

Potential role of the AMP-activated protein kinase in regulation of insulin action

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Abstract

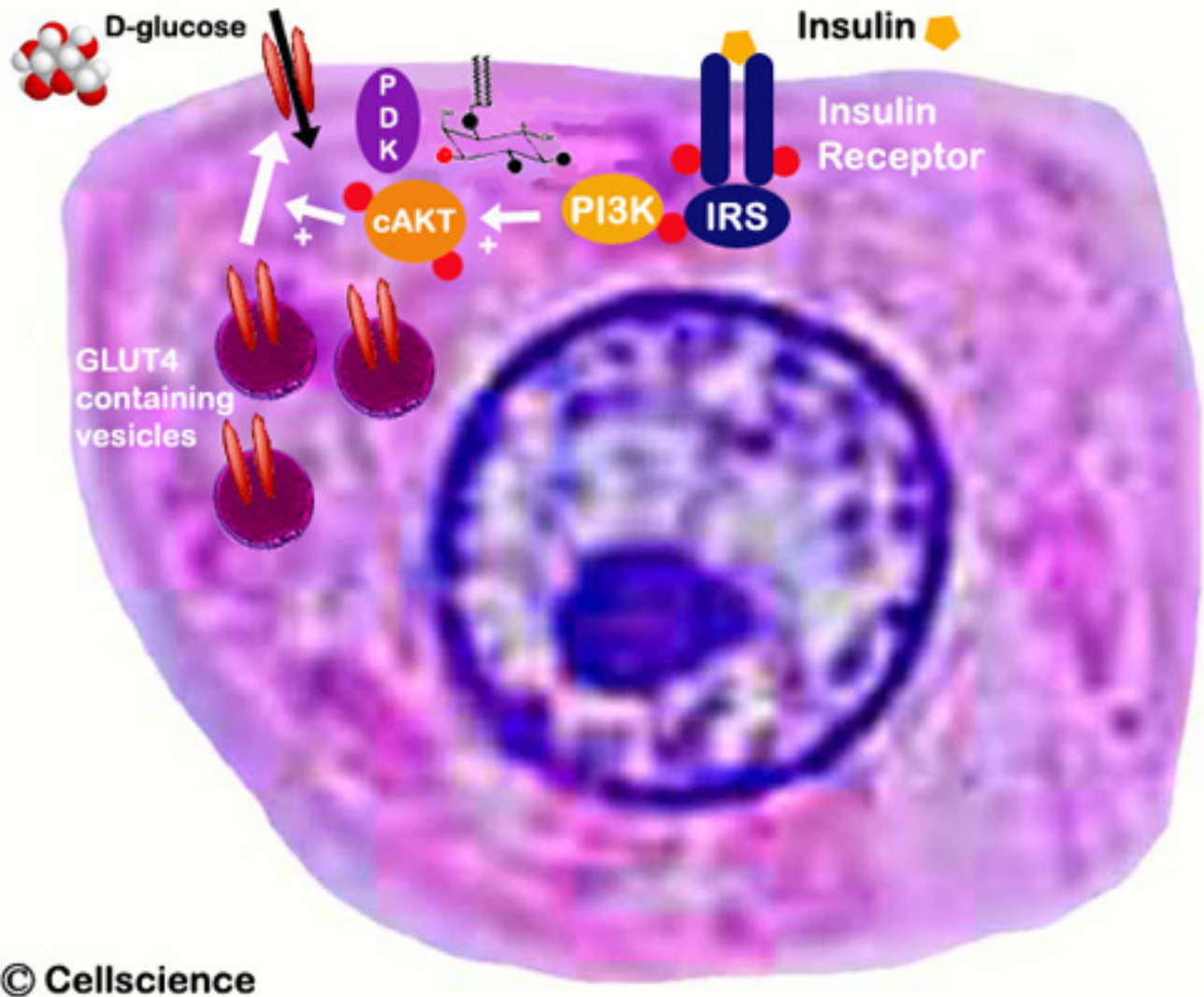
Because of the predominant role of skeletal muscle in insulin-stimulated clearance of blood glucose, understanding mechanisms for increasing the ability of muscle to respond to insulin could potentially lead to novel strategies for treatment or prevention of diabetes. Recently, the AMP-activated protein kinase (AMPK), a heterotrimeric serine/threonine kinase, has emerged as a promising candidate for the potentiation of insulin action. Several antidiabetic drugs have been shown to activate AMPK, cellular stresses such as exercise that increase AMPK activity also increase insulin action, and several downstream targets of AMPK seem to be involved in regulation of insulin action. Although the picture is currently incomplete, it seems possible that AMPK or one of its effectors is a positive regulator of insulin-stimulated glucose transport. In addition to a discussion of the latest literature regarding AMPK and insulin action, this review

includes a non-technical summary for students, academics from other fields, interested professionals, and the general public.

Introduction

Skeletal muscle is the primary depot for glucose cleared from the blood in the presence of insulin, and the resistance of skeletal muscle to the stimulation of glucose transport by insulin is a central characteristic of adult onset diabetes (DeFronzo, 1988; Petersen *et al.*, 1998; Cline *et al.*, 1999). Understanding mechanisms for increasing insulin sensitivity in skeletal muscle could elucidate potential therapeutic targets in the prevention or treatment of diabetes.

Leading authorities on the effects of insulin and metabolic stress on energy metabolism and glucose transport have published comprehensive reviews on these topics which provide a background for the central issue of the current review - the synergistic actions of AMPK and insulin.



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Figure 1. Some components of insulin signaling leading to glucose transport. Binding of insulin to its receptor starts a docking and phosphorylation (red circles) cascade involving insulin receptor substrate (IRS) and Akt and leads to stimulation of glucose transport. Phosphatidylinositol 3-kinase (PI3K) is a lipid kinase that phosphorylates membrane phosphatidylinositol, allowing recruitment of Akt to the membrane for subsequent phosphorylation and activation of Akt by phosphoinositide dependent kinases (PDKs). See reviews cited below for details.

Insulin stimulates glucose transport in skeletal muscle (Fig.1) by increasing cell surface localization of the glucose transporter GLUT4 and possibly by increasing the activity of individual transport proteins

(reviewed in Cushman *et al.*, 1998; Michelle, Poon, & Klip, 2003; Krook, Wallberg-Henriksson, & Zierath, 2004; Thong, Dugani, & Klip, 2005). Cellular stresses, including (but not limited to) exercise or muscle contractions, incubation of muscle in hyperosmotic medium, mitochondrial uncoupling, inhibition of cellular respiration, and hypoxic conditions, stimulate glucose transport in muscle in an insulin-independent fashion (Hayashi *et al.*, 2000). This insulin-independent stimulation of glucose transport appears to involve activation of AMPK, although (at least for the case of muscle contractions) there is a growing pool of evidence that AMPK may not be necessary for, or may only partially mediate these effects (Mu, Barton, & Birnbaum, 2003; Fujii, Aschenbach, Musi, Hirshman, & Goodyear, 2004; Fujii *et al.*, 2005; Jorgensen *et al.*, 2004). AMPK, introduced to the field of muscle glucose transport in 1998 by William Winder's and Grahame Hardie's groups (Merrill, Kurth, Hardie, & Winder, 1997) seems to be a metabolic fuel gauge that acts to combat cellular stress by coordinating suppression of non-essential ATP-dependent pathways and increasing means for ATP production (reviewed in Fryer & Carling, 2005; Carling, 2004; Winder & Hardie, 1999; Hardie, 2003).

Synergistic effects of cellular stress and insulin on glucose transport

It has been known for more than 20 years that insulin and cellular stress act synergistically in the stimulation of glucose transport. For example, DeFronzo *et al* found in 1981 that the stimulatory effect of insulin on glucose transport was much greater in exercising muscle than in resting muscle (DeFronzo, Ferrannini, Sato, Felig, & Wahren, 1981). Shortly afterward, Richter *et al* firmly established that exercise acutely increases insulin sensitivity in muscle (Richter, Garetto, Goodman, & Ruderman, 1982). For example, within 30 min after exercise, the stimulatory effect of low physiological insulin concentrations on glucose transport in skeletal muscle roughly doubles compared to effects in non-exercised muscle (Richter *et al.*, 1982).

The increased insulin sensitivity after exercise remains up to 18 hours (Cartee *et al.*, 1989) or until glycogen supercompensation occurs, and because of its prolonged presence is likely to be responsible for a large portion of the exercise effect on glucose transport into muscle.

Surprisingly, although a few candidate mechanisms for the exercise effect on potentiation of insulin action have come to prominence (*reviewed in* Wojtaszewski, Nielsen, & Richter, 2002; Holloszy, 2005; Christ-Roberts & Mandarino, 2004), the phenomenon seems somewhat understudied in comparison to its potential importance to the understanding of factors that increase insulin action.

Other beneficial effects of exercise that would lead to increases in insulin action occur on a longer time scale than the acute increase in insulin action after exercise. For example, there is a well-known exercise training effect that leads to increased expression of glucose transporters in skeletal muscle (Gulve & Spina, 1995). Additionally, exercise training is associated with decreased body fat content and increased insulin action (Arciero, Vukovich, Holloszy, Racette, & Kohrt, 1999). These long-term mechanisms for potentiation of insulin action (i.e. training effects, as opposed to immediate influences) will not be dealt with further in this review.

Is there a role for AMPK in the potentiation of insulin action?

A recently-developed hypothesis is that activation of AMPK increases sensitivity to insulin (Fig.2). For example, several agents that normalize blood glucose concentrations and/or improve insulin action, including the adipokines adiponectin (Yamauchi *et al.*, 2002) and leptin (Minokoshi *et al.*, 2002), metformin, (Zhou *et al.*, 2001), phenformin (Lizcano *et al.*, 2004), creatine (Ju, Smith, Oppelt, & Fisher, 2004), rosiglitazone (Fryer, Parbu-Patel, & Carling, 2002), troglitazone (Konrad *et al.*, 2005), and α -lipoic acid (Lee *et al.*, 2005)

have been shown to activate or phosphorylate AMPK. It's tempting to hypothesize that at least a portion of the effects of these agents are mediated through AMPK activation. Intriguingly, the AMPK activator AICAR (an adenosine analog) prevents insulin resistance associated with prolonged hyperglycemia (Kawanaka, Han, Gao, Nolte, & Holloszy, 2001).

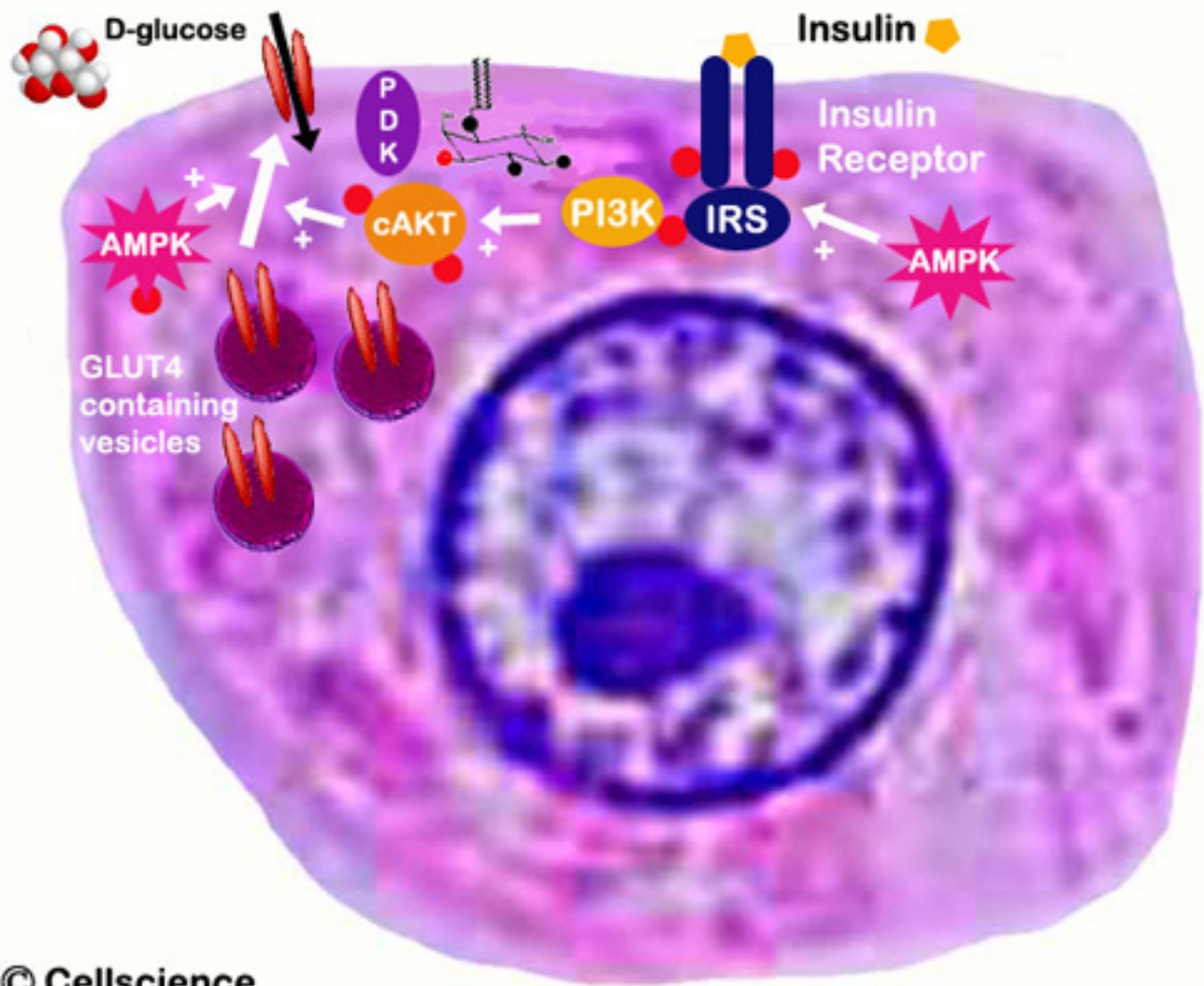


Figure 2. Is AMPK involved in the potentiation of insulin action?

Because several cellular stresses, chemical agents, and adipokines activate AMPK and also normalize blood glucose concentrations and/or improve insulin action, it seems possible that AMPK (shown activated by phosphorylation) could play a role in regulation of insulin action.

Two research groups independently found that AICAR, an AMPK activator, increases insulin sensitivity in skeletal muscle (Fisher, Gao, Han, Holloszy, & Nolte, 2002; Iglesias *et al.*, 2002). For the case of isolated muscle, the presence of serum during treatment of the tissue with AICAR is a requirement for the subsequent increase in insulin action (Fisher *et al.*, 2002). This requirement for serum is also necessary for induction of insulin sensitivity by hypoxia and muscle contractions (Gao, Gulve, & Holloszy, 1994; Fisher *et al.*, 2002), though the mechanism for the permissive effect of serum remains a mystery. Because hypoxia, exercise, muscle contractions, and AICAR all produce increases in insulin action and all activate AMPK, it seems possible that AMPK could be a mediator of increased insulin action (Fisher *et al.*, 2002). The exercise-related increase in insulin action is not dependent upon the synthesis of new proteins - it happens in a matter of a few hours and persists in the presence of the protein synthesis inhibitor cycloheximide (Fisher *et al.*, 2002). Thus, if AICAR acts through the same pathways as exercise to increase insulin sensitivity, AICAR effects on insulin action are most likely separate from the known effects of AICAR on increasing expression of the glucose transport and phosphorylation machinery (Ojuka, Nolte, & Holloszy, 2000; Holmes, Kurth-Kraczek, & Winder, 1999).

Unfortunately, AICAR is not necessarily a specific activator of AMPK. AICAR stimulates AMPK after the intracellular phosphorylation of AICAR to ZMP, an AMP analog (Corton, Gillespie, Hawley, & Hardie, 1995). Increased ZMP concentrations in AICAR-treated tissues could affect activities of any AMP-regulated enzymes and would not be limited to activation of AMPK. The recent characterizations of about a dozen AMPK-related kinases (Lizcano *et al.*, 2004), at least some of which are activated by AMP (Suzuki *et al.*, 2003; Lefebvre *et al.*, 2001; Lefebvre & Rosen, 2005), provides fertile ground for future study. A world-leading group of researchers on AMPK and its activation has not been able to find activation of AMPK-related kinases by AICAR, phenformin, or muscle contractions

(Sakamoto, Goransson, Hardie, & Alessi, 2004). However, researchers in my laboratory have found that AMPK-related kinase 5 (ARK5), also known as Nua Kinase 1 (Nuak1) is expressed in muscle and appears to be phosphorylated by Akt after exposure of muscle to insulin (Fisher *et al.*, 2005). ARK5 is so far the only AMPK-related kinase that has been reported to be activated by Akt (Suzuki *et al.*, 2003; Suzuki *et al.*, 2004). Whether or not ARK5 is expressed in muscle and is activated by cellular stress or insulin remains an open question, though at this time opinion should probably lean toward the careful and comprehensive study that demonstrated that in skeletal muscle AMPK itself is the only AMPK family member activated by AICAR, phenformin, and muscle contractions (Sakamoto *et al.*, 2004).

Jill L. Smith and Pankaj B. Patil recently worked out conditions for examining factors that regulate insulin action in C2C12 myotubes (Smith J.L., Patil P.B., & Fisher J.S., 2005). Some groups (*e.g.* Tortorella & Pilch, 2002) have not found C2C12 myotubes to be useful for studying insulin action. However, in our laboratory, C2C12 myotubes contain GLUT4 and are insulin-responsive (in terms of glucose transport) in a GLUT4-dependent manner (Smith J.L. *et al.*, 2005; Smith J.L., Patil, Minter, Lipsitz, & Fisher J.S., 2005). Under our conditions with C2C12 myotubes, AICAR and exposure to hyperosmotic stress both potentiate stimulation of glucose transport by insulin (Smith J.L. *et al.*, 2005). There needs to be a recovery from the hyperosmotic stress before a potentiation of insulin action occurs. Compound C (a somewhat-specific AMPK inhibitor) and iodotubercidin (a general kinase inhibitor that is effective against AMPK) prevented the increase in insulin action caused by hyperosmotic stress. Smith *et al.* (Smith J.L. *et al.*, 2005) were unable to use these AMPK inhibitors to probe for a role of AMPK in AICAR-associated increases in insulin action, because Compound C prevents AICAR uptake and iodotubercidin inhibits adenosine kinase, which is necessary for the conversion of AICAR to ZMP.

What lies beyond AMPK?

Unfortunately, this appears to be the extent of information implicating AMPK in the acute regulation (i.e. probably not requiring changes in gene expression) of insulin action. There is even some compelling data demonstrating that AMPK activation is not sufficient to increase insulin action (Kim, Solis, Arias, & Cartee, 2003; Al Khalili, Krook, Zierath, & Cartee, 2004). These findings suggest that the activation of another signaling pathway in addition to activation of AMPK may be necessary to impact insulin action.

If AMPK is really involved in the potentiation of insulin action, there seems to be no shortage of potential downstream effectors of AMPK's effect on insulin sensitivity. A few of the known or probable AMPK targets, and this is not necessarily an exhaustive list (Hardie, 2003), that could potentially alter insulin action after AMPK activation include IRS-1, the p38 mitogen-activated protein kinase (p38), nitric oxide synthase (NOS), acetyl coenzyme A carboxylase (ACC), the mammalian target of rapamycin (mTOR), and the 160 kDa Akt substrate protein (AS160).

IRS-1. AMPK phosphorylates S789 of IRS-1 *in vitro*, and in myotubes AICAR-stimulated phosphorylation of IRS-1 on S789 is reportedly associated with increased insulin-stimulated PI3K activity (Jakobsen *et al.*, 2001). However, the role of S789 phosphorylation of IRS-1 in insulin action is unclear. For example, phosphorylation of the same site seems to be associated with insulin resistance in liver (Qiao *et al.*, 2002). Interestingly, *in vitro* and in adipocytes, the site (or its equivalent) is phosphorylated by salt-inducible kinase 2 (SIK2), an AMPK-related kinase, and there is increased SIK2 expression in white fat of diabetic animals (Horike *et al.*, 2003).

p38. p38 has been implicated in the activation of GLUT4 by insulin (Somwar *et al.*, 2001; Somwar *et al.*, 2002), though this point remains

controversial (Turban *et al.*, 2005). Anisomycin, an activator of p38, has been reported to induce insulin sensitivity in isolated rat skeletal muscle (Geiger *et al.*, 2004). Inhibitors of p38 have been found to prevent AICAR-stimulated glucose transport, suggesting that p38 lies downstream of AMPK and is important in regulation of glucose transport (Lemieux *et al.*, 2003; Xi, Han, & Zhang, 2001). Consistent with the hypothesis that p38 lies downstream of AMPK, a dominant negative p38 mutant has been reported to prevent AICAR-stimulated glucose transport (Xi *et al.*, 2001). Regardless of whether or not p38 is downstream of AMPK, it appears to be activated by several stimulators of AMPK, including AICAR, mitochondrial uncoupling, and hyperosmotic stress (Xi *et al.*, 2001; Lemieux *et al.*, 2003; Taha *et al.*, 1997). Thus, it is possible that p38 could be a downstream mediator of AMPK-induced insulin sensitivity or else a co-activator of insulin sensitivity along with AMPK.

NOS. A few lines of evidence suggest that NOS lies downstream of AMPK. For example, AICAR treatment of mouse myotubes increases NOS activity, and L-NAME (a NOS inhibitor) prevents AICAR-stimulated glucose transport for skeletal muscle both *in vitro* and *in vivo* (Fryer *et al.*, 2000; Shearer *et al.*, 2004). Sodium nitroprusside (SNP, an NO donor that would lead to activation of guanylate cyclase), and 8-Bromo-cGMP (Br-cGMP, a cell-permeable cGMP analog) mimic the effects of AICAR on stimulation of glucose transport, while LY83583 (a guanylate cyclase inhibitor) blocks the stimulatory effect of AICAR on glucose transport (Fryer *et al.*, 2000). The activating phosphorylation of neuronal NOS (nNOS) occurs in muscle during exercise (Chen *et al.*, 2003), and AICAR reportedly activates both nNOS and endothelial NOS (Fryer *et al.*, 2000; Chen *et al.*, 1999). NOS may also play a role in insulin-stimulated glucose transport in muscle (Roy, Perreault, & Marette, 1998) and therefore seems a potential candidate for the mediation of insulin sensitivity after activation of AMPK.

ACC. AMPK phosphorylates and inactivates ACC, an enzyme which catalyzes the conversion of acetyl coenzyme A (CoA) to malonyl CoA. It has been suggested that inhibition by malonyl CoA of the translocation of long chain fatty acyl (LCFA) groups from the cytosol to the mitochondrial matrix leads to the subsequent buildup of LCFA CoAs within the cytosol, the activation of protein kinase C, and the serine phosphorylation of IRS-1 associated with insulin resistance (Ruderman *et al.*, 1999; Saha *et al.*, 1997; Ruderman & Dean, 1998; Ruderman *et al.*, 1999; Ruderman & Prentki, 2004). For example, glucose infusion causes insulin resistance in muscle that is related to increased malonyl CoA concentrations (Kraegen *et al.*, 2005). Conversely, transgenic animals that lack the form of ACC that is predominant in skeletal muscle have greater insulin sensitivity than their wild-type littermates (Abu-Elheiga, Oh, Kordari, & Wakil, 2003). Likewise, long-term treatment of rats with an ACC inhibitor increases insulin action (Harwood, Jr. *et al.*, 2003; Harwood, Jr., 2004). However, it does not appear that any studies have been performed regarding the acute effects (i.e. within a few minutes or hours) of ACC inhibition on insulin action.

mTOR. The mammalian target of rapamycin has been implicated as a negative regulator of insulin action through its (or its downstream effector S6K's) serine phosphorylation of insulin receptor substrate-1 (IRS-1) (Ozes *et al.*, 2001; Li, DeFea, & Roth, 1999; Um *et al.*, 2004). Recently, two binding partners of mTOR which appear to control substrate specificity have been characterized (Sarbasov *et al.*, 2005; Sarbasov *et al.*, 2004; Kim *et al.*, 2002). When mTOR is bound to raptor (rapamycin sensitive partner of mTOR), it is involved in phosphorylation of IRS-1. In contrast, when mTOR is associated with rictor (rapamycin insensitive companion of mTOR), serine 473 of Akt is one of its targets (Sarbasov *et al.*, 2005). Thus, mTOR•rictor is one of the PDKs shown in Figures 1 and 2. Phosphorylation of Akt on serine 473 is essential for the full activation of Akt (a key component of the insulin-signaling pathway, Sarbasov *et al.*, 2005), and

mTOR•rictor has been shown to be an insulin-stimulated Akt serine 473 kinase (Hresko & Mueckler, 2005). Thus, mTOR•rictor is a positive regulator of insulin action (through activation of Akt), while mTOR•rapTOR negatively affects insulin signaling (through serine phosphorylation of IRS-1). AMPK has been demonstrated to be an upstream negative regulator of mTOR (reviewed in Kahn *et al.*, 2005), so it seems likely that AMPK activation would decrease the serine phosphorylation of IRS-1 that impedes insulin action. Whether or not AMPK would differentially regulate mTOR•rictor and mTOR•rapTOR is an open question.

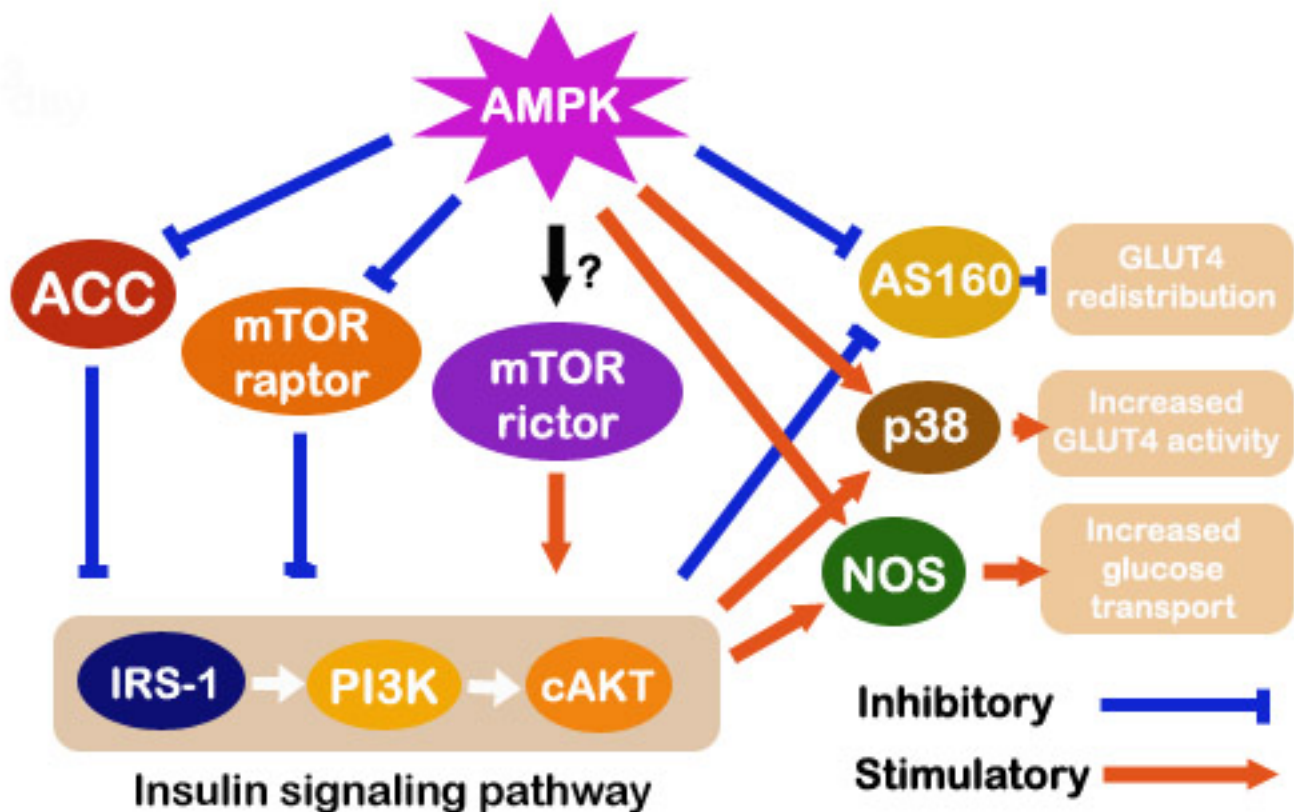


Figure 3. Some AMPK targets. NOS, p38, and AS160 appear to be regulated by both AMPK and insulin signaling, so it seems possible that they could mediate potential synergistic action between insulin signaling and AMPK. AMPK inhibits ACC, that may negatively impact insulin signaling through its production of malonyl CoA. AMPK is upstream of mTOR, that is a negative or positive modulator of insulin action, depending on its binding

partner.

AS160. Phosphorylation and inactivation of AS160, identified as an 160 kDa Rab GTPase activating protein that is an Akt target (Kane *et al.*, 2002), has been shown to be essential to stimulation of GLUT4 membrane localization by insulin in adipocytes (Sano *et al.*, 2003). While the mechanistic work characterizing the role of AS160 in GLUT4 trafficking has been performed in adipocytes (*e.g.* Eguez *et al.*, 2005; Larance *et al.*, 2005), it now appears that AS160 is phosphorylated in skeletal muscle in response to insulin, exercise, and treatment with AICAR (Brus *et al.*, 2005; Karlsson *et al.*, 2005; Plomgaard *et al.*, 2005). AS160's regulation by multiple pathways (*i.e.* insulin, muscle contractions, and AICAR) and its characterized role in regulation of glucose transport place it at the top of the list as a potential candidate in the control of insulin sensitivity.

Conclusions

Is AMPK indeed involved in the regulation of insulin action? The evidence at this point is circumstantial. However, molecular tools such as siRNAs against AMPK, constitutively active AMPK, dominant negative forms of AMPK, and transgenic animals, will most certainly help to determine whether AMPK activation is sufficient, or even necessary for the potentiation of insulin action that is associated with cellular stress. If AMPK is found to regulate insulin-stimulated glucose transport, researchers in the field will have plenty of work ahead determining which of the multitude of downstream effectors of AMPK mediates the potentiation of insulin action by AMPK.

Non-technical summary

Insulin, a natural hormone which is secreted into the bloodstream after the consumption of carbohydrate rich meals, causes some tissues, including skeletal muscle (the muscles that make the body move), fat,

and heart, to increase their uptake of glucose (blood sugar). Of these tissues, skeletal muscle is by far the most massive, comprising approximately 40% of body weight. The majority of glucose cleared from the bloodstream in response to insulin is stored in muscle. Therefore, muscle is of primary importance in the insulin-related control of blood glucose levels. Muscle that does not react properly to insulin often underlies the increased blood glucose concentrations which are the hallmark of diabetes.

It has been known for over two decades that a short time after exercise insulin works much better at stimulating glucose transport into muscle. Recently, evidence has emerged that a protein called the "AMP-activated protein kinase" (AMPK) could be responsible for increasing the sensitivity of muscle to insulin. AMPK appears to be a cellular fuel gauge that senses metabolic stresses or nutritional deficiency and subsequently controls several cellular processes to muster fuel resources and conserve energy. For example, it appears that AMPK can increase glucose transport into muscle but also increase the rate of fat burning by muscle. AMPK appears to activate several signaling pathways in muscle that are also stimulated by insulin, so it is possible that AMPK and insulin together could have synergistic effects on the signaling events that lead to glucose transport. AMPK also seems to prevent the negative effects on insulin's action of another cellular fuel gauge that senses nutrient sufficiency. Finally, it seems possible that the positive effect of AMPK on insulin's ability to stimulate glucose transport may simply be a result of increased fat burning that clears fat molecules (that inhibit insulin action), out of muscle cells.

Exercise robustly potentiates the action of insulin. However, some people at risk of developing diabetes are unable to exercise. Understanding the cellular mechanisms for increased sugar uptake into muscle is an important first step in developing drug strategies for treating or preventing diabetes when exercise is not a viable option.

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