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COMMENTARY

Can patients with type 2 diabetes be treated with 5′-AMP-activated protein kinase activators?

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Abbreviations

ACC	acetyl-CoA carboxylase
AICAR	5-aminoimidazole-4-carboxamide riboside
AMPK	5′-AMP-activated protein kinase
ERK	extracellular signal-regulated protein kinase
pAMPK	phosphorylated AMPK
R_a	rate of appearance
R_d	rate of disappearance
ZDF	Zucker diabetic fatty
ZMP	AICAR monophosphate

Why is 5′-AMP-activated protein kinase a reasonable therapeutic target?

5′-AMP-activated protein kinase (AMPK) has emerged as a key regulatory protein that is present in the majority of cells of the body. It is an energy-sensing protein that is activated in response to an increase in the AMP:ATP ratio, which occurs during hypoxia or muscle contraction [1]. It can also be activated by hormones such as adiponectin and leptin and the cytokine IL-6. AMPK must be phosphorylated at Thr172 of the α subunit by an upstream kinase before it can phosphorylate downstream targets. Three upstream kinases have been identified: calmodulin kinase kinase β (CaMKKβ); and TGFβ-activated kinase (TAK1); and a complex of serine-threonine kinase 11, Ste20-related adaptor and mouse protein 25 (LKB1-STRAD-MO25). Once phosphorylated, AMPK is also subject to allosteric activation by 5′-AMP. In general, activated AMPK phosphorylates proteins that increase ATP production and decrease ATP use. Processes such as cholesterol and fatty acid synthesis, protein synthesis and gluconeogenesis in liver are decreased, whereas glucose uptake and fatty acid oxidation in muscle and fatty acid oxidation in liver are enhanced. The levels of proteins involved in ATP production, including GLUT4, hexokinase and mitochondrial oxidative enzymes, are also increased. Specific transcription factors or coactivators (e.g. peroxisome proliferator-activated receptor γ coactivator 1α) are phosphorylated by activated AMPK. Insulin sensitivity is enhanced, evidenced by increased stimulation of glucose uptake by isolated skeletal muscle at submaximal insulin concentrations. Based on these consequences of its activation, AMPK has been targeted for the development of a new class of pharmaceutical agents for the prevention and treatment of type 2 diabetes.

Evidence from animal studies that AMPK activation could help in the treatment of type 2 diabetes

The first evidence suggesting that AMPK activators might be useful in the treatment of type 2 diabetes came from animal studies [2, 3]. AMPK was found to be activated in skeletal muscle during exercise and in response to electrical stimulation of contraction (Fig. 1). An increase in the phosphorylation/inactivation of acetyl-CoA carboxylase

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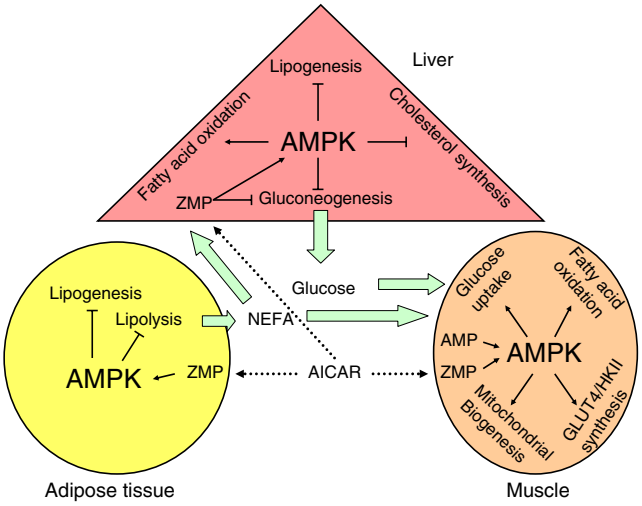

Fig. 1 Effects of AMPK activation in liver, adipose tissue and skeletal muscle. Phosphorylation of target proteins results in activation (represented by solid line arrow) or inhibition (represented by solid line with bar) of key metabolic processes in the cells. AMPK must be phosphorylated on Thr172 of the α subunit to be active. AMP levels increase in response to muscle contraction or hypoxia and can make AMPK a poorer substrate for the phosphatase, thus increasing the fraction that is phosphorylated. AMP also activates pAMPK allosterically. AICAR can be taken up by cells (dotted arrow) and phosphorylated to form ZMP, which can then mimic the effects of AMP in AMPK activation. ZMP can also inhibit gluconeogenesis independently of AMPK activation

(ACC), the first downstream target identified in skeletal muscle, accompanied this activation. An analogue of adenosine, 5-aminoimidazole-4-carboxamide riboside (AICAR), is taken up by muscle and phosphorylated to form AICAR monophosphate (ZMP), an analogue of AMP, which can thereby activate AMPK. Chemical activation of AMPK with AICAR in perfused rat hindlimbs not only stimulated fatty acid oxidation, but also increased glucose uptake [4]. Subsequent studies showed that AICAR stimulated the uptake of non-metabolisable glucose analogues into isolated epitrochlearis muscle and that stimulation by AICAR occurred through the increased translocation of GLUT4 into sarcolemmal membranes from a microsome fraction of perfused muscle [2]. Subcutaneous injection of AICAR into rats was shown to activate skeletal muscle AMPK, to induce an increase in muscle GLUT4 and hexokinase, and to cause an increase in glycogen content of the muscle [5].

Studies of AMPK activators in animal models of type 2 diabetes have also provided promising results [6–8]. Obese Zucker diabetic fatty (ZDF) rats, a model of type 2 diabetes, show marked decreases in plasma glucose and plasma insulin concentrations in response to the administration of AICAR for 7 weeks [6, 7]. Glucose tolerance was normalised 24 h after the last AICAR injection. These changes were accompanied by increases in GLUT4 content in muscle and improvements in 3-methylglucose uptake in incubated epitrochlearis and extensor digitorum longus muscles [6, 7]. Both exercise training and AICAR treatment prevented the usual rise in blood glucose at 9 weeks of age in the ZDF rat [7]. Deterioration of pancreatic islet cells was also attenuated in these rats by AICAR treatment [7]. Two mouse models of type 2 diabetes (KKAy-CETP and *ob/ob*) also show improvements in glucoregulation in response to treatment with AICAR [9, 10]. In all of these animal studies, relatively high doses of AICAR (0.5–1.0 mg/g body weight) were required, and the highest dose caused liver hypertrophy and lactic acidosis in rats [4].

Human studies on AMPK activation

After the beneficial effects of chemical AMPK activation were demonstrated in animal models, the next question was: 'Can these effects be seen in humans with tolerable doses of AICAR?' In the first study of this nature, conducted by Cuthbertson et al., healthy human volunteers were infused with AICAR at a rate of 10 mg [kg body weight]$^{-1}$ h^{-1} [11]. This produced a muscle ZMP concentration of 68 μmol/kg dry weight. Values of 200–500 μmol/kg wet weight, achieved in rat hindlimbs perfused with 0.5–2 mmol/l AICAR, were found to be effective for activation of AMPK [4]. In rats injected subcutaneously with AICAR, the concentration of ZMP achieved in muscle was in the 1 mmol/l range [5]. The infusion rate of 10 mg [kg body weight]$^{-1}$ h^{-1} stimulated a significant increase in infused 2-deoxyglucose uptake by muscle, without a detectable increase in AMPK levels. It is conceivable that the phosphorylated fraction of AMPK was allosterically activated by ZMP. This would not be detected either in the phosphorylated AMPK (pAMPK) western blot or in the AMPK activity assays that are conducted in the presence of saturating amounts of AMP. Phosphorylation of extracellular signal-regulated protein kinase 1/2 (ERK1/2) was increased in response to AICAR infusion. However, no cause–effect relationship could be established between 2-deoxyglucose uptake and ERK1/2 activation.

Major findings of the study conducted by Boon et al.

In the most recent study conducted by Boon et al. [12], the results of which are published in this issue of *Diabetologia*, patients with type 2 diabetes were infused with AICAR or saline at a rate of 45 mg [kg body weight]$^{-1}$ h^{-1}, a rate 3.5-fold higher than that used by Cuthbertson et al. [11]. This infusion rate produced a maximal plasma AICAR concentration of 0.16 mmol/l. Plasma glucose rates of appearance (R_a) and disappearance (R_d) were quantified using stable

isotope methodology. At this infusion rate, no effects were noted on R_d, but R_a was reduced during AICAR infusion, resulting in a greater rate of decline in blood glucose. The level of pAMPK in muscle biopsy samples was not changed, but the level of phosphorylated ACC was increased, possibly indicating that the prevailing pAMPK fraction was allosterically activated by ZMP. The AICAR-induced increase in blood lactate concentration could have resulted from increased glucose uptake by muscle and increased glycolytic flux or from muscle glycogenolysis, but there was no strong evidence for stimulation of glucose uptake by muscle. In rat studies, muscle glycogen was increased, not decreased after AICAR injection [5]. It is possible that glucose uptake was increased in muscle, but decreased in other tissues, resulting in a stable whole body R_d during the AICAR infusion. It appears that the major effect of AICAR infusion in this study was on the liver, decreasing the rate of glucose production. This effect on the liver could conceivably have resulted from AMPK-independent inhibition of fructose-1,6-bisphosphatase (a gluconeogenic enzyme) by ZMP [13]. NEFA R_a and R_d were both reduced in response to AICAR infusion, but R_d remained slightly higher than R_a, with the consequence that the plasma NEFA concentration decreased considerably. Although the available data do not permit the prediction of the mechanism involved, the authors suggested that an increased uptake and oxidation of fatty acids by the liver and inhibition of lipolysis in adipose tissue could be responsible.

Where do we go from here?

The work by Boon et al. represents a very important step in the development of AMPK activators for the treatment of type 2 diabetes. Glucose production was reduced in response to these relatively low doses of AICAR. The fact that this infusion rate is well tolerated by the patients with no untoward side effects is also encouraging. It would certainly be of interest to see whether doses effective in producing a detectable increase in muscle AMPK would be more effective in normalising glucoregulation in patients with type 2 diabetes. Some of the pharmaceutical agents (metformin and the thiazolidinediones) currently used for the treatment of type 2 diabetes have been shown to be AMPK activators, but it is questionable whether the concentrations required for AMPK activation in tissue incubation studies are actually achieved in muscle and liver of patients at pharmacological doses. A new AMPK activator (A-769662, a thienopyridone) that is effective in activating AMPK in the low micromolar range (EC_{50}= 0.8 μmol/l) has recently been developed. Treatment of *ob/ob* mice with A-769662 (30 mg/kg) decreased plasma glucose levels by 40% and decreased plasma and liver triacylglycerol levels [14].

The ideal AMPK activator will have several attributes. The drug will activate AMPK at a much lower concentration than AICAR does, and this activation will only occur in specific target tissues, such as liver, adipose tissue and skeletal muscle. The activator will be effective when administered by the oral route and will have minimal and tolerable side effects, even when used in the long term. It should be noted that exercise specifically activates AMPK in muscle and heart, and perhaps in liver at high work rates. For those who are able to exercise, it is not necessary to wait for the development of more potent AMPK activators to launch this strategy. The beneficial effects of exercise for the prevention and treatment of type 2 diabetes are well documented [15].

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References

1. Hardie DG (2007) AMP-activated protein kinase as a drug target. Annu Rev Pharmacol Toxicol 47:185–210
2. Winder WW, Thomson DM (2008) AMPK, the master switch for type 2 diabetes? In: Hawley JA, Zierath JR (eds) Physical activity and type 2 diabetes. Human Kinetics, Champaign, pp 177–185
3. Winder WW, Hardie DG (1999) AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. Am J Physiol 277:E1–E10
4. Merrill GF, Kurth EJ, Hardie DG, Winder WW (1997) AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. Am J Physiol 273:E1107–E1112
5. Holmes BF, Kurth-Kraczek EJ, Winder WW (1999) Chronic activation of 5′-AMP-activated protein kinase increases GLUT-4, hexokinase, and glycogen in muscle. J Appl Physiol 87:1990–1995
6. Buhl ES, Jessen N, Pold R et al (2002) Long-term AICAR administration reduces metabolic disturbances and lowers blood pressure in rats displaying features of the insulin resistance syndrome. Diabetes 51:2199–2206
7. Pold R, Jensen LS, Jessen N et al (2005) Long-term AICAR administration and exercise prevents diabetes in ZDF rats. Diabetes 54:928–934
8. Bergeron R, Previs SF, Cline GW et al (2001) Effect of 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside infusion on in vivo glucose and lipid metabolism in lean and obese Zucker rats. Diabetes 50:1076–1082
9. Song XM, Fiedler M, Galuska D et al (2002) 5-Aminoimidazole-4-carboxamide ribonucleoside treatment improves glucose homeostasis in insulin-resistant diabetic (ob/ob) mice. Diabetologia 45:56–65
10. Fiedler M, Zierath JR, Selen G, Wallberg-Henriksson H, Liang Y, Sakariassen KS (2001) 5-Aminoimidazole-4-carboxy-amide-1-β-D-ribofuranoside treatment ameliorates hyperglycaemia and hyperinsulinaemia but not dyslipidaemia in KKAy-CETP mice. Diabetologia 44:2180–2186

11. Cuthbertson DJ, Babraj JA, Mustard KJ et al (2007) 5-Amino-imidazole-4-carboxamide 1-β-D-ribofuranoside acutely stimulates skeletal muscle 2-deoxyglucose uptake in healthy men. Diabetes 56:2078–2084
12. Boon H, Bosselaar M, Praet SFE et al (2008) Intravenous AICAR administration reduces hepatic glucose output and inhibits whole body lipolysis in type 2 diabetic patients. Diabetologia doi:10.1007/s00125-008-1108-7
13. Vincent MF, Erion MD, Gruber HE, Van den Berghe G (1996) Hypoglycaemic effect of AICAriboside in mice. Diabetologia 39:1148–1155
14. Cool B, Zinker B, Chiou W et al (2006) Identification and characterization of a small molecule AMPK activator that treats key components of type 2 diabetes and the metabolic syndrome. Cell Metab 3:403–416
15. Hawley JA, Zierath JR (2008) Physical activity and type 2 diabetes. Human Kinetics, Champaign