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Akt isoforms and glucose homeostasis - the leptin connection

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Abstract

The serine/threonine kinase Akt, also known as protein kinase B (PKB), has attracted substantial attention, largely because it is frequently activated in human cancers. However, relatively little is known about the roles of Akt, particularly the individual isoforms of Akt, in glucose homeostasis in vivo. This review summarizes data on the role of Akt isoforms in glucose homeostasis and diabetes. Emphasis is given to the observation that certain combinations of whole-body Akt1 and Akt2 deficiencies reduce circulating levels of leptin and that restoration of leptin levels restores normal glucose homeostasis in diabetic Akt-deficient mice. The significance of these findings, together with recent observations suggesting that leptin emulates insulin action, is also discussed.

Prelude

The serine/threonine kinase Akt, also known as protein kinase B (PKB), was an anonymous kinase prior to the discovery that it is a downstream effector of PI3K and, subsequently, that it is the major effector of growth factor-mediated cell survival (reviewed in [1]). Over the years, Akt research has mainly focused on its role in cell survival and oncogenesis. However, genetic analysis in *C. elegans* indicates that the fundamental function of Akt involves the coupling of extracellular signals and metabolism. In both *C. elegans* and *Drosophila*, Akt is a transducer of insulin and insulin-like growth factor 1 (IGF1) action [2, 3]. The phenotype of mice with targeted deletions of both Akt1 and Akt2 resembles the phenotypes of insulin receptor (InsR) and IGF1 receptor (IGF1-R) knockout mice [4], suggesting that the function of Akt as a transducer of insulin/IGF1 action is conserved in mammals, at least during embryonic development.

While specific monogenic defects leading to type 2 diabetes remain rare, loss of function of either insulin receptor, certain insulin receptor substrate (IRS) proteins, or their downstream effector, Akt, have all been implicated in familial syndromes of diabetes and play an important role in the primary insulin response pathway that has been implicated in the genesis of more common forms of diabetes [5]. In fact, deficiency of IRS-2 or Akt2 alone is sufficient to elicit a diabetic phenotype in mice [6–8], and a loss-of-function mutation in Akt2 is associated with diabetes in one human family [9]. However, studies on the function(s) of Akt as a mediator of insulin metabolic action and in overall glucose homeostasis *in vivo* have been scarce. Particularly, relatively little is known about the role of different Akt isoforms in glucose homeostasis *in vivo*.

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Akt inhibitors have been developed for the treatment of cancer making it important to understand the effect of whole-body ablation of Akt isoforms on diabetes. Moreover, it is important to know whether diabetes can develop as a consequence of Akt inhibition during cancer therapy and how this development might be avoided.

Insulin regulation of Akt activity

Upon activation, the insulin receptor phosphorylates the insulin receptor substrate (IRS) proteins. Tyrosine-phosphorylated IRS activates the catalytic subunit of PI3K, p110, through the recruitment of the regulatory subunit of PI3K, p85. Once activated, the catalytic subunit, p110, phosphorylates phosphoinositides (PI) at the D3-position of the inositol ring to generate PI (3,4,5) P3 (PIP3) [10]. The rate-limiting step in Akt activation is the binding of PIP3 to the PH domain of Akt, which promotes subsequent translocation of Akt to the plasma membrane where it is phosphorylated at two residues, a process that is required for its full activation (Figure 1). The phosphorylated residues are a threonine in the catalytic domain and a serine in the C-terminal hydrophobic domain of Akt (Figure 1). The kinase that phosphorylates the threonine residue is PDK1; the kinase that phosphorylates the serine residue is mTORC2, a rapamycin-resistant complex containing mammalian target of rapamycin (mTOR), Rictor, SIN1, and mLST8 (for review, see [11]) (Figure 1). Antagonizing the activity of PI3K negatively regulates Akt activity. For example, PTEN (phosphatase and tensin homolog deleted from chromosome 10) [12] dephosphorylates the PIP3 that is generated by PI3K. Thus, PTEN is a negative regulator of Akt activity (Figure 1).

The signaling pathway that leads to Akt activation is highly conserved throughout evolution, from worms to mammals. In nematodes and flies, Akt is found exclusively downstream of IGF-1R and InsR. The most conserved function of this pathway is in cellular metabolism, particularly energy metabolism, a function that is coupled to lifespan in *C. elegans* and *Drosophila* [2, 3]. The most conserved downstream effectors of Akt are the forkhead transcription factors, FOXOs [13], and the target of rapamycin complex 1 (TORC1) [reviewed in [11], (Figure 1)]. Both the FoxOs and mammalian TORC1 (mTORC1) are implicated in glucose homeostasis and insulin resistance downstream of Akt (Box 1 and Box 2).

Box 1

FoxOs

FoxO proteins possess three highly conserved, putative Akt recognition motifs. FoxO proteins contain both a nuclear localization and nuclear export signal, which may mediate their import to and export from the nucleus, respectively. Upon Akt activation, FoxO proteins are phosphorylated on three conserved residues. These phosphorylation sites create docking sites for 14-3-3 proteins, which bind to the phosphorylated motifs and sequester FoxO in the cytoplasm, thereby inhibiting FoxO activity [13].

Four mammalian FoxO proteins have been identified: FoxO1, FoxO3, FoxO4, and FoxO6. All the FoxO proteins are regulated by growth factor and insulin signaling. FoxO6 is less related to the other FoxOs [13]. The activation of FoxO inhibits adipocyte and pancreatic β-cell differentiation [49, 60]. FoxO proteins are also implicated in myocyte differentiation and skeletal muscle atrophy [60]. Haplodeficiency of FoxO1 was shown to inhibit insulin resistance in InsR heterozygous mice, in part by decreasing hepatic glucose output. Mice expressing constitutively active FoxO1 in the liver develop diabetes, presumably as a result of increased glucose production [61]. The

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haplodeficiency of FoxO1 was also shown to rescue diabetes in IRS2 knockout mice that display β-cell dysfunction [62].

Box 2

mTOR

The rapamycin sensitive mTOR complex, mTORC1, comprises in addition to mTOR, Raptor, which is the predominant determinant of its activity, mLST8, as well as the accessory factors PRAS40 and Deptor [63]. One mechanism by which Akt activates mTORC1 is through direct phosphorylation of tuberous sclerosis complex 2 (TSC2), which otherwise inhibits mTORC1 activity [64]. However, TSC2 can be activated when intracellular levels of ATP are reduced and AMPK activity is elevated. AMPK directly phosphorylates TSC2, inducing its inhibition of mTORC1 [65]. Additionally, AMPK inhibits mTORC1 through direct phosphorylation of Raptor [66]. Akt also activates mTORC1 by maintaining intracellular ATP levels and reducing AMPK activity [67]. Tuberous sclerosis complex 1 (TSC1) and TSC2 form a heterodimer with GTPase activity that inhibits the activity of Rheb, a small GTPase required for mTOR activation [11]. In TSC2- or TSC1-null cells, mTORC1 is constitutively activated, independently of growth factors and Akt, consistent with an inhibitory role for TSC2. In contrast, Akt activity is markedly reduced in these cells. This reduction has been attributed to a negative feedback mechanism involving an inhibitory effect of S6 kinase (a downstream effector of mTORC1) on insulin receptor substrate-1 (IRS-1), which mediates PI3K activation by insulin and IGF-1 [23]. Additional negative regulatory loops elicited by mTORC1 that inhibit Akt activity may also exist [11].

The other mTOR complex, mTORC2, is formed of Rictor (Rapamycin insensitive companion of mTOR), mLST8, and mSin1 as well as the accessory factors Deptor and Protor-1. mTORC2 contributes to rapamycin-insensitive mTOR activity [63] and is the carboxy-terminus hydrophobic motif (HM) kinase for Akt and other AGC kinases.

The principal function of mTORC1 is to increase mRNA translation via the activation of S6 kinase and the inhibition of the eIF4E binding protein (4E-BP), a repressor of mRNA translation [64]. mTORC1 is required for adipogenesis [68]. Indeed, deletion of Raptor, the determinant of mTORC1 activity, impairs adipogenesis and the maintenance of adipose tissues [69]. Deletion of 4E-BP1, which is inhibited by mTORC1, accelerates adipocyte differentiation [58]. Thus, both the differentiation of preadipocytes and the function of differentiated adipocytes require mTORC1 activity. In addition, mTORC1 has been implicated in the regulation of β-cell mass and skeletal muscle atrophy. Both S6K1 and 4E-BP (or its target eIF4E) have been implicated in the induction of insulin biosynthesis following the feedback action of insulin on β-cells [27, 28].

Interestingly, a conserved mechanism of interplay between FoxO and mTORC1 has been recently uncovered in *Drosophila* and mammalian cells. In this interplay, FoxO transcriptionally induces Sestrin3, which in turn activates AMPK and inhibits mTORC1 [27, 28]. Thus, some of the functions generally attributed to FoxO might be due to its effect on mTORC1 activity.

The roles of Akt isoforms in glucose homeostasis and diabetes

Mammalian cells express three Akt isoforms (Akt1-3) encoded by three separate genes. The three isoforms share a high degree of amino acid identity and appear to have similar substrate specificity *in vitro* [31]. However, the relative expression of the isoforms varies in mammalian tissues. While Akt1 is the most abundantly expressed isoform in many

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mammalian tissues, Akt2 is expressed at the highest level in insulin-responsive tissues, and Akt3 is expressed at the highest level in the brain. Targeted deletion of the individual Akt isoforms in mice led to different phenotypes [7, 8, 14–17], which could be partially explained by the relative expression of the isoforms in various mammalian tissues. In addition, these findings could also suggest functional differences between the isoforms.

Unlike *Akt1*$^{-/-}$ [14, 15] or *Akt3*$^{-/-}$ [16, 17] mice, *Akt2*$^{-/-}$ mice display insulin resistance, manifested by high plasma insulin levels and an impaired ability to reduce glucose levels [7, 8]. This phenotype was attributed to the impairment of insulin action on liver and skeletal muscles [7]. The severity of this phenotype is dependent on the strain background. The deletion in the sv129/C57BL/6 hybrid or C57BL/6 backgrounds results in a relatively mild diabetic phenotype [7, 18] and resembles the pre-diabetic state in humans, while the phenotype is more severe in the DBA/1lacJ background [8]. The important role of Akt2 in glucose homeostasis was underscored by the discovery of a family in which type 2 diabetes was associated with an inherited loss-of-function mutation in Akt2. This mutant form of Akt2 might also act in a dominant-negative manner to inhibit wild type Akt2 and possibly other Akt isoforms, since its overexpression in 3T3L1 preadipocytes inhibits adipocyte differentiation [9].

Table 1 summarizes the phenotypes of individual as well as compound Akt-knockout mice. The genetic backgrounds that display a diabetic phenotype are indicated. As mentioned, *Akt1*$^{-/-}$ mice do not display a diabetic phenotype [32, 33]. However, in contrast to two independent reports using two different Akt1 knockout mice, a recent study using another *Akt1*$^{-/-}$ mouse showed that, paradoxically, Akt1 deficiency increased insulin sensitivity [19]. Since the authors of this recent report did not provide any mechanistic explanation for their observation, it is impossible to understand the discrepancy between this recent study and the previously published studies. This latter study also found that Akt1 is the major downstream effector of IRS-2 in β-cells, yet their *Akt1*$^{-/-}$ mice displayed improved glucose homeostasis, while mice with an IRS-2 deletion in the pancreas displayed impaired glucose homeostasis [20]. *Akt3*$^{-/-}$ mice exhibit a relative decrease in brain weight, consistent with the relatively high level of Akt3 expression in the brain. However, these mice do not display a diabetic phenotype [16, 17].

Analyses of compound Akt knockout mice revealed that *Akt1*$^{-/-}$*Akt2*$^{-/-}$ mice are neonatal lethal and display an approximately 50% percent decrease in body weight and size, largely due to skeletal muscle atrophy. They also display an almost complete inhibition of adipogenesis, a phenotype that was mirrored *in vitro* using their derived cells. In addition, the mice display impaired skin development and attenuated bone development [4]. *Akt1*$^{-/-}$*Akt3*$^{-/-}$ mice die in the uterus at midgestation, likely due to placental and vasculature defects [21]. On the other hand, *Akt2*$^{-/-}$*Akt3*$^{-/-}$ mice are born alive and display the combined phenotype observed in *Akt2*$^{-/-}$ and *Akt3*$^{-/-}$ mice. Finally, *Akt1*$^{+/-}$*Akt2*$^{-/-}$*Akt3*$^{-/-}$ mice are alive but are approximately 40% – 50% smaller in size relative to WT mice [22], and display a severe diabetic phenotype ([18], and below). Taken together, these results suggest that Akt1 is the most important, while Akt2 is the least important, isoform for embryonic development. Akt2 and Akt3 are dispensable for adult mouse viability, and only 50% of Akt1 is sufficient for mouse embryonic development and adult mouse viability.

The contributions of the three Akt isoforms to glucose homeostasis were assessed in compound Akt knockout mice. The haplodeficiency of Akt1 in *Akt2*$^{-/-}$ mice converted the pre-diabetic state of *Akt2*$^{-/-}$ mice to overt type 2 diabetes. The high blood insulin level in *Akt2*$^{-/-}$ mice was sufficient to maintain an almost normal level of blood glucose [7, 18]. However, the haplodeficiency of Akt1 in these mice induced severe hyperglycemia,

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concomitant with decreased insulin levels when compared to *Akt2*$^{-/-}$ mice, although insulin levels were still higher than those observed in wild type mice [18]. *Akt2*$^{-/-}$*Akt3*$^{-/-}$ mice and *Akt1*$^{+/-}$*Akt2*$^{-/-}$*Akt3*$^{-/-}$ mice displayed a diabetic phenotype similar to the one observed in *Akt2*$^{-/-}$ and *Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice, suggesting that Akt3 deficiency either does not affect or only minimally affects the diabetic phenotype observed in *Akt2*$^{-/-}$ and *Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice. Interestingly, *Akt1*$^{-/-}$*Akt2*$^{+/-}$ mice do not display a diabetic phenotype, suggesting that the pre-diabetic state is largely mediated by Akt2 deficiency. The hyperglycemic phenotype is observed when total Akt activity is further reduced in certain tissues of *Akt2*$^{-/-}$ mice via Akt1 deficiency [18]. The complementary effects of Akt1 and Akt2 resemble the complementary roles that the two PI3K catalytic subunit isoforms, p110α and p110β, play on insulin signaling *in vivo*. Heterozygous deletion of either p110α or p110β does not impair insulin metabolic actions, while the combined haplodeficiency of both isoforms impairs insulin metabolic actions [23].

Reciprocal experiments were conducted to determine whether activation of other Akt isoforms could compensate for Akt2 deficiency by rescuing the insulin resistance observed in *Akt2*$^{-/-}$ mice. *Akt2*$^{-/-}$ mice were crossed with *Pten*$^{+/-}$ mice to generate *Pten*$^{+/-}$*Akt2*$^{-/-}$ mice. These mice showed a substantially less severe diabetic phenotype than *Akt2*$^{-/-}$ mice when subjected to a glucose tolerance test (GTT) [18]. The high insulin levels observed in *Akt2*$^{-/-}$ mice were also markedly reduced in *Pten*$^{+/-}$*Akt2*$^{-/-}$ mice. Pten haplodeficiency rescued the phenotype observed in *Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice, as *Pten*$^{+/-}$*Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice did not exhibit hyperglycemia, and instead displayed an improved response to glucose injection, and showed high serum insulin levels under fed conditions (similar to those of *Akt2*$^{-/-}$ mice). Thus, haplodeficiency of Pten was sufficient to reverse the type 2 diabetes phenotype of *Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice to a pre-diabetic, insulin resistant state, similar to that observed in *Akt2*$^{-/-}$ mice [18]. These results are consistent with improved glucose homeostasis observed in mice with tissue-specific deletions of Pten in the pancreas [24]. They are also consistent with results showing that haplodeficiency of Pten in *IRS-2*$^{-/-}$ mice elevates Akt activity, and improves β-cell function and glucose homeostasis [25]. Taken together, these results suggest that activated Akt1, and possibly Akt3, can compensate for an Akt2 deficiency with respect to glucose homeostasis and diabetes. Thus, Akt isoforms can play both complementary and compensatory roles in glucose homeostasis in vivo.

In principle, the haplodeficiency of Pten could overcome the diabetic phenotype in *Akt2*$^{-/-}$ or *Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice through the activation of other non-Akt AGC kinases that are downstream effectors of PI3K. The activity of these kinases is dependent on the activity of PDK1 downstream of PI3K. However, PDK1 is much less sensitive than Akt to changes in the cellular content of PIP3 because the affinity of the PH domain of PDK1 for PIP3 is approximately one order of magnitude higher than the affinity of the Akt PH domain for PIP3 [26]. Therefore, it is unlikely that decreasing Pten activity by fifty percent could have a major impact on PDK activity. Most importantly it was reported that knock-in mice expressing PDK1 with a mutation in the PH domain, that lowered its affinity for PIP3, displayed insulin resistance. This insulin resistance was attributed exclusively to the reduction in Akt activity because other AGC kinases were not affected by the reduced ability of PDK1 to bind PIP3 [27]. These results further demonstrate, *in vivo*, that while Akt activity is affected by a small change in PIP3 content or PDK1 activity, the activity of other AGC kinases is not. Therefore, the possibility that haplodeficiency of Pten and the subsequent increase in PIP3 might exert their effect through AGC kinases other than Akt is very unlikely.

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How does haplodeficiency of Akt1 induce hyperglycemia in *Akt2*$^{-/-}$ mice?

One profound difference that distinguishes *Akt1*$^{+/-}$*Akt2*$^{-/-}$ from *Akt2*$^{-/-}$ mice, in addition to hyperglycemia, is the blunted insulin secretion in *Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice following glucose administration. The islets of *Akt2*$^{-/-}$ mice are larger in size than the islets of wild-type mice [7, 18]. This increase in islet size is probably due to a compensatory response intended to increase the blood insulin level. Morphological analyses of islets from *Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice showed that they are similarly increased in size, but unlike *Akt2*$^{-/-}$ islets, individual β-cells in *Akt1*$^{+/-}$*Akt2*$^{-/-}$ islets display profoundly reduced expression of insulin. Further, the total insulin content in these islets is markedly reduced compared to either *Akt2*$^{-/-}$ or wild-type islets [18]. Additionally, expression and membrane translocation of GLUT2 are substantially reduced in *Akt1*$^{+/-}$*Akt2*$^{-/-}$ β-cells compared to either wild-type or *Akt2*$^{-/-}$ β-cells. Interestingly, however, isolated *Akt1*$^{+/-}$*Akt2*$^{-/-}$ islets are not impaired in glucose sensing [18]. Thus, at least *in vitro*, the reduced GLUT2 expression observed in *Akt1*$^{+/-}$*Akt2*$_{-/-}$ islets is not sufficient to impair glucose sensing. However, the possibility that the reduced GLUT2 expression might affect glucose transport and sensing *in vivo* cannot be excluded. The reduction in insulin and GLUT2 levels is an early event that occurs even in 1 month old mice before the onset of islet size compensation [18]. Notably, haplodeficiency of PTEN in *Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice reverses the islet phenotype to that observed in *Akt2*$^{-/-}$ mice, further supporting the possibility of Akt isoform compensation.

Secretion of insulin from isolated islets following exposure to glucose is impaired in *Akt1*$^{+/-}$*Akt2*$^{-/-}$ islets compared to either wild-type or *Akt2*$^{-/-}$ islets. However, the reduction in insulin secretion is directly correlated with the reduced insulin content of *Akt1*$^{+/-}$*Akt2*$^{-/-}$ islets, raising the possibility that the reduced blood insulin level and the blunted secretion of insulin in response to glucose administration in *Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice is largely due to the reduced insulin content in β-cells. Apparently, the reduction of insulin and GLUT2 expression in *Akt1*$^{+/-}$*Akt2*$^{-/-}$ β-cells does not occur at the transcriptional level, since mRNA expression is not impaired. Instead, it appears that the reduction occurs at the level of protein synthesis [18]. Since mTORC1 activity is reduced in the islets, it is possible that the regulation of insulin and GLUT2 expression occurs at the level of mRNA translation. The reduction in mTORC1 activity is due to the decrease in Akt activity, which is also reflected by a reduction in FoxO phosphorylation [18]. Previous studies showing that insulin mediates a feed-forward positive regulatory loop that increases the translation of its own mRNA in β-cells through the activation of mTORC1 [28, 29] could explain, in part, the reduced insulin content of *Akt1*$^{+/-}$*Akt2*$^{-/-}$ β-cells. The role of mTORC1 in β-cells was underscored by the hyperactivation of mTORC1 in β-cells via β-cell-specific deletion of Tsc2 in mice [30, 31]. These mice display an increase in β-cell mass, and increased insulin secretion. On the other hand, mice that lack either S6K1 or the ribosomal protein S6 display reduced β-cell insulin content and exhibit impaired insulin secretion [30, 31]. These observations are consistent with the notion that the effect of Akt on β-cell function is largely mediated by mTORC1.

The studies described above employed global deletions of Akt isoforms. Therefore, it is unclear whether the phenotype of the β-cells observed in these studies is entirely cell autonomous. Certainly, the increased islet mass is a response to insulin resistance in liver and skeletal muscles, but are the negative effects on insulin secretion, insulin expression, and GLUT2 expression in *Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice cell autonomous?

A partial answer to this question could be derived from studies employing transgenic mice expressing a dominant-negative form of Akt (DN-Akt) specifically in β-cells, reducing β-cell Akt activity by about 80% [32]. These mice exhibited glucose intolerance as well as reduced basal and glucose-induced insulin levels, consistent with observations made in

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Akt1$^{+/-}$*Akt2*$^{-/-}$ mice. In a similar manner to *Akt1*$^{+/-}$*Akt2*$^{-/-}$ islets, glucose sensing was not impaired in DN-Akt islets. Although impaired insulin secretion was observed *in vitro* in islets expressing DN-Akt, reduced insulin content was not reported. Thus, these studies provided evidence that the effect of Akt deficiency on insulin secretion is cell autonomous.

Deletion of Pten specifically in the β-cells of mice, which increases total Akt activity, resulted in hypoglycemia, concomitant with an increase in β-cell number and islet mass [24]. Glucose-induced insulin secretion is elevated in these mice, and their β-cells express relatively high levels of GLUT2, consistent with the phenotype of *Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice. Similar to mice with this β-cell-specific deletion of Pten, transgenic mice expressing activated Akt1 in β-cells [33, 34] exhibit an increase in βincreased both *in vivo* and *in vitro* in isolated pancreata from these mice, correcting for the increase in β-cell mass indicates that there is actually no change (or even a decrease) in insulin secretion. Thus, identification of whether the phenotype of Akt-deficient β-cells is cell autonomous will require the generation of mice with specific deletions of Akt isoforms in β-cells.

The β-cell dysfunction observed in *Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice might lead one to assume that the severe hyperglycemia observed in these mice, as compared to *Akt2*$^{-/-}$ mice, is due to β-cell dysfunction and impaired insulin secretion. However, injection of insulin into *Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice did not significantly reduce the high blood glucose level in these mice [18]. Thus, what are the reasons for the severe hyperglycemia in *Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice?

The leptin connection

Members of a family displaying an autosomal-dominant inheritance of type 2 diabetes, expressing a dominant negative form of Akt2 were reported to also suffer from lipodystrophy [9]. Furthermore, the mutated Akt2 gene expressed by this family was shown to inhibit adipogenesis *in vitro* [9]. These observations, together with results showing impaired adipogenesis in *Akt1*$^{+/-}$*Akt2*$^{-/-}$ mice and in Akt-deficient cells *in vitro* [4], raised the possibility that diabetes developed in response to whole-body Akt deficiency in humans and mice might be related to lipodystrophy.

Lipodystrophy-mediated diabetes has been reported in mice and humans and was mitigated following administration of leptin [35]. Analyses of *Akt2−/−* and *Akt1+/−Akt2−/−* mice showed reduced body fat and a decrease in circulating levels of leptin [18]. Administration of leptin to *Akt1+/−Akt2−/−* mice was sufficient to restore normal glucose and insulin levels, and administration of leptin to *Akt2−/−* mice restored normal insulin levels [18]. In this respect, Akt-deficient mice resemble *Irs1−/−Irs3−/−* mice, which display lipoatrophy-mediated diabetes that can be corrected by leptin administration [54]. Interestingly, the combined deletions of Akt2 and leptin induced fasting hyperglycemia [36] similar to that observed in *Akt1+/−Akt2−/−* mice. This observation provides indirect support for the notion that hyperglycemia in *Akt1+/−Akt2−/−* mice is a result of leptin deficiency.

Leptin might reduce insulin resistance and hyperglycemia through its effect on the hypothalamus, resulting in a decrease in food intake. However, Akt-deficient mice did not show any significant decrease in food intake or body weight following the administration of leptin. Consistently, *Irs1−/−Irs3−/−* mice did not show a significant change in body weight following leptin administration [37], and caloric restriction did not recapitulate the action of leptin in lipoatrophic mice [35]. Thus, the remaining question is: How does leptin mitigate diabetes that is induced by Akt deficiency?

The ability of leptin to increase insulin sensitivity in Akt-deficient mice in the absence of reduced food intake suggests that the route of leptin action is not necessarily through its effect on the hypothalamus. Indeed, studies indicate alternative routes of leptin action. For

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instance, administration of leptin to *ob/ob* mice reverses hyperglycemia and hyperinsulinemia prior to weight loss [38–40]. It was also shown that under hyperinsulinemic conditions, leptin decreases hepatic glucose production, mainly by decreasing glycogenolysis and increasing glycogen production [41, 42].

Recent studies have shown that administration of leptin to rodents with STZ-induced type 1 diabetes emulates insulin action and restores a normal blood glucose level [43, 44]. Leptin administration in this model increased hepatic glycogen and reduced expression of gluconeogenic genes. These effects of leptin were attributed to its ability to suppress the high glucagon levels that would otherwise decrease hepatic glycogen storage. Another mechanism by which leptin may act was suggested by the recent findings that leptin restores normal glucose homeostasis in *ob/ob* mice and that this action of leptin is associated with the elevated circulating IGFBP2 levels in STZ-induced type 1 diabetes [45]. IGFBP2 levels are low in *ob/ob* mice but are elevated following leptin administration. Surprisingly, the elevation of IGFBP2 following injection of adenovirus expressing IGFBP2 into *ob/ob* mice was sufficient to restore normal glucose homeostasis and to reduce gluconeogenic gene expression in the liver of treated mice. The mechanism by which IGFBP2 restored normal glucose levels in *ob/ob* mice or STZ-treated mice is unknown. However, it is possible that IGFBP2 acts through an indirect mechanism independently of IGF1 [45].

How does leptin emulate the action of insulin? - Hypothesis

It is possible that leptin exerts its effect through the liver or other peripheral tissues. Indirect support for this possibility include the observations that liver-specific insulin receptor knockout mice display a greater than 10-fold increase in serum leptin levels as well as an approximately 35-fold increase in full-length leptin receptor levels in the liver [46]. Additionally, IRS1-deficient livers display an approximately 150-fold increase in the level of hepatic leptin receptors [47]. Liver-specific deletion of p85, the regulatory subunit of PI3K, elicits a 12-fold increase in soluble leptin receptor levels in the liver, though the increase in the full length receptor was not reported, with a concomitant 8-fold increase in serum leptin [48]. Thus, impairing insulin/PI3K/Akt signaling in the liver is coupled to a dramatic elevation of hepatic leptin receptor levels concomitant with an increase in the level of circulating leptin. These observations imply that when insulin signaling is impaired, a compensatory mechanism is exerted by leptin through its cognate receptor in the liver to reduce gluconeogenesis independently of insulin. This compensatory mechanism cannot occur in mice with global deletions of Akt1 and Akt2 because of their impaired adipogenesis and leptin deficiency.

Impairing Akt activity in the liver induces the expression of gluconeogenic genes, likely through its effects on FoxO and PGC1α [49, 50]. One possibility is that leptin suppresses hepatic expression of gluconeogenic genes through Stat3 activation via a mechanism independent of insulin and Akt in the liver. This hypothesis is based on the observations that Stat3 suppresses gluconeogenic gene expression in the liver through a PI3K/Akt-independent mechanism [51] and that leptin, through the leptin receptor, activates Stat3 [52]. In support of this possibility, Stat3 activation has been observed in the livers of STZ-treated mice following leptin therapy [43]. This potential compensation mechanism by leptin through its cognate receptor in the liver may occur only when hepatic insulin signaling is impaired.

Another non-exclusive possibility is that leptin, via its effect on the hypothalamus and the brain, subsequently increases insulin sensitivity in the liver by enhancing insulin signaling through other intermediates such as IGFBP2. This possibility is supported by the observation that in lipodystrophic mice, the intracerebroventricular administration of leptin

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that does not increase serum leptin levels, enhances insulin signaling in the liver with concomitant decrease in the expression of the gluconeogenic gene G6Pase [53].

Prospective

It is clear that a full understanding of the roles of Akt isoforms in glucose homeostasis *in vivo* requires the generation of mice with tissue-specific deletions of the Akt isoforms, both individually and in combination. Recently, it was shown that mice lacking Akt2 specifically in the liver are resistant to hepatic steatosis [36]. However, except for a mild increase in insulin levels, impaired glucose homeostasis was not reported in these mice. These results suggest that liver-specific deletion of Akt2 is not sufficient to recapitulate the insulin resistance phenotype observed in mice with whole body germ-line deletion of Akt2.

Since germ-line deletions may have developmental consequences, inducible whole-body deletions of Akt1, Akt2, and Akt1/Akt2 are required to determine their roles in glucose homeostasis. Whole-body deletion of Akt isoforms in adulthood also recapitulates the inhibition of Akt activity by drug therapy that is intended to cure cancer. Therefore, it is important to explore the extent to which glucose homeostasis is impaired after the induction of whole-body deletion of the Akt isoforms. It would also be interesting to determine whether glucose homeostasis in these mice can be restored by leptin therapy. Notably, mice treated with an Akt inhibitor develop diabetes that is untreatable with common anti-diabetic drugs [54]. The question remains whether this form of diabetes can be treated with leptin therapy.

The mechanism by which Akt regulates adipogenesis remains to be elucidated. Akt could be required for the differentiation process and for the functionality of adipocytes. Although both Akt1 and Akt2 are required for adipocyte differentiation [4], Akt2 is specifically required for GLUT4 translocation to the plasma membrane [55]. Interestingly, during adipocyte differentiation *in vitro* there is an isoform switch whereby Akt1 is expressed at a relatively high level in the early stages of adipogenesis but its expression is gradually decreased at later stages, while Akt2 expression is increased in later stages ([56], and D. Sunadrarajan and N. Hay, unpublished results). Thus, it is possible that Akt1 is required for the early stages of adipogenesis whereas Akt2 is required for the functionality of mature adipocytes.

mTORC1 could be a major downstream effector of Akt with respect to adipocyte differentiation since hyperactivation of mTORC1 in *Tsc2*$^{-/-}$ cells is sufficient to mediate adipocyte differentiation, despite the low activity of Akt in these cells [57]. Consistently the downstream effectors of mTORC1, 4E-BPs and S6K1, were implicated in adipogenesis. The deletion of 4E-BP1 and 4E-BP2 increases adipogenesis [58], and S6K1 was shown to be required for the commitment of embryonic stem cells to early adipocyte progenitors [59].

Finally, the role of leptin in glucose homeostasis and its mechanism of action remain unresolved. Why is hepatic leptin receptor expression dramatically elevated when hepatic PI3K/Akt signaling is impaired? Is it a compensatory mechanism that emulates insulin action through leptin signaling? Further studies such as liver-specific deletion of leptin receptor in mice with impaired hepatic PI3K/Akt signaling are required to address these questions.

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References

1. Kandel ES, Hay N. The regulation and activities of the multifunctional serine/threonine kinase Akt/PKB. Exp Cell Res. 1999; 253:210–29. [PubMed: 10579924]
2. Teleman AA. Molecular mechanisms of metabolic regulation by insulin in Drosophila. Biochem J. 2010; 425(1):13–26. [PubMed: 20001959]
3. Panowski SH, Dillin A. Signals of youth: endocrine regulation of aging in Caenorhabditis elegans. Trends Endocrinol Metab. 2009; 20(6):259–64. [PubMed: 19646896]
4. Peng XD, Xu PZ, Chen ML, Hahn-Windgassen A, Skeen J, Jacobs J, Sundararajan D, Chen WS, Crawford SE, Coleman KG, Hay N. Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development, and impeded adipogenesis in mice lacking Akt1 and Akt2. Genes Dev. 2003; 17(11):1352–65. [PubMed: 12782654]
5. Mauvais-Jarvis F, Kulkarni RN, Kahn CR. Knockout models are useful tools to dissect the pathophysiology and genetics of insulin resistance. Clin Endocrinol (Oxf). 2002; 57(1):1–9. [PubMed: 12100063]
6. Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, White MF. Disruption of IRS-2 causes type 2 diabetes in mice. Nature. 1998; 391(6670):900–4. [PubMed: 9495343]
7. Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB 3rd, Kaestner KH, Bartolomei MS, Shulman GI, Birnbaum MJ. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). Science. 2001; 292(5522):1728–31. [PubMed: 11387480]
8. Garofalo RS, Orena SJ, Rafidi K, Torchia AJ, Stock JL, Hildebrandt AL, Coskran T, Black SC, Brees DJ, Wicks JR, McNeish JD, Coleman KG. Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKB beta. J Clin Invest. 2003; 112(2):197–208. [PubMed: 12843127]
9. George S, Rochford JJ, Wolfrum C, Gray SL, Schinner S, Wilson JC, Soos MA, Murgatroyd PR, Williams RM, Acerini CL, Dunger DB, Barford D, Umpleby AM, Wareham NJ, Davies HA, Schafer AJ, Stoffel M, O'Rahilly S, Barroso I. A family with severe insulin resistance and diabetes due to a mutation in AKT2. Science. 2004; 304(5675):1325–8. [PubMed: 15166380]
10. Cantley LC. The phosphoinositide 3-kinase pathway. Science. 2002; 296(5573):1655–7. [PubMed: 12040186]
11. Bhaskar PT, Hay N. The two TORCs and Akt. Dev Cell. 2007; 12(4):487–502. [PubMed: 17419990]
12. Parsons R, Simpson L. PTEN and cancer. Methods Mol Biol. 2003; 222:147–66. [PubMed: 12710685]
13. Huang H, Tindall DJ. Dynamic FoxO transcription factors. J Cell Sci. 2007; 120(Pt 15):2479–87. [PubMed: 17646672]
14. Chen WS, Xu PZ, Gottlob K, Chen ML, Sokol K, Shiyanova T, Roninson I, Weng W, Suzuki R, Tobe K, Kadowaki T, Hay N. Growth retardation and increased apoptosis in mice with homozygous disruption of the Akt1 gene. Genes Dev. 2001; 15(17):2203–8. [PubMed: 11544177]
15. Cho H, Thorvaldsen JL, Chu Q, Feng F, Birnbaum MJ. Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J Biol Chem. 2001; 276(42):38349–52. [PubMed: 11533044]
16. Tschopp O, Yang ZZ, Brodbeck D, Dummler BA, Hemmings-Mieszczak M, Watanabe T, Michaelis T, Frahm J, Hemmings BA. Essential role of protein kinase B gamma (PKB gamma/Akt3) in postnatal brain development but not in glucose homeostasis. Development. 2005; 132(13):2943–54. [PubMed: 15930105]
17. Easton RM, Cho H, Roovers K, Shineman DW, Mizrahi M, Forman MS, Lee VM, Szabolcs M, de Jong R, Oltersdorf T, Ludwig T, Efstratiadis A, Birnbaum MJ. Role for Akt3/protein kinase Bgamma in attainment of normal brain size. Mol Cell Biol. 2005; 25(5):1869–78. [PubMed: 15713641]
18. Chen WS, Peng XD, Wang Y, Xu PZ, Chen ML, Luo Y, Jeon SM, Coleman K, Haschek WM, Bass J, Philipson LH, Hay N. Leptin deficiency and beta-cell dysfunction underlie type 2 diabetes in compound Akt knockout mice. Mol Cell Biol. 2009; 29(11):3151–62. [PubMed: 19289493]

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19. Buzzi F, Xu L, Zuellig RA, Boller SB, Spinas GA, Hynx D, Chang Z, Yang Z, Hemmings BA, Tschopp O, Niessen M. Differential effects of protein kinase B/Akt isoforms on glucose homeostasis and islet mass. Mol Cell Biol. 2010; 30(3):601–12. [PubMed: 19933838]
20. Cantley J, Choudhury AI, Asare-Anane H, Selman C, Lingard S, Heffron H, Herrera P, Persaud SJ, Withers DJ. Pancreatic deletion of insulin receptor substrate 2 reduces beta and alpha cell mass and impairs glucose homeostasis in mice. Diabetologia. 2007; 50(6):1248–56. [PubMed: 17393136]
21. Yang ZZ, Tschopp O, Di-Poi N, Bruder E, Baudry A, Dummler B, Wahli W, Hemmings BA. Dosage-dependent effects of Akt1/protein kinase Balpha (PKBalpha) and Akt3/PKBgamma on thymus, skin, and cardiovascular and nervous system development in mice. Mol Cell Biol. 2005; 25(23):10407–18. [PubMed: 16287854]
22. Dummler B, Tschopp O, Hynx D, Yang ZZ, Dirnhofer S, Hemmings BA. Life with a single isoform of Akt: mice lacking Akt2 and Akt3 are viable but display impaired glucose homeostasis and growth deficiencies. Mol Cell Biol. 2006; 26(21):8042–51. [PubMed: 16923958]
23. Brachmann SM, Ueki K, Engelman JA, Kahn RC, Cantley LC. Phosphoinositide 3-kinase catalytic subunit deletion and regulatory subunit deletion have opposite effects on insulin sensitivity in mice. Mol Cell Biol. 2005; 25(5):1596–607. [PubMed: 15713620]
24. Elghazi L, Bernal-Mizrachi E. Akt and PTEN: beta-cell mass and pancreas plasticity. Trends Endocrinol Metab. 2009; 20(5):243–51. [PubMed: 19541499]
25. Kushner JA, Simpson L, Wartschow LM, Guo S, Rankin MM, Parsons R, White MF. Phosphatase and tensin homolog regulation of islet growth and glucose homeostasis. J Biol Chem. 2005; 280(47):39388–93. [PubMed: 16170201]
26. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P. Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. Curr Biol. 1997; 7(4):261–9. [PubMed: 9094314]
27. Bayascas JR, Wullschleger S, Sakamoto K, Garcia-Martinez JM, Clacher C, Komander D, van Aalten DM, Boini KM, Lang F, Lipina C, Logie L, Sutherland C, Chudek JA, van Diepen JA, Voshol PJ, Lucocq JM, Alessi DR. Mutation of the PDK1 PH domain inhibits protein kinase B/Akt, leading to small size and insulin resistance. Mol Cell Biol. 2008; 28(10):3258–72. [PubMed: 18347057]
28. Leibiger IB, Leibiger B, Moede T, Berggren PO. Exocytosis of insulin promotes insulin gene transcription via the insulin receptor/PI-3 kinase/p70 s6 kinase and CaM kinase pathways. Mol Cell. 1998; 1(6):933–8. [PubMed: 9660977]
29. Leibiger IB, Leibiger B, Berggren PO. Insulin feedback action on pancreatic beta-cell function. FEBS Lett. 2002; 532(1–2):1–6. [PubMed: 12459452]
30. Shigeyama Y, Kobayashi T, Kido Y, Hashimoto N, Asahara S, Matsuda T, Takeda A, Inoue T, Shibutani Y, Koyanagi M, Uchida T, Inoue M, Hino O, Kasuga M, Noda T. Biphasic response of pancreatic beta-cell mass to ablation of tuberous sclerosis complex 2 in mice. Mol Cell Biol. 2008; 28(9):2971–9. [PubMed: 18316403]
31. Rachdi L, Balcazar N, Osorio-Duque F, Elghazi L, Weiss A, Gould A, Chang-Chen KJ, Gambello MJ, Bernal-Mizrachi E. Disruption of Tsc2 in pancreatic beta cells induces beta cell mass expansion and improved glucose tolerance in a TORC1-dependent manner. Proc Natl Acad Sci U S A. 2008; 105(27):9250–5. [PubMed: 18587048]
32. Bernal-Mizrachi E, Fatrai S, Johnson JD, Ohsugi M, Otani K, Han Z, Polonsky KS, Permutt MA. Defective insulin secretion and increased susceptibility to experimental diabetes are induced by reduced Akt activity in pancreatic islet beta cells. J Clin Invest. 2004; 114(7):928–36. [PubMed: 15467831]
33. Bernal-Mizrachi E, Wen W, Stahlhut S, Welling CM, Permutt MA. Islet beta cell expression of constitutively active Akt1/PKB alpha induces striking hypertrophy, hyperplasia, and hyperinsulinemia. J Clin Invest. 2001; 108(11):1631–8. [PubMed: 11733558]
34. Tuttle RL, Gill NS, Pugh W, Lee JP, Koeberlein B, Furth EE, Polonsky KS, Naji A, Birnbaum MJ. Regulation of pancreatic beta-cell growth and survival by the serine/threonine protein kinase Akt1/PKBalpha. Nat Med. 2001; 7(10):1133–7. [PubMed: 11590437]

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35. Gorden P, Gavrilova O. The clinical uses of leptin. Current Opinion in Pharmacology. 2003; 3(6): 655–659. [PubMed: 14644019]
36. Leavens KF, Easton RM, Shulman GI, Previs SF, Birnbaum MJ. Akt2 is required for hepatic lipid accumulation in models of insulin resistance. Cell Metab. 2009; 10(5):405–18. [PubMed: 19883618]
37. Laustsen PG, Michael MD, Crute BE, Cohen SE, Ueki K, Kulkarni RN, Keller SR, Lienhard GE, Kahn CR. Lipoatrophic diabetes in Irs1(−/−)/Irs3(−/−) double knockout mice. Genes Dev. 2002; 16(24):3213–22. [PubMed: 12502742]
38. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. Science. 1995; 269(5223):540–3. [PubMed: 7624776]
39. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature. 1998; 395(6704):763–70. [PubMed: 9796811]
40. Harris RB, Zhou J, Redmann SM Jr, Smagin GN, Smith SR, Rodgers E, Zachwieja JJ. A leptin dose-response study in obese (ob/ob) and lean (+/?) mice. Endocrinology. 1998; 139(1):8–19. [PubMed: 9421392]
41. Rossetti L, Massillon D, Barzilai N, Vuguin P, Chen W, Hawkins M, Wu J, Wang J. Short term effects of leptin on hepatic gluconeogenesis and in vivo insulin action. J Biol Chem. 1997; 272(44):27758–63. [PubMed: 9346919]
42. O'Doherty RM, Anderson PR, Zhao AZ, Bornfeldt KE, Newgard CB. Sparing effect of leptin on liver glycogen stores in rats during the fed-to-fasted transition. Am J Physiol. 1999; 277(3 Pt 1):E544–50. [PubMed: 10484368]
43. Yu X, Park BH, Wang MY, Wang ZV, Unger RH. Making insulin-deficient type 1 diabetic rodents thrive without insulin. Proc Natl Acad Sci U S A. 2008; 105(37):14070–5. [PubMed: 18779578]
44. Wang MY, Chen L, Clark GO, Lee Y, Stevens RD, Ilkayeva OR, Wenner BR, Bain JR, Charron MJ, Newgard CB, Unger RH. Leptin therapy in insulin-deficient type I diabetes. Proc Natl Acad Sci U S A. 2010; 107(11):4813–9. [PubMed: 20194735]
45. Hedbacker K, Birsoy K, Wysocki RW, Asilmaz E, Ahima RS, Farooqi IS, Friedman JM. Antidiabetic effects of IGFBP2, a leptin-regulated gene. Cell Metab. 2010; 11(1):11–22. [PubMed: 20074524]
46. Cohen SE, Kokkotou E, Biddinger SB, Kondo T, Gebhardt R, Kratzsch J, Mantzoros CS, Kahn CR. High circulating leptin receptors with normal leptin sensitivity in liver-specific insulin receptor knock-out (LIRKO) mice. J Biol Chem. 2007; 282(32):23672–8. [PubMed: 17556363]
47. Dong X, Park S, Lin X, Copps K, Yi X, White MF. Irs1 and Irs2 signaling is essential for hepatic glucose homeostasis and systemic growth. J Clin Invest. 2006; 116(1):101–14. [PubMed: 16374520]
48. Taniguchi CM, Kondo T, Sajan M, Luo J, Bronson R, Asano T, Farese R, Cantley LC, Kahn CR. Divergent regulation of hepatic glucose and lipid metabolism by phosphoinositide 3-kinase via Akt and PKClambda/zeta. Cell Metab. 2006; 3(5):343–53. [PubMed: 16679292]
49. Gross DN, van den Heuvel AP, Birnbaum MJ. The role of FoxO in the regulation of metabolism. Oncogene. 2008; 27(16):2320–36. [PubMed: 18391974]
50. Li X, Monks B, Ge Q, Birnbaum MJ. Akt/PKB regulates hepatic metabolism by directly inhibiting PGC-1alpha transcription coactivator. Nature. 2007; 447(7147):1012–6. [PubMed: 17554339]
51. Inoue H, Ogawa W, Ozaki M, Haga S, Matsumoto M, Furukawa K, Hashimoto N, Kido Y, Mori T, Sakaue H, Teshigawara K, Jin S, Iguchi H, Hiramatsu R, LeRoith D, Takeda K, Akira S, Kasuga M. Role of STAT-3 in regulation of hepatic gluconeogenic genes and carbohydrate metabolism in vivo. Nat Med. 2004; 10(2):168–74. [PubMed: 14716305]
52. Buettner C, Pocai A, Muse ED, Etgen AM, Myers MG Jr, Rossetti L. Critical role of STAT3 in leptin's metabolic actions. Cell Metab. 2006; 4(1):49–60. [PubMed: 16814732]
53. Asilmaz E, Cohen P, Miyazaki M, Dobrzyn P, Ueki K, Fayzikhodjaeva G, Soukas AA, Kahn CR, Ntambi JM, Socci ND, Friedman JM. Site and mechanism of leptin action in a rodent form of congenital lipodystrophy. J Clin Invest. 2004; 113(3):414–24. [PubMed: 14755338]

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54. Crouthamel MC, Kahana JA, Korenchuk S, Zhang SY, Sundaresan G, Eberwein DJ, Brown KK, Kumar R. Mechanism and management of AKT inhibitor-induced hyperglycemia. Clin Cancer Res. 2009; 15(1):217–25. [PubMed: 19118049]
55. Bae SS, Cho H, Mu J, Birnbaum MJ. Isoform-specific regulation of insulin-dependent glucose uptake by Akt/protein kinase B. J Biol Chem. 2003; 278(49):49530–6. [PubMed: 14522993]
56. Summers SA, Whiteman EL, Cho H, Lipfert L, Birnbaum MJ. Differentiation-dependent suppression of platelet-derived growth factor signaling in cultured adipocytes. J Biol Chem. 1999; 274(34):23858–67. [PubMed: 10446150]
57. Zhang HH, Huang J, Duvel K, Boback B, Wu S, Squillace RM, Wu CL, Manning BD. Insulin stimulates adipogenesis through the Akt-TSC2-mTORC1 pathway. PLoS One. 2009; 4(7):e6189. [PubMed: 19593385]
58. Le Bacquer O, Petroulakis E, Paglialunga S, Poulin F, Richard D, Cianflone K, Sonenberg N. Elevated sensitivity to diet-induced obesity and insulin resistance in mice lacking 4E-BP1 and 4E-BP2. J Clin Invest. 2007; 117(2):387–96. [PubMed: 17273556]
59. Carnevalli LS, Masuda K, Frigerio F, Le Bacquer O, Um SH, Gandin V, Topisirovic I, Sonenberg N, Thomas G, Kozma SC. S6K1 plays a critical role in early adipocyte differentiation. Dev Cell. 2010; 18(5):763–74. [PubMed: 20493810]
60. Accili D, Arden KC. FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. Cell. 2004; 117(4):421–6. [PubMed: 15137936]
61. Nakae J, Biggs WH 3rd, Kitamura T, Cavenee WK, Wright CV, Arden KC, Accili D. Regulation of insulin action and pancreatic beta-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1. Nat Genet. 2002; 32(2):245–53. [PubMed: 12219087]
62. Kitamura T, Nakae J, Kitamura Y, Kido Y, Biggs WH 3rd, Wright CV, White MF, Arden KC, Accili D. The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic beta cell growth. J Clin Invest. 2002; 110(12):1839–47. [PubMed: 12488434]
63. Laplante M, Sabatini DM. mTOR signaling at a glance. J Cell Sci. 2009; 122(Pt 20):3589–94. [PubMed: 19812304]
64. Hay N, Sonenberg N. Upstream and downstream of mTOR. Genes Dev. 2004; 18:1926–1945. [PubMed: 15314020]
65. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. Cell. 2003; 115(5):577–90. [PubMed: 14651849]
66. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE, Shaw RJ. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol Cell. 2008; 30(2): 214–26. [PubMed: 18439900]
67. Hahn-Windgassen A, Nogueira V, Chen CC, Skeen JE, Sonenberg N, Hay N. Akt activates the mammalian target of rapamycin by regulating cellular ATP level and AMPK activity. J Biol Chem. 2005; 280(37):32081–9. [PubMed: 16027121]
68. El-Chaar D, Gagnon A, Sorisky A. Inhibition of insulin signaling and adipogenesis by rapamycin: effect on phosphorylation of p70 S6 kinase vs eIF4E-BP1. Int J Obes Relat Metab Disord. 2004; 28(2):191–8. [PubMed: 14970836]
69. Polak P, Cybulski N, Feige JN, Auwerx J, Ruegg MA, Hall MN. Adipose-specific knockout of raptor results in lean mice with enhanced mitochondrial respiration. Cell Metab. 2008; 8(5):399–410. [PubMed: 19046571]
70. Du K, Herzig S, Kulkarni RN, Montminy M. TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver. Science. 2003; 300(5625):1574–7. [PubMed: 12791994]
71. Cheng KK, Iglesias MA, Lam KS, Wang Y, Sweeney G, Zhu W, Vanhoutte PM, Kraegen EW, Xu A. APPL1 potentiates insulin-mediated inhibition of hepatic glucose production and alleviates diabetes via Akt activation in mice. Cell Metab. 2009; 9(5):417–27. [PubMed: 19416712]
72. Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. Nature. 2002; 420(6913):333–6. [PubMed: 12447443]
73. Decker SJ, Saltiel AR. Staying in SHIP shape. Nat Med. 2005; 11(2):123–4. [PubMed: 15692594]
74. Brognard J, Newton AC. PHLiPPing the switch on Akt and protein kinase C signaling. Trends Endocrinol Metab. 2008; 19(6):223–30. [PubMed: 18511290]

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Box 3

Non-canonical regulators of Akt that are implicated in glucose homeostasis and diabetes

The *Drosophila* Tribbles homologue protein, TRB3, which belongs to a family of proteins in mammals, binds an inactive unphosphorylated Akt to inhibits its phosphorylation and activation [70]. In mice under fasting conditions, TRB3 expression is induced in the liver, and in obese diabetic mice the elevated expression of TRB3 in the liver interferes with Akt activation. Ectopic expression of TRB3 in the liver induces hyperglycemia and glucose intolerance. Thus, by interfering with Akt activation, TRB3 elicits insulin resistance [70]. More recently it was shown that the endosomal adaptor protein, APLL1, interacts with Akt and prevents its association with TRB3 [71]. The binding of APLL1 to Akt was shown to facilitate the interaction of Akt with the plasma membrane and endo-membranes for full activation. Hepatic overexpression of APLL1 in diabetic obese mice counteracts the inhibition of Akt activity by TRB3 and promotes insulin-mediated inhibition of hepatic glucose production [71]. Another negative regulator of Akt activity in diabetic obese mice is the jun kinase, JNK. JNK activity is elevated in obese mice and reduces Akt activity by the phosphorylation and inhibition of IRS1 [72].

The SH2-containing inositol phosphatase 2 (SHIP2) was implicated in glucose homeostasis through its effect on Akt activity. SHIP2 is a 5′-lipid phosphatase that can de-phosphorylate the phosphate at the position 5 in the inositol ring of PIP3, and thereby negate PI3K and Akt activity. Thus, SHIP2 can certainly affect glucose homeostasis through its effect on Akt activity, but the impact of SHIP2 on glucose homeostasis *in vivo* in mice is debated [73].

Finally, the Ser/Thr-specific PH domain leucine-rich phosphatases, PHLPP1 and PHLPP2, which de-phosphorylate the hydrophobic phosphorylation motif of Akt and attenuate Akt activity, could potentially affect glucose homeostasis. Indeed, high levels of PHLPP1 in skeletal muscles of type 2 diabetic patients, was found to be correlated with impaired Akt activity [74].

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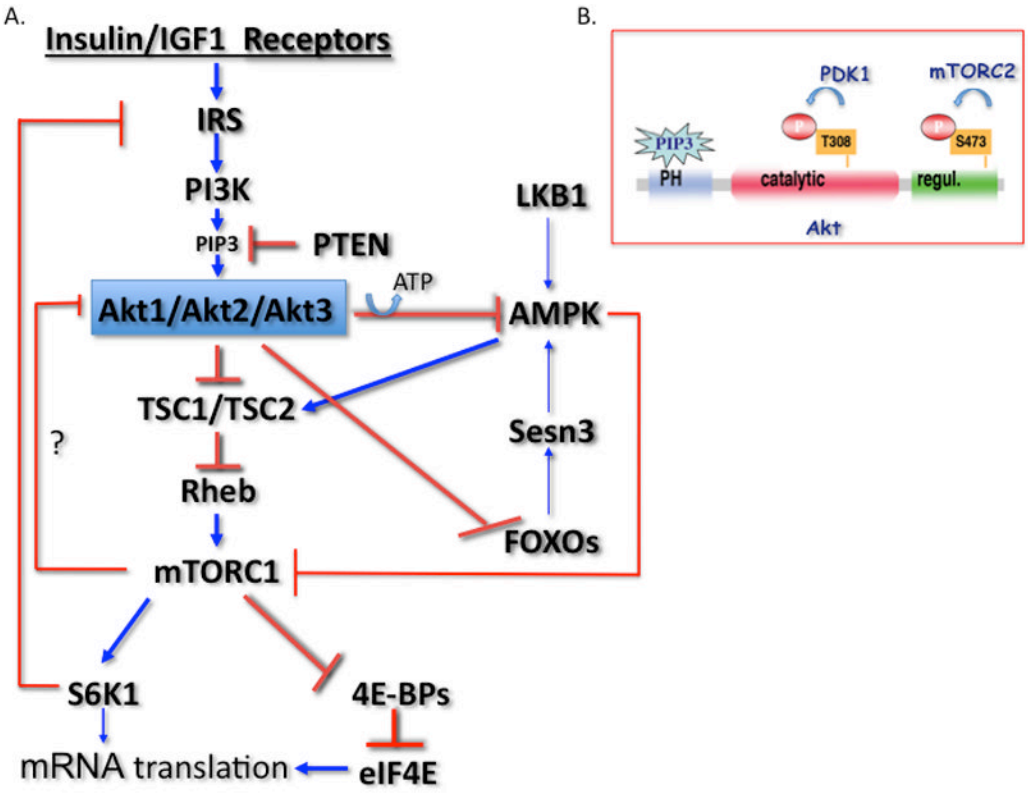

Figure 1. Schematic illustration depicting the pathway of Akt activation by insulin and IGF1, the downstream effectors and regulatory loops

(a) Insulin and IGF1 receptors activate IRS proteins, which in turn activate PI3 kinase. PI3K generates PIP3, which bind to the pleckstrin homology (PH) domain of the three Akt isoforms and translocate them to the membrane, where they are phosphorylated by PDK1 and mTORC2 for full activation. Akt phosphorylates and inactivates the FoxO transcription factors. Akt also phosphorylates Tsc2 and inhibits its activity, resulting in the activation of Rheb. Rheb activates mTORC1, which phosphorylates and activates S6K1, and phosphorylates and inactivates the 4E-BPs, resulting in the activation of eIF4E and 5′cap-dependent mRNA translation. Akt also can activate mTORC1 through the generation of intracellular ATP and the inactivation of AMPK, which otherwise inhibits mTORC1. The inhibition of FoxO by Akt prevents the induction of Sestrin 3, which otherwise activates AMPK and inhibits mTORC1. The activation of mTORC1 elicits a negative feedback loop that inhibits Akt activity either through S6K1 and the inactivation of IRS proteins or through other mechanisms. (b) The generic primary structure of Akt proteins is shown. Binding of PIP3 to the PH domain and the phosphorylations by PDK1 and mTORC2 are illustrated.

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Table 1

Summary of the genotypes and phenotypes of Akt knockout mice. Genotypes with diabetic phenotypes are in blue.

Genotype	Phenotype
Akt1-/-	Mild growth retardation + increased apoptosis
Akt2-/-	Insulin resistance (hyperinsulinemia); reduced level of circulating leptin
Akt3-/-	Smaller size brain
Akt1+/-Akt2-/-	Severe diabetes (hyperglycemia); leptin deficiency
Akt1-/-Akt2+/-	No diabetic phenotype was observed
Akt1-/-Akt2-/-	Neonatal lethal; 50% smaller; skeletal muscle atrophy; impaired adipogenesis
Akt1+/-Akt3-/-	Smaller size brain
Akt1-/-Akt3+/-	Viable
Akt1-/-Akt3-/-	Die in uterus at midgestation; placental and vasculature defects
Akt2-/-Akt3-/-	Insulin resistance (hyperinsulinemia); reduced level of circulating leptin + Smaller size brain
$Akt1^{+/-}Akt2^{-/-}Akt3^{-/-}$	40-50% smaller; Severe diabetes (hyperglycemia); leptin deficiency + Smaller size brain