

The role of AMP kinase in diabetes

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Type 2 diabetes is characterized by abnormal metabolism of glucose and fat, due in part to resistance to the actions of insulin in peripheral tissues. If untreated it leads to several complications such as blindness, kidney failure, neuropathy and amputations. The benefit of exercise in diabetic patients is well known and recent research indicates that AMP activated protein kinase (AMPK) plays a major role in this exercise related effect. AMPK is considered as a master switch regulating glucose and lipid metabolism. The AMPK is an enzyme that works as a fuel gauge, being activated in conditions of high energy phosphate depletion. AMPK is also activated robustly by skeletal muscle contraction and myocardial ischaemia, and is involved in the stimulation of glucose transport and fatty acid oxidation produced by these stimuli. In liver, activation of AMPK results in enhanced fatty acid oxidation and decreased production of glucose, cholesterol, and triglycerides. The two leading diabetic drugs namely, metformin and rosiglitazone, show their metabolic effects partially through AMPK. These data, along with evidence from studies showing that chemical activation of AMPK *in vivo* with 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) improves blood glucose concentrations and lipid profiles, make this enzyme an attractive pharmacological target for the treatment of type 2 diabetes and other metabolic disorders.

Key words AMP kinase - diabetes - thiazolidinediones

Type 2 diabetes is characterized by the abnormal metabolism of glucose and fat, due in part to resistance to the actions of insulin in skeletal muscle, liver and fat. In the natural history of type 2 diabetes, pancreatic β -cells initially compensate for insulin resistance by secreting excess insulin. However with time, progressive β -cell failure leads to insulin

deficiency and overt hyperglycaemia. Progression in diabetes leads to the development of chronic complications such as retinopathy, neuropathy and nephropathy, *etc.* At present, oral therapy for type 2 diabetes relies on several approaches targeted to reduce hyperglycaemia namely sulphonyl ureas, which increase insulin release from pancreatic islets; α -glucosidase

inhibitors, which inhibit gut glucose absorption; metformin, which acts to reduce hepatic glucose output through inhibition of gluconeogenesis, peroxisome proliferators-activated receptor- γ activator thiazolidinediones (TZDs), which promote insulin sensitization. These therapies have either limited efficacy or significant mechanism based side effects like hypoglycaemia, flatulence, body weight gain or enhancement of gastrointestinal problems. Recently launched glucagon like peptide-1 (GLP-1) mimetic peptides cannot be used for oral treatment and there are concerns of its undesirable effects such as nausea, vomiting, *etc*¹. In such a scenario, there is an acute unmet need for an oral anti-diabetic drug without side effects. Pharma companies across the world are exploring several novel targets for this purpose. Evidence accumulated over the past few years indicates that the AMP activated protein kinase (AMPK) may be a good target for the pharmacologic treatment of type 2 diabetes. Current concepts of the mechanisms by which AMPK influences glucose and fat metabolism are discussed in this review.

AMP activated protein kinase (AMPK)

The AMPK belongs to the family of energy sensing enzymes that are activated by cellular stresses resulting in ATP depletion, thus acting like a 'fuel gauge'². Upon activation, AMPK functions to restore cellular ATP by both inhibiting ATP consumption processes as well as accelerating ATP generation processes (Fig. 1). The cascade is activated by stresses such as prolonged exercise³, electrical stimulation in skeletal muscle⁴, ischaemia in heart muscle⁵, heat shock⁶ as well as through inhibition of tricarboxylic acid cycle or oxidative phosphorylation⁷. AMPK is activated by an increase in AMP/ATP and creatine/phosphocreatine (pCr) ratios, resulting allosteric modification or/and through mechanism involving

phosphorylation of the α subunit by upstream kinases (AMPKKs)^{2,8}.

AMPK is a heterotrimeric protein consists of three subunits namely α , β and γ . The regulatory subunit plays an important role in maintaining stability of the heterotrimer complex^{2,8}. Both the α and β subunits have two isoforms each, namely $\alpha 1$ & $\alpha 2$ and $\beta 1$ & $\beta 2$, while the γ subunit exists in three isomeric forms, *i.e.*, $\gamma 1$, $\gamma 2$ and $\gamma 3$. AMPK $\alpha 1$ is widely expressed, whereas the $\alpha 2$ subunit isoform is mainly found in the heart, skeletal muscle, and liver⁹. AMPK α consists of 548 amino acids (aa) and it contains catalytic domain (1-312 aa), an autoinhibitory domain (312-392 aa) and a subunit binding domain (392-548 aa). The catalytic domain has a site of phosphorylation at Thr¹⁷² which is required to be phosphorylated for its activation by AMPKK. Removal of both autoinhibitory domain and subunit binding domains leads to constitutive active AMPK retaining one third of activity compared to full AMPK complex whereas removal of subunit binding domain leads to complete loss of activity^{10,11}. The subunit domain contains PEST sequences which are masked by β and α subunits resulting in slow turnover of this enzyme in native state¹¹. The β subunit contains the glycogen binding domain whereas each γ subunit contains four Bateman domain or cystathione β -synthase (CBS) domains and each pair of CBS domain functions as prospective AMP-binding sites¹⁰.

Role of AMPK in skeletal muscle

Skeletal muscle is the main site for glucose disposal in the body and there are two ways to stimulate glucose uptake in skeletal muscle: insulin dependent and insulin independent¹². Insulin resistance is one of the early defects detected in the muscle of diabetic patients and insulin resistance is caused mainly due to defect in insulin signaling

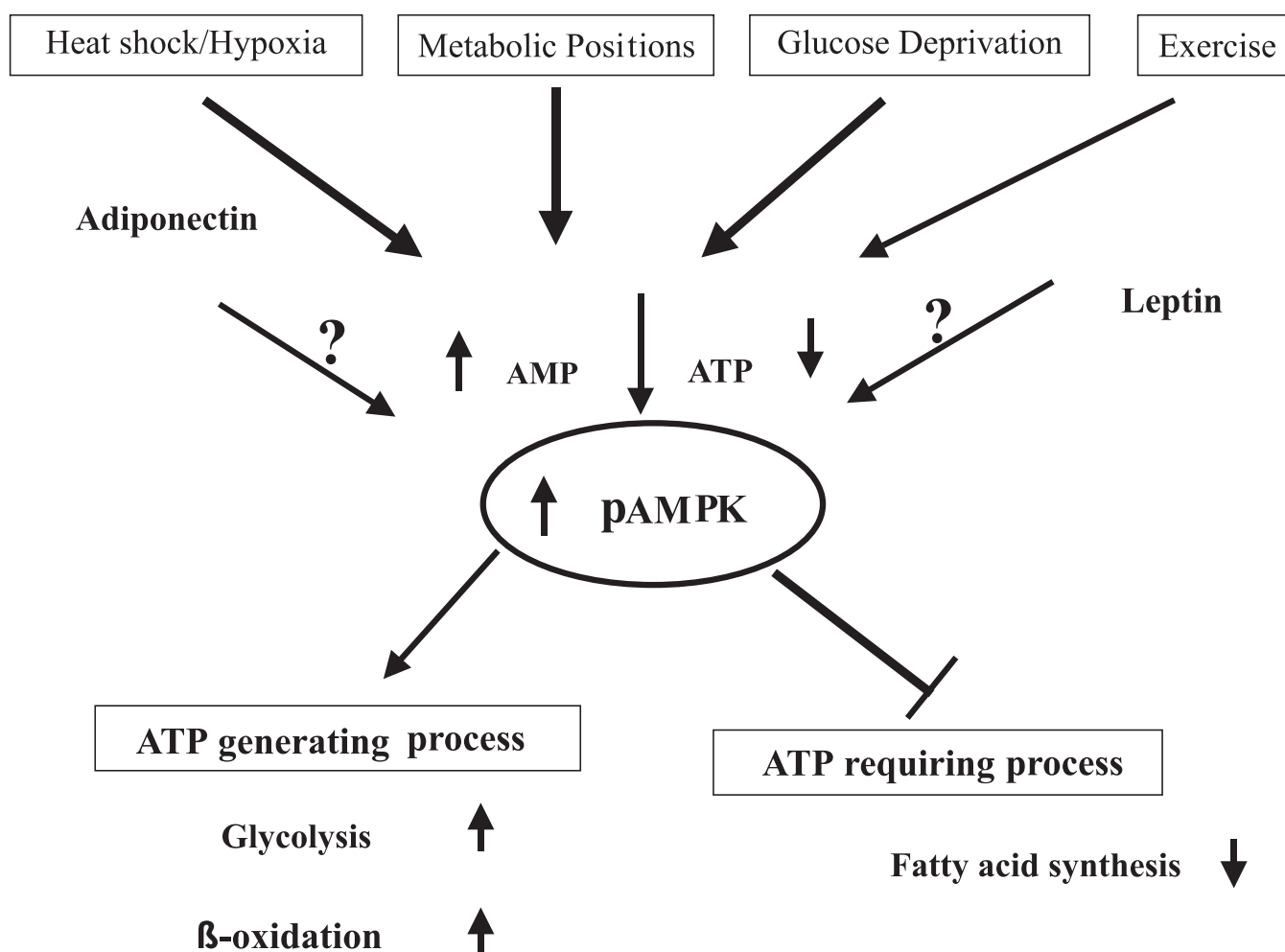


Fig. 1. Mechanism of AMPK activation. AMPK is activated by different stimuli due to increase in the AMP/ATP ratios resulting in ATP depletion, thus acting like a 'fuel gauge'. Upon activation, AMPK functions to restore cellular ATP by inhibiting ATP consumption processes (fatty acid synthesis) but accelerating ATP generation processes (glycolysis & β -oxidation of fat).

pathways. Decrease in insulin-stimulated tyrosine phosphorylation of the insulin receptor and insulin receptor-1 substrate (IRS-1) and in IRS-1-associated phosphatidylinositol-3 kinase (PI-3 kinase) activity leading to the problem in translocation of glucose transporter (GLUT4) from microvesicle to membrane¹³⁻¹⁷. Currently no pharmacological approach is being pursued to correct these defects in insulin signaling pathway. Therefore, targeting insulin independent pathway to restore glucose disposal can be explored as an alternative approach. There are sufficient data available to support the

hypothesis that exercise enhances muscle glucose disposal in diabetic patients through an insulin independent mechanism. For example, acute exercise does not increase insulin receptor and IRS-1 tyrosine phosphorylation or PI3-kinase activity^{14,18}. Moreover, blockade of the insulin signaling pathway does not alter exercise-stimulated muscle glucose transport¹⁹⁻²¹. Efforts are ongoing to unravel the molecular mechanism by which exercise functions. Mimicking exercise like effect through drug could be an attractive approach to improve blood glucose level.

Muscle contraction leads to increase in AMP/ATP and Cr/ pCr levels leading to robust increase in AMPK activity which is well correlated with contraction mediated glucose uptake in muscle²². A similar effect has been observed with AICAR, an AMP analogue and known AMPK activator suggesting that AMPK plays an important role in contraction mediated glucose disposal²³. Activation of AMPK by AICAR in rat or muscle cells overexpressing constitutively active AMPK increases glucose uptake and causes translocation of the glucose transporters GLUT1 and GLUT4 from microvesicle to plasma membrane establishing the link between AMPK activation, glucose transport and the translocation of glucose transporters²⁴.

Systematic studies using AMPK knockout and transgenic animals helped to clarify the molecular mechanisms of regulation of glucose transport in

muscle by AMPK. In AMPK α 2 knockout mice (whole body deletion), AICAR stimulated glucose transport is blocked in muscles but insulin stimulated glucose transport is normal in the contracting muscles isolated from these animals²⁵. There is overexpression of the AMPK α 1 subunit in these animals indicating that though AICAR mediated glucose transport is mainly through AMPK α 2 but it is not indispensable as there is a compensatory mechanism provided by AMPK α 1²⁵. On the other hand, in transgenic mice overexpressing muscle specific domain negative-AMPK α 2, which blocks the activities of both α 1 and α 2 endogenous subunits, AICAR failed to show any stimulation of glucose uptake. Contraction mediated glucose uptake is partially although not completely affected in the isolated muscles of these animals indicating that apart from AMPK mediated mechanism some other unknown mechanisms may be involved in this process²⁶.

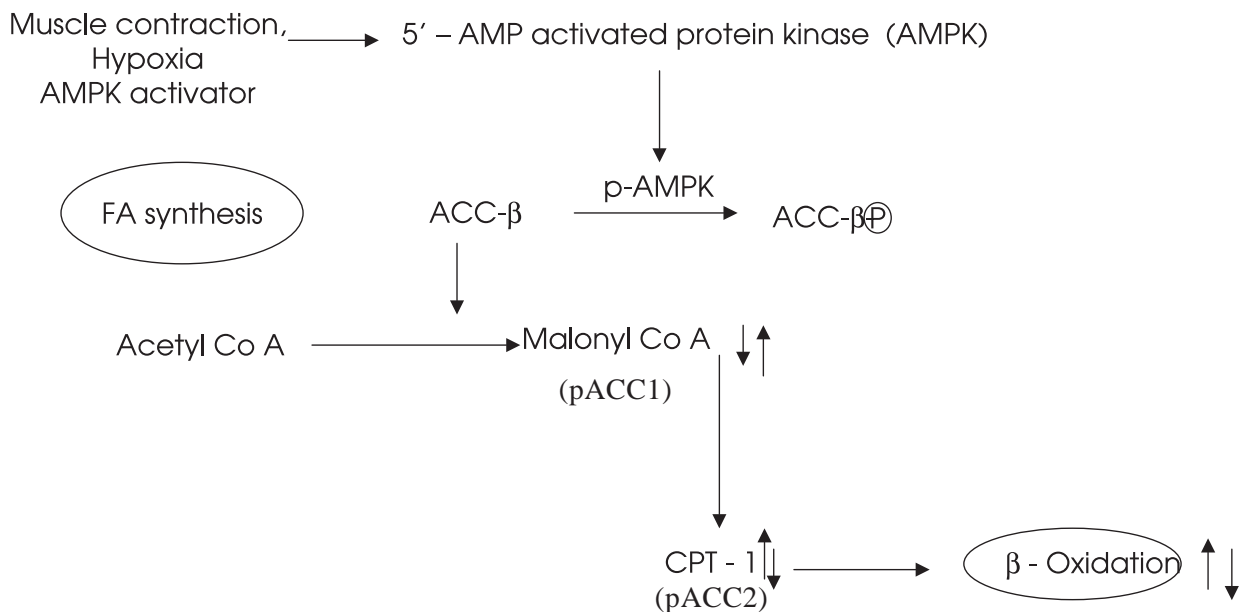


Fig. 2. Mechanism of AMPK mediated fat metabolism. Upon activation either by exercise or contraction, AMPK decreases the activity of acetyl COA carboxylase (ACC) by inhibiting its transcription and also through phosphorylation. This leads to a decrease in muscle malonyl COA (pACC1) content and thus relieving its inhibitory activity on CPT-1 (pACC2). Higher CPT-1 activity enhances the entry of fatty acids into the mitochondria for oxidation.

The above reports proved the role of non insulin mediated glucose disposal in muscle. A series of *in vivo* and *ex vivo* experiments also showed that AMPK is involved in insulin dependent direct glucose transport in muscle. Soleus muscle isolated from AICAR-treated rat shows insulin mediated glucose uptake through translocation of glucose transporter GLUT4²⁷. Soleus muscle isolated from normal rats followed by incubation with AICAR and insulin leads to additive increase in glucose uptake through glucose transporter²⁸. In insulin resistant Zucker *fa/fa* rats, a single dose of AICAR injection improves insulin sensitivity measured using an insulin clamp study²⁹. Taken together these *in vivo* and *ex vivo* data prove that AMPK mediated glucose uptake is an additive effect of both insulin dependent and independent mechanisms.

Apart from increasing glucose transport in muscle, AMPK also plays an important role in muscle fat metabolism. Upon activation either by exercise or contraction, AMPK decreases the activity of acetyl CoA carboxylase (ACC) by inhibiting its transcription and also through phosphorylation^{4,30-32}. This leads to a decrease in muscle malonyl CoA content and thus relieving its inhibitory activity on carnitine palmitoyl transferase 1 (CPT-1)³³. Higher CPT-1 activity enhances the entry of fatty acids into the mitochondria for oxidation. So, in summary, activated AMPK decreases free fatty acid synthesis (pACC1) and increases mitochondrial β -oxidation (pACC2), thereby reduces elevated free fatty acid level and thus ameliorates insulin resistance (Fig. 2).

Role of AMPK in liver

In the liver, activated AMPK inactivates ACC at transcriptional and post translational level and also inhibits HMG CoA reductase, the rate limiting

enzyme in cholesterol synthesis by phosphorylation^{34,35}. Like skeletal muscle, in liver also, activated AMPK decreases malonyl CoA synthesis resulting in increased β -oxidation through enhanced CPT-1 activity³⁶. Type 2 diabetic patients are often associated with hypertriglyceridaemia and high cholesterol, the potential risk factors for cardiovascular problems. Activated AMPK could reduce this risk by controlling elevated level of free fatty acid, TG and cholesterol³⁷.

The elevation of fasting plasma glucose is associated with type 2 diabetes and it is regulated by gluconeogenesis, a process which makes glucose from non-carbohydrate source in liver. Gluconeogenic enzymes like phosphoenol pyruvate carboxy kinase (PEPCK) and glucose-6-phosphate dehydrogenase are the major players in this process^{38,39}. In HepG2 cells and primary rat hepatocytes, AICAR inhibits the transcription of PEPCK indicating the role of AMPK in inhibition of gluconeogenesis^{38,39}. AICAR inhibits hepatic glucose output in rat liver when perfused *in vivo*²⁴. Thus, AMPK, by inhibiting hepatic glucose output and increasing muscle glucose uptake, could control elevated blood glucose level in the body.

Role of AMPK in adipocytokine signaling

Adiponectin and leptin, the two adipocytokines produced and secreted from adipose tissues play an important role in the pathogenesis of type 2 diabetes. Adiponectin increases free fatty acid oxidation through ACC, an AMPK target gene and also enhances insulin sensitivity both in skeletal muscle and liver⁴⁰⁻⁴². There is an inverse relationship with the level of blood plasma adiponectin and insulin resistance. These results indicate that adiponectin behaves like an ideal AMPK activator.

The role of leptin in the pathogenesis of insulin resistance is well known. Leptin has both central

and peripheral mechanisms through which it controls energy expenditure. Being secreted from adipose tissue, leptin binds to its receptor in brain and exhibits its effect through JAK-STAT (Janus Kinases-signal Transducers and Activators of Transcription) pathway. *Ex vivo* study conducted in mice indicated that some of its peripheral actions might be through AMPK^{43,44}. The activation of AMPK by leptin causes inhibition of ACC, which in turn stimulates skeletal muscle fatty acid oxidation indicating that its insulin sensitization action might be through reduction in free fatty acid level, the potential precursor of insulin resistance.

Peroxisome proliferator activated receptor gamma (PPAR γ) and AMPK regulate lipid metabolism differently

PPAR γ , an anabolic regulator induces adipogenesis leading to the storage of TG in adipose tissue and thereby decreases blood TG level, but this process results in increased body weight. Activated AMPK, a catabolic regulator on the other hand, decreases TG level by inhibiting fatty acid synthesis and also increasing β -oxidation, through ACC. Mice lacking ACC β have lower fat stored compared with wild-type mice despite higher food intake indicating that AMPK activation does not lead to increase in body weight⁴⁵. Additionally, activated AMPK phosphorylates hormone sensitive lipase (HSL) and activated HSL can metabolise stored fat in the adipose tissue⁴⁶. Taken together, activation of AMPK will be a preferable option over activation of PPAR α with respect to controlling dyslipidaemia.

Role of co-activator p300 in AMPK mediated signal regulation

AMPK inhibits PEPCK through transcription, ACC both at transcriptional and post-translational

levels and HMG CoA reductase through post-translational phosphorylation of the enzyme (p - HMGCoA reductase). This differential modulation raises several questions: why does AMPK regulate genes differentially ? Why is ACC being inhibited in both transcriptional and post-translational levels ? Is differential activation of AMPK required for regulating different genes ? So far, it is not clear how AMPK inhibits different genes at the transcriptional level. Yang *et al*⁴⁷ first showed that p300, a universal transcriptional co-activator is a substrate of AMPK. Being phosphorylated at Ser89 by AMPK, pP300 gets detached from nuclear transcription factors leading to the inhibition of the transcription of the genes⁴⁷. The potential role of p300 in the transcriptional regulation of these genes is not known. Systematic research is required to unravel the mechanism behind the differential regulation of different proteins by AMPK

AMPK as a pharmacological target - Present and promise

AMPK is a global target as it regulates different diversified signals in metabolic pathways. On the basis of the merits associated with this target, an ideal AMPK activator is expected to increase muscle glucose transport and muscle insulin sensitivity; enhance fat oxidation in muscle and liver; inhibit hepatic gluconeogenesis; decrease cholesterol and triglyceride synthesis in liver and should be devoid of problems associated with present antidiabetic drugs (gastrointestinal problem, body weight increase, *etc.*). Three different kinds of AMPK activators have been reported so far. First, PPAR γ activators, rosiglitazone and pioglitazone, which activate AMPK without direct binding but by increasing cellular AMP/ATP ratio. Second, AICAR, an analogue of natural activator AMP, which activates AMPK through direct binding followed by allosteric modification. Lastly, metformin, an

AMPK activator which does not affect AMP/ATP ratios or bind to AMPK, but acts through an unknown mechanism⁴⁸. PPAR γ agonist rosiglitazone, the leading anti-diabetic drug, although an activator of AMPK, is associated with PPAR γ related side effects, like weight gain and oedema. The anti-diabetic effect of metformin is explained partially through AMPK but it also has several deficiencies. AICAR showed promise in preclinical model as an anti-diabetic drug but failed in phase 1 clinical trial (100 mg/kg)⁴⁹. Although it showed good plasma glucose and TG reduction, it produced uric acid and lactic acidosis due to the formation of purine metabolite⁴⁹. Therefore, it is a great challenge for the pharmaceutical companies to get a safe but efficacious AMPK activator. We also need to remember that there are certain difficulties associated with AMPK, which makes it a difficult pharmacological target, (i) AMPK is a heterotrimeric protein and so far no crystal structure is available, (ii) each subunit contains two or more isoforms, and (iii) the AMP binding site is not well defined. Despite these limitations, several pharmaceutical companies are working on this target and have reported several AMPK activators in preclinical studies.

Conclusion

Our understanding on the role of AMP kinase on different metabolic processes have increased several folds in recent years. Its activation can cause insulin sensitization, modulate plasma glucose level through its action on liver and muscle, which can have beneficial effect in type 2 diabetes patients. AMPK activation also regulates both lipid oxidation and synthesis and thereby can be beneficial in controlling dyslipidaemia. Extensive research is in progress to discover novel AMPK activators for the treatment of type 2 diabetes and its associated disorders.

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