

Activation of AMP-activated protein kinase in the liver: a new strategy for the management of metabolic hepatic disorders

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It is now becoming evident that the liver has an important role in the control of whole body metabolism of energy nutrients. In this review, we focus on recent findings showing that AMP-activated protein kinase (AMPK) plays a major role in the control of hepatic metabolism. AMPK integrates nutritional and hormonal signals to promote energy balance by switching on catabolic pathways and switching off ATP-consuming pathways, both by short-term effects on phosphorylation of regulatory proteins and by long-term effects on gene expression. Activation of AMPK in the liver leads to the stimulation of fatty acid oxidation and inhibition of lipogenesis, glucose production and protein synthesis. Medical interest in the AMPK system has recently increased with the demonstration that AMPK could mediate some of the effects of the fat cell-derived adiponectin and the antidiabetic drugs metformin and thiazolidinediones. These findings reinforce the idea that pharmacological activation of AMPK may provide, through signalling and metabolic and gene expression effects, a new strategy for the management of metabolic hepatic disorders linked to type 2 diabetes and obesity.

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Introduction

All living organisms possess mechanisms for repeatedly reassessing the status of long-term energy stores and, when the need arises, making compensatory adjustments to adapt their metabolism to the nutritional environment. Mammals have developed a complex series of nutrient cues, relieved by hormones or impulses from the central nervous system, to regulate whole-body metabolism in response to acute deficiencies or to prolonged excess or shortage of nutrient supply. Hepatic metabolism plays a key role in the regulation of whole-body energy status since the liver is the major site for storage and release of carbohydrates and for fatty acid synthesis. Indeed, during the postprandial period, net glucose uptake permits the repletion of hepatic glycogen stores, the excess dietary carbohydrates being then converted into triglycerides to promote long-term energy storage. By contrast, in the fasted state, a series of metabolic modifications induces

net glucose output and lipid breakdown in the liver to maintain whole body homeostasis.

AMP-activated protein kinase (AMPK) is a phylogenetically conserved serine/threonine protein kinase which has been proposed to act as a 'metabolic master switch' mediating the cellular adaptation to environmental or nutritional stress factors. Once activated, AMPK leads to a concomitant inhibition of energy-consuming biosynthetic pathways, such as fatty acid and sterol synthesis, and activation of ATP-producing catabolic pathways, such as fatty acid oxidation. In the last few years, AMPK has come to be regarded as a point of conversion of regulatory signals monitoring systemic and cellular energy status. Among its identified roles, AMPK has been implicated in the control of hepatic glucose and lipid homeostasis by many additional effects both on genes and on short-term regulation of specific enzymes. In the light of recent observations suggesting that early changes in hepatic metabolism could

initiate the subsequent development of insulin resistance, type 2 diabetes and obesity, it has been proposed that AMPK could provide a link in metabolic diseases underlying defects in energy metabolism. In the present review, we update those topics and discuss new findings that suggest that AMPK may be a promising pharmacological target for the treatment of type 2 diabetes and obesity.

Structure and regulation of AMPK by upstream kinases in liver

AMPK exists as a heterotrimeric complex consisting of a catalytic subunit α and two regulatory subunits β and γ (Hardie, 2004). Two to three isoforms of each subunit ($\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$, $\gamma 1$, $\gamma 2$, and $\gamma 3$) encoded by different genes are known, giving rise to a large variety of heterotrimeric combinations. It has been demonstrated that $\alpha 1$ - and $\alpha 2$ -containing complexes account each for about half of total AMPK activity in liver extracts, $\beta 1$ - and $\beta 2$ -containing complexes for 95% and 5%, respectively, and the $\gamma 1$ -containing complexes for 90%, the $\gamma 2$ -containing complexes for 10% and $\gamma 3$ -containing complexes for only minor activity (Cheung *et al.* 2000). There is no evidence for any selective association between $\alpha 1$ and $\alpha 2$ isoforms and the various β or γ isoforms in the liver. Nevertheless, the AMP dependence of the different isoform combinations is markedly affected by the identity of the α and γ isoforms present in the heterotrimeric complex suggesting that the allosteric AMP-binding site might involve the γ subunit, as well as the α subunit (Cheung *et al.* 2000).

In response to stresses that deplete ATP, the intracellular AMP : ATP ratio increases due to the action of adenylate kinase, resulting in the activation of AMPK (Hardie, 2004). AMPK is stimulated allosterically by AMP, which binds to the cystathionine β -synthase tandem repeats in the γ subunit, but is also activated by phosphorylation on residue Thr¹⁷², which lies within the activation loop of the kinase domain on the α -subunit (Hardie, 2004). The discovery of AMPK kinases came from the identification of upstream kinases for the sucrose non-fermenting 1 (SNF1) complex, the yeast orthologue of AMPK. The nearest relative kinases in mammals were LKB1 and calmodulin-dependent protein kinase kinase β (Hardie, 2004; Birnbaum, 2005). The major AMPK kinase activity in liver seems to correspond to LKB1 since it has been shown that deletion of LKB1 in the liver results in a proportional decrease of AMPK phosphorylation at Thr¹⁷² rendering AMPK insensitive to stimuli which normally activate it (Shaw *et al.* 2005).

Activators of hepatic AMPK

Numerous studies have reported AMPK activation in hepatic cells by intracellular changes in AMP : ATP ratio,

resulting from any stress that depletes cellular ATP such as metabolic poisons targeting mitochondria (arsenite, antimycin A, azide, oligomycin and 2,4-dinitrophenol), heat shock and hypoxia (Hardie, 2004). Since the liver seldom suffers from hypoxia and the liver ATP concentration changes little under physiological conditions, hepatic AMPK activation should only occur as an ultimate cellular protection in these cases. However, evidence showing that hepatic AMPK could be activated in response to physiological stimuli such as exercise and nutrient deprivation (Hardie, 2004), and to physiopathological situations such as prolonged starvation (Assifi *et al.* 2005; Dentin *et al.* 2005), ischaemia–reperfusion injury (Peralta *et al.* 2001) and chronic alcohol consumption (You *et al.* 2004) suggests that AMPK might have a wider role in the liver. Moreover, the recent findings showing that hepatic AMPK is activated by adiponectin (Yamauchi *et al.* 2002) and by two different antidiabetic drugs, metformin (Zhou *et al.* 2001) and thiazolidinediones (TZDs) (Saha *et al.* 2004), further reinforced therapeutical interest, identifying AMPK as a potential target for the treatment of metabolic diseases such as obesity and type 2 diabetes. Most of these effects, including those of metformin, are believed to be independent of any apparent changes in the AMP : ATP ratio, but this assumption is mainly based on results obtained in other tissues than liver. Furthermore, the AMP : ATP ratio was also systematically calculated using total adenine nucleotide concentrations. This latter should be taken with caution, given that AMPK is only regulated by the cytosolic AMP : ATP ratio. Finally, since mitochondrial oxidative phosphorylation is crucial for liver energetics, subtle modifications of the cellular energetic state could be sufficient to activate AMPK.

Among chemical methods for activating AMPK in the liver, the use of 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside (AICAR), a cell-permeant compound which is phosphorylated to the AMP analogue 5-aminoimidazole-4-carboxamide ribotide (ZMP), has been reported both *in vitro* (Corton *et al.* 1995) and *in vivo* (Pencek *et al.* 2005; Reiter *et al.* 2005). The accumulation of ZMP leads to AMPK activation by mimicking the effects of AMP on the kinase, i.e. direct allosteric activation and promotion of phosphorylation by upstream AMPK kinase (Corton *et al.* 1995). While AICAR is still the most widely used pharmacological activator of AMPK, it has been demonstrated that some of its actions could be independent of AMPK. All enzymes whose activities are influenced by AMP, such as fructose-1,6-bisphosphatase and 6-phosphofructo-2-kinase or glucokinase, are potential targets for ZMP. Furthermore, it has been reported that AICAR treatment decrease intracellular ATP concentration both *in vitro* (Corton *et al.* 1995) and *in vivo* (Pencek *et al.* 2005). It has been recently demonstrated using mice lacking both $\alpha 1$ and $\alpha 2$ catalytic subunits in

Table 1. Hepatic targets of AMPK

Target	Enzymatic properties	Phosphorylation site(s)	Biological effect(s)	References
ACC1	↓ Activity	Ser ⁷⁹	↓ Lipogenesis	Munday <i>et al.</i> (1988)
ACC2	↓ Activity	Ser ²¹⁸	↑ Oxidation	Winder <i>et al.</i> (1997)
eEF2Kinase	↑ Activity	Ser ³⁹⁸	↓ Protein synthesis	Horman <i>et al.</i> (2002) Browne <i>et al.</i> (2004)
GPAT	↓ Activity	?	↓ Triglyceride synthesis	Muoio <i>et al.</i> (1999)
HMG-CoA reductase	↓ Activity	Ser ⁸⁷²	↓ Cholesterol synthesis	Clarke & Hardie (1990)
MCD	↑ Activity	?	↓ Malonyl CoA	Assifi <i>et al.</i> (2005)
PFK-2/FBPase-2	Weak kinetic effects	Ser ²² , Ser ³²	?	Guigas <i>et al.</i> (2006)
mTOR	↓ Activity	Thr ²⁴⁴⁶	↓ Protein synthesis	Cheng <i>et al.</i> (2004)
TSC2	↑ Activity	Thr ¹²²⁷ , Ser ¹³⁴⁵	↓ Protein synthesis	Inoki <i>et al.</i> (2003)

the liver (AMPK α 1 α 2^{LS}^{-/-}) that AICAR inhibits hepatic glucose phosphorylation by an AMPK-independent impairment of the glucose-induced translocation of GK following ATP depletion (Guigas *et al.* 2006). Lastly part of the detrimental effect of AICAR is likely to be due to an AMPK-independent inhibition of mitochondrial oxidative phosphorylation induced by a concomitant effect of ZMP on the mitochondrial respiratory-chain and a drop of adenine nucleotides and inorganic phosphate following its phosphorylation (B. Guigas, L. Hue & B. Viollet, unpublished results). Among the other AMPK activators previously described, metformin could also exert AMPK-independent effects in the liver, notably by its mild and specific inhibition of the mitochondrial respiratory-chain complex, which may be responsible for part of its therapeutic action.

Metabolic action of AMPK in liver

Lipid homeostasis. AMPK phosphorylates multiple targets in the liver in order to acutely switch on alternative catabolic pathways and switch off anabolic pathways (Table 1). Acetyl-CoA carboxylase (ACC) and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) were the first enzymes shown to be downstream targets for AMPK (Hardie, 2004). ACC and HMG-CoA reductase are, respectively, key enzymes in fatty acid and cholesterol synthesis, known as ATP-consuming biosynthetic pathways. ACC is an important rate-controlling enzyme for the synthesis of malonyl-CoA, which is both a critical precursor in the biosynthesis of fatty acids and a potent inhibitor of mitochondrial fatty acid oxidation. In the liver, two separate genes encode major isoforms of ACC, ACC1 and ACC2, which display distinct cellular distribution leading to the compartmentalization of cellular malonyl-CoA production. The malonyl-CoA synthesized by ACC1 is used in fatty acid synthesis, whereas the malonyl-CoA generated by ACC2 is involved in the control of fatty acid oxidation (Abu-Elheiga *et al.* 2001). Inhibition of

ACC by AMPK leads to a fall in malonyl-CoA content and a subsequent decrease in fatty acid synthesis and increase in mitochondrial fatty acid oxidation via the allosteric regulation of carnitine palmitoyltransferase-1 (CPT-1), which catalyses the entry of long-chain fatty acyl-CoA into mitochondria. The transition from fasted to fed states is associated with nutritional and hormonal changes that lead to increased hepatic glycerolipid and fatty acid synthesis and decreased fatty acid oxidation and ketogenesis (Assifi *et al.* 2005). In re-fed rat, the activity of ACC increases dramatically in the first 1–3 h after re-feeding, which results in changes in hepatic malonyl-CoA concentration, and is associated with a coordinate reduction of AMPK activity, which remains low for at least 24 h (Assifi *et al.* 2005). Accordingly, insulin activates ACC to promote malonyl-CoA synthesis by inhibiting AMPK (Witters & Kemp, 1992). In addition, malonyl CoA decarboxylase (MCD), an enzyme involved in the turnover of malonyl-CoA, has been shown to be activated by AMPK in response to energy depletion, resulting in reduced malonyl-CoA levels and increased fatty acid oxidation (Assifi *et al.* 2005). Thus, in the liver, AMPK coordinates the changes in the activity of enzymes of lipid metabolism and regulates the partitioning of fatty acid between oxidative and biosynthetic pathways (Fig. 1). This has been illustrated by AMPK α 2^{-/-} mice and liver-specific AMPK α 2^{-/-} mouse models, which both exhibit hypertriglyceridaemia (Viollet *et al.* 2003; Andreelli *et al.* 2006). Furthermore, AICAR infusion resulted in a decrease in plasma triglyceride concentrations in both lean and obese Zucker rats, which can likely be attributed to an inhibition of hepatic lipogenesis (Bergeron *et al.* 2001). These results are consistent with *in vitro* findings demonstrating AICAR-induced inhibition of mitochondrial glycerol-3-phosphate acyltransferase (GPAT) activity and subsequent inhibition of triacylglycerol synthesis (Muoio *et al.* 1999). Since mitochondrial GPAT and CPT1 are both located on the outer mitochondrial membrane, mitochondrial GPAT competes directly with CPT1 for acyl-CoA substrates and AMPK would regulate acyl-CoA channelling towards

β -oxidation and away from glycerolipid biosynthesis (Fig. 1).

Glucose homeostasis. Since AMPK is usually considered as part of a mechanism involved in energy sparing, a potential role for AMPK in the regulation of the energy-consuming process of hepatic gluconeogenesis (*de novo* synthesis of glucose from three-carbon precursors) has been considered. Results obtained with pharmacological compounds and adenovirus-mediated AMPK activation/inactivation strategies have demonstrated that AMPK plays a role in the control of glucose production by the liver. It has been first shown that systemic infusion of AICAR in normal and insulin-resistant obese rats leads to the inhibition of hepatic glucose production (Bergeron *et al.* 2001). In addition, it has been also reported that metformin, which activates AMPK, is able to suppress glucose production in primary cultured hepatocytes (Zhou *et al.* 2001). These findings were supported by the observation that treatment of hepatocytes in primary culture with adenovirus expressing a constitutively active form of AMPK α 2 (AMPK α 2-CA) reduced glucose output (Foretz *et al.* 2005). The potent effects of circulating adipocyte-derived hormones on whole-body glucose metabolism recently highlighted the involvement of AMPK in the control of glucose output by the liver. Indeed, a physiological link has been established between circulating resistin levels and hepatic AMPK activity in the maintenance of blood glucose levels (Satoh *et al.* 2004). Furthermore, it was recently demonstrated that the hypoglycaemic effects of adiponectin appear to be mediated by hepatic AMPK activation (Yamauchi *et al.* 2002). This was corroborated

by the incapacity of adiponectin to regulate hepatic glucose production in the absence of the AMPK α 2 subunit in the liver (Andreelli *et al.* 2006).

Recent results with animal models confirm the physiological importance of hepatic AMPK for whole-body glucose homeostasis. First, short-term activation of AMPK specifically in the liver by adenovirus-mediated expression of AMPK α 2-CA is sufficient for controlling hyperglycaemia in murine models of diabetes (Foretz *et al.* 2005). Second, liver-specific AMPK α 2 $^{-/-}$ mice, which exhibited hyperglycaemia and glucose intolerance, presented increased fasted hepatic glucose production (Andreelli *et al.* 2006), demonstrating that the hepatic AMPK α 2 isoform is essential to suppress hepatic glucose production and maintain fasting blood glucose levels in the physiological range. Furthermore, these results indicate that the remaining AMPK α 1 in the liver is not sufficient to control hepatic glucose production. Third, AMPK α 1 α 2 $_{LS}^{-/-}$ mice are resistant to the hypoglycaemic effect of AICAR indicating that hepatic AMPK has a crucial role in the control of blood glucose levels (B. Viollet & F. Andreelli, unpublished results). Lastly, in mice lacking LKB1 in the liver, AMPK was almost completely inactive and fasting blood glucose levels were highly increased (Shaw *et al.* 2005). In these mice, the antidiabetic drug metformin no longer normalized blood glucose levels providing the apparent possibility that LKB1-mediated activation of AMPK in the liver might be required to lower blood glucose levels in diabetic mice (Shaw *et al.* 2005). Nevertheless, recent studies have shown that LKB1 phosphorylates and activates at least 12 AMPK-related kinases (Lizcano *et al.* 2004) which could contribute to the action of metformin in the liver. In

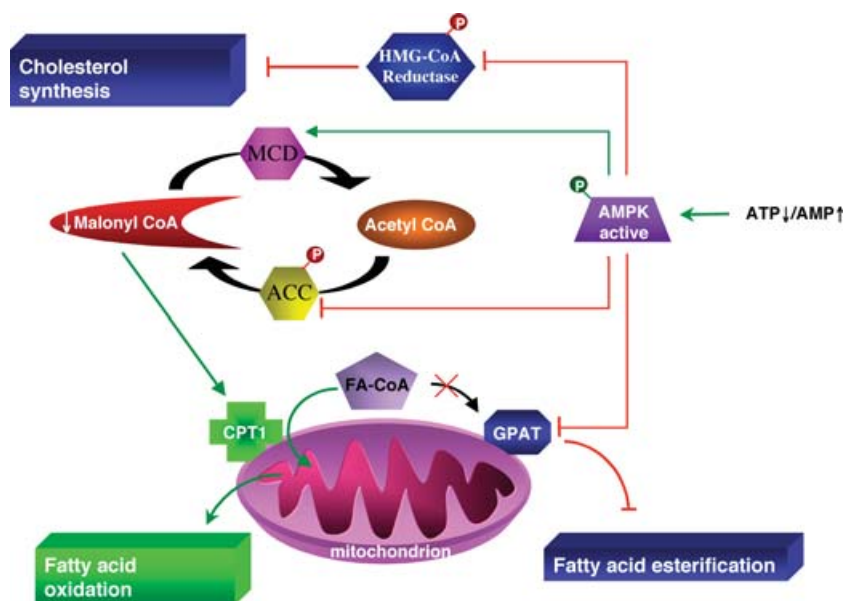


Figure 1. Regulation of lipid metabolism by hepatic AMPK

Activation of AMPK leads to the inhibition of cholesterol synthesis by phosphorylation of HMG-CoA reductase. By inhibiting ACC and activating MCD, AMPK increases fatty acid oxidation via the regulation of levels of malonyl-CoA, which is both a critical precursor for biosynthesis of fatty acids and a potent inhibitor of CPT-1, the enzyme that controls the transfer of long-chain fatty acyl-CoA into the mitochondria. AMPK inhibits also GPAT, the first committed enzyme in glycerolipid synthesis. The net resulting effect of AMPK activation is to inhibit energy-consuming lipogenic pathways (fatty acid, triglyceride and sterol synthesis) in favour of fatty acid oxidation. FA-CoA: fatty acyl-CoA.

recent studies, overexpression of the salt-induced kinases (SIK) of the AMPK-related kinase family were found to inhibit hepatic gluconeogenesis (Screaton *et al.* 2004; Koo *et al.* 2005). These data raised the question whether the glucose-lowering function of LKB1 is mediated by AMPK-related kinases rather than AMPK itself.

Protein synthesis and cell proliferation. Protein synthesis is an anabolic pathway consuming a significant amount of ATP (4 ATP equivalents per peptide bond during the elongation step of protein synthesis). So it is logical that AMPK plays a fundamental role in the inhibition of protein synthesis (Fig. 2A). The control of protein synthesis is complex and involves (de)phosphorylation of several translation factors and ribosomal proteins (Proud, 2004) (Fig. 2B). One of the main targets of AMPK amongst these regulatory elements is the mammalian target of rapamycin (mTOR) (Sarbasov *et al.* 2005). mTOR is a protein kinase known to be activated in the liver by different anabolic agents, including insulin and certain amino acids like glutamine and leucine (Krause *et al.* 2002b). The mTOR pathway positively regulates cell growth by modulating a large number of processes including protein translation, autophagy, cell survival and proliferation (Sarbasov *et al.* 2005). Once activated, mTOR is able to phosphorylate two regulatory proteins, the p70 ribosomal S6 protein kinase (p70S6K) and the eukaryotic initiation factor 4E-binding protein-1 (4E-BP1). The phosphorylation of 4E-BP1 relieves its inhibitory action on eukaryotic initiation factor 4E (eIF-4E), which can then bind the mRNA cap and stimulate protein synthesis initiation. p70S6K phosphorylates several substrates including the S6 ribosomal protein and the translational regulator eukaryotic elongation factor-2 kinase (eEF2K), involved in the control of initiation and elongation steps, respectively (Proud, 2004). eEF2K is a dedicated calcium- and calmodulin-dependent kinase controlling the phosphorylation and inactivation of eukaryotic elongation factor-2 (eEF2). p70S6K phosphorylates directly and inactivates eEF2K. The resulting decrease in phosphorylation state of eEF2 observed in this condition leads to a stimulation of protein synthesis.

AMPK activation can inhibit the mTOR pathway (Krause *et al.* 2002a; Reiter *et al.* 2005). Indeed, AMPK is able to directly phosphorylate mTOR on Thr²⁴⁴⁶ leading to its inactivation (Cheng *et al.* 2004). Moreover, upstream of mTOR, AMPK can also phosphorylate and activate the tuberous sclerosis complex 2 (TSC2) (Inoki *et al.* 2003). TSC2 is a GTPase activating protein that stimulates the intrinsic GTPase activity of the G protein Rheb, thereby promoting its conversion from the GTP-bound active state to the GDP-bound inactive state. Rheb, in its GTP-bound active state, activates mTOR by a not-well-defined mechanism. Therefore, AMPK-induced

TSC2 activation promotes Rheb and mTOR inactivation, thereby inhibiting the initiation step of protein synthesis. Moreover, AMPK can directly phosphorylate and activate eEF2K, leading to eEF2 inhibition (Horman *et al.* 2002; Browne *et al.* 2004).

The ability to increase the protein synthesis capacity of the cell is responsible for the ability of mTOR to drive cell growth and proliferation. Indeed, the signals that up-regulate mTOR activity are frequently activated in human cancers (Rao *et al.* 2004). AMPK is an anti-growth molecule because of its relationship with two tumour suppressor genes: *LKB1* and *TSC2* (Luo *et al.* 2005). *LKB1* mutations result in Peutz-Jeghers syndrome and predisposition to cancers of the colon, pancreas, breast and other sites. Mutations of *TSC1/TSC2* cause tuberous sclerosis which is associated with increased risk of cancers. Recently, the AMPK–TSC2–mTOR–eEF2 pathway has been linked to cell survival under hypoxic conditions (Liu *et al.* 2006). Moreover, AMPK activators, such as AICAR and metformin, inhibit the growth or the survival of some cancer cells, including hepatoma cells. The downstream targets of AMPK linked to these effects seem to include the mTOR pathway but also the tumour suppressor p53 and the cell-cycle inhibitor p21. These observations further suggest that AMPK provides a link between regulators of cellular metabolism and cell proliferation in cancer, which could be exploited for cancer treatment and/or prevention. Interestingly, a recent epidemiological study has revealed that diabetic patients treated with metformin are less prone to develop cancers (Evans *et al.* 2005). However, it remains to be demonstrated that this noticeable effect is mediated by AMPK.

Long-term effects of hepatic AMPK activation (regulation of gene transcription)

Although the action of AMPK in systemic energy balance is achieved by rapid and direct phosphorylation of metabolic enzymes, long-term effects have also been clearly demonstrated on gene expression. This has come from the finding that proteins related to all three AMPK subunits have been characterized in the yeast SNF1 complex, which is involved in the derepression of glucose-regulated genes through the modulation of the transcriptional activity of nuclear factors. Very interestingly, AMPK α 2-containing complexes are found in both the nucleus and the cytoplasm (Salt *et al.* 1998) raising the possibility that AMPK might regulate gene expression, at least in part, by the phosphorylation of nuclear factors (Table 2).

Glycolytic/lipogenic gene expression. It is now clearly established that AMPK plays an important role in the repression of glycolytic and lipogenic gene expression in the liver. AMPK activation by AICAR or by the

use of AMPK α 1-CA inhibits insulin/glucose-induced transcriptional stimulation of liver-type pyruvate kinase (L-PK), fatty acid synthase (FAS), Spot14 and ACC genes in primary cultured hepatocytes (Ferre *et al.* 2003).

Expression of AMPK α 2-CA in the liver by adenovirus-mediated gene transfer considerably decreased the refeeding-induced transcriptional activation of glycolytic and lipogenic genes and their upstream regulators,

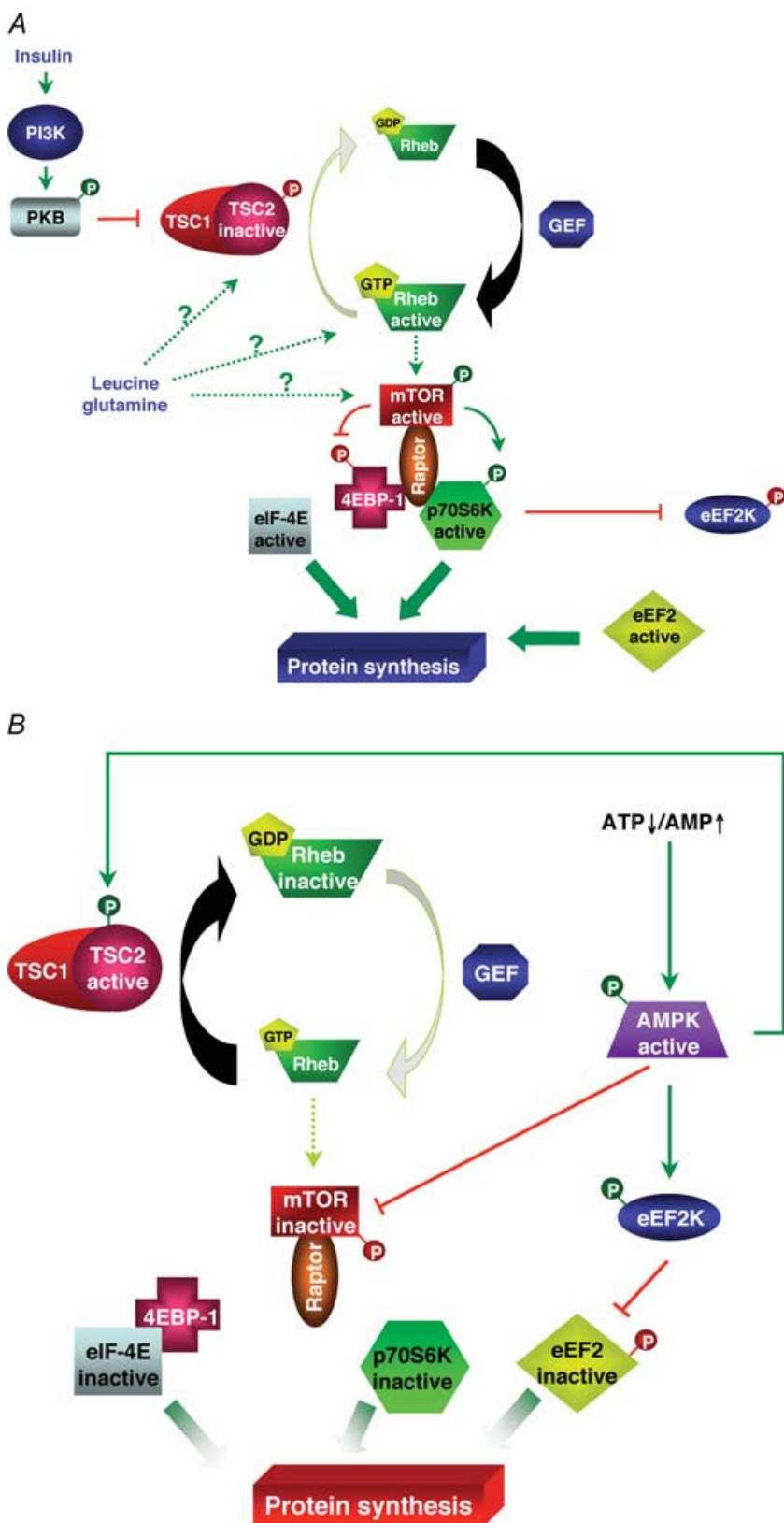


Figure 2. Diagrammatic representation of signalling involved in the regulation of the mTOR/p70S6K pathway by AMPK

A, both insulin and amino acids stimulate the mTOR/p70S6K pathway to promote protein synthesis. Insulin, by activating the phosphatidylinositol-3-kinase/protein kinase B pathway, inhibits TSC2, and so, via the activation of Rheb, induces mTOR activation. On the other hand, the pathway used by amino acids like leucine or glutamine to activate the mTOR pathway is still not well defined. Once activated, mTOR, with the participation of Raptor, is able to phosphorylate 4EBP-1 and p70S6K. B, activated AMPK is able to phosphorylate and activate both TSC2 and eEF2K. Moreover, AMPK can inactivate directly mTOR. GEF: guanylate exchange factor.

Table 2. AMPK-targeted transcription factors

Target	Phosphorylation site	Phosphorylation effect	Target gene(s)	References
ChREBP	Ser ⁵⁶⁸	↓ DNA binding	Glycolytic and lipogenic genes	Kawaguchi <i>et al.</i> (2002)
FoxO1	?	↓ Protein stability	G6Pase	Barthel <i>et al.</i> (2002)
HNF4	Ser ³⁰⁴	↓ Protein stability	L-PK, ApoCIII, ...	Leclerc <i>et al.</i> (2001); Hong <i>et al.</i> (2003)
SREBP1c	—	↓ Gene expression	Glycolytic and lipogenic genes	Zhou <i>et al.</i> (2001); Foretz <i>et al.</i> (2005)
TORC2	Ser ¹⁷¹	↑ Cytoplasmic sequestration	Gluconeogenic genes	Koo <i>et al.</i> (2005)

SREBP-1c (sterol regulatory element-binding protein-1c) and ChREBP (carbohydrate response element-binding protein) (Foretz *et al.* 2005). Similarly, activation of AMPK by metformin in the liver suppresses the expression of SREBP1c during the fasted–fed transition (Zhou *et al.* 2001). In addition, it has been demonstrated that activation of AMPK leads to the phosphorylation of ChREBP on Ser⁵⁶⁸, which caused a decrease in its DNA binding activity and subsequent transcriptional inhibition of glucose responsive genes (Kawaguchi *et al.* 2002). The nuclear hormone receptor HNF4 α involved in L-PK gene transcription has been also described as a target of AMPK (Leclerc *et al.* 2001; Hong *et al.* 2003). AMPK can phosphorylate HNF4 α on Ser³⁰⁴, a residue located in the dimerization domain of the protein, reducing the ability to form homodimers and to bind DNA. Phosphorylation of HNF4 α leads to decreased protein stability and probably increases its degradation rate (Hong *et al.* 2003).

Polyunsaturated fatty acids (PUFAs) are known to play pivotal roles as ‘fuel partitioners’ in the liver via their unique ability to partition fatty acids away from lipid synthesis towards fatty acid oxidation. As AMPK exhibited the same properties, it has been hypothesized that PUFAs may directly regulate AMPK activity. PUFAs exert their effects by a concomitant up-regulation of gene expression involved in fatty acid oxidation and down-regulation of glycolytic and lipogenic gene expression by acting through the inhibition of SREBP1c gene transcription, mRNA stabilization and proteolytic cleavage and the suppression of ChREBP nuclear translocation. To address the role of AMPK in the inhibitory effect of PUFAs on glycolytic and lipogenic gene expression, a series of experiments have been performed in AMPK α 1^{−/−}, AMPK α 2^{−/−} (Dentin *et al.* 2005) and AMPK α 1 α 2^{LS}^{−/−} mice (Fig. 3A). As shown in Fig. 3A, the inhibition of L-PK, FAS and ChREBP expression was sustained in livers of AMPK α 1 α 2^{LS}^{−/−} mice fed with a PUFA-enriched diet. The lack of both AMPK α 1 and α 2 isoforms did not affect ChREBP subcellular translocation into the nucleus under HCHO feeding and retention in the cytosol under PUFA conditions (Fig. 3B). Together, these data demonstrate that PUFAs inhibit ChREBP nuclear translocation and repress glycolytic/lipogenic gene expression by an AMPK-independent mechanism.

Gluconeogenic gene expression. Activation of AMPK in primary culture of hepatocytes was shown to reduce gene expression of the key gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) (Foretz *et al.* 2005). Administration of full-length adiponectin is known to reduce both blood glucose level and the expression of gluconeogenic genes. The concomitant injection of adenovirus encoding a dominant negative form of AMPK (AMPK-DN) prevented the inhibition of PEPCK and G6Pase gene expression by adiponectin (Yamauchi *et al.* 2002) suggesting that AMPK may regulate directly transcriptional regulators of the gluconeogenic programme in hepatocytes. Recently, TORC2 (transducer of regulated CREB activity 2) has emerged as a critical regulator of gluconeogenesis in mice (Koo *et al.* 2005). Under fasting conditions, glucagon triggers the transcription of gluconeogenic genes via the cAMP-responsive factor CREB (CRE binding protein) and subsequent recruitment of the coactivator CBP and TORC2. This leads to the expression of the coactivator PGC-1 α , which in turn drives the transcription of PEPCK and G6Pase genes in association with the transcription factor HNF4 α and the forkhead family activator FoxO1 (Fig. 4A). TORC2 activity is tightly regulated by a series of phosphorylation events blocking its nuclear accumulation. Interestingly, AMPK and the AMPK-related kinases SIK1 and -2 were found to phosphorylate Ser¹⁷¹ of TORC2, promoting binding to the 14-3-3 proteins and its sequestration out of the nucleus (Screaton *et al.* 2004; Koo *et al.* 2005) (Fig. 4B). The S171A mutant partially rescued SIK1- and AICAR-mediated suppression of G6Pase, PEPCK and PGC-1 α expression in primary hepatocytes, suggesting that TORC2 might be a critical downstream target of both AMPK and AMPK-related kinase SIK1 in the regulation of hepatic gluconeogenesis (Koo *et al.* 2005). Thus, the regulation of both nuclear import and nuclear export has emerged as one of the most efficient mechanisms to adapt gene expression to the cell environment by restricting the access of transcriptional regulators to their target genes. It is also noteworthy that glucose starvation and AICAR treatment strongly repress G6Pase expression due to the complete disappearance of the transcription factor FoxO1 (Barthel *et al.* 2002), indicating that AMPK may also

control transcription by regulating protein stability or degradation (Table 2).

Cytochrome P450 gene expression. AMPK has been implicated in the regulation of the drug-metabolizing enzyme cytochrome P450 (CYP) gene family (Rencurel *et al.* 2005). The CYP gene plays a crucial role in the transformation of xenobiotics by the liver and their expression is strongly induced in response to phenobarbital (PB) administration. Incubation of hepatoma cells with PB caused a dose-dependent increase in AMPK activity that was accompanied by a decrease in intracellular ATP levels. Expression of AMPK α 1-CA mimics the phenobarbital induction of CYP2B gene expression in HepG2 and primary human hepatocytes. Conversely, expression of AMPK-DN inhibits the induction of these genes in response to phenobarbital treatment. These data support a role for AMPK in the induction of CYP gene expression by barbiturate drugs. The action of AMPK could be mediated by changes in the nuclear localization or activity of the nuclear receptor constitutive androstane receptor (CAR) responsible for the PB induction of CYP2B genes (Kodama

et al. 2004). Interestingly, CAR is also involved in the PB-induced transcriptional repression of the PEPCK gene (Kodama *et al.* 2004).

Hepatic AMPK as a therapeutic target for metabolic disorders

AMPK activation for hyperglycaemic states. Because of its favourable global metabolic effects when activated, it is tempting to consider AMPK as a possible therapeutic target in the prevention and treatment of type 2 diabetes and insulin resistance. An important hallmark of type 2 diabetes is an increase in hepatic glucose production. Suppression of gluconeogenesis, a key metabolic pathway for hepatic glucose output, has been shown to improve overall glycaemic control in both human patients and type 2 diabetic animal models. It has been demonstrated that hepatic AMPK activation abolishes hyperglycaemia in diabetic *ob/ob* and STZ-induced diabetic mice by suppression of gluconeogenesis (Foretz *et al.* 2005). These results demonstrate that AMPK activation may have an effect under conditions of severe insulin resistance or

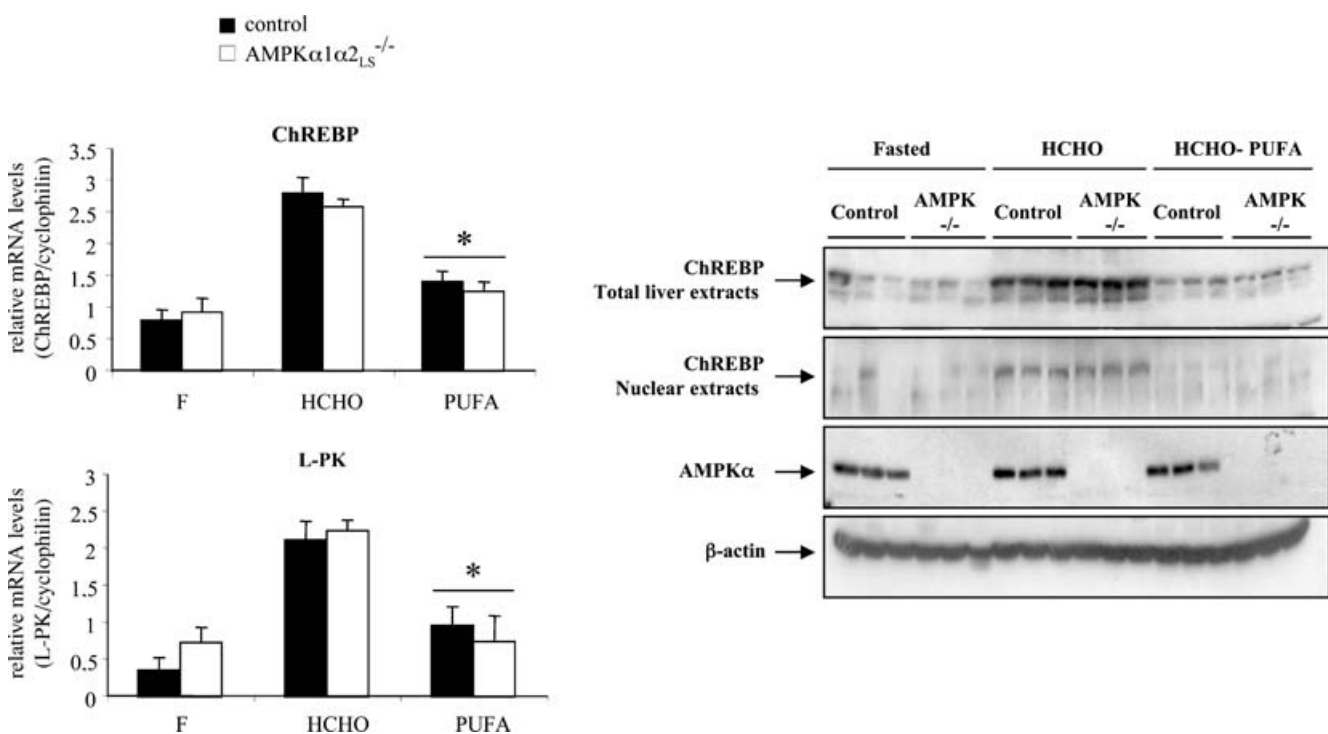


Figure 3. Effect of dietary PUFAs on L-PK and ChREBP gene expression and ChREBP localization in livers of AMPK α 1 α 2 $L_5^{-/-}$ mice

A, quantitative RT-PCR analysis of L-PK and ChREBP gene expression from livers of 24 h-fasted mice (F) and mice re-fed for 18 h with a high carbohydrate diet (HCHO) supplemented or not with PUFAs (PUFA) performed in control (filled bars) and AMPK α 1 α 2 $L_5^{-/-}$ (open bars) mice. Results are means $\pm$ S.E.M.; $n = 3$ /group. *Significantly different from mice re-fed with HCHO diet for 18 h ($P < 0.005$). **B**, cytosolic and nuclear ChREBP content from livers of 24 h-fasted control and AMPK α 1 α 2 $L_5^{-/-}$ mice re-fed for 18 h upon HCHO diet supplemented or not with PUFAs. Expression of AMPK α catalytic subunits has been measured by using anti-pan-AMPK α antibodies. β -Actin protein levels are presented as loading control. A representative Western blot is shown; $n = 3$ /group.

insulinopenia and that insulin signalling is therefore not required for this effect. Metformin is now a mainstay of therapy in the treatment of type 2 diabetes and is also an effective agent to decrease the risk of development of the disease. The ability of metformin to suppress hepatic glucose production and to lower blood glucose levels requires LKB1/AMPK signals (Zhou *et al.* 2001; Shaw *et al.* 2005). TZDs are a class of antidiabetic drugs that increase systemic insulin sensitivity both in rodents and humans. TZDs are ligands for PPAR γ , a member of the nuclear hormone receptor family of transcription factors, which is expressed in adipose tissue and to some extent

in liver and other tissues. The molecular pathway of AMPK activation by TZDs is still unclear, but activation has been recently attributed to their ability to increase adiponectin plasma levels, since activation of AMPK by rosiglitazone treatment is diminished in adiponectin KO mice (Nawrocki *et al.* 2006). Alternatively, this effect could also be due to decreased intracellular ATP levels in liver in response to TZD treatment (Saha *et al.* 2004).

Circulating levels of adiponectin are decreased in individuals with obesity, atherosclerosis and insulin resistance, suggesting that its deficiency may have a causal role in the etiopathogenesis of these diseases. Therefore,

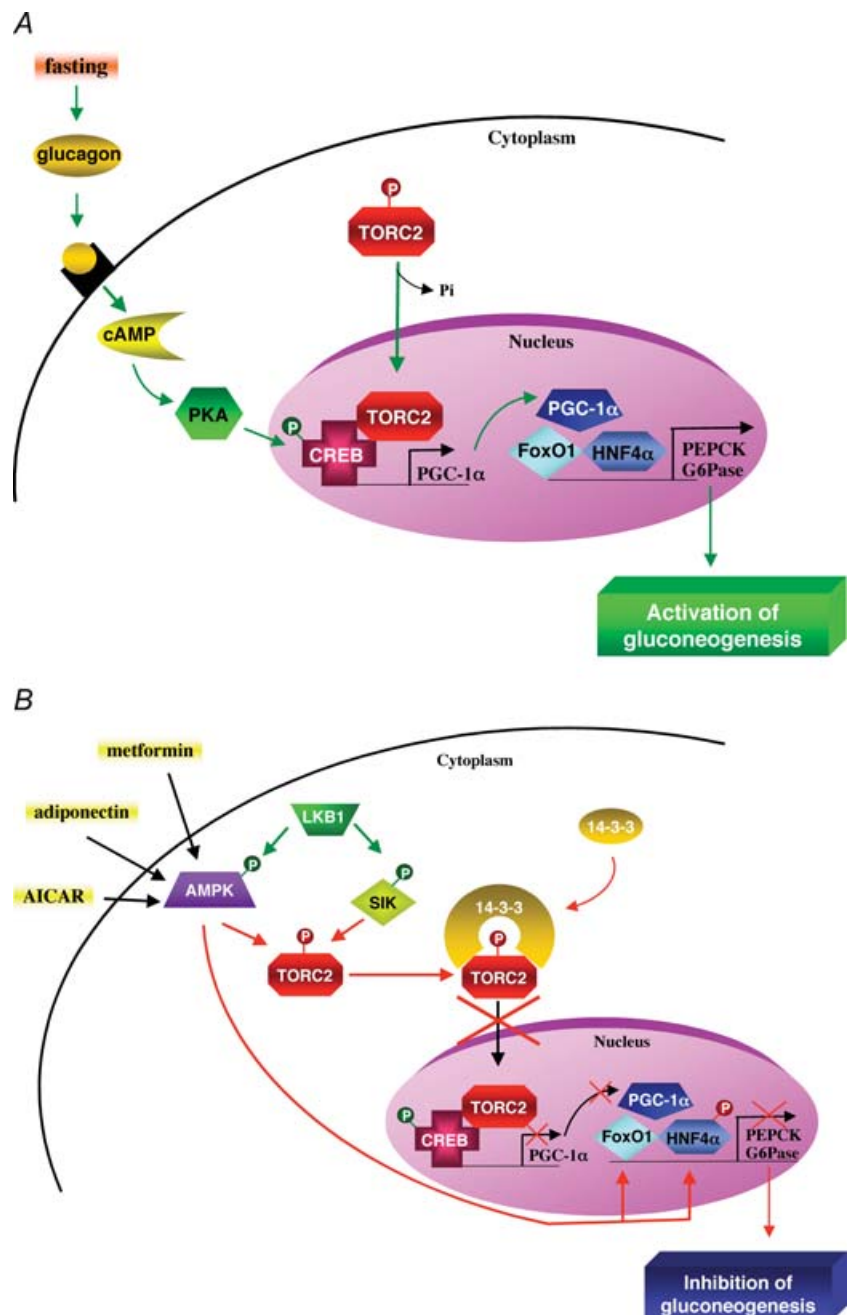


Figure 4. Transcriptional control of gluconeogenesis by TORC2 and AMPK

A, in response to fasting, the cAMP-responsive CREB coactivator TORC2 controls the gluconeogenic programme in liver via its nuclear translocation and association with CREB transcription factor, driving the expression of the PGC1 α coactivator. Expression of the coactivator PGC1 α in turn drives the transcription of key gluconeogenic enzymes such as PEPCK and G6Pase in association with the transcription factor HNF4 α and the forkhead family activator FoxO1. **B**, activity of TORC2 is controlled by AMPK and AMPK-related kinase SIK phosphorylation, which determines whether TORC2 becomes localized in the nucleus. Phosphorylated TORC2 is sequestered in the cytoplasm via a phosphorylation-dependent interaction with 14-3-3 proteins. Moreover, AMPK can also control gluconeogenic gene transcription by regulating stability or degradation of HNF4 α and FoxO1 transcription factors.

adiponectin replacement in humans may represent a promising approach to prevent and/or treat obesity, insulin resistance and type 2 diabetes. It has been reported that an acute increase in circulating adiponectin levels triggers a transient decrease in basal glucose levels by inhibiting both the expression of hepatic gluconeogenic enzymes and the rate of endogenous glucose production in both wild-type mice and a type 2 diabetes mouse model (Berg *et al.* 2001). The action of full length adiponectin on hepatic glucose production is dependent on phosphorylation and activation of AMPK in the liver (Yamauchi *et al.* 2002). Lack of action of adiponectin on hepatic glucose production when AMPK α 2 catalytic subunit is missing strongly supports the concept that adiponectin's effect is strictly dependent on AMPK (Andreelli *et al.* 2006).

AMPK for hepatic steatosis management. Non-alcoholic fatty liver disease is a clinicopathological term that encompasses a disease spectrum ranging from simple triglyceride accumulation in hepatocytes (hepatic steatosis) to hepatic steatosis with inflammation (steatohepatitis), fibrosis and cirrhosis. Metformin and TZDs markedly reduced hepatic steatosis both in rodents (Lin *et al.* 2000) and humans (Bajaj *et al.* 2003), presumably acting through hepatic AMPK activation.

Chronic ethanol administration in mice, a model mimicking the chronic alcohol consumption in humans, leads to hepatic steatosis and is associated with a significant increase in the abundance of the mature (active) form of SREBP1c in the liver, an increased expression of lipogenic genes and an inhibition of AMPK and peroxisome proliferator-activated receptor α (PPAR α), two critical signalling molecules controlling the pathways of hepatic fatty acid oxidation (You *et al.* 2004). Transcriptional control by AMPK may be important for the treatment of liver steatosis because AMPK activation suppresses expression of SREBP1c (Zhou *et al.* 2001; Foretz *et al.* 2005). In addition, chronic ethanol consumption significantly decreased circulating concentrations of adiponectin in mice (Xu *et al.* 2003). Delivery of recombinant full-length adiponectin into these mice alleviated liver steatosis and injury through activation of fatty acid oxidation and by decrease of lipogenesis.

AMPK for protective effects of ischaemic preconditioning in liver transplantation. Hepatic ischaemia–reperfusion injury associated with liver transplantation and hepatic resections is a critical problem in clinical practice. Preconditioning (a short period of ischaemia and reperfusion) is associated with a beneficial effect during major hepatic surgery in patients subjected to complex hepatic resections in which long periods of ischaemia

are necessary. Ischaemic preconditioning prevents ATP degradation and concomitant intracellular accumulation of AMPK induced by subsequent ischaemia (Peralta *et al.* 2000). Increases in AMP levels during ischaemia activate AMPK while inhibition of AMPK abolishes completely the effects of preconditioning, indicating that AMPK is essential to promote the preconditioning effect (Peralta *et al.* 2001). So, activation of AMPK in liver by preconditioning may represent a new strategy to reduce the hepatic injury associated with ischaemia–reperfusion in humans.

Conclusion

The liver is highly sensitive to changes in metabolic demands and variation in nutritional and hormonal signals will act on the hepatic AMPK system to regulate whole body energy metabolism. The AMPK system plays a major role in the regulation of glucose and lipid metabolism through its acute effects on energy metabolism pathways and long-term effects involving changes in gene expression. The relationship between AMPK activation and beneficial metabolic effects in diabetic rodent models has provided the rationale for the development of new therapeutic strategies based on pharmacological but also nutritional use of AMPK activators in order to prevent or reverse hepatic disorders linked to type 2 diabetes and obesity.

Perspectives

AMPK activation in the liver entails metabolic consequences that are beneficial for the diabetic patients. This holds true for the inhibition of gluconeogenesis, which helps to maintain glycaemia. Many other known or potential effects of AMPK on liver metabolism remain, however, to be investigated. This is the case for glycogen and cholesterol metabolism. A decrease in the latter would mimic the effects of statins and benefit those patients already prone to cardiovascular complications. By contrast, the expected stimulation of fatty acid oxidation and ketogenesis by AMPK might lead to ketoacidosis, a dreadful complication of diabetes. The inhibition of protein synthesis by AMPK could favour protein degradation and lead to a negative nitrogen balance together with enhanced urea synthesis, a pathway known to consume 4 mol of ATP per mol of urea. Clearly, these effects of AMPK on protein metabolism and on the detoxifying capacity of the liver deserve serious consideration. In conclusion, care should be taken that the well-documented beneficial effects of pharmacological activation of AMPK on glucose metabolism do not mask other metabolic effects that could be detrimental for diabetic patients.

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