic substrates

During periods of starvation, the main glucose precursors are lactate, pyruvate, glycerol, alanine and other glucogenic amino acids [69–72]. GNG indeed enables the recycling of valuable metabolites, such as (i) lactate generated mainly by glycolysis in exercising muscle and in red blood cells, (ii) amino acids such as alanine and glutamine from muscle protein degradation and (iii) glycerol from breakdown of triglycerides in adipose tissue. Indeed, during prolonged starvation GNG from glycerol accounts for up to 60% of the overall glucose production and becomes predominant in T2D due to increased lipolysis [73]. A recent study using ^{13}C isotope tracing found an even greater contribution of glycerol to GNG, with over 75% of all glucose carbons labelled when (^{13}C)glycerol, pyruvate/lactate and glutamine were present in the same time, making glycerol the preferred substrate for glucose production under physiological conditions [74]. Therefore, the potential of targeting glycerol metabolism to treat T2D has been somewhat underestimated.

In hepatocytes from starved rats, the maximal rate of glucose production from lactate/pyruvate is close to 1 μmol of glucose/min/g of wet weight and ∼0.5 μmol of glucose/min/g of wet weight from glycerol and amino acids [71]. These *in vitro* rates of glucose production depend on the concentrations of precursors, with half maximal rates observed at concentrations that exceed their usual *in vivo* plasma concentrations, thus allowing for a stimulation of GNG by increased substrate supply from extrahepatic tissues. A normal diet contains several gluconeogenic precursors, such as fructose and dihydroxyacetone. The gluconeogenic rates from these substrates are about twice as high as from lactate/pyruvate [71]. In addition, the heart consumes a substantial proportion of plasma lactate as preferential energy-providing substrate.

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The gluconeogenic pathway

GNG is functional mainly in liver and, during prolonged starvation, in renal cortex and to a lesser extent in jejunum [75]. These are the only tissues possessing the full set of enzymes needed to carry out glucose synthesis, including the key enzymes phosphoenolpyruvate carboxykinase (PEPCK), FBPase-1 and glucose-6-phosphatase (G6Pase) (see Figure 1).

GNG is an energy costly process, requiring 6 ATP equivalents for the synthesis of 1 molecule of glucose from pyruvate. The energy cost is even greater with amino acid substrates such as alanine, where an extra 4 ATP equivalents are needed for disposal nitrogen the in form of urea. During starvation, the liver obtains most energy from the oxidation of fatty acids released from adipose tissue by lipolysis.

The gluconeogenic pathway involves substrate transport across membranes and provision of reducing equivalents for the synthesis of triose phosphates (Figure 1). Pyruvate is produced in the cytosol from lactate or alanine. The transport of pyruvate across the mitochondrial membrane by the mitochondrial pyruvate carrier is driven by the proton motive force and in hepatocytes GNG is potently inhibited by α-cyano-4-hydroxycinnamate, suggesting that pyruvate transport may be rate limiting [76]. In mitochondria, pyruvate carboxylase converts pyruvate to oxaloacetate (OAA), which has to be transported back to the cytosol where most PEPCK is located. However, the efflux of OAA to the cytosol is too slow and, as an alternative, conversion of mitochondrial OAA to malate and transport by the bidirectional malate/aspartate shuttle is an obligatory step in GNG from lactate and pyruvate [77] (Figure 1). With pyruvate as a substrate, OAA is reduced to malate, which in turn is transported to the cytosol for regeneration of OAA and NADH. With lactate as a substrate, pyruvate and NADH are generated in the cytosol by lactate dehydrogenase and no additional NADH is required. In this case, OAA produced in mitochondria is converted to aspartate, which can readily cross the mitochondrial membrane and regenerate OAA in the cytosol. Alternatively, OAA can be directly converted into phosphoenolpyruvate (PEP) by mitochondrial PEPCK, and PEP is then transported out of mitochondria [78]. Thus, the carbons derived from lactate/pyruvate leave the mitochondria via the malate–aspartate redox shuttle or as PEP via an anion transporter depending on PEPCK isoform expression and the redox state ($NADH/NAD^+$ ratio) of the cytosol and the mitochondrial matrix. With glycerol as a substrate, reducing equivalents are formed in the cytosol and are transferred to mitochondria by GPD2, which is coupled to coenzyme Q and complex III of the mitochondrial electron transport chain, since no NADH is required for GNG from substrates entering the pathway beyond GAPDH. Of note, under 'normal' conditions the glycerol phosphate shuttle has a rather low relative significance compared with the malate–aspartate shuttle for the transfer of electrons (as NADH) from the cytosol to the mitochondria. However, when the reduced substrate lactate is used for GNG, the malate–aspartate shuttle turns in the 'opposite' direction (see above). Even more strikingly, when glycerol becomes a major substrate for GNG during prolonged starvation and in T2D due to increased lipolysis in white adipose tissue, electron transfer to mitochondria by GPD2 increases considerably, and so the glycerol phosphate shuttle represents a significant contribution to cytosolic redox state under these conditions.

Key steps and control of GNG

Glycolysis and GNG share several enzymes that catalyze reactions close to equilibrium and have little influence on the net direction of metabolic flux. The uni-directionality of either glycolysis or GNG is ensured by a handful of enzymes that catalyze key opposite exergonic reactions. When these opposite non-equilibrium reactions operate simultaneously and at similar rates, 'futile' cycling occurs, the net balance of which is a wasteful expenditure of energy. Three such cycles exist in the glycolytic/gluconeogenic pathway: the glucose/Glc-6-P cycle, the Fru-6-P/fructose-1,6-bisphosphate (Fru-1,6-P_2) cycle and the PEP/pyruvate cycle. These cycles are functional in liver of well-fed animals but are greatly reduced during starvation. Therefore, net gluconeogenic flux prevails when gluconeogenic enzymes are increased/stimulated, when glycolytic enzymes are inhibited or a combination of both (for detailed reviews, see [79,80]).

GNG is controlled by the supply and availability of gluconeogenic substrates, by glucose its end product, and by hormonal and nutritional status. In the animal, global control is integrated and hormones can affect the supply of gluconeogenic substrates as well as the gluconeogenic capacity of the liver. Control is complex and exerted at several levels in space and time. Regulatory mechanisms include effects of allosteric ligands, stimulators and inhibitors, covalent modification of enzymes responsible for (in)activation, and long-term control by induction or repression of enzymes.

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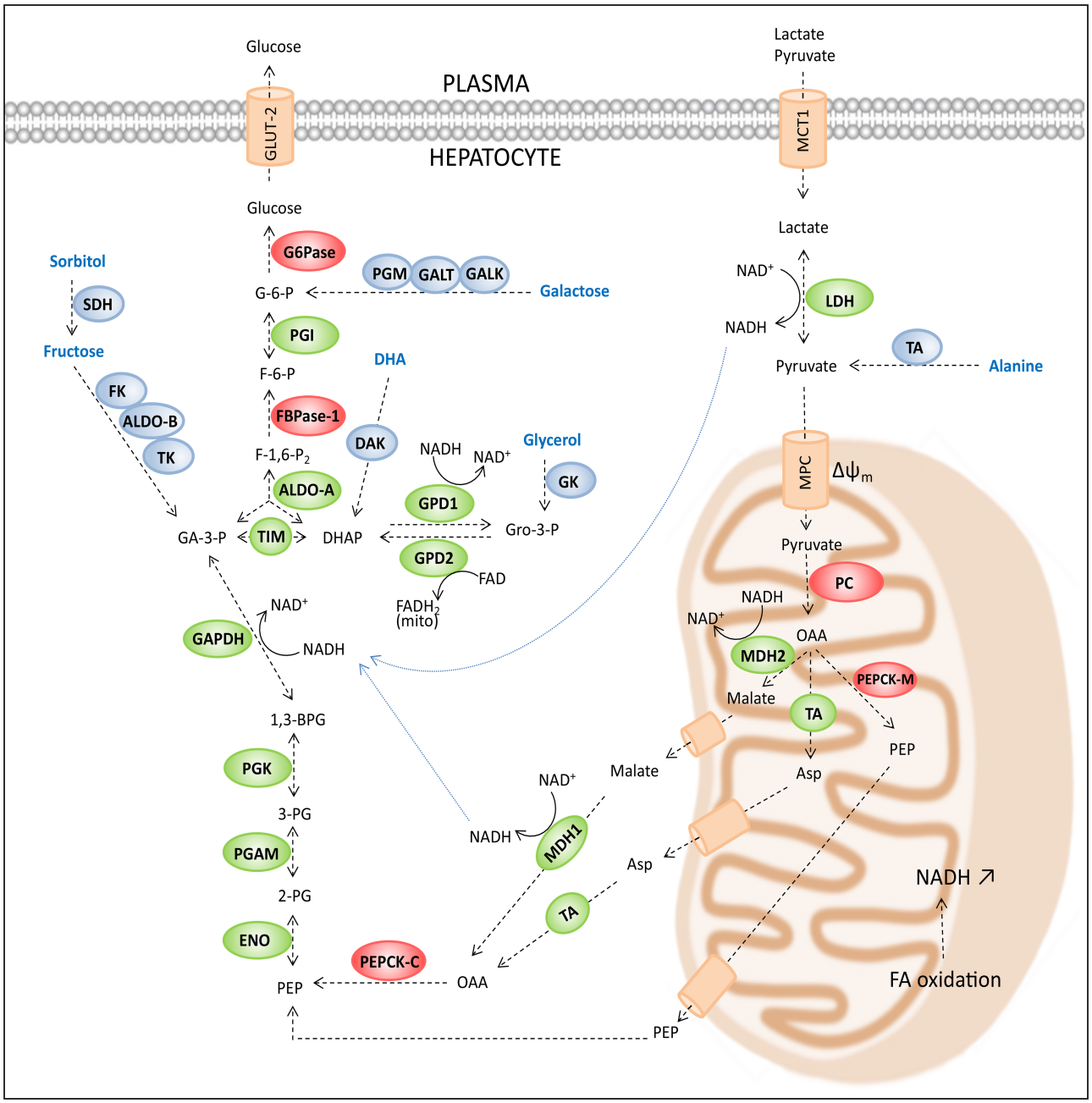

Figure 1. Hepatic GNG from different substrates.

Important gluconeogenic substrates are lactate (from glycolysis), alanine (from proteolysis), glycerol (released during lipolysis), galactose (from lactose), fructose (from sucrose) and dietary sorbitol. Of note, when lactate is the only substrate, the majority of carbons exit mitochondria as aspartate, whereas malate prevails when pyruvate is the sole substrate. Enzymes catalyzing the main reactions of the GNG pathway are represented in green. Those catalyzing unidirectional/rate-limiting reactions are indicated in red. Accessory pathways for glucose production from alternative substrates are indicated in blue. Abbreviations: GALK, galactokinase; GALT, galactose-1-phosphate uridyltransferase; PGM, phosphoglucomutase; G6Pase, glucose-6-phosphatase; PGI, phosphoglucose isomerase; FBPase-1, fructose-1,6-bisphosphatase; ALDO-A/B, aldolase A/B; SDH, sorbitol dehydrogenase; FK, fructokinase; TK, triosekinase; DAK, dihydroxyacetone kinase; GK, glycerol kinase; GPD1/2, glycerol-3-phosphate dehydrogenase-1/2; TIM, triosephosphate isomerase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PC, pyruvate carboxylase; PGK, phosphoglycerate kinase; PGAM, phosphoglycerate mutase; ENO, enolase; PEPCK-C/M, cytosolic/mitochondrial phosphoenolpyruvate carboxykinase; LDH, lactate dehydrogenase; TA, transaminase or aminotransferase; PC, pyruvate carboxylase; MDH1/2, malate dehydrogenase-1/2; MCT1, monocarboxylate transporter-1; MPC, mitochondrial pyruvate carrier; FA, fatty acid.

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Control by glucagon and AMPK

As a general rule, GNG is active when glucagon secretion is increased, as is the case during periods of starvation, or under pathological conditions such as T2D [81], when increased GNG participates in the establishment of pathological hyperglycaemia. The control of GNG by glucagon is mediated by increased 3′,5′-cyclic adenosine monophosphate (cAMP) and cAMP-dependent protein kinase (PKA) activation. The control of GNG by protein phosphorylation and changes in gene expression is summarized in Table 1.

Glucagon exerts no direct short-term effects on glucokinase (GK) (see however [119]) or on G6Pase activity. In contrast, the hepatic content of these two enzymes changes in the long-term, GK being down-regulated while G6Pase becomes up-regulated by glucagon. Fructose-2,6-bisphosphate (Fru-2,6-P_2) levels are crucial in the control of glycolysis/GNG. Fru-2,6-P_2 is a potent allosteric stimulator of 6-phosphofructo-1-kinase (PFK-1), and thus of glycolysis, as well being an inhibitor of FBPase-1, one of the key gluconeogenic enzymes. The hepatic isoenzyme of the bifunctional 6-phosphofructo-2-kinase (PFK-2)/fructose-2,6-bisphosphatase (FBPase-2), called PFKFB1, controls the intracellular concentration of Fru-2,6-P_2. In response to glucagon, PKA phosphorylates PFKFB1 causing PFK-2 inactivation and FBPase-2 activation, thereby decreasing Fru-2,6-P_2 levels and favouring gluconeogenic flux. In addition, the liver isoform of pyruvate kinase is inactivated by PKA-mediated phosphorylation to prevent cycling by pyruvate carboxylase and PEPCK, whose activities are not regulated by PKA in the short-term. However, the liver content of pyruvate carboxylase and of PEPCK is greatly increased in the long-term by glucagon due to increases in transcription. Glucagon activates several transcription factors via cAMP and Ca^{2+} signalling pathways [124] including cAMP response element (CRE)-binding protein (CREB) and forkhead box class O members (FOXOs). These transcription factors need co-activators such as CREB binding protein (CBP)/p300, CREB regulated transcription co-activator 2 (CRTC2), formerly known as transducer of regulated CREB activity 2 (TORC2), and peroxisome proliferator-activated receptor (PPAR)-γ co-activator 1α (PGC-1α) for full transcriptional activity, all of which are up-regulated during starvation [125]. In response to glucagon, PKA activation leads to the phosphorylation of CREB at Ser133 and CRTC2 dephosphorylation at Ser171 [126]. This leads to the formation of a CREB-CBP-CRTC2 complex thereby increasing the transcription of PEPCK, G6Pase and PGC-1α [97]. The FBPase-1 and pyruvate carboxylase genes also contain CREs in their promotor regions. PGC-1α is transcriptionally induced by CREB to increase the transcription of gluconeogenic genes by co-activating FOXO1. Insulin inhibits these transcriptional machineries, for example via protein kinase B (PKB)-induced phosphorylation of FOXO1 promoting its association with 14-3-3 proteins, cytoplasmic sequestration and subsequent proteasomal degradation [127].

As far as we know, there is no convincing evidence to show that AMPK directly affects the activity of the same key enzymes controlled by PKA-induced phosphorylation in response to glucagon. Although AMPK phosphorylates PFKFB1 at two Ser residues, one of which (Ser32) is phosphorylated by PKA, there was no change in PFK-2 activity [106]. Indeed, liver-type PFK-2/FBPase-2 and L-pyruvate kinase were not phosphorylated at significant rates by AMPK [128].

In contrast, AMPK phosphorylates and activates hepatic cyclic nucleotide phosphodiesterase 4B (PDE4B) [129], the main phosphodiesterase isoenzyme responsible for reducing cAMP levels in liver. This important observation offers a convincing explanation for the anti-glucagon effect of AMPK, which is pertinent to T2D (see Figure 2 and text below). AMPK-induced PDE4B activation might have broader implications for controlling cyclic nucleotide concentrations, for example in response to other hormones such as catecholamines, and the AMPK sites in PDE4B are conserved in all PDE4 isoenzymes, which exhibit broad tissue expression patterns.

AMPK phosphorylates and inactivates ACC, thereby stimulating fatty acid oxidation, which provides energy required for liver GNG as well as acetyl-CoA, to allosterically stimulate pyruvate carboxylase. AMPK also inhibits biosynthetic energy-consuming processes, such as glycogen and protein synthesis, by phosphorylating liver glycogen synthase [8] and increasing eukaryotic elongation factor-2 phosphorylation [130,131], respectively. In addition, AMPK is known to phosphorylate CRTC2 resulting in interaction with 14-3-3 proteins, cytosolic sequestration and neutralization of CREB co-activation [97]. However, the LKB1-salt-inducible protein kinase (SIK) pathway rather than AMPK was shown to play a key role as the main suppressor of GNG in the liver [132]. SIKs are AMP-related kinases downstream of LKB1 and SIK3 could be the physiologically important kinase for liver gluconeogenic gene suppression [133]. AMPK has been reported to directly phosphorylate hepatic nuclear factor 4α (HNF4α) to repress its transcriptional activity [134]. HNF4α regulates gene transcription in liver, intestine and endocrine pancreas and its mutation in maturity onset diabetes of the young

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Table 1 Control of enzymes and proteins involved in GNG

Enzyme/protein	Control by Phosphory-lation	Stimulatory ligands	Inhibitory ligands	Change in expression	Refs
L-PYK (PKLR)	(–) by PKA at Ser43 (–) by AMPK?	PEP Fru-1,6-P_2 Low pH	ATP Amino acids (Ala) High pH	↓ Starvation ↓ Glucagon/cAMP/PKA ↓ AMPK via HNF4 ↑ Carbohydrate-rich diet ↑ Insulin	[82] [83] [84] [85] [86] [87]
L-FBP1 (FBP1)	(–) by AMPK? (+) by PKA at Ser338/341	Mg^{2+} Fru-1,6-P_2	Zn^{2+} AMP, (ZMP) Fru-2,6-P_2	↑ Nutrient excess	[88] [89] [90] [91] [92]
G6Pase	No evidence	Glc-6-P Ca^{2+} Mg^{2+}	Fatty acyl-CoA Phosphoinositides (Glucose)	↓ Insulin ↓ AICAR ↓ AMPK? ↓ Metformin ↑ Starvation ↑ Glucagon/cAMP PKA via CREB, CRTC2 ↑ Diabetes ↑ Glucocorticoids	[93] [94] [95] [96] [97] [98]
L-PFK1 (PFKL)	(±) by PKA?	AMP ADP Fru-2,6-P_2 Fru-1,6-P_2 Fru-6-P Pi	ATP Gro-3-P Citrate	↓ Starvation ↑ Insulin	[99] [100–102]
PFKFB1 L-PFK2 L-FBP2	(–) by PKA (Ser32) (±) by AMPK at Ser33 (+) by PKA at Ser32 (±) by AMPK at Ser33	Fru-6-P AMP Pi Gro-3-P	Gro-3-P Citrate PEP Fru-6-P	↑ Insulin ↑ Glucocorticoids ↑ Hypoxia	[103] [79] [104] [105] [106] [107]
PEPCK-M (PEPCK2)	No evidence	Ca^{2+}, Mg^{2+} OAA		↓ AMPK? ↓ Insulin ↑ Glucagon/cAMP/PKA via CREB, CRTC2 ↑ Starvation ↑ Glucocorticoids	[108] [109] [78]
PC	No evidence But (+) by glucagon	Acetyl-CoA ATP:ADP↑	Glutamate	↑ Glucagon	[110–113]
MPC	No evidence But (+) by glucagon	Pyruvate		No evidence	[114–118]
GK (HK4) GKRP	No evidence But (–) by glucagon, AICAr, Metformin AMPK?	Via GKRP: High glucose Fru-1-P	Via GKRP: Low glucose Fru-6-P	↑ Insulin/PKB via FOXO,SREBP1C ↓ Diabetes	[106] [119] [120] [121] [122] [123] [119]

Abbreviations: L-PYK, liver pyruvate kinase; L-PFK1, liver 6-phosphofructo-1-kinase; L-FBP1, liver fructose-1,6-bisphosphatase; L-PFK-2, liver 6-phosphofructo-2-kinase; L-FBP2, liver fructose-2,6-bisphosphatase; GK, glucokinase; GKRP, glucokinase regulatory protein. For other abbreviations, see legend to Figure 1 and main text.

(MODY) is associated with insufficient insulin secretion, but without apparent defects in the liver. Bile acids are important for regulating hepatic GNG by binding to the nuclear hormone farnesoid X receptor (FXR) [135,136]. AMPK activators, including metformin, inhibit FXR agonist-induced target gene transcription in

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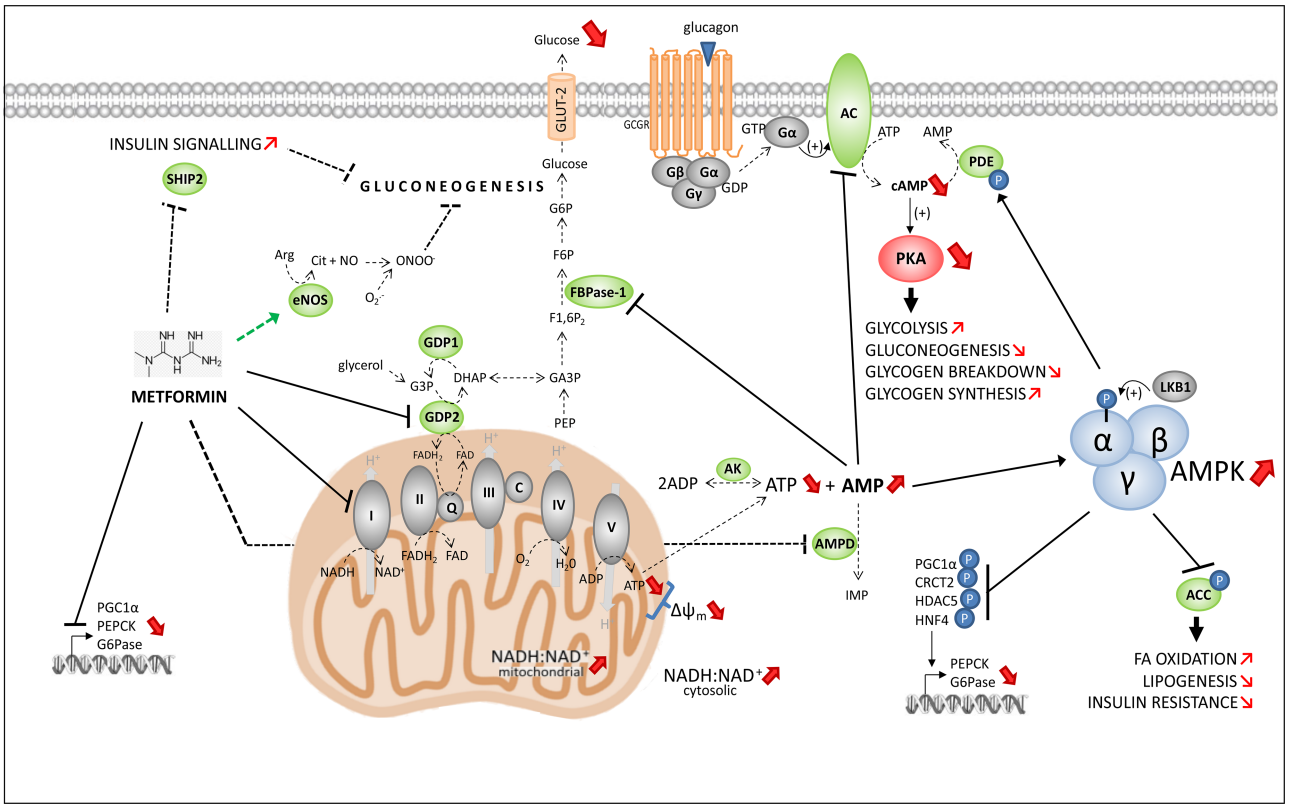

Figure 2. Inhibition of HGP by metformin and AMPK.

AMPK inhibits hepatic GNG in the short-term mainly by antagonizing glucagon signalling via phosphorylation-induced activation of a PDE that degrades cAMP. The most convincing explanation for metformin-mediated inhibition of hepatic GNG in the short-term is inhibition of FBPase-1 by increased AMP. Of note, the insulin-sensitizing effect of metformin and AMPK by decreased lipogenesis only applies to long-term/chronic models. Also, inhibition of AMP deaminase (AMPD) and adenylate cyclase (AC) and activation of endothelial nitric oxide synthase (eNOS) only occur at supra-pharmacological doses of metformin. Effects of both metformin treatment and/or AMPK activation are indicated by red arrows (↗, increase; ↘, decrease). For abbreviations see the main text and the legend to Figure 1.

liver and AMPK directly phosphorylates FXR to regulate its activity [137]. On another note, while AMPK and mammalian target of rapamycin (mTOR) are interlinked, they have opposing roles in the control of nutrient metabolism [138]. In fact, mTOR complex 2 (mTORC2) rather than mTORC1 controls GNG in the liver through a number of transcription factors including FOXO1 [139]. Lastly, glucose metabolism in various tissues including liver GNG is under direct control of the molecular clock and exhibits rhythmic patterns in a circadian manner [140]. It is not surprising that there is a complex interplay between AMPK and the intrinsic circadian clock, where AMPK directly targets core clock components such as cryptochrome (CRY) and period circadian protein homologue (PER) proteins while being itself under the control of the clock [141], thereby creating an obvious reciprocal link between energy balance and biological rhythms. Despite the multiple sites of gluconeogenic control by AMPK, hepatic GNG has been reported to function almost normally in the absence of AMPK, at least in basal conditions (see below).

Models and techniques for studying effects of AMPK activation on hepatic GNG

Many different *in vivo* and *in vitro* models coupled with different experimental techniques to measure metabolic parameters have been used to study the role of AMPK in controlling hepatic GNG. Animal models that have been used include congenital or inducible, full-body [142–146] or liver-specific [65,147–150] KO of different AMPK subunits, LKB1 KO [151,152] or constitutively active AMPK expressing mice [153–155].

Experimental measurements *in vivo* include oral (OGTT) or intraperitoneal glucose (IPGTT), pyruvate (IPPTT) or insulin (IPITT) tolerance tests and hyperinsulinaemic euglycaemic clamp via the jugular or portal vein. *In vitro*, preparations of freshly isolated hepatocytes, primary cultures of hepatocytes and immortalized cell lines such as human HepG2, Huh7, IHH, HepaRG or murine AML12 have been exploited. Experimental conditions that vary are nutritional status (starved or fed), experimental duration (minutes or hours for glucose production), GNG substrates (reduced such as lactate, oxidized such as pyruvate, or both in combination to preserve the redox balance, glycerol circumventing mitochondrial transit, isotopically labelled or unlabelled substrates, concentrations in the normal range or supraphysiological to maximize flux, consideration of blanks in the absence of exogenous substrates), the presence or absence of exogenous glucose and amino acids in experimental solutions, the presence or absence of hormones (insulin, glucagon), the route of administration for treatment (oral gavage, drinking water, solid chow, intraperitoneal injection, intravenous infusion), as well as species (mainly rat, mouse or human). In addition, the crucial distinction between short-term/acute and long-term/chronic effects of manipulating AMPK or pharmacological treatment on enzymes and metabolic fluxes have not always been thoroughly considered. This is particularly true for *in vivo* studies where complexity increases due to systemic interactions. As such, conclusions that have been drawn might only be pertinent to a particular species studied under a defined set of conditions.

Lastly, it is important to point out that adaptive changes might occur in long-term genetic models. Even though these models have been precious tools, some of the data should be interpreted with caution. For example, AMPK activation [154] and metformin treatment [146] both down-regulate expression of the gluconeogenic enzyme PEPCK. Although mice lacking hepatic PEPCK are unable to synthesize glucose from lactate/pyruvate, thus presenting substantially impaired hepatic glucose production, their glycaemia was close to normal even during starvation [156]. It turned out that these mice had altered their metabolism to increase extrahepatic gluconeogenesis while diminishing whole-body glucose utilization as part of an adaptive pro-survival response. Similar adaptive responses might occur in other transgenic models or after chronic pharmacological treatment, for example with AMPK activators.

Anti-diabetic drugs

AMPK has emerged as a drug target for T2D because its activation in muscle during exercise is beneficial for management of the disease and metformin, the most prescribed anti-diabetic drug, activates AMPK [157]. Other molecules used clinically to treat T2D are thiazolidinediones (TZDs) such as rosiglitazone which, as well as acting as PPAR-γ agonists improving insulin resistance, also activate AMPK [158,159].

Metformin: a Pandora's box?

The biguanide metformin is the front-line drug used worldwide for the treatment of T2D. Its anti-hyperglycaemic properties are mainly due to the suppression of excessive HGP without affecting peripheral glucose uptake in the short-term [160]. Metformin also exerts beneficial effects on the gut by modulation of duodenal AMPK signalling [161], possibly increasing glucagon-like peptide-1 (GLP-1) secretion [162] known to have an anti-hyperglycaemic effect, and via diverse effects on the intestinal microbiome [163]. Metformin has been reported to have anti-cancer properties and cardiovascular benefits, as well as anti-inflammatory and antioxidant effects (for recent reviews, see [164–166]). The anti-hyperglycaemic action of metformin has been amply demonstrated in T2D patients but without causing hypoglycaemia [167]. Strikingly, metformin does not alter normal glucose homeostasis in non-diabetic subjects [168–174]. This important restriction should be considered when analyzing the mechanisms of action of the drug. Another caveat is linked to the multifaceted mechanisms of metformin action, which are complicated to study, since the various experimental models and species employed yield different results and effects of the drug are dependent on the nutritional status of the animals studied, dose of the drug, route of administration *in vivo* and chronicity of the treatment. A number of different mechanisms have been proposed to explain the anti-hyperglycaemic effect of metformin, which is mainly due to the inhibition of hepatic GNG [157,175]. These mechanisms are briefly summarized below and in Figure 2.

Inhibition of mitochondrial respiratory chain complex I

This inhibitory effect of metformin was first reported independently by two groups in 2000 [176,177] and repeatedly confirmed (for reviews, see [157,175]). Acting via this mechanism, metformin treatment increases the mitochondrial redox state ($NADH:NAD^+$) by reducing NADH oxidation and decreases oxygen

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consumption. *In vitro*, millimolar concentrations, but not therapeutic concentrations (below 0.1 mM), of metformin are required for direct inhibition of complex I activity in isolated mitochondria, although lower concentrations are effective by pre-incubation with the drug (for a detailed discussion, see [178]). However, therapeutic concentrations of metformin did induce a dose- and time-dependent inhibition of complex I in hepatocytes as well as in various cell lines [157,175]. This difference could result from the slow accumulation of metformin, a positively charged molecule, in the mitochondrial matrix driven by the electrochemical mitochondrial membrane potential ($\Delta\psi_m$). Therefore, it has also been proposed that low doses of metformin could decrease $\Delta\psi_m$ independent of complex I inhibition by analogy with uncoupling agents such as dinitrophenol [179]. It has been questioned whether complex I activity (and therefore the mitochondrial redox state) and $\Delta\psi_m$ can really be dissociated [167]. Nevertheless, the weak and reversible inhibition of mitochondrial complex I activity by metformin could lead to the following metabolic changes:

i. Perturbation of nucleotide concentrations by decreasing ATP synthesis and increasing ADP and AMP levels leading to a rise in the AMP:ATP ratio with several important consequences. A fall in ATP concentration could inhibit GNG, which is an energy-costly pathway. Accordingly, treatment of hepatocytes with the complex I inhibitor rotenone increased AMP levels and decreased glucose production [180], apparently independent of AMPK activation.
ii. Increased AMP levels lead to AMPK activation [2]. It was argued that supra-pharmacological doses of metformin (>250 μM) are required to affect adenine nucleotide concentrations in hepatocytes [146], at least in short incubations up to 24 h. However, AMPK activation occurs at pharmacological doses (≤80 μM in the portal vein of rodents) [181,182]. It is worth mentioning that nucleotides are difficult to measure *in vivo* since any ischemia/hypoxia must be avoided during tissue removal and freeze-clamping. In addition, most studies measure total adenine nucleotide concentrations in perchloric acid extracts. Therefore, small changes in AMP, ADP and ATP might not be detected. Moreover, it is *free* concentrations of adenine nucleotides that are sensed by AMPK and which are much lower than total concentrations due to binding to intracellular proteins and sequestration by Mg^{2+} in the case of ATP. Whether AMPK activation suffices to explain the inhibition of GNG by metformin is strongly debated and discussed below.
iii. AMP strongly inhibits the key gluconeogenic enzyme FBPase-1, acting synergistically with Fru-2,6-P_2 [88]. Interestingly, knock-in (KI) of a FBPase-1 mutant insensitive to inhibition by AMP rendered mice largely insensitive to the blood glucose lowering (anti-hyperglycaemic) effect of metformin *in vivo* [92]. These results support the proposal that mitochondrial complex I inhibition by metformin mainly decreases glucose production independent of AMPK [180]. However, in these FBPase-1 KI mice that were insulin-resistant due to HFD feeding, the reduction in blood glucose levels by oral metformin treatment was blunted, but not completely abolished [92], suggesting additional mechanisms independent of AMP-mediated inhibition of FBPase-1 to explain the anti-hyperglycaemic effect of metformin, that might well be due to AMPK.
iv. Elevated concentrations of AMP after treatment with rather high doses of metformin could inhibit adenylate cyclase and thereby reducing glucagon-induced increases in cAMP and PKA signalling [183]. However, it should be emphasized that the high concentrations of *free* AMP needed to inhibit adenylate cyclase would probably never be reached *in vivo*.
v. Mitochondrial respiratory chain inhibition would be expected to increase the cytosolic NADH:NAD ratio and inhibit GNG from reduced substrates such as lactate. This mechanism has been invoked to mediate an effect of metformin independent of complex I inhibition and is summarized below.

Effects of metformin independent of complex I inhibition

i. Mitochondrial GPD2 was shown to be directly inhibited *in vitro* by metformin concentrations from 50–250 μM [184]. Moreover, acute and chronic low-dose metformin treatment effectively reduced endogenous glucose production, while increasing cytosolic redox and decreasing mitochondrial redox states [185]. GPD2 is part of the Gro-3-P redox shuttle, transferring reducing equivalents from the cytosol to the mitochondrial matrix. Inhibition of GPD2 should thus lead to impaired GNG from reduced precursors such as lactate, but not from oxidized substrates such as pyruvate. However, as pointed out [186], the malate–aspartate redox shuttle is responsible for most of the total redox flux from the cytosol to the mitochondria, at least under basal conditions (see above). However when GNG is high, this shuttle turns in the other

direction and the Gro-3-P shuttle becomes more important. In the studies of Madiraju et al. [184], free concentrations of cytosolic NADH were not measured directly, but were calculated from the lactate:pyruvate ratio. On the other hand, the effects of low doses of metformin to inhibit GNG from oxidized (dihydroxyacetone) and reduced (xylitol) substrates was attributed to partitioning of substrates towards glycolysis by allosteric regulation of PFK-1 and/or FBPase-1 together with a decrease in Gro-3-P, an inhibitor of PFK-1, rather than via inhibition of transfer of reducing equivalents [179]. Also, the effect of metformin to lower Glc-6-P concentrations in hepatocytes challenged with high glucose was attributed to AMPK-independent stimulation of glycolysis via changes in allosteric effectors of PFK-1 and FBPase-1 including AMP and Gro-3-P [187].

ii. Concentrations of metformin as high as 10 mM were shown to inhibit AMP deaminase (AMPD), thereby increasing AMP levels to activate AMPK [188]. However, the concentrations of metformin used were well above therapeutic doses and the study was performed on L6 rat skeletal muscle cells, not liver. In fact skeletal muscle expresses AMPD1, whereas AMPD2 is the isoenzyme expressed in liver. Another target proposed to be directly inhibited by metformin treatment is the Src homology 2 domain-containing phosphatidylinositol-5-phosphatase 2 (SHIP2), thereby improving insulin resistance by stimulating phosphatidylinositol 3,4,5-trisphosphate (PIP3) signalling leading to increased skeletal muscle glucose uptake [189]. Also, metformin was proposed to decrease protein phosphatase 1 regulatory subunit 3C (PPP1R3C) expression, thereby inhibiting cAMP-induced CRTC2 dephosphorylation and GNG [190].

iii. A non-canonical energy charge-independent mechanism of AMPK activation by glucose retrieval but also by metformin was proposed. The mechanism involves complex formation between vATPase-Ragulator-Axin-LKB1 on the surface of the lysosome to activate AMPK [191,192].

iv. Metformin was reported to promote AMPKαβγ heterotrimer formation and activation by LKB1-induced AMPKα Thr172 phosphorylation by direct binding to the AMPK complex [193].

v. Production of reactive nitrogen species by endothelial nitric oxide synthase (eNOS) was proposed to mediate metformin action [194]. Metformin-induced $ONOO^{(-)}$ generation, AMPK activation and GNG suppression were only seen in hepatocytes from wild-type mice but were abolished in eNOS-deficient mice. Moreover, metformin lowered blood glucose levels after starvation in diabetic wild-type but not in eNOS-deficient mice.

Involvement of AMPK in the control of hepatic GNG

Arguments in favour

Mice with a whole-body KO of AMPKα2 were hyperglycaemic, probably due to increased adrenergic signalling [144], whereas deletion of AMPKα1 resulted in no significant metabolic phenotype [195]. Interestingly, when AMPKα2 was deleted specifically in liver, increased HGP was also observed [106,147], supporting a direct link between hepatic AMPK and GNG. Mice bearing a liver-specific deletion of AMPKα1 and AMPKα2 were partly resistant to the hypoglycaemic effect of AICAr administration [196]. Also, specific deletion of LKB1 in the liver of mice resulted in hyperglycaemia, increased expression of hepatic gluconeogenic enzymes [151] and increased glucose output by primary hepatocytes [152]. However, these effects might also have been due to AMPK-related kinases, most of which are activated by LKB1. In addition, adaptive mechanisms might occur in all of these genetically manipulated animal models.

After administration of an adenovirus carrying a constitutively active form of AMPKα2 by tail vein injection, mice were slightly hypoglycaemic due to impaired HGP and glycogen synthesis [153]. Moreover, hepatocytes from mice bearing a liver-specific mildly activating mutant of AMPKγ1 displayed reduced basal and glucagon-stimulated glucose production [154], but glycaemia in these mice was not measured. Interestingly, this liver specific AMPK activating mutation prevented hepatic steatosis induced on a high-fructose diet by decreasing lipogenesis. High fructose initially activates AMPK in hepatocytes because of ATP-depletion [197], however a prolonged fructose load leads to a drop in inorganic phosphate relieving inhibition of AMPD2 that degrades AMP [198]. Also, fructose surges have been proposed to decrease hepatic AMPK activity through increased methylglyoxal that chemically modifies arginine residues damaging the AMP binding sites of the AMPKγ-subunits [199], the net result of which is increased lipogenesis and hepatic insulin resistance. More recently, a new elegant inducible liver-specific constitutively active AMPK expressing mouse model was developed [155]. The mice exhibited reduced glycaemia during starvation and in an IPPTT, suggesting decreased HGP.

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Metformin effects were impaired in mice injected with adenovirus carrying a dominant-negative form of AMPKα1 [200] or shRNAs against AMPKα1/α2 [181]. Whole-body deletion of AMPKβ1 in mice unexpectedly protected against HFD-induced diabetes [142], but this effect might have been due to reduced appetite and food intake due to the absence of hypothalamic AMPK, thereby preventing obesity, fatty liver and hepatic insulin resistance. Strikingly, whole-body deletion of neither AMPKβ1 [142] nor AMPKβ2 [143] altered basal (fasting) glycaemia in mice. However, antipsychotic- as well as glucagon-induced hyperglycaemia was exacerbated in AMPKβ1$^{-/-}$ mice, as measured in the starved state during an IPPTT or after a glucagon bolus [145]; the effect being reversed by treatment with the AMPKβ1 activator A769662. Unfortunately, no liver-specific models to manipulate AMPKβ exist so far to focus on HGP.

AMPK activation by different compounds including metformin led to increased cAMP phosphodiesterase (PDE) activity in primary mouse hepatocytes [129]. The underlying mechanism involved phosphorylation-induced activation of PDE4B by AMPK, leading to reduced cyclic AMP levels, decreased PKA activation and subsequent antagonism of glucagon signalling (see Figure 2). These effects were strictly AMPK-dependent since they were abolished in hepatocytes from liver-specific AMPKα1/α2 double KO mice. Interestingly, glucagon levels are elevated in individuals with T2D [201] and, as stated above, metformin treatment only efficiently reduces glycaemia in diabetic but not in normo-glycaemic individuals [174]. Elevated glucagon levels might thus be a prime cause for the onset of hyperglycaemia in T2D and targeting glucagon signalling could have therapeutic potential.

Lastly, hepatic AMPK could have an indirect effect in the long-term to normalize glycaemia in insulin-resistant models, such as mice on a HFD, by improving liver insulin sensitivity. Lipid accumulation in peripheral organs such as liver can lead to lipotoxicity and is associated with insulin resistance, possibly involving novel protein kinase C isoforms [202]. AMPK favours fatty acid oxidation over lipogenesis in many tissues including liver, mainly by phosphorylation-induced inhibition of ACC. This could be sufficient to prevent hepatic lipid accumulation/steatosis on a HFD [148,203]. The reduced fat content of the liver could improve responsiveness to insulin to decrease glucose output, thereby participating in the normalization of glycaemia. Accordingly, genetic liver-specific AMPK activation protects against HFD-induced obesity and fatty liver associated with insulin resistance [155]. It should be mentioned that whole body manipulation of AMPK would affect AMPK activity in the hypothalamus, thereby influencing food intake with secondary (potentially opposite) effects on liver AMPK activity and basal GNG [204–206].

Arguments against

Metabolic flux analysis revealed that liver AMPK is not required for maintaining GNG rates but preserves cellular energy status during starvation [150]. AMPK is clearly not required for the down-regulation of gluconeogenic enzyme gene expression by metformin in primary mouse hepatocytes and, in mice lacking AMPK in the liver, the anti-hyperglycaemic effect of metformin was maintained [146]. As mentioned above, perhaps the most convincing study on the mechanism of metformin action indicated that mice carrying a point mutation in FBPase-1, rendering this key gluconeogenic enzyme insensitive to inhibition by AMP (as well as ZMP), were mostly resistant to glycaemic control by metformin and AICAr [92]. These results are in agreement with the observation that complex I inhibition decreases glucose production in hepatocytes independent of AMPK [180] to improve glycaemia in a rodent model for T2D. Direct inhibition of GPD2 by metformin is another mechanism to explain the anti-hyperglycaemic effect of metformin that does not require AMPK and could be mediated by increases in cytoplasmic NADH:NAD$^+$ ratio and Gro-3-P levels [184]. Inhibition of GPD2 and the mitochondrial pyruvate carrier (MPC) by AMPK activator 991 are AMPK-independent mechanisms by which the compound decreases GNG [38].

In conclusion, it appears that AMPK is neither necessary nor sufficient to fully explain the effect of metformin to reduce hepatic glucose output, but might well counteract the glucagon-induced increases in GNG pertinent to T2D. It must be stressed that the ideal transgenic model to address the role of AMPK in controlling hepatic GNG would require not only a spatial component, namely organ-targeted alterations, but also a temporal component in the form an inducible expression/knockdown system in the adult animal for comparing short-term versus long-term effects of AMPK manipulation in the setting of T2D in response to drug administration *in vivo*.

Other AMPK activating anti-diabetic treatments

Although many compounds used to treat diabetes, such as metformin, berberine and TZDs all activate AMPK [159,207], whether this underlies their mode of action is also controversial [146]. Many AMPK

activators are natural plant products or derivates thereof, some of which are used in traditional medicine [207,208]. Although precise mechanisms of AMPK activation by plant extracts and plant-purified or plant-derived molecules are often lacking, many have anti-diabetic (blood glucose-lowering) properties with clinical potential (for detailed reviews, see [209] and [207]), including berberine [210,211] and salicylate [212]. Inhibition of mitochondrial respiration by herbal compounds may be a common mechanism, but direct AMPK activators exist in the natural world. Salicylate [27] and the dihydrophenathrene lusianthridin [213] are examples of such compounds that directly activate AMPK both allosterically and by protecting against AMPKα Thr172 dephosphorylation by binding to its ADaM site. Another herbal alkaloid, hernandezine, also seems to directly protect against AMPKα Thr172 dephosphorylation [214]. Interestingly, long-chain fatty acyl-CoA esters such as palmitoyl-CoA were shown to act via the ADaM pocket to allosterically activate AMPKβ1 [215].

Conclusions

As a general conclusion, genetically manipulated mouse models indicate that there is an inverse link between liver AMPK activity and HGP, but that AMPK probably plays a rather minor role in both long-term and short-term control of HGP by the anti-diabetic drug metformin. Inhibition of liver GNG by AMPK might seem at odds with its role in controlling whole body energy balance, even though GNG is an energy-costly pathway. Circulating glucose levels need to be maintained for tissues and cells that rely on glucose for energy production, especially during starvation. In the starved state, AMPK activation probably plays a role in the switch towards fatty acid oxidation by most organs and tissues, reducing their glucose dependency. However, excessive glucose production by the liver has to be kept in check since this can lead to the development of insulin resistance/T2D. Thus in the short-term, the anti-glucagon effect of AMPK activation would be (patho)physiologically relevant. As pointed out above, glucagon levels are abnormally elevated in T2D, which exacerbates hepatic glucose production. It is noteworthy in this respect that drug targeting glucagon signalling was proposed for treating T2D [216]. Interestingly, glucagon itself has been shown to activate hepatic AMPK [46,48,129] via changes in adenine nucleotide concentrations [47], possibly representing an important physiological inhibitory feedback loop to prevent excessive HGP. The insulin-sensitizing effects of liver AMPK activation in HFD-induced diabetes, by preventing lipid accumulation/steatosis and subsequent insulin-resistance, could also be beneficial. The experimental presence of glucagon is probably the key to see effects of AMPK activation and, although mostly absent from *in vitro* hepatocyte incubations, the hormone would be present in animals during starvation and during the development of diet-induced insulin resistance. Chronic oral administration of a small-molecule AMPK activator ('C24') reduced blood glucose and lipid levels in plasma, reduced hepatic expression of PEPCK and G6Pase and improved glucose tolerance in diabetic db/db mice [217], while glucose production by primary hepatocytes was decreased upon incubation with C24. However, based on the administration of direct AMPK activators PF739 and MK8722 to diabetic animals *in vivo*, it was concluded that AMPK activation in the liver would not be significant for glycaemic control and that the only beneficial effect of AMPK activation would be in skeletal muscle to increase glucose uptake, responsible for the observed decrease in (fasting) glycaemia [30,31]. However, anti-glucagon effects were not studied and, even in starved mice, timing would be crucial for drug administration since glucagon levels rise and fall transiently. Also, inclusion of an IPPTT as well as directly measuring HGP, for example by euglycaemic clamp in diabetic animals, would have been preferable.

Therefore, supported by our recent work and that of others [38,129,145,155], we believe there is still a case for AMPK activation in the liver to oppose the effects of elevated circulating glucagon levels and improve (hepatic) insulin resistance that characterizes T2D. Ideally, drugs should be pan AMPK activators mainly targeting liver and muscle but also subcutaneous fat [218], while avoiding AMPK activation in hypothalamus and heart, which could have adverse effects on food intake and hypertrophy, respectively. Also, AMPK activation should not be too strong or of too long duration to avoid risks of shutting down protein synthesis and causing cell cycle arrest. Indeed, treatment with metformin or the novel direct activator O304 meets these criteria for mild pan-AMPK activation and was shown to have only few adverse but many beneficial effects, some of which still require proof to be attributed to AMPK.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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CRediT Author Contribution

Mark H. Rider: Conceptualization, Funding acquisition, Writing — original draft, Writing — review and editing. **Manuel Johanns:** Conceptualization, Writing — original draft, Writing — review and editing. **Louis Hue:** Conceptualization, Writing — original draft, Writing — review and editing.

Abbreviations

ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; cAMP, cyclic 3′,5′-AMP; FBPase-1, fructose-1,6-bisphosphatase; G6Pase, glucose-6-phosphatase; GNG, gluconeogenesis; Gro-3-P, *sn*-glycerol 3-phosphate; GPD2, Gro-3-P dehydrogenase-2; HGP, hepatic glucose production; IPPTT, intraperitoneal pyruvate tolerance test; KO, knock-out; LKB1, liver kinase B1; OGTT, oral glucose tolerance test; PDE, cAMP phosphodiesterase; PEPCK, phosphoenolpyruvate carboxykinase; PKA, cAMP-dependent protein kinase; T2D, type 2 diabetes.

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