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Phosphoinositides: Key modulators of energy metabolism☆

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Abstract

Phosphoinositides are key players in many trafficking and signaling pathways. Recent advances regarding the synthesis, location and functions of these lipids have dramatically improved our understanding of how and when these lipids are generated and what their roles are in animal physiology. In particular, phosphoinositides play a central role in insulin signaling, and manipulation of PtdIns(3,4,5)P_3 levels in particular, may be an important potential therapeutic target for the alleviation of insulin resistance associated with obesity and the metabolic syndrome. In this article we review the metabolism, regulation and functional roles of phosphoinositides in insulin signaling and the regulation of energy metabolism. This article is part of a Special Issue entitled Phosphoinositides.

Keywords

Phosphatidylinositol; PtdIns(3,4,5)P_3; Akt; mTORC1; Phosphorylated phosphatidylinositides; GLUT4

1. Introduction

The regulation of cellular metabolism by hormones and biogenic amines is central to normal metabolic homeostasis. Disruption of these signaling pathways is a key molecular theme during pathophysiological conditions such as metabolic syndrome and obesity [1–3]. Phosphoinositides are key molecular mediators of the anabolic functions of insulin and as such, are key regulators of metabolism. This review will discuss the role of phosphoinositides in insulin action and secretion, and how their dysregulation can provide insights into the pathogenesis of metabolic diseases.

☆This article is part of a Special Issue entitled Phosphoinositides.

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2. How are phosphoinositide levels controlled?

Inositol phospholipids (PtdIns) can exist in one of eight molecular species, via phosphorylation at the 3, 4 or 5 position of the inositol head-group. Polyphosphoinositides are enzymatically generated by a series of phosphorylation or dephosphorylation reactions. In general, phosphoinositide kinases and phosphatases are able to specifically phosphorylate or dephosphorylate only one position on the inositol ring, though the substrate (and therefore product) may vary. The local levels of phosphoinositide species are regulated by the activity of these kinases and phosphatases, as well as the subcellular restriction of the enzymes. In most cases, this is accomplished via adaptor proteins that can regulate the activity and direct the enzyme to the correct subcellular location. Since phosphoinositides are embedded in lipid bilayers, they cannot move through the cytoplasm unless their lipid microenvironment is changed as well. This restricts these lipids to specific locations in cellular membranes. Once generated, phosphorylated phosphoinositides often function by recruiting adaptor proteins, which propagate molecular interactions to initiate phosphorylation cascades. This review will focus on the molecular roles of these lipids in regulating pathways that control glucose, lipid and protein metabolism.

Inmost cells, PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 are the lowest abundance phosphoinositides, and are generated primarily by phosphorylation of PtdIns(4,5)P_2 the four Class I PI3-Kinase enzymes (α, β, δ, and γ; encoded by the genes *PIK3CA*, *PIK3CB*, *PIK3CD* and *PIK3CG*). Each of these kinases interacts with subunits that regulate their activity and localization. The Class IB PI3K, p110γ, is activated by GPCR signaling and plays an important role in inflammation (reviewed in [4,5]). The other three Class I PI3K isoforms (named Class IA), are present in complexes with the regulatory subunits p55 and p85. These regulatory subunits contain phosphotyrosine-binding SH2 domains, which recruit the catalytic p110 enzymes to receptors or receptor-associated adapter proteins upon tyrosine phosphorylation [6,7]. The PI3K regulatory subunits may also be activated by other protein–protein interactions including the small GTPases Ras [8,9] and Rab5 [10], as well as the lipid phosphatase PTEN [11–13]. Once recruited to tyrosine phosphorylated receptors, the Class I PI3K enzymes phosphorylate PtdIns(4,5)P_2 to produce PtdIns(3,4,5)P_3 and initiate downstream signaling events.

In the case of insulin signaling, the adaptor proteins that link the regulatory subunits to the activated receptors are the Insulin Receptor Substrates (*IRS1*, *IRS2* and *IRS4*). In the case of insulin resistance, due to either inflammation or mTORC1 activation, these subunits may be serine phosphorylated on several sites [14–20]. This prevents tyrosine phosphorylation of IRS proteins, reducing the recruitment of PI3K and preventing the formation of PtdIns(3,4,5)P_3 [21–25]. Importantly, this means that the pathophysiological states associated with insulin resistance involve reduced PtdIns(3,4,5)P_3 synthesis.

PtdIns(3,4,5)P_3 increases are typically transient in nature as the phospholipid is rapidly degraded following its synthesis. There are three mechanisms by which levels of PtdIns(3,4,5)P_3 can be reduced, each of which depends on the activity of a lipid phosphatase acting at the 3, 4 or 5 position of the inositol ring. The best studied negative regulator of PtdIns(3,4,5)P_3 is PTEN, a 3-phosphatase that catalyzes the conversion of PtdIns(3,4,5)P_3 to

PtdIns(4,5)P_2. PTEN was first identified as a tumor suppressor in 1997 [26,27], then as a PtdIns(3,4,5)P_3 3-phosphatase in 1998 [28]. PtdIns(3,4,5)P_3 levels can be also reduced by the 5-phosphatases such as SHIP-2 (encoded by *INPPL1*) or SKIP (*INPP5K*) [29]. In contrast to PTEN, the 5-phosphatases generate PtdIns(3,4)P_2 rather than PtdIns(4,5)P_2 from PtdIns(3,4,5)P_3. Depletion of either SHIP or INPP5K results in increased PtdIns(3,4,5)$_3$ levels, while enhancing Akt signaling in both cultured cells and animals [30–36].

Although PtdIns(3,4)P_2 is a degradation product of PtdIns(3,4,5)P_3, some data indicates that this lipid can also support Akt activation via interactions with the PH domain of the kinase [37]. Furthermore, PtdIns(3,4)P_2 can itself be degraded by the 4-phosphatases *INPP4A* and *INPP4B* [38,39]. Work by several groups has shown that like PTEN, INPP4B functions as a tumor suppressor, and that depletion of this enzyme leads to increased Akt signaling [40,41]. These data suggest that sustained levels of PtdIns(3,4)P_2 are able to maintain Akt signaling, though whether this is functionally different from PtdIns(3,4,5)P_3-supported Akt activity has not yet been established. Notably, in the case of sustained Akt activation in the absence of INPP4B, the end product of this phosphoinositide pathway is PtdIns(3)P rather than PtdIns(4,5)P_2. It remains uncertain whether negative regulation of Akt by 4-phosphatases differs from negative regulation by 5-phosphatases, and this point is important for insulin-sensitizing interventions that aim to inhibit these catalytic activities.

3. Regulation of Akt signaling pathways by PtdIns(3,4,5)P_3

The protein kinase Akt was first identified as the homolog of a viral oncogene v-Akt [42], and has three isoforms in mammalian genomes. In addition to a carboxy-terminal AGC kinase domain, each isoform of Akt has an amino-terminal Pleckstrin homology (PH) domain which is able to bind PtdIns(3,4)P_2 or PtdIns(3,4,5)P_3 [37]. Correspondingly, the activation and phosphorylation of Akt by growth factors are dependent on the synthesis of these phospholipids, and can be blocked by inhibitors of PtdIns(3,4,5)P_3 synthesis such as wortmannin [43–45].

The activation of Akt is a multi-step process involving the recruitment of Akt to the plasma membrane via direct binding to PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2, bringing the enzyme within proximity of two activating protein kinases, PDK1 and mTORC2 (mTOR Complex 2), which phosphorylate Akt on Threonine 308 and Serine 473, respectively. Phosphorylation of both of these residues is required for complete activation of Akt [46,47]. The role of phosphatidylinositol-3-kinases in mTORC2 activity is unclear, and the degree to which mTORC2 function is increased by insulin remains uncertain. However, PDK1 has a PH domain which is itself able to bind PtdIns(3,4,5)P_3, PtdIns(3,4)P_2, and more weakly PtdIns(3)P and PtdIns(4,5)P_2 [48]. Ablation of the PDK1 lipid binding activity results in a complete blockade in Akt phosphorylation in embryonic fibroblasts [49]. Once activated, Akt is able to phosphorylate substrates essential for suppressing gluconeogenesis, enhancing glucose uptake, glycogen and lipid synthesis (Fig. 1).

4. Regulation of mTORC1 signaling by PI(3,4,5)P_3 and PI(3,5)P_2

The nutrient- and growth factor-responsive protein kinase complex TOR complex 1 (TORC1) is regulated by phosphoinositides via at least two separate mechanisms. Early studies revealed that the PI3K inhibitor wortmannin blocks activation of mTORC1 by insulin [50,51], and it was later shown that this inhibition was at least in part due to the Akt-mediated inactivation of the Rheb GAP complex TSC1/2 [52–56].

The localization of mTORC1 to the lysosome is also as an important component of activation. A second set of small GTPases, the Rag GTPases, along with the vacuolar ATPase are also important regulators of TORC1 activation and lysosomal localization [57–61]. In addition to this, recent work has supported the hypothesis that the TSC1/2 complex also must be recruited to the lysosome [62]. The localization of mTORC1 to the lysosome is inhibitory, and its release from the lysosome is essential for its activation [63]. The molecular events that modulate the binding and release of TORC1 are still not completely understood, but work by our group and others has implicated PtdIns(3)P and PtdIns(3,5)P_2 as playing a role in this process [64–69]. The loss of PtdIns(3,5)P_2 disrupts the localization of TORC1 to the lysosome in both yeast and mammalian systems potentially via direct binding of Raptor to this lipid [64,66]. The loss of either PtdIns(3)P or PtdIns(3,5)P_2 has been associated with reduced TORC1 activity in both yeast and mammalian systems [64–69]. Alternately, the PtdIns(3)P binding protein phospholipase D has also been identified as another activator of TORC1 [68,70–73]. The precise molecular events by which these lipids modulate the activation cascade of TORC1 are still under intense investigation. Once activated, mTORC1 plays an essential role in the regulation of protein, lipid and glycogen synthesis (see below).

5. Regulation of GLUT4 trafficking by multiple phosphoinositides

Insulin stimulates glucose uptake into peripheral tissues, primarily fat and muscle. In adipocytes and muscle cells, this is largely dependent on the facilitative glucose transporter GLUT4 (encoded by *SLC2A4*; [74,75]). This transporter is normally sequestered in internal compartments, but upon insulin stimulation, these vesicles fuse with the plasma membrane, allowing glucose to travel down a concentration gradient into cells (this was recently reviewed in [76–78]). The recycling, translocation and fusion of GLUT4-containing vesicles in response to insulin can be broken down into several general steps, each of which is regulated by several distinct phosphoinositide species.

Studies from the early 1990s showed that the PI3-Kinase inhibitor wortmannin blocks insulin stimulated glucose uptake and GLUT4 translocation in multiple cell types [79–81]. Further work employing Akt inhibitors and knockdowns has suggested that the effects of wortmannin may be mediated through a PtdIns(3,4,5)P_3–Akt signaling pathway [82–86]. Once activated by PtdIns(3,4,5)P_3, Akt has several important targets in GLUT4 trafficking. The Rab10 family GTPase activating protein (GAP) AS160 (encoded by *TBC1D4*) is phosphorylated and inactivated by insulin, allowing for translocation of GLUT4 storage vesicles (reviewed in [76,78,87,88]). Inactivation of AS160 leads to the increased activity of its targets, the Rab10 family GTPases. However, the direct activation of these G proteins by

insulin has not been demonstrated, and the downstream effectors of Rab10 have not been defined [89,90].

Another recently described Akt substrate in GLUT4 translocation is the RGC1/2 complex [91–94]. Akt works in concert with 14–3–3 proteins to inhibit this RalGAP allowing for activation of that GTPase on Glut4-containing vesicles [94]. Once activated, RalA targets these vesicles to the plasma membrane by interacting with the exocyst complex, which is assembled in response to insulin and is required for efficient fusion of GLUT4 vesicles [93,95,96].

The final fusion step of GLUT4 trafficking has been proposed to be regulated by two other Akt substrates. Synip (encoded by *STXBP4*), a masking protein for the targeting SNARE syntaxin-4 has been proposed to be phosphorylated and dis-inhibited by Akt as an important positive regulator of GLUT4 fusion [97,98], but this finding has been controversial [99]. A more recent report has implicated CDP138 (*CDCD5*) in the regulation of GLUT4 fusion. Loss of this Akt substrate appears to have no effect on translocation of vesicles, but blocks fusion events [100]. Interestingly, the C2 domain of this protein has been shown to interact with mixed intracellular lipids, but whether it interacts with specific phosphoinositides is currently unknown.

Aside from PtdIns(3,4,5)P_3-dependent Akt substrates, PtdIns(4,5)P_2 has also been proposed to be necessary for vesicle exocytosis. This phospholipid is essential for clustering and activation of t-SNAREs in a variety of exocytic contexts [101–103], and also interacts directly with a number of exocyst components. Both Exo70 [104,105] and Sec3 [106] interact with PtdIns(4,5)P_2, but the relevance of these interactions to insulin-stimulated GLUT4 trafficking has not yet been examined.

In addition to its role in GLUT4 exocytosis, PtdIns(4,5)P_2 is also an essential mediator of endocytosis (reviewed in [107]). Since GLUT4 must be retrieved from the plasma membrane and sorted, endocytosis is an essential compartment of GLUT4 biology. Cargo is internalized first into compartments that are PtdIns(3,4,5)P_3 and PtdIns(4,5)P_2 positive. These very early endosomal structures are also positive for the small GTPase Rab5 and the adaptor protein APPL1. As these vesicles mature, the levels of PtdIns(3,4,5)P_3 and PtdIns(4,5)P_2 decrease, where as the levels of PtdIns(3)P increase [108,109]. In most cells, PtdIns(3)P exists primarily on early endosomal structures. Along with Rab5, PtdIns(3)P serves as a coincidence detector for EEA1 and plays an essential role in homotypic endosomal fusion. This aggregation process feeds into the recycling endosomeand is important for the internalization and sorting of membrane components, including GLUT4.

PtdIns(3)P is likely generated by the recruitment of the two Rab5 PI3-Kinase effectors, p110β and Vps34 [110,111]. These very early, APPL1 positive endosomal structures also serve as signaling platforms for Akt and EGF signaling [109,112–114]. The effect of these transient structures appears to be context dependent. In some instances, the loss of APPL1 leads to decreased Akt signaling [113,114], whereas in other instances, such as phagocytosis and EGF signaling, APPL1 serves as a negative regulator of signaling [109,115]. These studies have been largely performed in undifferentiated dividing cells, and it will be

interesting to test the role of APPL1 and PtdIns(3)P positive endosomal structures in GLUT4 internalization. Ablation of APPL1 is inhibitory towards insulin-stimulated glucose uptake in cultured adipocytes, but it is difficult to decouple the effects of this knockdown on Akt signaling from other potential trafficking effects [114]. GLUT4 continuously traffics between the plasma membrane and various pools of internalized, recycled and pre-exocytic vesicles. Therefore maintenance of internal pools of PtdIns(3)P is likely essential for the efficient movement of GLUT4 through these stages.

In addition to the roles of PtdIns(3)P in endosomal trafficking, cultured myocytes and adipocytes also have a second major pool of this lipid at or near the plasma membrane [116–119]. The precise role of this PtdIns(3)P pool is currently unclear, but reductions in this pool are co-incident with reductions in insulin-stimulated GLUT4 trafficking and glucose transport [116–120]. Furthermore, exogenous transfection of this lipid into cultured cells results in increased translocation, but not fusion of GLUT4 with the plasma membrane [121]. These data suggest that in insulin responsive tissues, PtdIns(3)P may play a role in the recruitment of GLUT4 to a location proximal to sites of fusion whereas PtdIns(3,4,5)P_3 may play a more dominant role in vesicle fusion.

In addition to the well documented roles of PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 in GLUT4 trafficking, there have also been reports of positive roles of PtdIns(3,5)P_2 or PtdIns(5)P in the regulation of insulin-stimulated glucose transport. Synthesis of PtdIns(3,5)P2 and PtdIns(5)P obligatorily requires a single kinase PIKFYVE [122,123]. Overexpression of dominant interfering mutants, and knockdown experiments suggests a correlation between reduced PtdIns(3,5)P_2 or PtdIns(5)P levels with reduced insulin-stimulated GLUT4 translocation and glucose uptake [124–128]. A muscle-specific *Pikfyve* knockout mouse exhibited modest insulin resistance and reduced glucose disposal in muscle tissues, and potentially secondary to that, increased adiposity on normal chow diet [129]. These data implicate *PIKFYVE* as a positive regulator of GLUT4 trafficking, though the exact stages of GLUT4 trafficking and the specific molecular targets of PtdIns(3,5)P_2 or PtdIns(5)P are still not yet known.

6. Insulin secretion

Phosphoinositides also play a role in the glucose-dependent secretion of insulin from pancreatic beta cells. Similar to its role in t-SNARE assembly for GLUT4 exocytosis, PtdIns(4,5)P_2 has also been shown to be essential for insulin release from pancreatic beta cells. Direct application of PtdIns(4)P or PtdIns(4,5)P_2 to cultured beta cells promotes the priming of insulin granules [130,131]. Furthermore, either sequestration of PtdIns(4,5)P_2 by overexpression of the PtdIns(4,5)P_2-binding GFP-PLCδ1 [131,132], or by anti-PtdIns(4,5)P_2 antibodies [130] reduces insulin secretion from beta cells. Finally siRNA-mediated depletion of either PI4Kβ (which generates PtdIns(4)P) or PIP5Kγ (which generates PtdIns(4,5)P_2 from PtdIns(4)P) also reduces insulin secretion in cultured beta cells [131]. In addition to this, PtdIns(3,4,5)P_3-dependent Akt signaling also plays a positive feedback role in insulin secretion, both via beta cell proliferation, and direct effects on insulin release [133–135]. Finally, PtdIns(3)P may also play a role in regulated exocytosis of insulin vesicles. Recent work has uncovered a positive role for PI3K-C2α, which generates PtdIns(3)P, in insulin

exocytosis [136], perhaps reflecting a general pro-exocytic role of this phospholipid, such as has been described in neurosecretory cells [137,138].

7. Akt-dependent regulation of gluconeogenesis

The major function of insulin in the liver is to suppress gluconeogenesis. This is accomplished primarily via PtdIns(3,4,5)P3/Akt-dependent regulation of the pro-gluconeogenic transcription factor FOXO1 [139–142]. Once this transcription factor is phosphorylated, it exits the nucleus and is not transcriptionally active. Interestingly, in cases of obesity-induced insulin resistance, gluconeogenesis is impaired but lipogenesis is still active, implying either differential sensitivity of these Akt-dependent processes, or alternative Akt-independent mechanisms that are activated in obese states [139,143,144].

8. Glycogen and lipid synthesis

Both glycogen and lipid storage in metabolically-responsive tissues are largely dependent on Akt signaling pathways. Inmuscle and adipose tissue, both substrate availability (via glucose uptake), and lipid and glycogen synthesis are sensitive to wortmannin [145,146]. The direct pathways by which PtdIns(3,4,5)P_3 regulates lipid synthesis involve both Akt and mTORC1 dependent effects, as well as transcriptional and post-translational changes [147–151]. For example, Akt inactivates GSK3, which is a negative regulator of glycogen synthase [152,153]. However, recent models wherein Akt is unable to inhibit GSK3 have called into question the physiological relevance of GSK inhibition on glycogenesis [154–156]. Our group and others have implicated mTORC1-dependent activation of SREBP1 as a novel regulator in liver glycogen synthesis [151,157]. Similarly, a mTORC1/SREBP1 pathway plays an important role in lipogenesis in concert with the effects of mTORC1 on the phosphatidic acid phosphatase Lipin [143,148,158–160].

9. *In vivo* studies regarding the role of phosphoinositides in metabolism

Due to the key role of phosphoinositides in several metabolically relevant biological processes, there have been intensive studies directed towards understanding their roles in whole-animal physiology. This has been done primarily using knockout mouse or inhibitor studies as detailed below.

10. Decreases in PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 levels lead to insulin resistance

The majority of animal models describing phosphoinositide disruption of enzymes involve the synthesis and degradation of PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2, both of which are generated by Class IA PI3-kinases. Ablation of the most widely expressed catalytic isoforms (α and β) both result in embryonic lethality [161,162]. Somewhat surprisingly, a kinase-dead knock-in for *Pik3cb* (p110β) is not embryonic lethal, suggesting that it is the absence of the protein rather than the loss of catalytic activity that leads to the lethality [163]. Transgenic mice expressing kinase-dead *Pik3cb* have mild peripheral insulin resistance. Expression of an inactivating kinase domain mutation in *Pik3ca* is embryonic lethal when homozygous,

and peripherally insulin resistant as a heterozygote [164]. While heterozygotes mutants of *Pik3ca* and *Pik3cb* have not been shown to have a metabolic phenotype, compound double *Pik3ca*/*Pik3c* heterozygotes have mild glucose and insulin intolerance [165]. Together, these data suggest that global reductions of *Pik3ca*, and to a lesser extent *Pik3cb* lead to peripheral insulin resistance, likely due to an inability of insulin to activate Akt-dependent processes.

To test tissue-specific roles of these isoforms, floxed alleles have been generated for both *Pik3ca* and *Pik3cb*. Liver-specific knockout of *Pik3ca*, either through use of albumin-Cre or transfection with adenoviral Cre ablates insulin-stimulated PI3K activity, with associated reductions in Akt phosphorylation in the liver. Based on both insulin-tolerance tests, and hyperinsulinemic/euglycemic-clamp studies, insulin suppression of endogenous glucose production is impaired [166]. This is consistent with the phenotype of hepatic insulin resistance for both acute and chronic depletion of *Pik3ca* in the liver. Interestingly, chronic reductions of liver *Pik3ca* on a normal chow diet does not cause significant reductions in the serum lipid profile [166,167], as is the case for acute reduction of liver *Pik3ca* or liver insulin-receptor knockout mice [166,168]. There were, however, reductions in high-fat diet induced hypercholesterolemia and hepatosteatosis [167].

Although p110β is reported to provide only a small fraction of insulin-stimulated PtdIns(3,4,5)P_3, liver-specific knockout of this enzyme also resulted in substantial hepatic insulin resistance, but surprisingly has only very limited effects on Akt phosphorylation [167,169]. A single nucleotide polymorphism in the promoter of *PIK3CB* is associated with improved hepatic but not peripheral insulin sensitivity [170]. Liver-specific *Pik3cb* knockout mice had no detectable differences in serum or hepatic lipid profiles, even when challenged by a high fat diet [167]. Together these data strongly implicate PtdIns(3,4,5)P_3 and/or PI(3,4)P_2 in the regulation of gluconeogenesis, but the physiological relevance of this pathway in lipid steatosis is not as clear.

11. Increasing the levels of PtdIns(3,4,5)P_3 causes insulin sensitization

Pathophysiological conditions such as insulin resistance cause impaired production of PtdIns(3,4,5)P_3. Since decreased PtdIns(3,4,5)P_3 reduces insulin signaling, insulin resistance could be potentially ameliorated by increasing PtdIns(3,4,5)P_3 levels. Therefore, inhibition of PtdIns(3,4,5)P_3 degradation has been explored as a means by which the deleterious effects of high fat diet-induced insulin resistance may be corrected. Converse to the PtdIns(3,4,5)P_3 reductions present in Class IA PI3K knockout models, *Pten* knockout mice have increased levels of this phosphoinositide, due to an inability to dephosphorylate PtdIns(3,4,5)P_3 into PtdIns(4,5)P_2.

Homozygous deletion of *Pten* results in embryonic lethality [171,172], but heterozygotes have reduced fasting glucose levels and elevated insulin sensitivity [173]. Antisense oligonucleotides have also been used to probe PTEN function in whole animals. Reduction of PTEN levels by 70–90% in liver and adipose tissue caused normalization of blood glucose and insulin in ob/ob mice [174]. PTEN has been extensively studied in tissue-specific knockout models (reviewed in [175]). Mice with a targeted knockout of *Pten* in skeletal and cardiac muscle using *Ckmm-Cre* were resistant to high fat diet-induced insulin

resistance [176], but there were no detectable differences in body weight either under normal or high fat diet fed conditions. These mice were also characterized by enhanced Akt phosphorylation and glycogen accumulation in their soleus muscles. It is unclear in this case why enhanced insulin sensitivity of these mice is only observed after a high-fat diet challenge and not under normal diet conditions, and may underlie a role for PTEN/ PtdIns(3,4,5)P_3 in the muscle response to high fat feeding.

Two groups have independently generated *Alb-Cre* driven liver-specific *Pten* knockout mice [177,178]. Both groups report substantial hepatomegaly, suppressed fasting glucose levels and increased insulin sensitivity. These data are consistent with a positive role for PtdIns(3,4,5)P_3 in cell division and the suppression of gluconeogenesis. One group also reported substantial hepatosteatosis and accumulation of glycogen in the liver-specific PTEN knockout mice, likely due to accelerated insulin-stimulated lipogenesis and glycogenesis [177]. Some of these mice also developed hepatocellular carcinomas, underlying the important role of PTEN both in normal physiology and as a tumor suppressor.

Insulin leads to increased glucose uptake and lipid/glycogen storage in adipose tissue. Targeted deletion of PTEN from white adipose tissue using the *Ap2-Cre* promoter revealed increased Akt signaling, an accumulation of lipids in white adipose tissue depots, and an increase in whole-body insulin sensitivity [179]. This phenotype is consistent with other models of increased insulin action in adipose tissue [180].

PtdIns(3,4,5)P_3 and PTEN have also been implicated in the regulation of pancreatic function. Two groups have explored the phenotype of beta-cell specific knockouts of *Pten*, driven by RIP (Rat Insulin Promoter)-Cre [135,181]. Both groups report hyperplasia of islets, increased glucose-induced insulin secretion, decreased fasting glucose and reduced whole-animal insulin sensitivity. These data highlight a role for PTEN in suppressing islet growth and dampening glucose-induced insulin secretion from islet cells.

The increased insulin sensitivity in *Pten* loss of function models led to the hypothesis that pharmacological inhibition of PTEN may improve insulin resistance by elevating PtdIns(3,4,5)P_3 levels. Bisperoxovanadium compounds inhibit PTEN in vitro and result in increased Akt activation in cells [182,183]. These vanadate derivatives also have dramatic positive effects on the stimulation of glucose transport in cells [184–188]. In animals, these drugs cause reductions in circulating glucose levels consistent stimulation of glucose uptake and suppression of gluconeogenesis [189,190]. Complicating these results however is the insight that bisperoxovanadium compounds increase tyrosine phosphorylation of the insulin receptor and IRS proteins [185,186]. These findings suggest that these drugs also inhibit the Protein Tyrosine Phosphatase family of tyrosine phosphatases, another important set of negative regulators of insulin signaling that work upstream of PI3K activity.

PtdIns(3,4,5)P_3 levels are also negatively regulated by a 5-phosphatases such as SHIP-2 or SKIP. Whole-body *Inppl1* (SHIP-2) knockout mice are viable although animals were smaller than littermate controls [32,191]. On normal chow diet, these mice had no apparent improvements in glucose homeostasis, but these animals were resistant to high-fat diet induced hyperglycemia and hyperinsulinemia [32]. An inhibitor of SHIP-2 was developed

by Astellas Pharmaceuticals and has shown some promise, as it also increases Akt phosphorylation and glucose uptake in cells, as well as reducing glucose levels in mice [192].

Another 5-phosphatase that has been suggested to negatively regulate PtdIns(3,4,5)P_3 levels is *Inpp5k* (also known as SKIP). Whereas homozygous deletion of this enzyme is embryonic lethal, heterozygotes are insulin sensitive with modest resistance to diet-induced obesity. These data are consistent with a negative role of INPP5K in Akt signaling [30,31,34,193]. In each of these cases (for PTEN, SHIP-2 or INPP5K) the presumed effect of loss or inhibition is through hyperactivation of Akt, but this has not been formally established, as other PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2 dependent processes may also play a role. The PtdIns(3,4)P_2 phosphatases *INPP4A* and *INPP4B* have been identified as playing a role in Akt signaling in the context of tumor growth, but their role in regulating metabolism has not yet been explored.

Related to this point, the benefits of inhibiting PTEN or other PtdIns(3,4,5)P_3 phosphatases, either genetically or pharmacologically should be cautioned due to the important roles of PTEN and INPP4A/B potent tumor suppressors [26,40,41,171]. Careful balancing of the insulin-sensitizing effects associated with PtdIns(3,4,5)P_3 phosphatase inhibition with the potential transformation potential of this idea would be necessary for this to be effective.

12. Key questions in the field

Since their discovery as major modulators of insulin action, phosphoinositides have been under intensive investigation. The study of these lipids has led to major advances in our understanding of intracellular trafficking, signal transduction and organelle biogenesis.

Blockade of phosphoinositide synthesis in insulin resistance is an important, yet underappreciated aspect of our understanding of the pathophysiology of obesity. The majority of insulin-resistance inducing interventions are associated with serine phosphorylation of IRS, which occurs upstream of PtdIns(3,4,5)P_3 synthesis. This makes the re-establishment of PtdIns(3,4,5)P_3 levels an important insulin-sensitizing and glucose lowering possibility. This idea though should be taken with caution, for two reasons. One point is that promoting glucose storage in an obese person may reduce circulating glucose levels, but has the confounding problem of exacerbating lipid synthesis and other obesity associated complications. This has been a concern with several insulin sensitizing interventions including insulin therapy [194–197], thiazolidinediones [198–200] and sulfonylureas [195].

Complicating this problemis the observation that in insulin resistant states, alternative mechanisms of nutrient disposition are upregulated including mTORC1 activation [143,151,201], counter-inflammatory processes [202,203] and a downregulation of catabolic signaling processes [204–207]. This is especially true for glycogenic and lipogenic processes, which are not substantially suppressed in obesity-associated insulin resistance [2,144]. These data suggest that re-sensitization of PtdIns(3,4,5)P_3 dependent processes may not alleviate obesity-associated comorbidities if these other counter-regulatory processes are

not also suppressed (see Fig. 2). It is not clear from current studies the role that phosphoinositides play in these important counter-regulatory actions associated with obesity but understanding these mechanisms are extremely important to developing better treatments for obesity/insulin resistance.

That being said, the extent to which re-sensitization of PtdIns(3,4,5)P_3 dependent signaling is associated with global improvements in energy balance has not been thoroughly explored. As obesity is associated with dramatic decreases in peripheral energy expenditure, the role of phosphoinositides, especially in muscle and brown adipose tissue, associated with these decreases is a ripe area for investigation. The in vivo tools for the manipulation of phosphoinositides in a temporal and tissue specific manner are just starting to emerge and we expect that these studies will shed light on the complex inter-relationships involved in the balance between anabolic and catabolic action at an organismal level. Understanding the effects of manipulation of these lipids in health and disease will play a key role in combating the co-morbidities associated with obesity and Type 2 diabetes.

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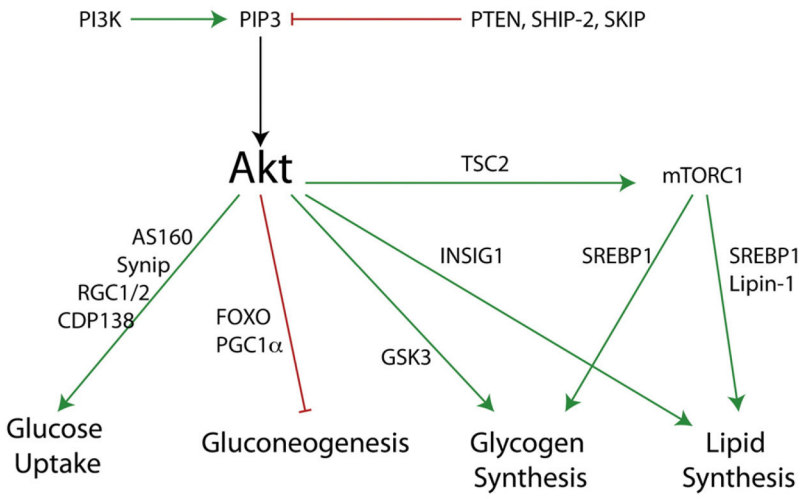

Fig. 1.

Roles of the PtdIns(3,4,5)P_3/Akt pathway on insulin signaling in liver fat and muscle tissue with representative substrates indicated.

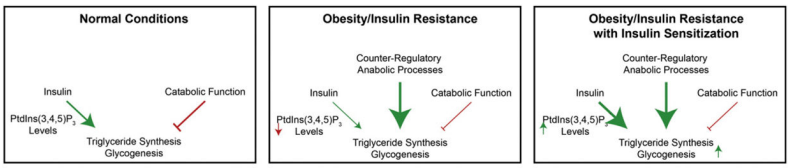

Fig. 2.

Inter-relationship between sensitization of insulin action, counter-regulatory actions and lipid deposition in obesity.