MINIREVIEW

Insulin-Sensitive Protein Kinases (Atypical Protein Kinase C and Protein Kinase B/Akt): Actions and Defects in Obesity and Type II Diabetes

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Glucose transport into muscle is the initial process in glucose clearance and is uniformly defective in insulin-resistant conditions of obesity, metabolic syndrome, and Type II diabetes mellitus. Insulin regulates glucose transport by activating insulin receptor substrate-1 (IRS-1)–dependent phosphatidylinositol 3-kinase (PI3K) which, *via* increases in PI-3,4,5-triphosphate (PIP_3), activates atypical protein kinase C (aPKC) and protein kinase B (PKB/Akt). Here, we review (i) the evidence that both aPKC and PKB are required for insulin-stimulated glucose transport, (ii) abnormalities in muscle aPKC/PKB activation seen in obesity and diabetes, and (iii) mechanisms for impaired aPKC activation in insulin-resistant conditions. In most cases, defective muscle aPKC/PKB activation reflects both impaired activation of IRS-1/PI3K, the upstream activator of aPKC and PKB in muscle and, in the case of aPKC, poor responsiveness to PIP_3, the lipid product of PI3K. Interestingly, insulin-sensitizing agents (e.g., thiazolidinediones, metformin) improve aPKC activation by insulin *in vivo* and PIP_3 *in vitro*, most likely by activating 5′-adenosine monophosphate–activated protein kinase, which favorably alters intracellular lipid metabolism.

Differently from muscle, aPKC activation in the liver is dependent on IRS-2/PI3K rather than IRS-1/PI3K and, surprisingly, the activation of IRS-2/PI3K and aPKC is conserved in high-fat feeding, obesity, and diabetes. This conservation has important implications, as continued activation of hepatic aPKC in hyperinsulinemic states may increase the expression of sterol regulatory element binding protein-1c, which controls genes that increase hepatic lipid synthesis. On the other hand, the defective activation of IRS-1/PI3K and PKB, as seen in diabetic liver, undoubtedly and importantly contributes to increases in hepatic glucose output. Thus, the divergent activation of aPKC and PKB in the liver may explain why some hepatic actions of insulin (e.g., aPKC-dependent lipid synthesis) are increased while other actions (e.g., PKB-dependent glucose metabolism) are diminished. This may explain the paradox that the liver secretes excessive amounts of both very low density lipoprotein triglycerides and glucose in Type II diabetes.

Previous reviews from our laboratory that have appeared in the Proceedings have provided essentials on phospholipid-signaling mechanisms used by insulin to activate several protein kinases that seem to be important in mediating the metabolic effects of insulin. During recent years, there have been many new advances in our understanding of how these lipid-dependent protein kinases function during insulin action and why they fail to function in states of insulin resistance. The present review will attempt to summarize what we believe are some of the more important advances. Exp Biol Med 230:593–605, 2005

Key words: insulin; glucose transport; atypical protein kinase C; protein kinase B; muscle; liver; glucose; lipids; obesity; diabetes

Introduction

Throughout the past decade, our laboratory has focused on the role of atypical protein kinase C (aPKC) isoforms as mediators of insulin-stimulated glucose transport in muscle and adipocytes. In recent years, our interest has been further whetted by the finding that there are consistent defects in aPKC activation by insulin in muscles and adipocytes in insulin-resistant states of obesity and Type II diabetes, as seen in a variety of laboratory animals and humans. Also

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intriguing is our even more recent finding (unpublished) that, as in skeletal muscle, aPKC activation by insulin is defective in heart muscle of high-fat–fed mice (i.e., a surrogate for obesity), Type II diabetic Goto-Kakizaki (GK) rats, *ob/ob* obese/diabetic mice, and rats rendered diabetic by the streptozotocin (STZ)-induced destruction of islet β-cells. Thus, abnormalities seen in skeletal muscle in insulin-resistant conditions may have similar counterparts in heart muscle.

Defects in aPKC activation by insulin in skeletal and heart muscle appear to be explained not only by diminished activation of insulin receptor substrate (IRS)–dependent phosphatidylinositol 3-kinase (PI3K), which functions upstream of aPKC, but also by the diminished ability of the lipid product of PI3K (i.e., PI-3,4,5-triphosphate [PIP_3]) to directly activate aPKC.

Unlike the situation in muscle and adipocytes, aPKC activation has not been found to be impaired in the livers of all insulin-resistant animals that we have examined to date including high-fat–fed mice, *ob/ob* diabetic mice, GK-diabetic rats, STZ-diabetic rats, and obese monkeys. This surprising finding may be particularly important because aPKCs are major mediators for insulin-induced increases in the expression of sterol regulatory element binding protein-1c (SREBP-1c) in the liver. Also, this transcription factor activates a battery of genes, including fatty acid synthase (FAS) and acetyl-coenzyme A carboxylase, that control lipid synthesis in the liver. Thus, the unabated or heightened activation of aPKC by insulin in the liver may importantly contribute to the pathogenesis of the dyslipidemia (i.e., very low density lipoprotein [VLDL]–associated hypertriglyceridemia and reciprocal decreases in cardioprotective high-density lipoprotein lipids) that is characteristic of hyperinsulinemic states of obesity, metabolic syndrome, and Type II diabetes.

In view of the tissue-specific differences in whether aPKC activation is compromised or conserved, it is essential to recognize the factors that control PI3K and aPKC activation by insulin in various types of insulin-sensitive tissue. In this regard, data from mice in which IRS-1 or IRS-2 has been knocked out suggest that aPKCs in the liver are controlled by IRS-2/PI3K, rather than IRS-1/PI3K (Figs. 1 and 2). This is very different from muscle, wherein IRS-1/PI3K, rather than IRS-2/PI3K, is the primary activator of aPKCs.

In addition to aPKC, it is essential to recognize the factors that control the activation of protein kinase B (PKB/Akt) which, like aPKC, is activated by PI3K and is required for insulin-stimulated glucose transport in muscle and adipocytes. However, unlike aPKC, PKB is also required for the insulin regulation of glycogen synthesis in the muscle, adipocytes, and liver and gluconeogenesis and glucose release in the liver. In this regard, in muscle, like aPKC, PKB activation is largely controlled by IRS-1; in the liver, unlike aPKC, both IRS-1/PI3K and IRS-2/PI3K control the activation of PKB during insulin action (Figs. 1 and 2).

Of further note, we have recently found that insulin signaling to IRS-1/PI3K, IRS-2/PI3K, aPKC, and PKB is intact in livers of both high-fat–fed mice and spontaneously occurring obesity in monkeys. Thus, conserved PKB activation in the liver most likely contributes to maintaining relatively normal glucose homeostasis in these states. On the other hand, because insulin resistance in muscle leads to hyperinsulinemia in states of obesity and high-fat feeding, increases in SREBP-1c expression and hepatic lipid synthesis are expected to ensue from heightened activation of IRS-2/PI3K and aPKC by insulin.

We also recently found that, whereas the activation of IRS-1/PI3K and PKB is impaired in the livers of GK-diabetic rats, STZ-diabetic rats, and *ob/ob* diabetic mice, the activation of both IRS-2/PI3K and aPKC is fully or well conserved in this organ. Thus, as in states of obesity, conserved activation of IRS-2/PI3K and aPKC in the liver in these diabetic rodents is expected to increase the expression of SREBP-1c and hepatic lipid synthesis in response to the hyperinsulinemia seen in GK-diabetic rats and *ob/ob* diabetic mice. However, unlike obesity, the defect in IRS-1/PI3K and PKB activation seen in these diabetic rodents would be expected to increase hepatic glucose output and contribute to their hyperglycemia (Fig. 1).

As has been previously alluded to, it appears that defects in aPKC activation in muscle and adipocytes are seen throughout the spectrum of obesity and Type II diabetes. Moreover, these defects in muscle aPKC activation most likely importantly contribute to the development of insulin resistance in these syndromes. Thus, it is essential to understand the mechanisms that increase aPKC activity in muscle and adipocytes. To this end, it is now recognized that several insulin-sensitizing agents (i.e., 5-amino-imidazole-4-carboxamide-1-β-D-riboside [AICAR], adiponectin, thiazolidinediones [TZDs], metformin) activate 5′-adenosine monophosphate–activated protein kinase (AMPK) in muscle and other types of tissue. Moreover, preliminary findings suggest that each of these agents activates and/or improves the activation of aPKCs in skeletal and/or heart muscle by insulin and/or PIP_3. These findings provide important insight into how presently used therapeutic agents improve insulin effectiveness, and this insight should pave the way for developing new therapeutic agents.

General Aspects of Insulin Signaling and Control of Metabolic Functions. Insulin effects are initiated through the insulin receptor, which phosphorylates tyrosine residues on IRS-1, IRS-2, Cbl, and other proteins including IRS-3, IRS-4, Shc, Gab-1, SIRPs, adapter protein containing PH and SH2 domains (APS), and FAK (1–3). Tyrosine phosphorylation of IRS-1, IRS-2, Cbl, and other proteins leads to binding to SH2 domains of (i) the p85 subunit of PI3K which, *via* activation of the p110 catalytic subunit, converts PI-4,5-$(PO_4)_2$ to PIP_3; (ii) GRB2, which

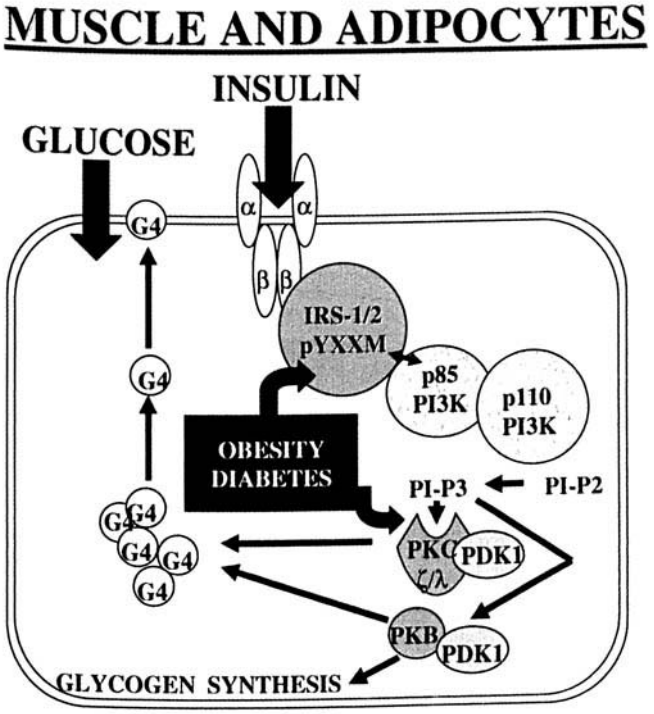

Figure 1. Insulin signaling in muscle and adipocytes. In skeletal muscle, IRS-1, *via* PI3K, controls aPKC and PKB activation. In adipocytes, both IRS-1 and IRS-2, *via* PI3K, control aPKC and PKB activation. In both muscle and adipocytes, aPKC and PKB stimulate the translocation of Glut4 (G4) glucose transporters to the plasma membrane. Insulin signaling to IRS-1, PI3K, aPKC, and PKB is impaired in simple obesity and diabetes. In addition, the activation of aPKC by PIP3 is impaired in simple obesity and diabetes.

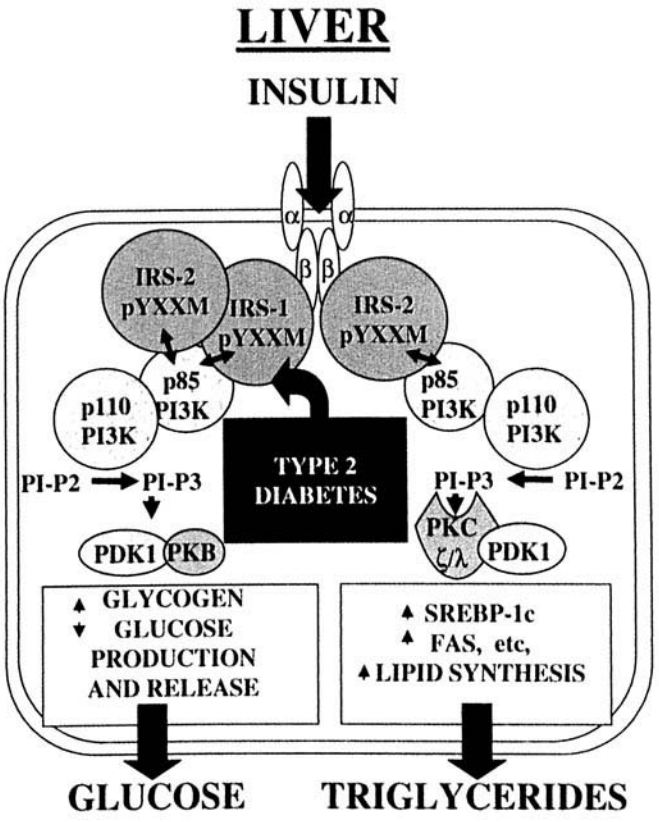

Figure 2. Insulin signaling in the liver. In the liver, IRS-2, *via* PI3K, controls aPKC activation. In contrast, both IRS-1 and IRS-2, *via* PI3K, control PKB activation. The expression of SREBP-1c, which transactivates many genes that are active in fat synthesis including FAS, is largely, but not exclusively, controlled by aPKC. Increases in lipid synthesis lead to increases in the secretion of VLDL triglycerides. With respect to liver handling of glucose, PKB (and, possibly, other undefined factors, but not aPKC), increases glycogen synthesis and diminishes glucose production and release. In simple obesity, insulin signaling is grossly intact in the liver. With the onset of diabetes, IRS-1 signaling to PI3K and PKB is diminished, but IRS-2 signaling to PI3K and aPKC is better or fully conserved. Thus, in hyperinsulinemic states of simple obesity and Type II diabetes, increased IRS-2 signaling to aPKC leads to increases in SREBP-1c expression, lipid synthesis, and VLDL-triglyceride secretion. In diabetes, diminished signaling to IRS-1 and PKB leads to increases in hepatic glucose output.

activates the extracellular receptor–activated kinase (ERK) pathway; and/or (iii) Crk, which activates C3G and TC10.

In conjunction with 3-phosphoinositide–dependent protein kinase-1 (PDK1), PIP_3 activates downstream effectors that control various metabolic processes including glucose transporter 4 (Glut4) translocation to the plasma membrane and glucose transport in muscle and adipocytes; glycogen synthesis in muscle, adipocytes, and liver; and lipid synthesis in liver (Figs. 1 and 2).

Although PI3K is required, simple increases in PI3K activity do not necessarily increase Glut4 translocation/glucose transport, suggesting that PI3K must be activated in specific subcellular compartments or other factors are required. It is generally accepted that IRS-1 and IRS-2 function upstream of PI3K pools required for activation of aPKCs and PKB which, in turn, are thought to control glucose transport and other metabolic processes. As we later discuss, Cbl-dependent PI3K may also be required for aPKC activation and may also function in localizing signaling complexes to appropriate areas of the plasma membrane that are required for Glut4 translocation and glucose transport.

Independently of PI3K, the insulin receptor phosphorylates tyrosine residues of Cbl and APS which, along with Cbl-associated protein (CAP) and flotillin, localize to the caveolin-rich microdomains of the plasma membrane. The Cbl also forms a signaling complex with SH2/SH3 adapter protein Crk, GTP/GDP exchange factor C3G, small Rho G protein TC10, and other factors needed for insulin-stimulated Glut4 translocation/glucose transport (3). Although this signaling complex forms independently of PI3K, Cbl, like IRS-1 and IRS-2, activates PI3K in adipocytes (4, 5) and is required for the insulin activation of aPKC and glucose transport in adipocytes, but probably not in muscle (6). Although much work supports the importance of the Cbl/Crk/C3G/TC10 pathway, this view has recently been challenged because silencing RNA (SiRNA) can diminish levels of these factors without inhibiting insulin-stimulated glucose transport in 3T3/L1 adipocytes (7). On the other hand, a similar study in human adipocytes in which SiRNA was used to knock down Cbl suggested that Cbl is required for insulin-stimulated glucose

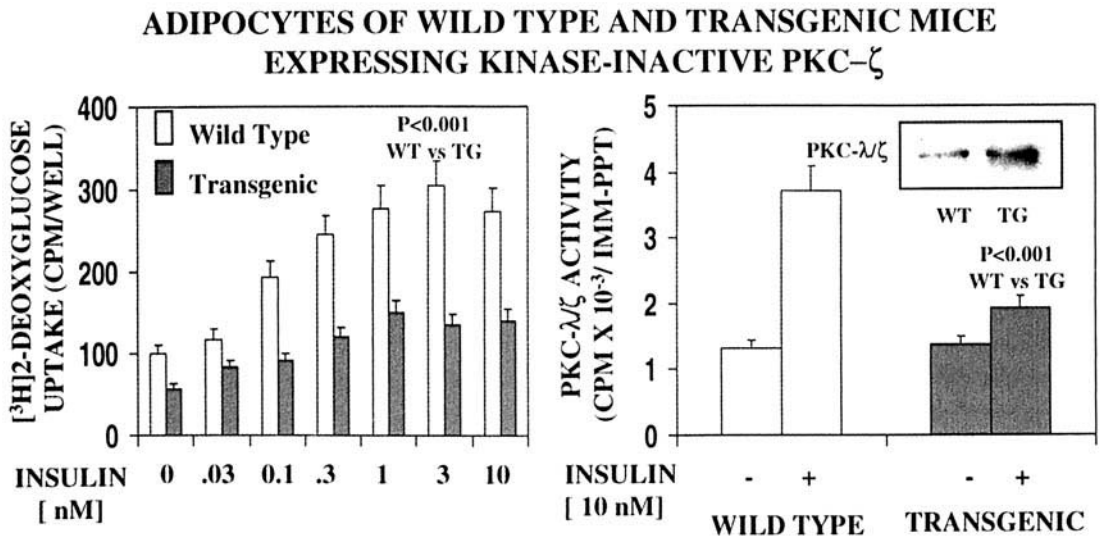

Figure 3. Impaired activation of PKC-λ and PKC-ζ and diminished effects of insulin on glucose transport in adipocytes harvested from transgenic mice expressing kinase-inactive PKC-ζ. Adipocytes were isolated from littermate wild-type mice or transgenic mice expressing kinase-inactive PKC-ζ and incubated with increasing concentrations of insulin for 30 mins for studies of glucose transport (left; [^{3}H]-2-deoxyglucose uptake over 1 min) or with 10 n*M* insulin for 15 mins for studies of activation of total atypical PKCs (precipitated with antiserum recognizing both PKC-λ and PKC-ζ). As is apparent, there was excellent agreement between defects seen in aPKC activation and insulin-stimulated glucose transport.

transport (8). Caveats associated with the use of SiRNA and other methods of requirement analysis are discussed later.

In addition to glucose transport in muscle and adipocytes, insulin controls glucose metabolism by increasing glycogen synthesis and decreasing glycogenolysis in muscle, adipocytes, and liver and by decreasing gluconeogenesis and glucose release in the liver and kidney. Whereas both aPKC and PKB are thought to regulate glucose transport, PKB appears to be more important than aPKC for regulating glycogen synthesis in muscle, adipocytes, and liver and gluconeogenesis and glucose release in the liver. On the other hand, aPKC, operating downstream of PI3K, seems to be a major regulator of expression of the transcription factor SREBP-1c; thus, aPKC appears to importantly contribute to insulin effects on lipid synthesis in the liver (9). Whether PKB coregulates SREBP-1c is debated (9).

Different from the PI3K and the Cbl/Crk/C3G/TC10 pathways, the GRB2/SOS/RAS/RAF/MEK1/ERK1/2 pathway is not required for insulin effects on glucose metabolism, but nevertheless importantly functions for insulin effects on protein synthesis, gene expression, cell differentiation, and proliferation. On the other hand, ERK1 and ERK2, functioning in a different context (i.e., in a signaling complex with AMPK and proline-rich tyrosine kinase-2 [PYK2]), may be important for mediating AMPK-dependent increases in aPKC activity and glucose transport (as is later discussed).

Downstream Effectors of PI3K During Insulin-Stimulated Glucose Transport

PDK1. Operating immediately downstream of PI3K is PDK1 which, like PI3K, is required for insulin-stimulated Glut4 translocation/glucose transport (10). The activation loop phosphorylation of not only aPKCs (11, 12) and PKB (13), but also of p70S6 kinase, protein kinase C–related kinase-1 and -2 (protein kinase N) and conventional and novel protein kinase Cs (PKCs), is analogously controlled by PDK1 (10, 11). Of these, aPKCs and PKB are thought to be required for insulin-stimulated Glut4 translocation/glucose transport. In activating aPKCs and PKB, PDK1, like PI3K, needs to be localized in specific plasma membrane domains, as simple insertion of PDK1 into the plasma membrane does not activate glucose transport (14).

PKB/Akt. Several studies using expression of constitutively acting or kinase-inactive forms of PKB provided the initial evidence that PKB is required for insulin-stimulated Glut4 translocation and/or glucose transport in rat adipocytes, 3T3/L1 adipocytes, and L6 myotubes (15–18). On the other hand, adenoviral-mediated expression of an activation-resistant PKBα double alanine (AA) mutant (T308A/S473A) failed to inhibit Glut4 translocation/glucose transport, despite effectively inhibiting other PKB-dependent effects of insulin in 3T3/L1 adipocytes (19). Similarly, in rat adipocytes, plasmid-mediated expression of kinase-inactive PKB only mildly inhibited insulin-stimulated hemagglutinin antigen (HA)-Glut4 translocation (20). We too found that adenoviral-mediated expression of a kinase-inactive, activation-resistant, triple-AAA mutant form of PKB effectively inhibits both PKBα and PKBβ activation by insulin in 3T3/L1 adipocytes, L6 myotubes, and cultured human adipocytes, but does not inhibit insulin-stimulated Glut4 translocation/glucose transport (21). Others have also found that these dominant-negative forms of PKB do not inhibit insulin-stimulated glucose transport in 3T3/L1

adipocytes and L6 myotubes. In addition, knockout of PKBα is without effect on glucose transport in mouse tissue (22), and knockout of PKBβ inhibits the effects of submaximal insulin, but not the effects of maximally effective concentrations of insulin on glucose transport in isolated skeletal muscles (23). This dose dependency of inhibitory effects of PKBβ knockout most likely reflects the inhibitory effects of the diabetic state present in these knockout mice (resulting from increased hepatic output of glucose) on initial insulin-signaling mechanisms involving the insulin receptor or early signaling steps that are present in excess or "spare," with respect to the relationship between receptor occupancy and glucose transport effects of insulin.

In other approaches, recent SiRNA knockdown studies have suggested that PKBα and PKBβ are required for insulin-stimulated Glut4 translocation/glucose transport in 3T3/L1 adipocytes (24, 25). As we later discuss, however, rigorous controls needed for fully accepting this conclusion with this approach have not been provided. In this regard, it is important to note that we have recently found (unpublished) that the SiRNA construct, as employed in the concentrations used to knock down PKBβ in one study of 3T3/L1 adipocytes (24), also diminished PKC-λ levels in these cells; thus, the findings in this particular study cannot be accepted as truly suggesting that PKB is required for insulin-stimulated glucose transport.

Perhaps the most convincing evidence that PKB is required for insulin-stimulated Glut4 translocation/glucose transport comes from studies of mouse adipocytes engineered to lack both PKBα and PKBβ in which insulin effects on glucose transport are absent and, perhaps more important, rescued by introduction of PKBα or PKBβ into these cells by adenoviral gene transfer (26).

aPKC. As with PKB, multiple lines of evidence have suggested that aPKCs are required for insulin-stimulated Glut4 translocation/glucose transport in muscles and adipocytes. First, in stably transfected 3T3/L1 adipocytes (27) and L6 myotubes (28), expression of kinase-inactive aPKC inhibits, and constitutively active aPKC mimics, insulin effects on Glut4 translocation/glucose transport. Second, the plasmid-mediated expression of kinase-inactive PKC-ζ and PKC-λ (which are highly homologous in functionally important molecular regions and function interchangeably, as we later discuss) inhibits insulin-stimulated HA-Glut4 translocation in transiently transfected rat adipocytes (29, 30). Third, adenoviral-mediated expression of kinase-inactive PKC-ζ or PKC-λ inhibits insulin effects on Glut4 translocation and glucose transport in 3T3/L1 adipocytes (4, 21, 27, 31), L6 myotubes (21, 32, 33), and human adipocytes (21). Fourth, plasmid-mediated expression of activation-resistant PKC-ζ (i.e., mutated at the Thr-410 activation loop site [15, 34] or Thr-560 autophosphorylation site [34]), inhibits insulin-stimulated HA-Glut4 translocation in rat adipocytes. Fifth, expression of wild-type aPKC stimulates or potentiates insulin effects on Glut4 translocation/glucose transport in 3T3/L1 adipocytes (27), rat adipocytes (29, 30), L6 myotubes (32, 33), and rat skeletal muscles (35). Sixth, expression of constitutively active aPKC mimics insulin effects on Glut4 translocation/glucose transport in rat adipocytes (29, 30), L6 myotubes (32), 3T3/L1 adipocytes (27, 31), and cultured human adipocytes (21). Seventh, inhibitory effects of kinase-inactive PKC-ζ on insulin-stimulated HA-Glut4 translocation can be rescued by expression of wild-type forms of PKC-ζ or PKC-λ (30, 32).

As with PKB, the most convincing evidence that aPKCs are required for insulin-stimulated Glut4 translocation/glucose transport derives from studies of mouse embryonic stem (ES) cells and adipocytes that are differentiated from these ES cells (36), in which insulin stimulates glucose transport in wild-type PKC-$\lambda^{+/+}$ ES cells and Glut4-containing PKC-$\lambda^{+/+}$ adipocytes derived by differentiation of these ES cells, but not in PKC-$\lambda^{-/-}$ ES cells and ES-derived PKC-$\lambda^{-/-}$ adipocytes in which PKC-λ has been knocked out by gene-targeting methods (mouse ES cells and derived adipocytes contain primarily PKC-λ and only very small amounts of PKC-ζ (36). Moreover, and perhaps most important, insulin-stimulated glucose transport is rescued by the expression of wild-type PKC-λ or PKC-ζ in both PKC-$\lambda^{-/-}$ ES cells and PKC-$\lambda^{-/-}$ adipocytes (36).

Finally, in very recent studies, we have found that insulin effects on aPKC activity and glucose transport are markedly diminished in adipocytes isolated from transgenic mice that express kinase-inactive aPKC (Fig. 2). In addition, we now have mice in which the PKC-λ gene has been floxed, and we are in the process of crossing these mice with transgenic mice in which Cre-recombinase is controlled by muscle-specific MCK-1 or adipocyte-specific AP2 promoters. Thus, unless PKC-λ is critical for the development of muscle or adipose tissue, we hope to soon be able to test the requirement for aPKCs in muscle (skeletal and heart) and adipocytes of mice in which PKC-λ, the major aPKC in mouse muscle and adipocytes, is knocked out by recombinant methods.

Despite strong evidence from many sources suggesting that aPKCs are required for insulin-stimulated Glut4 translocation/glucose transport, it was recently reported that SiRNA-induced 80% knockdown of PKC-λ only slightly diminished insulin-stimulated Glut4 translocation/glucose transport in 3T3/L1 adipocytes (37). In contrast, Ugi and colleagues, using microinjection methods in 3T3/L1 adipocytes, found that SiRNA targeting of PKC-λ markedly diminished insulin-stimulated Glut4 translocation (25). The reason for these widely differing results is uncertain, but it may be due to the use of different SiRNAs and/or methods that result in different levels of aPKC depletion. Unfortunately, there is no information on how much aPKC is required to support insulin-stimulated glucose transport or the related question of how efficient are the localizing mechanisms (to offset decreases in aPKC levels) that recruit aPKCs to cell compartments that participate in Glut4

translocation and glucose transport. In this respect, note that aPKCs serve many cellular functions throughout the animal and plant kingdoms and, in the case of glucose transport, aPKCs are intensely localized in caveolin-rich lipid rafts of the plasma membrane that form during insulin action in 3T3/L1 adipocytes (38). Also, as another caveat, just as PKBα seems to substitute for PKBβ during insulin action in cells in which PKBβ is knocked out (22–24, 26, 37), other conventional/novel PKCs may substitute for aPKCs when they are deficient. Additionally, in Western analyses, we have been able to blot a faster migrating (shorter, presumably) form of PKC-ζ in 3T3/L1 adipocytes that is recognized by N-terminal, but not C-terminal, anti–PKC-ζ antibodies (unpublished). It is uncertain if this PKC-ζ–like moiety is clipped or otherwise altered on its C-terminus, or if an alternative splice product of PKC-ζ is present in 3T3/L1 adipocytes. It is also uncertain if this PKC-ζ–like moiety is functional in insulin-stimulated glucose transport. Finally, the cells used and the methods of introduction of SiRNA were very different in these studies. Thus, whereas Ugi and colleagues used microinjection (25), Jiang and colleagues used unattached 3T3/L1 adipocytes that were electroporated and subsequently selected by reattachment of "more competent" cells (24).

With respect to the SiRNA studies, we have independently confirmed (using oligofectamine to proficiently transfect a large numbers of cells) that the SiRNA construct used by Ugi and colleagues (25) to knock down PKC-λ inhibits insulin-stimulated glucose transport in 3T3/L1 adipocytes. We have also found that SiRNA constructs that target and knock down PKC-ζ in L6 myotubes inhibit insulin-stimulated glucose transport in these cells that primarily contain PKC-ζ and have little or no PKC-λ. Most important, we have found that the SiRNA that targets and knocks down PKC-λ in 3T3/L1 adipocytes has no effect in L6 myotubes, and the SiRNA that targets and knocks down PKC-ζ in L6 myotubes has no effect in 3T3/L1 adipocytes. Furthermore, expression of wild-type PKC-ζ in 3T3/L1 adipocytes rescues insulin effects on glucose transport in which PKC-λ has been knocked down, and expression of wild-type PKC-λ in L6 myotubes rescues insulin effects on glucose transport in which PKC-λ has been knocked down. Thus, the SiRNAs used in our studies and those of Ugi and colleagues (25) appear to be very specific in knocking down only the targeted aPKC. Accordingly, we believe that the SiRNA data of both Ugi and colleagues (25) and our group strongly support the hypothesis that aPKCs are required for insulin-stimulated glucose transport in both 3T3/L1 adipocytes and L6 myotubes.

PKC-ζ/PKC-λ Interchangeability. Much of the evidence that we have alluded to suggests that PKC-ζ and PKC-λ can function interchangeably during insulin-stimulated glucose transport, including:

1. Expression of either kinase-inactive PKC-ζ or PKC-λ inhibits insulin-stimulated glucose transport in all tested cell types, regardless of which aPKC is most prevalent (rat and human muscles and adipocytes contain primarily PKC-ζ, whereas mouse muscles and adipocytes contain primarily PKC-λ).

2. Wild-type forms of either aPKC can (i) reverse inhibitory effects of either of these kinase-inactive aPKCs, (ii) restore glucose transport in mouse PKC-$\lambda^{-/-}$ ES cells (36), or (iii) mimic or potentiate insulin effects on glucose transport in muscles and adipocytes.

3. The PKC-ζ restores insulin effects on glucose transport in 3T3/L1 adipocytes in which PKC-λ has been knocked down, and PKC-λ can restore insulin effects on glucose transport in L6 myotubes in which PKC-ζ has been knocked down by SiRNA.

This interchangeability probably reflects the fact that, presumably reflecting evolutionary necessity, the functional regions of the aPKCs have been conserved in mouse, rat, and human tissue. Thus, note that (i) PKC-λ is 72% homologous to PKC-ζ, (ii) PKC-λ and PKC-ι (the human counterpart of mouse PKC-λ) are 98% homologous, (iii) the autoinhibitory pseudosubstrate sequence is identical in all three aPKCs, (iv) the C-terminal sequence is nearly identical in all three aPKCs, (v) each aPKC has homologous threonine (Thr) residues in its activation loop (PDK1 dependent) and autophosphorylation (PDK1 independent) sites, and (vi) PIP_3 activates both PKC-ζ and PKC-λ (34, 39). Also, note that the inhibitory effects of expression of kinase-inactive or other mutated forms of aPKCs cannot be explained by the inhibition of PDK1 or PKB (32, 34, 39). Although it is not presently germane to insulin action, shortened forms of PKC-ζ have been found in mouse tissue, particularly in the brain, where it appears to be important in memory processes.

Caveats in Experimental Approaches That Examine Protein Kinase Dependence

From the aforesaid considerations, it is clear that, at least at this point, no single experimental approach can be considered to have provided an entirely convincing argument that either aPKC or PKB is required for insulin-stimulated glucose transport in adipocytes and, even more so, in myocytes.

With respect to studies that rely on the overexpression of wild-type forms or mutated forms of signaling factors, it may be argued that excessive quantities of specific proteins may have untoward or unphysiologic effects, regardless of whether the expressed protein is normal or mutated. On the other hand, the finding that the wild-type protein enhances insulin effects or rescues inhibitory effects of mutated forms of the protein being tested, in our view, provides strong, if not compelling, evidence that this caveat is probably more theoretical than real. As may be surmised, the approach of using an enzymatically inactive protein kinase that is mutated in a single key amino acid (usually in the adenosine-triphosphate–binding site) rests on the hypothesis that, except for the ability to phosphorylate the usual specific substrates, this mutated protein performs all other functions exactly as the

wild-type protein and, therefore, competes with, and thereby inhibits, the wild-type protein, presumably on a one-to-one basis. In this situation, it is the kinase activity of the targeted protein, rather than its ability to function in other ways (e.g., in critical binding or scaffolding in a signaling complex), that is being tested by expression of a kinase-inactive dominant negative protein. Stated differently, this approach depends on molecular dilution and subsequent inhibition, rather than depletion, of the targeted protein.

With respect to studies that rely on protein depletion for testing protein kinase requirements, such as SiRNA and gene knockout studies, there are two caveats that must be considered: (i) the knockdown or knockout must be complete or sufficient to severely limit the availability of the protein to function in the specific cellular process under study, and (ii) other related signaling factors must not be capable or available to substitute for the targeted depleted protein. In general, gene knockout studies are likely to achieve the degree of protein depletion that is necessary to test the requirement for specific protein. However, in the case of SiRNA, there is a lingering question as to whether the degree of protein depletion is adequate. Unfortunately, there is no available information on how much aPKC or PKB protein is required for the specific cellular process of Glut4 translocation (or, for that matter, any cellular process). In this regard, it must be remembered that these kinases have evolved over long periods of time and have diverse cellular functions which, depending on the effectiveness of localizing mechanisms for each specific function, may vary considerably in cellular concentration requirements. In the case of aPKCs, these proteins are required for very basic aspects of cellular polarity and structural somatic development in many species and genera throughout the plant and animal kingdoms. In addition, it is likely that more specialized cellular functions, such as insulin-stimulated glucose transport, may have evolved relatively late in the course of evolution.

Of course, the introduction of high concentrations of oligonucleotides into cells in SiRNA studies carries the added caveat that these substances may be toxic and produce untoward effects or they may target mRNAs that code for other proteins. Indeed, we have encountered considerable toxicity problems with the use of antisense oligonucleotides in studies on insulin-stimulated glucose transport, and we no longer employ this experimental approach. Moreover, as previously discussed, we have found that the SiRNA construct used to knock down PKBβ in one study of 3T3/L1 adipocytes (24) potently knocks down PKC-λ levels in these cells. Thus, relevant target specificity of the SiRNA must be ascertained before drawing conclusions regarding requirements for insulin-stimulated glucose transport or other functions. In this regard, it should also be noted that the use of a scrambled RNA may not be a sufficient control for SiRNA-mediated protein knockdown studies. In the case of glucose transport, whenever possible there should be demonstration that the SiRNA does not inhibit glucose-transport effects of agents that stimulate Glut4 translocation by mechanisms that do not require the targeted protein (e.g., for studies of PKB requirements, it would be relatively easy to show that glucose transport effects of factors that do not require PKB, such as sorbitol, are intact in SiRNA-treated cells). [In this regard, we found (unpublished) that the SiRNA construct used to knock down PKBβ (but which simultaneously knocked down PKC-λ levels, as previously mentioned) in one study of 3T3/L1 adipocytes (24) inhibited sorbitol-stimulated, as well as insulin-stimulated, glucose transport.] Furthermore, for truly compelling evidence, it would be desirable to show that glucose transport function can be rescued by the introduction of wild-type protein into the SiRNA-treated cell; this can most elegantly be achieved by using a cDNA that uses a degenerate/alternate code that can restore the wild-type protein but, because of mismatches, not be recognized by the SiRNA that targets the endogenous mRNA. Alternatively, a different isoform that does not share the same SiRNA-targeted coding sequence as the native protein kinase that is being knocked down can be used to rescue function, as we have done for studies of aPKC requirements during insulin action in 3T3/L1 adipocytes and L6 myotubes, as previously mentioned.

In our view, as has been previously alluded, one of the better experimental approaches in demonstrating kinase dependency is to have a complete or nearly complete knockout of the targeted gene (or a "knock in" of an altered dominant-negative gene to replace the normal gene) that embarrasses the cellular function under question and restore such function by introduction of the normal wild-type kinase into the knockout cell. Such studies, as previously noted, have in fact been performed for both aPKC in PKB in cultured mouse adipocytes in which these protein kinases have been knocked out by recombinant methods. Thus, in our view, it seems clear that both proteins are required for insulin-stimulated glucose transport, at least in these cultured cells. Whether this approach will prove to be feasible in cultured muscle cells is uncertain. Also, whether this approach of knockout and rescue will be feasible in muscles and/or adipocytes of intact animals is uncertain.

Tissue-Specific Differences in aPKC and PKB Activation by IRS-1 and IRS-2

Evidence that IRS-1 operates upstream of aPKC and PKB during insulin action in skeletal muscle derives from the observation of diminished activation of aPKC and PKB in IRS-1 knockout mice (40). In contrast, initial studies in IRS-2 knockout mice suggest that IRS-2 is not required for aPKC or PKB activation in skeletal muscle (unpublished).

In contrast to muscle, whereas PKB activation is markedly diminished in the livers of IRS-1 knockout mice, aPKC activation is fully intact (40). However, aPKC activation is diminished in IRS-2–deficient hepatocytes (41). Thus, it may be surmised that aPKCs are largely controlled by IRS-2/PI3K, rather than IRS-1/PI3K, in the

liver. On the other hand, PKB activation in the liver appears to be dependent on the activation of both IRS-1/PI3K (40) and IRS-2/PI3K (41), as knockout of either IRS-1 or IRS-2 inhibits PKB activation.

Different from muscle and liver, knockout of IRS-1 in white adipocytes results in partial decreases in aPKC activation (and glucose transport), but no apparent loss of PKB activation (42). Similarly, in immortalized brown adipocytes in which either IRS-1 or IRS-2 has been knocked out, there is a 60%–70% loss of aPKC activation and glucose transport but, as in white adipocytes, PKB activation is not compromised with knock out of either IRS-1 or IRS-2 (42). Therefore, it appears that (i) both IRS-1 and IRS-2 are required for activating aPKCs and glucose transport in white and brown adipocytes, (ii) either IRS-1 or IRS-2 (± IRS-3, possibly) is sufficient for fully activating PKB in white and brown adipocytes, and (iii) defects in glucose transport in IRS-1– or IRS-2–deficient adipocytes are explicable by deficient activation of aPKC, but not PKB.

In keeping with an important role for IRS-1 during aPKC activation in muscle and adipocytes by insulin is the finding of concomitant defects in activation of both IRS-1/PI3K and aPKC in adipocytes (43) and skeletal muscles (44, 45) of GK-diabetic rats, adipocytes (unpublished), and skeletal muscles (45) of *ob/ob* mice and high-fat–fed mice (45), skeletal muscles of Type II diabetic humans (46, 47) and monkeys (48), and cultured adipocytes of obese humans (49). Interestingly, despite impaired aPKC and IRS-1/PI3K activation, PKB activation by maximally effective concentrations of insulin is only mildly, if at all, impaired in muscles of Type II diabetic GK rats (43, 44), monkeys (48), and humans (46, 47). This suggests that PKB is activated at levels of IRS-1/PI3K activation that are lower than those needed for aPKC activation or factors other than IRS-1/PI3K activate PKB in these conditions.

Activation of aPKCs *via* Cbl During TZD and Insulin Action in Adipocytes

In 3T3/L1 adipocytes, TZDs increase tyrosine phosphorylation of Cbl (3, 4, 6) and activate Cbl-dependent PI3K, which participates in activating aPKC (but not PKB) and Glut4 translocation/glucose transport (4, 5). Note that this is not the only effect of TZDs on glucose transport in adipocytes, as TZDs also potentiate insulin activation of IRS-1/PI3K and IRS-2/PI3K in 3T3/L1 adipocytes (4). With respect to activating Cbl, TZDs, apparently *via* peroxisomal proliferator-activated receptors, induce increases in CAP, which assists in coupling Cbl to tyrosine kinases.

Unlike adipocytes, Cbl/PI3K activation does not appear to occur in muscle (6). Nevertheless, TZDs activate aPKC and glucose transport in muscle *via* other mechanisms, undoubtedly at least in part by altering the release of adiponectin and other factors (e.g., TNFα, resistin, free fatty acids) from adipose tissue. In this regard, adiponectin is known to activate muscle AMPK, thereby altering general insulin signaling mechanisms or specifically increasing aPKC responsiveness to PIP_3, as later discussed.

In addition to TZDs, insulin increases tyrosine phosphorylation of Cbl and activates Cbl-dependent PI3K and Cbl binding to both the p85 subunit of PI3K and Crk which, in turn, activates Crk, C3G, and TC10 (3, 6, 42). The activation of Cbl-dependent PI3K, along with IRS-1– and IRS-2–dependent PI3K, appears to be required for insulin-stimulated Glut4 translocation/glucose transport (6, 42). In this regard, it seems likely that there is an integrated signaling complex and interdependence among IRS-1/2/PI3K, Cbl/PI3K, and Cbl/Crk/C3G/TC10 signaling pathways in adipocytes. Whether comparable signaling complexes are operative in muscle cells is unknown.

Tissue-Specific Differences in Activation of aPKCs, PKB, IRS-1, and IRS-2 in Skeletal Muscle, Adipocytes, Liver, and Heart Muscle in Models of Obesity and Diabetes

Muscle and Adipocytes. There are both tissue- and species-specific differences in the activation of aPKC and PKB in various forms of obesity and diabetes. In some cases, differences in aPKC and PKB activation reflect differences in the activation of IRS-1/PI3K and IRS-2/PI3K. This is not true, however, in skeletal muscle of Type II diabetic rats (44), monkeys (48), and humans (46, 47); adipocytes of Type II diabetic rats (43); and cultured adipocytes and myocytes of obese humans (49), wherein there are defects in activation of IRS-1/PI3K and aPKC, but not PKB, at least at maximally effective insulin concentrations. On the other hand, defects in PKB activation accompany defects in aPKC and IRS-1/PI3K activation in the skeletal muscle of *ob/ob* (45) and *db/db* (50) mice and high-fat–fed mice (45, 51), adipocytes of *ob/ob* mice (unpublished), and adipose tissue of obese monkeys (unpublished). The finding that IRS-1/PI3K and aPKC, but not PKB, activation is impaired most likely reflects the fact that PKB activation occurs at relatively low levels of IRS-1/PI3K activation; alternatively, factors other than IRS-1/PI3K may substitute for PKB activation. In any event, in a number of instances the defect in aPKC activation is the only post-PI3K defect that seems to account for defects in glucose disposal and/or glucose uptake into muscle observed in the experimental conditions used in these cases.

Unlike the situation in high-fat–fed mice (45, 51), both feeding rats the same high-fat diet (40% fat calories) and STZ-induced diabetes in rats led to defects in aPKC activation and glucose transport in skeletal muscle, in the absence of defects in activation of IRS-1/PI3K, IRS-2/PI3K, and PKB (52). In these cases, an isolated defect in aPKC responsiveness to PIP_3 appears to be the major mechanism for diminished activation of aPKC and glucose transport.

Of further note, acute administration of iv lipids over 5 hrs to rats leads to an impairment in insulin-stimulated glucose disposal in clamp studies, and this is accompanied

by defects in activation of IRS-1/PI3K, aPKC, and PKBα, but no significant change in activation of PKBβ or phosphorylation of its target, glycogen synthase kinase-3β in rat muscle (53). Because PKBβ has been proposed to be at least quantitatively more important than PKBα for insulin-stimulated glucose transport, it may be surmised that the impaired activation of aPKC is likely to be responsible for diminished effects of insulin on glucose disposal in this experimental paradigm.

As has been previously alluded to, in a number of cases defects in muscle aPKC activation, but not PKB activation, have been observed, particularly in studies in which relatively maximally effective insulin concentrations were employed. In these cases, the observed defects in insulin-stimulated glucose transport and/or disposal would appear to reflect the defect in aPKC activation, rather than PKB activation. On the other hand, we have recently found (unpublished) that, in human diabetic muscle, despite being unable to observe a defect in PKB activation at higher (probably supraphysiologic) levels of serum insulin (200–500 μU/ml), there is clear-cut defect in PKB activation at lower insulin concentrations (50–100 μU/ml; i.e., high physiologic or approximately half-maximal for insulin-stimulated glucose disposal). In contrast to PKB activation, there are defects in aPKC activation at both higher and lower insulin concentrations. We interpret these findings as suggesting that (i) the dose-*dependent* defect in PKB activation is reflective of an upstream-signaling defect(s) involving the insulin receptor or immediate postreceptor factors, which are known to have "spareness" (i.e., to be present in excess of that degree of saturation needed to elicit maximal biologic effects); and (ii) the dose-*independent* defect in aPKC activation is reflective of defects directly involving aPKCs that would be apparent regardless of the insulin concentration and activation of upstream-signaling factors. Stated differently, to use a parlance that was previously used to describe findings in studies of receptor function, the defect in PKB activation would be classified as a "receptor" defect, whereas the latter defect in aPKC activation would be classified as a "postreceptor" defect. The defect in aPKC activation is most likely reflective of the poor responsiveness of aPKCs to PIP_3.

Liver. Considering that aPKC activation is consistently diminished in muscle and adipocytes in each of the previously stated forms of high-fat feeding, obesity, and Type II diabetes, it was most surprising to find that aPKC activation is fully intact or enhanced in the livers of Type II diabetic GK rats, *ob/ob* diabetic mice, high-fat–fed mice (45), and STZ-diabetic rats (submitted for publication). It was equally surprising to find that, regardless of whether PKB activation is preserved or impaired in muscle, PKB activation is diminished in the livers of GK-diabetic rats and *ob/ob* mice (45) and STZ-diabetic rats (submitted for publication). Subsequent studies in GK-diabetic and STZ-diabetic rats have further revealed that the activation IRS-1/PI3K, but not IRS-2/PI3K, is diminished in the liver. Thus, it seems likely that in GK-diabetic and STZ-diabetic rats (i) defects in hepatic PKB activation are due to diminished IRS-1/PI3K activation, and (ii) the conservation of hepatic aPKC activation is due to the apparently intact activation of IRS-2/PI3K in livers of these diabetic rats. As previously discussed, this postulation is in keeping with findings in IRS-1 (40) and IRS-2 (unpublished) knockout mice. However, the postulation that a fully intact IRS-2/PI3K maintains aPKC activation in the liver, as seen in GK-diabetic and STZ-diabetic rats, may not be applicable in the livers of *ob/ob* mice, wherein activation of both IRS-1/PI3K and, to a lesser extent, IRS-2/PI3K is reported to be impaired (54). On the other hand, in preliminary studies we have seen marked defects in IRS-1/PI3K, but not IRS-2/PI3K, activation in livers of *ob/ob* mice.

In contrast to models of Type II diabetes, the activation of IRS-1/PI3K, IRS-2/PI3K, aPKC, and PKB is apparently normal (or increased) in livers of high-fat–fed mice (45), high-fat–fed rats (55), and obese monkeys (submitted for publication). As previously discussed, because of skeletal muscle–dependent insulin resistance and hyperinsulinemia, conserved insulin signaling in the liver in these conditions may result in excessive synthesis of liver-derived lipids, along with essentially normal insulin signaling to factors controlling hepatic glucose handling. On the other hand, impairments in insulin-regulated processes that influence hepatic glucose output in obese or high-fat–fed animals have been observed, but this probably reflects alterations in metabolites (e.g., free fatty acids or other factors such as glucagon which may influence, oppositely to insulin, gluconeogenesis and/or glycogen metabolism rather than altered insulin-signaling mechanisms).

Heart. In preliminary (unpublished) studies of heart muscle, we have found that there are defects in aPKC activation in high-fat–fed mice, GK-diabetic rats, *ob/ob* diabetic mice, and STZ-diabetic rats. Defects in PKB activation are also evident in hearts of GK-diabetic rats, but not in hearts of high-fat–fed mice or STZ-diabetic rats. In association with defects in aPKC and PKB activation, a defect in IRS-1/PI3K activation, but not IRS-2/PI3K activation, is seen in heart muscle of GK-diabetic rats. However, in hearts of STZ-diabetic rats, there are no defects in activation of IRS/1/PI3K, IRS-2/PI3K, or PKB (56). Although further studies are in progress to examine other signaling factors, it appears that there are consistent defects in insulin signaling to aPKC in heart muscle that are similar in many respects to those observed in skeletal muscle. The clinical importance of defects in insulin signaling to aPKC and, in some cases, to PKB, and the subsequent defect in insulin-stimulated glucose transport in the heart, is presently uncertain.

Activation of aPKCs *via* AMPK

Certain agents including sorbitol, high concentrations of glucose, AICAR, dinitrophenol (DNP), and exercise

activate Glut4 translocation/glucose transport independent of PI3K and PKB. The effects of AICAR, DNP and, perhaps to a lesser but still undefined extent, exercise on glucose transport are at least partly (more likely largely) mediated *via* AMPK, whereas the effects of sorbitol and glucose may involve an "osmotic stress" sensor. Interestingly, high glucose (57), AICAR (58), and sorbitol (59) provoke increases in phosphorylation (activation) of the nonreceptor tyrosine kinase (i.e., PYK2), which activates the GRB2/SOS/RAS/RAF/MEK1/ERK pathway and apparently thereby activates phospholipase D (PLD) to generate phosphatidic acid (PA) which, like PIP_3, directly activates aPKCs. Indeed, more recently we have found that the adenoviral-mediated expression of dominant-negative PYK2 (but not wild-type PYK2) blocks AICAR-induced activation of ERK, PLD, aPKCs, and Glut4 translocation/glucose transport in L6 myotubes (unpublished). Further, from studies of expression of kinase-inactive aPKCs, it appears that aPKCs are required for sorbitol-, glucose-, and AICAR-dependent increases in Glut4 translocation/glucose transport (57–59).

How AICAR activates PYK2 and thereby activates ERK, PLD, aPKCs, and glucose transport is uncertain. Presumably, these activations require AMPK activation because the expression of kinase-inactive AMPKα2 blocks the effects of AICAR on ERK, aPKC, and glucose transport (unpublished).

In addition to AICAR, other substances that activate AMPK include TZDs (60), adiponectin (which is released from adipocytes in response to TZDs; Ref. 61), and metformin (60, 62). Interestingly, these substances, *via* AMPK, inhibit lipid synthesis and promote glucose uptake, glycolysis, and fatty-acid oxidation (59–61), each of which may improve aPKC activation.

Interestingly, we have found that TZDs increase aPKC activation by insulin in muscles of Type II diabetic humans (46), Type II diabetic rats (44), and obese monkeys (submitted for publication). In the latter two cases, TZD-induced increases in muscle aPKC activation occur without improvement in either IRS-1/PI3K or PKB activation and, moreover, in obese monkeys such increases in aPKC activation are at least partly due to increased responsiveness to PIP_3. Moreover, the administration of adiponectin to mice for 4 hrs provokes increases in basal- and insulin-stimulated aPKC activity in muscle, without change in activation of IRS-1/PI3K or PKB (unpublished).

In addition to adiponectin, we have found that the AMPK activator metformin initially activates basal aPKC activity and, with more prolonged treatment, improves the defect in insulin-stimulated aPKC activation in skeletal muscles of human Type II diabetic subjects without altering IRS-1/PI3K or PKB activation (submitted for publication). Of further note, aPKC responsiveness to PIP_3 is improved by metformin. In addition to aPKC, metformin concomitantly activates basal-stimulated ERK, but not insulin-stimulated ERK, in these muscles, which may be relevant to metformin-induced increases in basal aPKC activity (i.e., *via* AMPK or PYK2).

From these findings, it appears that AICAR, TZDs, metformin, and adiponectin operate *via* AMPK to directly activate aPKCs and improve aPKC activation by both insulin and PIP_3. The increase in aPKC responsiveness to PIP_3 is most likely due to AMPK-dependent decreases in inhibitory intramyocellar lipids and, possibly, to the diminished activation of conventional and novel PKCs. This diminished activation can inhibit insulin actions (most likely at several levels and, perhaps directly, at the levels of aPKCs, the insulin receptor, and IRS-1), particularly when chronically activated by excesses of lipids and/or glucose.

The Role of aPKCs During Exercise-Stimulated Glucose Transport

Along with others, we have reported that exercise activates aPKCs and ERK in mouse muscle (58) and human muscle (46, 63). Unlike AICAR, exercise does not activate PYK2 and may largely use an AMPK-independent mechanism to stimulate glucose transport (64, 65). However, the issue of the importance of AMPK in exercise-stimulated glucose transport is still unsettled and, regardless of the role of AMPK, exercise, like AICAR, may use ERK, PLD, and PA to activate aPKCs and glucose transport, particularly in fast-twitch muscles.

Defects in Activation of aPKCs by Insulin and PIP_3 in Obesity and Type II Diabetes

Insulin-stimulated aPKC activation is defective in adipocytes (43) and muscles of Type II diabetic rats (44); muscles of Type II diabetic monkeys (48) and humans (46, 47); cultured myocytes of obese, glucose-intolerant humans (49, 66); muscles of obese, glucose-intolerant humans (46); muscles of obese, glucose-tolerant women (47, 67); muscles of obese women who have polycystic ovary syndrome (67); and cultured adipocytes of obese humans (49). Whereas defects in muscle aPKC activation in overt diabetes are associated with defects in IRS-1/PI3K activation (46–48), significant defects in IRS-1/PI3K activation are not readily apparent in at least some cases (e.g., in muscles of obese, glucose-intolerant humans [46]; obese, glucose-tolerant humans [47]; and cultured myocytes of obese, glucose-intolerant humans [66]).

In view of the failure to find consistent, readily demonstrable defects in IRS-1/PI3K activation in all insulin-resistant conditions, it was interesting to find that PIP_3 poorly activates aPKCs immunoprecipitated from muscle lysates obtained from Type II diabetic humans (46) and monkeys (48), obese humans (67) and monkeys (48), and cultured adipocytes and myocytes of obese humans (49). This defect in aPKC responsiveness to PIP_3 in these muscles is in marked contrast to the insulin-like increases in aPKC activity seen with PIP_3 treatment of aPKCs immunoprecipitated from muscles of normal sub-

jects (46, 48). Of further note, defects in aPKC responsiveness to PIP_3 are also seen in muscles of high-fat–fed, insulin-resistant rats (53) and mice (submitted for publication) and STZ-diabetic rats (53).

Collectively, the aforementioned findings in clamp/muscle biopsy studies in humans (46, 47) and monkeys (48), and studies in cultured myocytes and adipocytes of obese humans (49), strongly suggest that the impaired activation of aPKCs importantly contributes to the pathogenesis of defects in insulin-stimulated glucose transport and systemic insulin resistance as seen in obesity, the associated metabolic syndrome, and Type II diabetes.

In addition to causing signaling defects that limit glucose transport in skeletal muscle and adipocytes, preliminary findings suggest that there is a similar defect in aPKC activation in heart muscle that limits glucose uptake in this vital organ in states of obesity and diabetes.

Remarkably, it appears that, unlike muscle, aPKC activation in the liver is conserved in both obesity and Type II diabetes. Because aPKCs mediate, at least in part, insulin effects on hepatic lipid synthesis, this conservation of hepatic aPKC activation may explain the overproduction of lipids (i.e., VLDL triglycerides) by the liver in obesity, metabolic syndrome, and diabetes. Moreover, coupled with the fact that PKB activation in the liver appears to be compromised as obesity progresses to diabetes, the conservation of aPKC activation could explain how the diabetic liver generates excessive amounts of both glucose and VLDL lipids, which is a particularly lethal combination for abetting the development of macrovascular disease.

Finally, given the importance of defective muscle aPKC activation in causing systemic insulin resistance, it is imperative to better understand the mechanisms whereby insulin-sensitizing agents improve aPKC activation in muscle. We believe that AMPK will prove to be important in this regard.

Conclusions

We believe that the large number of positive findings and presently available evidence weigh heavily in support of the hypothesis that aPKC and PKB are corequired for insulin-stimulated glucose transport. It also seems likely that the Cbl/Crk/TC10 pathway is required, very possibly to localize and activate aPKC in specific cell organelles that function in insulin-stimulated Glut4 translocation. Although there are some experimental findings that could be construed to suggest that neither aPKC nor PKB nor Cbl is required for insulin-stimulated glucose transport, particularly in 3T3/L1 adipocytes, the findings in these studies are not compelling and appear to be more than adequately counterbalanced by a large number of positive findings independently observed by multiple investigators. Moreover, unless aPKC, as well as PKB, is required for insulin-stimulated glucose transport, particularly in muscle, we would have to accept that we are presently no closer to understanding the post-PI3K defect in insulin action in insulin-resistant states of high-fat feeding, obesity, and Type II diabetes mellitus. Indeed, a defect in aPKC activation is the most consistent defect that has been found to be present in most, if not all, insulin-resistant conditions thus far studied. In many cases, PKB activation, at least as measured at high insulin levels (e.g., as used in many clamp studies), is not or is only marginally defective and it is, therefore, unlikely that the defective activation of PKB *per se* is responsible for the defects in insulin-stimulated glucose disposal observed in these clamp studies and other studies. Similarly, there are some instances in which defects in glucose transport appear to be explained by the impaired activation of PKB, rather than aPKC. Stated differently, the defects in insulin action in insulin-resistant states are presently understandable only if we accept positive roles for both aPKC and PKB in insulin-stimulated glucose transport. Without this acceptance, we are forced to deny the importance of very large amounts of data that are otherwise very coherent and consistent, and we would have to acknowledge that we presently are no closer to understanding why insulin fails to act effectively in insulin-resistant syndromes than we were 4 decades ago when the insulin receptor was discovered.

At this point, we are working under the conviction that our opinions on aPKC and PKB are correct, and we optimistically believe that we are at, or close to, an understanding of why insulin fails to work effectively in stimulating glucose uptake in muscle and, on the other hand, is overactive in stimulating lipid synthesis in the liver in insulin-resistant hyperinsulinemic states. Accordingly, we believe that we are on the threshold for devising better programs for treating the "metabolic" or "insulin resistance" syndrome seen in obesity and Type II diabetes mellitus.

1. White MF, Kahn CR. The insulin signaling system. J Biol Chem 269:1–4, 1994.
2. Withers DJ, White M. The insulin signaling system. Endocrinology 141:1917–1921, 2000.
3. Pessin JE, Saltiel AR. Signaling pathways in insulin action. J Clin Invest 106:165–169, 2000.
4. Standaert ML, Kanoh Y, Sajan MP, Bandyopadhyay G, Farese RV. Cbl, IRS-1 and IRS-2 mediate effects of rosiglitazone on phosphatidylinositol (PI) 3-kinase, protein kinase C-λ and glucose transport in 3T3/L1 adipocytes. Endocrinology 143:1705–1716, 2002.
5. Miura A, Sajan MP, Standert ML, Bandyopadhyay G, Franklin DM, Lea-Currie R, Farese RV. Cbl pYXXM motifs activate the p85 subunit of phosphatidylinositol 3-kinase, atypical protein kinase C and glucose transport during thiazolidinedione action in 3T3/L1 and human adipocytes. Biochemistry 42:14335–14341, 2003.
6. Standaert ML, Sajan MP, Miura A, Bandyopadhyay G, Farese RV. Requirements for pYXXM motifs in Cbl for binding to the p85 subunit of PI3K and Crk, and activation of atypical protein kinase C and glucose transport during insulin action in 3T3/L1 adipocytes. Biochemistry 43:15494–15502, 2004.
7. Mitra P, Zheng X, Czech MP. RNA-based analysis of CAP, Cbl, and CrkII in the regulation of GLUT4 by insulin. J Biol Chem 279:37431–37435, 2004.

8. Ahn M-Y, Katsanakis KD, Bheda F, Pillay TS. Primary and essential role of the adapter protein APS for recruitment of both c-Cbl and CAP in insulin signaling. J Biol Chem 279:21526–21532, 2004.
9. Matsumoto M, Ogawa W, Akimoto K, Inoue H, Miyaki K, Furukawa K, Hayashi Y, Iguchi H, Marsuki Y, Hiramatsu R, Shimano H, Yamada N, Ohno S, Kasuga M, Noda T. PKCλ in liver mediates insulin-induced SREBP-1c expression and determines both hepatic lipid content and overall insulin sensitivity. J Clin Invest 112:935–944, 2003.
10. Bandyopadhyay G, Standaert ML, Sajan MP, Karnitz LM, Cong L, Quon MJ, Farese RV. Dependence of insulin-stimulated glucose transporter 4 translocation on 3-phosphoinositide-dependent protein kinase-1 and its target threonine-410 in the activation loop of protein kinase-C-ζ. Mol Endocrinol 13:1766–1772, 1999.
11. Le Good JA, Ziegler WH, Parakh DB, Alessi DR, Cohen P, Parker PJ. Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1. Science 281:2042–2045, 1998.
12. Chou MM, How W, Johnson J, Graham LK, Lee MH, Chen CS, Newton AC, Schaffhause BS, Toker A. Regulation of protein kinase C zeta by PI 3-kinase and PDK-1. Curr Biol 8:1069–1077, 1998.
13. Alessi D, James S, Downes C, Cohen P. Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Bζ. Curr Biol 7:261–269, 1997.
14. Egawa K, Maegawa H, Shi K, Nakamura T, Obata T, Yoshizaki T, Morino K, Shimizu S, Nishio Y, Suzuki E, Kashiwagi A. Membrane localization of 3-phosphoinositide-dependent protein kinase-1 stimulates activities of Akt and atypical PKC, but does not stimulate glucose transport and glycogen synthesis in 3T3/L1 adipocytes. J Biol Chem 277:38863–38869, 2002.
15. Kohn AD, Summers SA, Birnbaum MJ, Roth RA. Expression of a constitutively active Akt Ser/Thr kinase in 3T3/L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. J Biol Chem 271:31372–31378, 1996.
16. Tanti J, Grillo S, Gremeaux T, Coffer PJ, Van Obberghen E, Le Marchand-Brustel Y. Potential role of protein kinase B in glucose transporter 4 translocation in adipocytes. Endocrinology 138:2005–2009, 1997.
17. Wang Q, Somwar R, Bilan PJ, Liu Z, Jin J, Woodgett JR, Klip A. Protein kinase B/Akt participates in GLUT4 translocation in L6 myoblasts. Mol Cell Biol 19:4008–4018, 1999.
18. Hill MM, Clark SF, Tucker DF, Birnbaum MJ, James DE, Macaulay SL. A role for protein kinase Bβ/Akt2 in GLUT4 translocation in adipocytes. Mol Cell Biol 19:7771–7781.
19. Kitamura T, Ogawa W, Sakaue H, Hino Y, Kuroda S, Takata M, Matsumoto M, Maeda T, Konishi H, Kikkawa U, Kasuga M. Requirement for activation of the serine-threonine kinase Akt (protein kinase B) in insulin stimulation of protein synthesis but not of glucose transport. Mol Cell Biol 18:3708–3717, 1998.
20. Cong L, Chen H, Li Y, Zhou L, McGibbon MA, Taylor SI, Qoun MJ. Physiological role of Akt in insulin-stimulated translocation of GLUT4 in transfected rat adipose cells. Mol Endocrinol 11:1881–1889, 1997.
21. Bandyopadhyay G, Sajan MP, Yoshinori Kanoh Y, Standaert ML, Quon MJ, Lea-Currie RL, Sen A, Farese RV. Protein kinases C-ζ mediates insulin effects on glucose transport in cultured human adipocytes. J Clin Endocrinol Metab 87:716–723, 2002.
22. Cho H, Thorvaldsen JL, Chu Q, Feng F, Birnbaum MJ. Akt1/PKBζ is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J Biol Chem 276:38349–38352, 2001.
23. Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB III, Kaestner KH, Bartolomei MS, Shulman GI, Birnbaum MJ. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase AKT2 (PKB-β). Science 292:1728–1731, 2001.
24. Jiang ZY, Zhou QL, Coleman KA, Chouinard M, Boese Q, Czech MP. Insulin signaling through Akt/PKB analyzed by small interfering RNA-mediated gene silencing. Proc Natl Acad Sci U S A 100:7569–7574, 2003.
25. Ugi S, Imamura T, Maegawa H, Egawa K, Yoshizaki T, Shi K, Obata T, Ebina Y, Kashiwagi A, Olefsky JM. Protein phosphatase 2A negatively regulates insulin's metabolic pathway by inhibiting Akt (protein kinase B) activity in 3T3/L1 adipocytes. Mol Cell Biol 24:8778–87789, 2004.
26. Bae SS, Cho H, Mu J, Birnbaum MJ. Isoform-specific regulation of insulin-dependent glucose uptake by Akt/protein kinase B. J Biol Chem 278:49530–49536, 2003.
27. Bandyopadhyay G, Standaert ML, Zhao L, Yu B, Avignon A, Galloway L, Karnam P, Moscat J, Farese RV. Activation of protein kinase C (α, β and ζ) by insulin in 3T3/L1 cells: transfection studies suggest a role for PKC-ζ in glucose transport. J Biol Chem 272:2551–2558, 1997.
28. Bandyopadhyay G, Standaert ML, Galloway L, Moscat J, Farese RV. Evidence for involvement of protein kinase C (PKC)-ζ and noninvolvement of diacylglycerol-sensitive PKCs in insulin-stimulated glucose transport in L6 myotubes. Endocrinology 138:4721–4731, 1997.
29. Standaert ML, Galloway L, Karnam P, Bandyopadhyay G, Moscat J, Farese RV. Protein kinase C-ζ as a downstream effector of phosphatidylinositol 3-kinase during insulin stimulation in rat adipocytes: potential role in glucose transport. J Biol Chem 272:30075–30082, 1997.
30. Bandyopadhyay G, Standaert ML, Kikkawa U, Ono Y, Moscat J, Farese RV. Effects of transiently expressed atypical (ζ, λ), conventional (α, β) and novel (δ, ε) protein kinase C isoforms on insulin-stimulated translocation of epitope-tagged GLUT4 glucose transporters in rat adipocytes: specific interchangeable effects of protein kinases C-ζ and C-λ. Biochem J 337:461–470, 1999.
31. Kotani K, Ogawa W, Matsumoto M, Kitamura T, Sakaue H, Hino Y, Miyake K, Sano W, Akimoto K, Ohno S, Kasuga M. Requirement of atypical protein kinase Cλ for insulin stimulation on glucose uptake but not for Akt activation in 3T3/L1 adipocytes. Mol Cell Biol 18:6971–6982, 1998.
32. Bandyopadhyay G, Kanoh Y, Sajan MP, Standaert ML, Farese RV. Effects of adenoviral gene transfer of wild-type, constitutively-active, and kinase-defective protein kinase C-λ on insulin-stimulated glucose transport in L6 myotubes. Endocrinology 141:4120–4127, 2000.
33. Condorelli G, Vigliotta G, Trencia A, Maitan MA, Caruso M, Miele C, Oriente F, Santopietro S, Formisano P, Beguinot F. Protein kinase C(PKC)-ζ activation inhibits PKC-ζ and mediates the action of PED/PEA-15 on glucose transport in L6 muscle cells. Diabetes 50:1244–1250, 2001.
34. Standaert ML, Bandyopadhyay G, Kanoh Y, Sajan MP, Farese RV. Insulin and PIP_3 activate PKC-ζ by mechanisms that are both dependent and independent of phosphorylation of activation loop (T410) and autophosphorylation (T560) sites. Biochemistry 40:249–255, 2001.
35. Etgen GJ, Valasek KM, Broderick CL, Miller AR. In vivo adenoviral delivery of human protein kinase C-ζ stimulates glucose transport activity in rat skeletal muscle. J Biol Chem 274:22139–22142, 1999.
36. Bandyopadhyay G, Standaert ML, Sajan MP, Kanoh Y, Miura A, Braun U, Kruse F, Leitges M, Farese RV. Protein kinase C-λ knockout in embryonic stem cells and adipocytes impairs insulin-stimulated glucose transport. Mol Endocrinol 18:373–383, 2004.
37. Zhou QL, Park JG, Jiang ZY, Holik JJ, Mitra P, Semiz S, Powelka AM, Tang X, Virbasius J, Czech MP. Analysis of insulin signaling by RNAi-based gene silencing. Biochem Soc Trans 32:817–821, 2004.
38. Kanzaki M, Mora S, Hwang JB, Saltiel AR, Pessin JE. Atypical protein kinase C (PKCζ/λ) is a convergent downstream target of insulin-stimulated phosphatidylinositol 3-kinase and TC10 signaling pathways. J Cell Biol 164:279–290, 2004.
39. Standaert ML, Bandyopadhyay G, Perez L, Price D, Galloway L, Poklepovic A, Sajan MP, Cenni V, Sirri A, Moscat J, Toker A, Farese RV. Insulin activates protein kinases C-ζ and C-λ by an autophosphorylation-dependent mechanism and stimulates their translocation to

GLUT4 vesicles and other membrane fractions in rat adipocytes. J Biol Chem 274:25308–25316, 1999.
40. Sajan MP, Standaert ML, Miura A, Kahn RC, Farese RV. Tissue-specific differences in activation of atypical protein kinase C and protein kinase B in muscle, liver and adipocytes of insulin receptor substrate-1 knockout mice. Mol Endocrinol 18:2513–2521, 2004.
41. Valverde AM, Burks DJ, Fabregat I, Fisher TL, Carretero J, White MF, Benito M. Molecular mechanisms of insulin resistance in IRS-2-deficient hepatocytes. Diabetes 52:2239–2248, 2003.
42. Miura A, Sajan MP, Standaert ML, Bandyopadhyay G, Kahn CR, Farese RV. Insulin substrates 1 and 2 are required for activation of atypical protein kinase C and Cbl-dependent phosphatidylinositol 3-kinase during insulin action I immortalized brown adipocytes. Biochemistry 43:15503–15509, 2004.
43. Kanoh Y, Bandyopadhyay G, Sajan MP, Standaert ML, Farese RV. Thiazolidinedione treatment enhances insulin effects on protein kinase C-ζ/λ activation and glucose transport in adipocytes of nondiabetic and Goto-Kakizaki type II diabetic rats. J Biol Chem 275:16690–16696, 2000.
44. Kanoh Y, Bandyopadhyay G, Sajan MP, Standaert ML, Farese RV. Rosiglitazone, insulin treatment and fasting correct defective activation of protein kinase C-ζ by insulin in vastus lateralis muscles and adipocytes of diabetic rats. Endocrinology 142:1595–1605, 2001.
45. Standaert ML, Sajan MP, Miura A, Kanoh Y, Chen HC, Farese RV Jr, Farese RV. Insulin-induced activation of atypical protein kinase C, but not protein kinase B, is maintained in diabetic (*ob/ob* and Goto-Kakizaki) liver. J Biol Chem 279:24929–24934, 2004.
46. Beeson M, Sajan MP, Dizon M, Kanoh Y, Bandyopadhyay G, Standaert ML, Farese RV. Activation of protein kinase C-ζ by insulin and PI-3,4,5-$(PO_4)_3$ is defective in muscle in type 2 diabetes and impaired glucose tolerance: amelioration by rosiglitazone and exercise. Diabetes 52:1926–1934, 2003.
47. Kim YB, Kotani K, Ciaraldi TP, Farese RV, Henry RR, Kahn BB. Insulin-stimulated PKC C-λ/ζ activity is reduced in skeletal muscle of humans with obesity and type 2 diabetes. Diabetes 52:1935–1942, 2003.
48. Standaert ML, Ortmeyer HK, Hansen BC, Sajan MP, Kanoh Y, Bandyopadhyay G, Farese RV. Skeletal muscle insulin resistance in obesity-associated type 2 diabetes mellitus in monkeys is linked to a defect in insulin activation of protein kinase C-ζ/λ/ι. Diabetes 51:2936–2943, 2002.
49. Sajan MP, Standaert ML, Miura A, Bandyopadhyay G, Vollenweider P, Franklin DM, Lea-Currie R, Farese RV. Impaired activation of protein kinase C-ζ by insulin and phosphatidylinositol-3,4,5-$(PO_4)_3$ in cultured adipocytes and myotubes of obese subjects. J Clin Endocrinol Metab 89:3994–3998, 2004.
50. Hori H, Sasaoka T, Ishihara H, Wadu T, Murakami S, Ishiki M, Kobayashi M. Association of SH2-containing inositol phosphatase 2 with the insulin resistance of *db/db* mice. Diabetes 51:2387–2394, 2002.
51. Chen HC, Rao M, Sajan MP, Standaert ML, Kanoh Y, Miura A, Farese RV Jr, Farese RV. Role of adipocyte-derived factors in enhancing insulin signaling in skeletal muscle and white adipose tissue of mice lacking acyl CoA:diacylglycerol acyltransferase 1. Diabetes 53:1445–1451, 2004.
52. Kanoh Y, Sajan MP, Bandyopadhyay G, Miura A, Standaert ML, Farese RV. Defective activation of protein kinase C ζ and λ by insulin and phosphatidylinositol-3,4,5-$(PO_4)_3$ in skeletal muscle of rats following high-fat feeding and streptozotocin-induced diabetes. Endocrinology 144:947–954, 2003.
53. Kim Y-B, Shulman GI, Kahn BB. Fatty acid infusion selectively impairs insulin action on Akt1 and protein kinase C λ/ζ but not on glycogen synthase kinase-3β. J Biol Chem 277:32915–32922, 2002.
54. Kerouz NJ, Horsch D, Pons S, Kahn CR. Differential regulation of insulin receptor substrates-1 and -2 (IRS-1 and IRS-2) and phosphatidylinositol 3-kinase in liver and muscle of the obese diabetic (*ob/ob*) mouse. J Clin Invest 100:3164–3172, 1997.
55. Anai M, Funaki M, Ogihara T, Kanda A, Onishi Y, Sakoda H, Inukai K, Nawano M, Fukushima Y, Yazaki Y, Kikuchi M, Oka Y, Asano T. Enhanced insulin-stimulated activation of phosphatidylinositol 3-kinase in the liver of high-fat-fed rats. Diabetes 48:158–169, 1999.
56. Laviola L, Belsanti G, Davalli AM, Napoli R, Perrini S, Weir GC, Giorgino R, Giorgino F. Effects of streptozotocin diabetes and diabetes treatment by islet transplantation on in vivo insulin signaling in rat heart. Diabetes 50:2709–2720, 2001.
57. Bandyopadhyay G, Sajan MP, Kanho Y, Standaert ML, Quon MJ, Reed BC, Dikic I, Farese RV. Glucose activates protein kinase C-ζ/λ through proline-rich tyrosine kinase-2, extracellular signal-regulated kinase, and phospholipase D. J Biol Chem 276:35537–35545, 2001.
58. Chen HC, Bandyopadhyay G, Sajan MP, Kanoh Y, Standaert ML, Farese RV Jr, Farese RV. Activation of the ERK pathway and atypical protein kinase C isoforms in exercise- and AICAR-stimulated glucose transport. J Biol Chem 277:23554–23562, 2002.
59. Sajan MP, Bandyopadhyopadhyay G, Kanoh Y, Standaert ML, Quon MJ, Reed BC, Dikic I, Farese RV. Sorbitol activates atypical PKCs and GLUT 4 glucose transporter translocation/glucose transport through PYK2, the ERK pathway and phospholipase D. Biochem J 362:665–674, 2002.
60. Fryer LGD, Parbu-Patel A, Carling D. The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. J Biol Chem 277:25226–25232, 2002.
61. Yamouchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 8:1288–1295, 2002.
62. Musi N, Hirshman MF, Nygren J, Svanfeldt M, Bavenholm P, Rooyackers O, Zhou G, Williamson JM, Ljunqvist O, Efendic S, Moller DE, Thorell A, Goodyear LJ. Metformin increases AMPK activity in muscle of subjects with type 2 diabetes. Diabetes 51, 2074–2081, 2002.
63. Perrini S, Henriksson J, Zierath J, Widegren U. Exercise-induced protein kinase C isoform-specific activation in human skeletal muscle. Diabetes 53:21–24, 2004.
64. Mu J, Brozinick JT Jr, Valladares O, Bucan M, Birnbaum MJ. A role of AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. Mol Cell 7:1085–1094, 2001.
65. Wojtaszewski JFP, Nielsen JN, Jorgensen SB, Frosig C, Birk JB, Richter EA. Transgenic models—a scientific tool to understand exercise-induced metabolism: the regulatory role of AMPK (5′-AMP-activated protein kinase) in glucose transport and glycogen synthase activity in skeletal muscle. Biochem Soc Trans 31:1290–1294, 2003.
66. Vollenweider P, Menard B, Nicod P. Insulin resistance, defective IRS-2-associated phosphatidylinositol-3′ kinase activation, and impaired atypical protein kinase C (ζ/λ) activation in myotubes from obese patients with impaired glucose tolerance. Diabetes 51:1052–1059, 2002.
67. Beeson M, Sajan MP, Gomez-Daspet J, Luna V, Dizon M, Grebenev D,. Powe JL, Lucidi S, Miura A, Kanoh Y, Bandyopadhyay G, Standaert ML, Yeko TR, Farese RV. Defective activation of protein kinase C-ζ by insulin and phosphatidylinositol 3-kinase in obesity and polycystic ovary syndrome. Metab Syndr Rel Disord 2:49–56, 2004.