

Akt/PKB activation and insulin signaling: a novel insulin signaling pathway in the treatment of type 2 diabetes

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Abstract: Type 2 diabetes is a metabolic disease categorized primarily by reduced insulin sensitivity, β -cell dysfunction, and elevated hepatic glucose production. Treatments reducing hyperglycemia and the secondary complications that result from these dysfunctions are being sought after. Two distinct pathways encourage glucose transport activity in skeletal muscle, ie, the contraction-stimulated pathway reliant on $\text{Ca}^{2+}/5'$ -monophosphate-activated protein kinase (AMPK)-dependent mechanisms and an insulin-dependent pathway activated via upregulation of serine/threonine protein kinase Akt/PKB. Metformin is an established treatment for type 2 diabetes due to its ability to increase peripheral glucose uptake while reducing hepatic glucose production in an AMPK-dependent manner. Peripheral insulin action is reduced in type 2 diabetics whereas AMPK signaling remains largely intact. This paper firstly reviews AMPK and its role in glucose uptake and then focuses on a novel mechanism known to operate via an insulin-dependent pathway. Inositol hexakisphosphate (IP6) kinase 1 (IP6K1) produces a pyrophosphate group at the position of IP6 to generate a further inositol pyrophosphate, ie, diphosphoinositol pentakisphosphate (IP7). IP7 binds with Akt/PKB at its pleckstrin homology domain, preventing interaction with phosphatidylinositol 3,4,5-trisphosphate, and therefore reducing Akt/PKB membrane translocation and insulin-stimulated glucose uptake. Novel evidence suggesting a reduction in IP7 production via IP6K1 inhibition represents an exciting therapeutic avenue in the treatment of insulin resistance. Metformin-induced activation of AMPK is a key current intervention in the management of type 2 diabetes. However, this treatment does not seem to improve peripheral insulin resistance. In light of this evidence, we suggest that inhibition of IP6K1 may increase insulin sensitivity and provide a novel research direction in the treatment of insulin resistance.

Keywords: type 2 diabetes, insulin resistance, Akt/PKB, $5'$ -monophosphate-activated protein kinase

Metabolic dysfunction in type 2 diabetes

Type 2 diabetes is a multifactorial metabolic disease characterized by defects in β -cell function and insulin action and increased hepatic glucose production.¹ Metabolic dysfunction in type 2 diabetes is also the product of reduced glucose effectiveness or the ability of glucose to transport itself by a mass action effect.² Central to this metabolic condition is altered glucose and lipid metabolism resulting from the combined effects of insulin resistance in skeletal muscle, hepatic, renal, and adipose tissue. The resulting hyperglycemia is the primary cause of the secondary complications associated with type 2 diabetes. Thus, treatments that target glucose uptake while reducing gluconeogenesis are key in the management of type 2 diabetes.

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Faced with an increased prevalence, which is predicted to reach 6.1% of the world's population by 2025,¹ advances in the treatment of type 2 diabetes are clearly being sought after. There are three main approaches to treatment of diabetes, ie, lifestyle modifications requiring patient-led metabolic responsibility, gastric bypass surgery, and pharmaceutical interventions. A current and effective target for the pharmaceutical approach to treatment of type 2 diabetes centers around upregulation of the metabolic master switch, ie, 5'-monophosphate-activated protein kinase (AMPK),³ with the use of metformin.

AMPK: a current target in management of type 2 diabetes

AMPK is a key regulatory protein ubiquitously expressed in cells throughout the human body. In mammalian tissues, AMPK is a heterotrimer consisting of a catalytic α subunit with two further regulatory subunits, β and γ . Each of these subunits has two or more isoforms.^{4,5} The γ subunit consists of 4-cystathionine- β -synthase domains, which are required for binding of adenosine triphosphate (ATP) or adenosine monophosphate (AMP). The binding of two AMPs to the γ subunit leads to conformational changes in the complex that result in activation of AMPK through exposure and phosphorylation of the Thr¹⁷² binding site on the catalytic α subunit.^{6,7} Binding of ATP to the γ subunit operates in an opposing manner by decreasing AMPK activity, while the binding of adenosine diphosphate (ADP) to the regulatory γ subunit protects AMPK against dephosphorylation.⁸

A working hypothesis is that upon stimulation, AMPK mediates contraction-stimulated GLUT-4 translocation and glucose uptake in skeletal muscle via phosphorylation of the Rab guanosine triphosphatase (GTPase)-activating protein (GAP), AS160, in a manner similar to insulin.^{9–11} AS160 is a proposed downstream target of AMPK and it is suggested that phosphorylation of AS160 by AMPK results in decreased Rab-GTPase activity, leading to an increase in GTP loading on intracellular GLUT-4 storage sites, promoting GLUT-4 translocation and cell membrane fusion while stimulating glucose uptake in adipocytes and skeletal muscle.^{12–14}

Using an *in vivo* electroporation/gene delivery technique that prevents phosphorylation on four regulatory phospho-Akt substrate sites on AS160, Kramer et al⁹ demonstrated that both insulin-stimulated and contraction-stimulated glucose transport was impaired in skeletal muscle (tibialis anterior). In the same study, overexpression of mutant AS160 with abolished Rab-GAP activity showed normal elevations in contraction-stimulated glucose uptake.⁹ Furthermore, transgenic mouse

models lacking AMPK α 2 activity in muscle show complete inhibition of AS160 phosphorylation and glucose transport on stimulation with the AMPK activator, 5-aminoimidazole-4-carboxamide ribonucleoside. Taken together, these data suggest a prominent role of both AMPK and AS160 in glucose transport, uptake, and utilization.¹⁵

Metformin is extensively used in the treatment and management of type 2 diabetes. Metformin improves glycemic control primarily via suppression of hepatic glucose production, and to a lesser extent, but still metabolically important, increased peripheral glucose uptake.¹⁶ This pharmaceutical agent activates hepatocyte-specific AMPK, resulting in reduced acetyl-CoA carboxylase activity, increased fatty acid oxidation, and suppression of lipogenic enzyme expression.¹⁶ However, recent work suggests that inhibition of gluconeogenesis by metformin acts independently of the AMPK pathway because hepatic glucose production remains blunted in AMPK-depleted hepatocytes despite treatment with metformin.¹⁷

Metformin is also known to activate AMPK and stimulate glucose uptake in isolated rodent skeletal muscle, presumably via the ability of metformin to increase the intracellular AMP/ATP ratio.¹⁸ In addition, administration of metformin is known to increase AMPK activity in human skeletal muscle, promote GLUT-4 membrane translocation, and stimulate insulin-independent glucose uptake,¹⁸ in a manner similar to muscle contraction. Although inhibition of hepatic glucose production by metformin is considered to be the primary mechanism by which AMPK lowers hyperglycemia, its ability to increase glucose uptake in peripheral tissue is no less important. This action is of particular relevance given that peripheral insulin resistance is considered to occur prior to hepatic insulin resistance.¹⁹ However, it has been noted in more recent work using a high-fat mouse model that insulin resistance in the liver precedes reduced insulin action in skeletal muscle.²⁰ Recent work carried out in type 2 diabetic humans found that administration of metformin 2,550 mg/day for 3–4 months increased glucose disposal but did not alter insulin receptor substrate-1, class IA phosphatidylinositol-3 (PI3) kinase, or Akt/protein kinase B (PKB) activity in skeletal muscle.²¹ These results suggest that metformin can stimulate glucose transport activity in type 2 diabetics, but does so via a mechanism that is independent of the traditional insulin signaling pathway in skeletal muscle.

Pathways in type 2 diabetes stimulated by insulin or contraction

When forming an understanding of the progression of this disease, it is important to recognize two points. First, peripheral

glucose uptake into skeletal muscle (the main disposal site for glucose) can be promoted via two distinct pathways, ie, insulin-dependent mechanisms resulting in recruitment and activation of Akt/PKB and contraction-mediated stimulation²² or hypoxia-mediated stimulation²³ of AMPK (Figure 1). It has been consistently shown that PI3 kinase is necessary for insulin-stimulated but not for contraction-stimulated glucose uptake,²⁴⁻²⁷ while Akt2 knockout mice demonstrate normal basal and contraction-stimulated glucose uptake.²⁸ Indeed, glucose transport is additive when either hypoxia or contractile activity are coupled with insulin, whereas hypoxia and contractile activity are not.^{29,30} In support of this is the observation that wortmannin, a selective inhibitor of PI3 kinase, completely blocks insulin-stimulated glucose transport but has no effect on contraction-mediated or hypoxia-mediated 2-deoxy-D-glucose uptake in rat skeletal muscle.²⁵

Thus, insulin-stimulated glucose transport is thought to occur independently of AMPK activity,^{31,32} and when defects in the Akt/PKB pathway occur, the likely outcome is peripheral insulin resistance.³³ In addition, it is worth noting that Akt/PKB and target proteins sitting upstream and downstream thereof are defective in type 2 diabetes whereas AMPK activation remains largely intact.^{23,34} This latter point is important because peripheral insulin resistance is a catalyst for

β -cell dysfunction, increased hepatic glucose production, and progression of the disease,³⁵ so any intervention that improves peripheral insulin resistance may conceivably reverse β -cell function and reduce hepatic glucose production.

Hypoxia and contractile activity are known stimulators of AMPK, and upon activation, AMPK is widely accepted to increase glucose transport activity and GLUT translocation via an insulin-independent mechanism. Azevedo et al³⁰ demonstrated that insulin stimulation of 2-deoxyglucose transport is impaired in insulin-resistant tissue. These investigators further demonstrated no differences in hypoxia-stimulated glucose transport rates between lean and obese diabetic groups, suggesting that this insulin-independent (or AMPK-dependent) pathway is intact in insulin-resistant muscle tissue.³⁰ The muscle protein content of AMPK α 1, α 2, and γ 3 is similar in type 2 diabetics when compared with healthy controls.²² In addition, no difference was found in basal phosphorylated AMPK activity when the same comparison was made between insulin-resistant and lean muscle tissue.²² Furthermore, the basal activity of AMPK α 1, α 2, and Thr¹⁷² AMPK- α phosphorylation are all normal in type 2 diabetes.^{35,36} In support of this, normal AMPK activity was found despite decreased insulin-stimulated glucose transport when measured during euglycemic-hyperinsulinemic clamp in the same population.³⁶

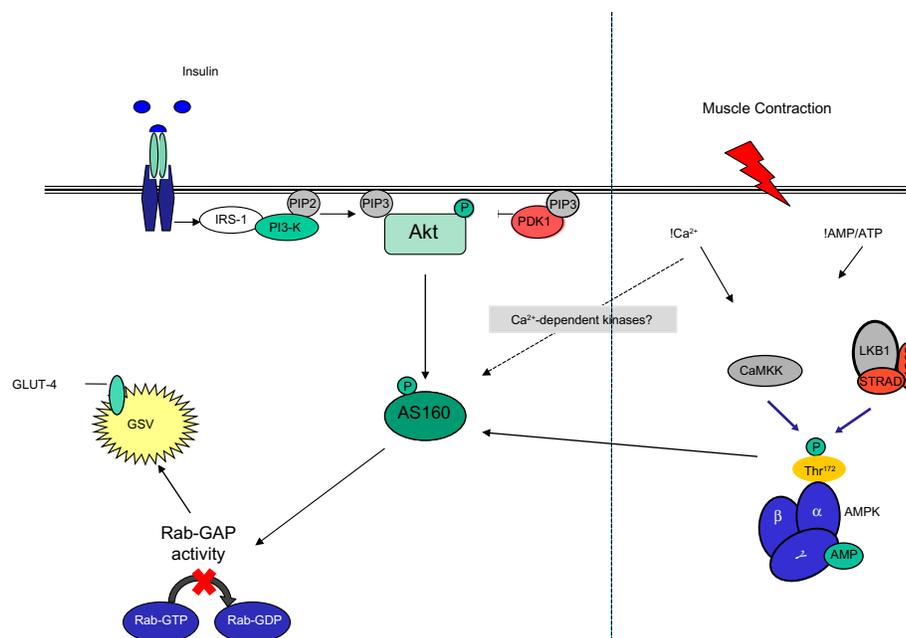


Figure 1 Insulin and contraction signaling pathways during GLUT-4 recruitment and translocation.

Note: Data from Sakamoto et al.¹⁴

Abbreviations: IRS, insulin receptor substrate; PI3-K, class IA phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol (4,5)-biphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PDK1, phosphoinositide-dependent protein kinase-1; Akt, serine/threonine protein kinase; AS160, 160 kDa Akt substrate; GLUT-4, glucose transporter 4; GSV, GLUT-4 storage vesicle; Rab-GAP, Rab-GTPase-activating protein; Rab-GDP, guanosine-50-diphosphate-loaded Rab; Rab-GTP, guanosine-50-triphosphate-loaded Rab; CaMKK, Ca²⁺/calmodulin-dependent protein kinase kinase; LKB1, Serine/threonine kinase 11; STRAD, putative kinase; MO25, mouse protein 25/scaffold protein; AMPK, 5'-monophosphate-activated protein kinase; Thr¹⁷², phosphorylated AMPK α at threonine 172; AMP, adenosine monophosphate; ATP, adenosine triphosphate; P, phosphorylated site.

The data reported by Højlund et al,³⁶ along with the finding that type 2 diabetics have normal AMPK activation during exercise³⁵ while showing metformin-induced increases in AMPK phosphorylation,³⁴ suggest again that AMPK function is intact in type 2 diabetes. The conclusion drawn from the work of both Musi et al³⁴ and Højlund et al³⁶ is that normal AMPK activity and reduced insulin sensitivity are metabolic aspects of insulin resistance, indirectly supporting the notion that two separate pathways operate to facilitate glucose transport. Further, Højlund et al³⁶ also demonstrated that AMPK activity under basal conditions in human skeletal muscle is not regulated by normal physiological concentrations of insulin. These findings taken together may suggest that future therapeutic targets should focus not on AMPK specifically, but also on prominent proteins associated with the insulin-signaling pathway for the treatment of insulin resistance. However, the targeting of AMPK for insulin resistance cannot be completely disregarded, with McBride et al³⁷ proposing a novel role for the AMPK β subunit in glycogen sensing and synthesis. Further, AMPK α 2 knockout mice show whole body insulin resistance, suggesting that AMPK may play a role in modulating insulin sensitivity.³⁸ Insulin sensitivity is not altered in a dominant-negative mutant AMPK rodent model (Tg-KD-AMPK α 2),³⁹ although it is more susceptible to high-fat diet-induced insulin resistance,⁴⁰ suggesting that AMPK is not an important mediator of insulin sensitivity in skeletal muscle.⁴¹ The potential role of AMPK in insulin-stimulated glucose transport may be complicated by the recent findings from Sakamoto's laboratory.⁴² 5-aminoimidazole-4-carboxamide ribonucleoside-induced activation of AMPK in knockin mice expressing a glucose-6-phosphate-insensitive glycogen synthase mutant demonstrates elevated glycogen accumulation through allosteric activation of glycogen synthase, caused by elevated glucose uptake and intracellular glucose-6-phosphate levels. This evidence suggests that AMPK may be able to promote glycogen synthesis in skeletal muscle independent of the traditional insulin-signaling nexus.⁴² More work is clearly needed to identify how, or even if, AMPK affects insulin-induced glucose uptake, given that a direct mechanism via which AMPK is activated by insulin is yet to be determined.

AMPK is clearly a major target for the treatment of type 2 diabetes. However, AMPK activation is known to be largely normal in this population^{22,43} and provides a rationale for the prescription of exercise in the treatment of type 2 diabetes. Although AMPK activation produces a number of desirable effects in metabolically responsive tissue, it seems to bypass the problem, at least in skeletal muscle. It therefore

seems logical to focus future research on the activation/inhibition of signaling mechanisms known to be defective in insulin-resistant tissue in the pursuit of future pharmaceutical treatment.

Akt/PKB isoforms

Akt, also known as PKB, is a serine/threonine-specific protein kinase that plays a key role in multiple cellular processes, including cell growth, survival, proliferation, and metabolism.⁴⁴ Three homologous isoforms have been identified, ie, Akt1/PKB α , Akt2/PKB β , and Akt3/PKB γ .⁴⁵ Research conducted over the past decade has identified distinct roles for each isoform, with Akt1/PKB α linked to cell survival, Akt2/PKB β with cell-substrate metabolism,⁴⁶ and Akt3/PKB γ with brain development.⁴⁷

Akt1/PKB α is known to inhibit apoptosis via activation of NF- κ B and regulation of I κ B kinase, resulting in upregulation of prosurvival genes.⁴⁸ The same isoform is also implicated in stimulating skeletal muscle hypertrophy by promoting protein synthesis and inhibiting protein degradation pathways, providing evidence for its roles in muscle homeostasis.⁴⁹

Hyperglycemia and reduced glucose transport in muscle is present in knockout Akt2/PKB β mice,⁵⁰ but is not apparent with deletion of the Akt1/PKB α and Akt3/PKB γ isoforms. Involvement of the Akt3/PKB γ isoform in brain development comes from evidence examining Akt3 knockout mice, which shows reduced cerebral cell numbers and seemingly increased protein degradation and reduced synthesis. Further, the same Akt3/PKB γ knockout mice show diminished mTOR (mammalian target of rapamycin) signaling,⁵¹ highlighting its role in cell death and growth inhibition.

Akt/PKB activation

PI3 kinase, following upstream stimulation by the insulin receptor substrate, causes recruitment of phosphatidylinositol (4,5)-bisphosphate (PIP2) and production of the second lipid messenger, phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 then binds to the pleckstrin homology domain of Akt/PKB, allowing for its translocation and binding to the cell membrane. Following this membrane translocation, Akt/PKB can then be phosphorylated and activated⁵² by two further kinases, PDK1 and mTOR complex 2, within the T-loop of the catalytic domain (Thr³⁰⁸) and the carboxyl terminal hydrophobic domain (Ser⁴⁷³), respectively, resulting in phosphorylation of many downstream targets involved in cellular growth and metabolism.⁴⁴

Akt/PKB and insulin signaling

It is clear that Akt signaling plays a central role in insulin-stimulated glucose uptake in both muscle and adipose tissue while inhibiting glucose release from hepatocytes.⁵ The effect of insulin on glucose uptake in peripheral tissue via Akt/PKB is through its ability to translocate GLUTs to the cell membrane, thereby facilitating glucose uptake. Binding of insulin to its cell surface protein receptors causes subsequent tyrosine phosphorylation, resulting in phosphorylation of insulin receptor substrates on specific tyrosine residues and activation and recruitment of PI3 kinase and its downstream target Akt/PKB.⁵² The Akt substrate of 160 kDa (AS160), also known as TBC1D4, is an established candidate in Akt/PKB-induced GLUT-4 translocation in skeletal muscle. AS160 functionally maintains Rab-GTPase(s) in an inactive form via guanosine-50-diphosphate-loading, thereby retaining GLUT-4 within GLUT storage vesicles.¹⁴ Upon activation, Akt/PKB phosphorylates AS160, leading to a reduction in Rab-GAP activity, promoting GLUT-4 translocation and glucose uptake.^{14,53,54} Thus, any defects in the PI3 kinase/Akt/AS160 transduction pathway would ultimately reduce glucose uptake in skeletal muscle. Similarly, hepatic deletion of the Akt1 and Akt2 isoform causes glucose intolerance, insulin resistance, and a defective insulin transcriptional response to feeding in hepatocytes.⁵⁵

Akt/PKB and insulin resistance

Evidence from both human and animal work links insulin resistance with defects to both upstream and downstream targets of Akt/PKB in the form of dephosphorylation of protein side chains on insulin receptor substrates as well as complete loss of insulin receptor substrates from the cell surface membrane,⁵⁶ reduced PI3 kinase activity⁵⁷ and impaired phosphorylation of the Akt/PKB substrate, AS160, in type 2 diabetic skeletal muscle.⁵⁸ In addition, knockdown or depletion of the Akt2/PKB β isoform in mice causes insulin resistance and diabetic-like symptoms, with Akt2/PKB β knockout rodents also demonstrating hepatic insulin resistance.⁵⁹ This work has been extended to humans, with the finding that a mutation in the gene encoding Akt2/PKB β results in severe insulin resistance,⁶⁰ establishing Akt2/PKB β as a key protein in the maintenance of euglycemia, given that defects in this important mediator would presumably result in reduced AS160-induced GLUT translocation.

In substituting the AS160-Thr⁶⁴⁹ phosphorylation/14-3-3 binding residue with a nonphosphorylatable alanine, Chen et al were able to show that insulin-stimulated AS160 binding to 14-3-4 is completely abolished.¹¹ The same work

also showed that insulin-stimulated glucose uptake and cell surface GLUT-4 content were reduced in this mutated knockin model.¹¹ These results demonstrate the key role played by Akt/PKB and its downstream targets in insulin sensitivity and normal glucose tolerance, and may therefore represent an argument for its targeting as an intervention in the treatment of insulin resistance.

Inositol pyrophosphate and Akt/PKB signaling: targeting peripheral insulin resistance

Research in the field highlights Akt/PKB as a major signaling intermediate in insulin-stimulated glucose transport in type 2 diabetes characterized by reduced insulin signaling of Akt/PKB.^{45,58} Akt/PKB is known to upregulate S6 kinase 1 (S6K1) via activation of mTOR complex 1, leading to phosphorylation of the serine residues on insulin receptor substrate-1 and resulting in Akt/PKB inhibition and a reduction in insulin-stimulated glucose uptake.⁶¹ PDK1-mediated phosphorylation of Akt at Thr³⁰⁸ is dramatically increased by binding of PIP3 to the pleckstrin homology domain on Akt.⁶² Recent work from Chakraborty et al³³ suggests a separate and seemingly novel feedback mechanism in the regulation of insulin signaling, whereby specific inositol pyrophosphates can also have an inhibitory effect on insulin stimulation of Akt/PKB by competing with PIP3 at the pleckstrin homology domain.

Diphosphoinositol polyphosphates, also known as inositol pyrophosphates, are a family of water-soluble inositol phosphates.⁶³ The most notable of these is diphosphoinositol pentakisphosphate (IP7),⁶⁴ which has been implicated in insulin secretion⁶⁵ and peripheral insulin signaling (Figure 2).⁴⁵ Inositol pyrophosphates were first discovered in 1993 and have received a great deal of attention since due to their “high energy” pyrophosphate bond, which can be synthesized by a family of three inositol hexakisphosphate (IP6) kinases (IP6Ks).⁶⁶ The inositol hexakisphosphate kinase-1 (IP6K1) isoform produces a pyrophosphate group at the position of IP6 to generate a further inositol pyrophosphate, IP7. Production of IP7 results in its binding to the pleckstrin homology domain of Akt/PKB, preventing its translocation to the cell membrane and reducing its subsequent phosphorylation by PDK1. The evidence for this comes from the finding that IP7 fails to prevent PDK1 Thr³⁰⁸ phosphorylation of Akt/PKB lacking a pleckstrin homology domain.³³ The consequence of this is a reduction in insulin (Akt/PKB)-stimulated glucose uptake in muscle and adipose tissue (Figure 2), with a potential increase in accumulation of hepatic fat.³³

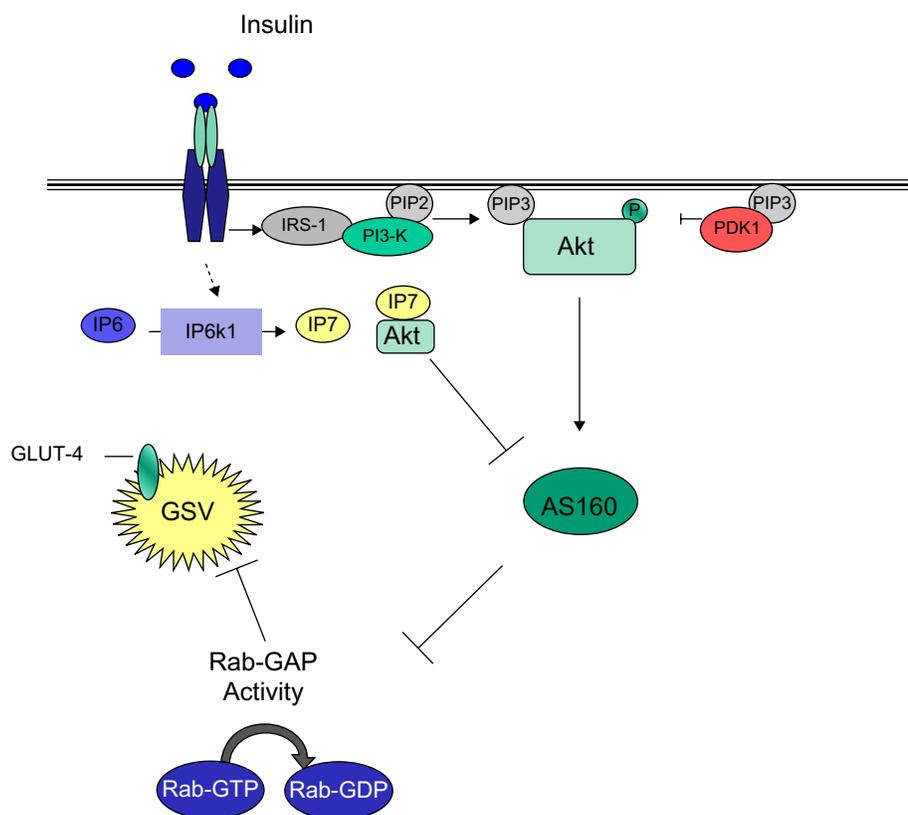


Figure 2 Inositol phosphates and Akt/PKB inhibition, a potential Akt/PKB feedback mechanism.

Note: Adapted from Cell, 143(6), Manning BD, Insulin signaling: inositol phosphates get into the Akt, 861–863. Copyright © 2010, with permission from Elsevier.

Abbreviations: IRS, insulin receptor substrate; PI3-K, class IA phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol (4,5)-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PDK1, phosphoinositide-dependent protein kinase-1; Akt, serine/threonine protein kinase; AS160, 160 kDa Akt substrate; IP6K1, inositol hexakisphosphate kinase 1; IP6, inositol hexakisphosphate; IP7, diphosphoinositol pentakisphosphate; GLUT-4, glucose transporter 4; GSV, GLUT-4 storage vesicle; Rab-GAP, Rab-GTPase-activating protein; Rab-GDP, guanosine-50-diphosphate-loaded Rab; Rab-GTP, guanosine-50-triphosphate-loaded Rab; P, phosphorylated site.

Unpublished data from the authors' laboratory shows that IP6K1 is detectable in human skeletal muscle and correlates with one-compartment measures of insulin sensitivity (S_I) in glucose-intolerant individuals. It is well known that calorie restriction in humans improves insulin sensitivity,⁶⁷ while starvation reduces IP7 formation in wild-type embryonic fibroblasts, again highlighting a potential mechanism whereby IP7 may play a role in insulin resistance.³³

Does inhibition of IP7 formation restore normal Akt? Implications for obesity and insulin resistance

It seems that depletion or chemical inhibition of IP6K1 may improve peripheral insulin action and hepatic function. Specific IP6K1 inhibitors have been shown, at least in cell culture, to reduce the conversion of IP6 to IP7, and so increase PIP3 binding to the pleckstrin homology domain of Akt/PKB.^{33,68} Padmanabhan et al⁶⁸ showed that the selective inhibitor, N2-(m-(trifluoromethyl)benzyl) N6-(p-nitrobenzyl) purine (TNP), reduces IP6K1 activity in a dose-dependent

manner in vitro while reducing IP7 synthesis in vivo, providing the potential for decreased IP7-Akt/PKB interaction and an increase in Akt/PKB membrane translocation. At least in the HeLa cell line, IP7 formation is returned to near baseline concentrations 2 hours post removal of TNP, suggesting that the action of TNP on IP6K1 activity is both rapid and reversible.⁶⁸ Using IP6K1 knockout hepatocytes,³³ a 60% reduction in IP7 and elevations in Akt/PKB and GSK3 β are seen in response to insulin stimulation. Similarly, these same hepatocytes show enhanced age-dependent phosphorylation of Akt at Thr³⁰⁸,³³ suggesting a role for IP7 in age-related insulin resistance, with either depletion or knockout resulting in improved glucose tolerance by a presumable decrease in hepatic glucose production. The work on IP6K1 knockout and improved hepatic insulin resistance is a novel research topic with very little current published work, so provides an exciting avenue for future research.

This finding has been extended in IP6K1 knockout mouse models which have elevated Akt/PKB activity in response to insulin stimulation, accompanied by increased

glucose transport rates in skeletal muscle.³³ IP6K1 mice also display diminished circulating insulin levels, suggesting increased insulin sensitivity or efficiency.⁶⁹ These data are of note because IP6K1 knockout mice have a two-fold increase in insulin sensitivity when compared with their wild-type counterparts, and phosphorylated Akt/PKB at sites Thr³⁰⁸ and Ser⁴⁷³ were both increased while phosphorylated GSK3 β was reduced in skeletal muscle.³³ This is an important discovery because phosphorylated GSK3 β Ser⁹ renders glycogen synthesis active, resulting in the potential for increased glucose uptake and glycogenesis. These data were accompanied by a significant elevation in muscle glycogen content in IP6K1 knockout models versus wild-type mice,³³ with low muscle glycogen content being an inherent metabolic characteristic of type 2 diabetes.⁷⁰ These data are important because they suggest that IP6K1 deletion may result in insulin hypersensitivity, a known physiological consequence of elevated Akt/PKB activity.⁷¹ Further, a reduction in IP7 by 10 μ M TNP increased phosphorylation of Akt/PKB at Thr³⁰⁸ without affecting phosphorylation of Ser⁴⁷³.³³ This latter finding is of clinical significance given that it is not likely that future pharmaceutical intervention acting via IP7 inhibition will negatively affect muscle growth and development through inhibition of mTOR signaling.

Interestingly, IP6K1 knockout mice also exhibit a lean phenotype,⁷² presumably as a result of increased fatty acid metabolism via β oxidation⁴⁵ and reduced adipogenesis.⁷³ Akt has been referred to as lipogenic, with double knockout of the Akt1 and Akt2 isoforms displaying reduced fat mass.⁷⁴ Thus, it seems that IP7 formation, as synthesized by IP6K1 in mice, is implicated in obesity and insulin resistance via inhibition of Akt and increased GSK3 β activity.³³ Indeed, increased Akt activity and resistance to obesity are defined characteristics in genetic models of insulin hypersensitivity (ie, protein phosphatase 1B, S6K1, and JNK mutants).^{33,75}

IP6K1 inhibition and β -cell function

Decreased β -cell function is critical to the development of overt type 2 diabetes.¹ IP6K1 knockout mice were found to have lower fasting serum insulin when compared with their wild-type counterparts, which the authors suggested represents a loss of β -cell function.⁶⁹ Depletion of IP6K1 seems to inhibit insulin exocytosis in β -cells,⁶⁹ while maintenance of IP7 levels in the same cell type may enhance insulin secretion.⁷⁶ Indeed, IP6K1 knockout rodents demonstrate a 65%–70% reduction in circulating insulin concentrations,⁶⁹ suggesting that mice lacking IP6K1 in their pancreatic β -cells have reduced insulin release. However, the mutant model

used by Bhandari et al⁶⁹ also showed signs of improved insulin-stimulated glucose uptake. The reduction in circulating insulin levels may therefore be a product of the hyperbolic relationship that exists between insulin sensitivity and insulin secretion,⁷⁷ ie, a lower insulin requirement due to a more efficient rate of glucose disposal. Further, IP6K1 knockout mice with low physiological insulin concentrations do not demonstrate diabetic characteristics, with normal fasting glucose and glycosylated hemoglobin levels, and show reduced body weight over a 10-week period⁶⁹ and resistance to obesity.³³ These findings are particularly exciting in light of the positive effect that inhibition of IP6K1 might have on the sensitivity of peripheral tissue to insulin via increased Akt/PKB activity. Inhibition of IP6K1 in β -cells requires more research because incubation of pancreatic Min6 cells with TNP results in direct inhibition of insulin release,⁶⁸ and more importantly, this reduction in insulin release is not a product of increased peripheral insulin action.

Targeting isoforms: β -cell function, cancer, and cell growth

The work of Bhandari et al⁶⁹ in a mouse model is clearly exciting, given that these authors assessed the effects of targeted depletion of the coding sequence of IP6K1 exon 6 on glucose control and insulin secretion. However, the IP6K1 mutant mice used in this study⁶⁹ were smaller than their wild-type counterparts despite the same food intake, suggesting a negative consequence for protein synthesis and cell growth. This is surprising, given that a reduction in IP7 using the IP6K1 inhibitor TNP does not affect phosphorylation of Akt/PKB at Ser⁴⁷³.³³ The work of Bhandari et al⁶⁹ may be countered by the knowledge that IP6K1 depletion may increase muscle protein synthesis while reducing fat accumulation,³³ decreasing adipogenesis via GSK3 β ,⁷⁸ and increasing Akt/PKB-induced fat oxidation.⁷⁹ These results offer indirect evidence that reduced animal size⁶⁹ may be due to increased fat loss rather than muscle atrophy, particularly given that increased p-Akt/PKB at Ser⁴⁷³, as seen in IP6K1 knockout mice,³³ may increase protein synthesis via mTOR activation.

Hyperactivity of Akt/PKB is a hallmark of most human forms of cancer, given that it modulates cell proliferation, growth, survival, and transit metabolism. In addition, overexpression of Akt/PKB is noted in various cancers and is possibly related to specific Akt/PKB isoforms.⁴⁴ Indeed, Akt1/PKB α is linked to breast cancer,⁸⁰ Akt2/PKB β is reported in pancreatic cancer,³³ and Akt3/PKB γ mutations are associated with human melanoma.⁸¹ A detailed description of Akt/PKB and its link to cancer is beyond the scope of this paper,

but this area has been comprehensively reviewed by others.⁴⁴ However, it is clear that blanket inhibition of IP6K1, with resulting hyperactivity of Akt/PKB in all cell types and/or in the whole body, cannot be advised at this stage.

Concluding remarks

Activation of AMPK has a clear beneficial effect in metabolically responsive tissue in type 2 diabetics. We suggest that future research and potential therapies, in parallel with continued research into the role of AMPK in glucose control, should be directed towards mechanisms known to be involved in insulin resistance. Recent published work provides exciting and novel data that may lead to a pharmaceutical agent capable of promoting insulin-stimulated glucose transport in insulin-resistant tissue via inhibition of IP6K1. Although early in development, use of IP6K1 inhibitors provides an interesting area of research in the fight against insulin resistance and type 2 diabetes. Due to the varied response of Akt/PKB in different tissue types and the resulting individual and/or combined effects this kinase may have on whole body metabolism, cell growth, and human cancers, blanket inhibition of IP6K1 and reduced production of IP7 is not recommended at this point. Indeed, while AMPK activity promotes growth of prostate cancer cells,⁸² use of metformin *in vivo* reduces the occurrence of pancreatic and hepatic malignancies.⁸³ Further, it is important to point out that even AMPK, the current target protein, which is induced by metformin, is implicated in some cancers,⁸² but use of metformin is inversely correlated with other types of cancer in a dose-dependent manner.⁸³

The introduction of Akt1/PKB α , Akt2/PKB β , and Akt3/PKB γ knockout rodents has suggested different physiological functions for specific isoforms, but there is now some evidence pointing towards compensatory effects in a given isoform when another is knocked out. In terms of insulin resistance, it appears that future work should focus on pharmaceutical inhibition of IP6K1 in established animal models of diabetes (for example, Ob/Ob) before human trials can be considered. Likewise, the roles of IP6K1 depletion in pancreatic β -cells and in insulin production and release, warrant further investigation.

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