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Atypical Protein Kinase C in Cardiometabolic Abnormalities

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Abstract

Purpose—Review aberrations of insulin signaling to atypical protein kinase C (aPKC) in muscle and liver that generate cardiovascular risk factors, including, obesity, hypertriglyceridemia, hypercholesterolemia, insulin resistance and glucose intolerance in type 2 diabetes mellitus (T2DM), and obesity-associated metabolic syndrome (MetSyn).

Recent Findings—aPKC and/or Akt mediate insulin effects on glucose transport in muscle, and synthesis of lipids, cytokines and glucose in liver. In T2DM, whereas Akt and aPKC activation are diminished in muscle, and hepatic Akt activation is diminished, hepatic aPKC activation is conserved. Imbalance between muscle and hepatic aPKC activation (and expression of PKC- α in humans) by insulin results from differential downregulation of insulin receptor substrates that control phosphatidylinositol 3-kinase. Conserved activation of hepatic aPKC in hyperinsulinemic states of T2DM, obesity and MetSyn is problematic as excessive activation of aPKC-dependent lipogenic, gluconeogenic and proinflammatory pathways increases cardiovascular risk factors. Indeed, selective inhibition of hepatic aPKC by adenoviral-mediated expression of kinase-inactive aPKC, or newly-developed small-molecule biochemicals, dramatically improves abdominal obesity, hepatosteatosis, hypertriglyceridemia, hypercholesterolemia, insulin resistance and glucose intolerance in murine models of obesity and T2DM.

Summary—Hepatic aPKC is a unifying target for treating multiple clinical abnormalities that increase cardiovascular risk in insulin-resistant states of obesity, MetSyn and T2DM.

Keywords

Atypical Protein Kinase C; Obesity; Metabolic Syndrome; Type 2 Diabetes Mellitus; Insulin Signaling in Liver and Muscle

Introduction

Much cardiovascular disease in Western/Westernized populations results from metabolic abnormalities in obesity, the “metabolic syndrome” (MetSyn) and type 2 diabetes mellitus (T2DM). Unfortunately, the prevalence of these disorders is increasing exponentially. Clearly, new approaches are needed to contain this costly pandemic.

Obesity, MetSyn and T2DM are characterized by insulin resistance, which underlies emergence of cardiovascular risk factors. Fortunately, we now know the major signaling factors used by insulin to regulate metabolic processes in two important target tissues,

muscle and liver, how these signaling factors are altered in obesity, MetSyn and T2DM, and ways to reverse these alterations.

Text

Insulin Signaling

Insulin binds to receptors that sequentially activate insulin receptor substrates (IRSs), 1 and 2, and phosphatidylinositol (PI) 3-kinase (PI3K), which converts PI-4,5-(PO₄)₂ to PI-3,4,5-(PO₄)₃ (PIP₃) in plasma and other cellular membranes. PIP₃ binds to Akt and aPKCs and facilitates phosphorylation, first by phosphoinositide-dependent kinase-1 (PDK1), and second, for Akt, by “PDK2” (target of rapamycin C2, TORC2), and, for aPKCs, by auto(trans)phosphorylation and molecular unfolding [1].

In muscle, IRS-1/PI3K controls Akt and aPKC activation by insulin [2,3]; thus, as muscle IRS-1/PI3K is downregulated in obesity and T2DM, activation of both Akt and aPKC is diminished. In liver, whereas IRS-1/PI3K is the major albeit not exclusive regulator of Akt [2,3,4], aPKCs are controlled by IRS-2/PI3K [3,4,5]. Moreover, in livers of T2DM rodents, whereas IRS-1/PI3K activation is diminished, IRS-2/PI3K activation is maintained [6,7]; this explains how Akt activation is impaired while aPKC activation is conserved in T2DM liver [6,7]. Accordingly, in hyperinsulinemic forms of T2DM [7] and obesity [8], insulin effects on hepatic aPKC are exaggerated, even when Akt activation is diminished.

Insulin also activates conventional (c) and novel (n) PKCs in liver by activating glycerol-3-phosphate/acyltransferase and subsequent generation of diacylglycerol (DAG) via the de novo phospholipid synthesis pathway.

Metabolic Effects of Insulin

Akt and aPKC mediate most metabolic effects of insulin. In muscle, both Akt and aPKC regulate glucose transport [1]. The aPKC requirement in mouse muscle seems clear, as knockout of PKC-λ, the major aPKC, impairs glucose transport effects of both insulin [9] and metformin, which activates aPKC independently of PI3K/PIP₃ [10]. Similarly in rat muscle and L6 myotubes, PKC-ζ, the major aPKC, is required for glucose transport effects of insulin and metformin [10–13].

In liver, insulin increases activity and expression of sterol receptor element binding protein-1c (SREBP-1c) by a mechanism that appears to require both aPKC [7,8,14,15] and Akt [16,17]. Activation of SREBP-1c is a multi-step process that involves proteolytic release of a fragment that translocates to the nucleus and increases transcription of SREBP-1c itself, which in turn transactivates multiple lipogenic genes, including, fatty acid synthase (FAS) and acyl-CoA-carboxylase (ACC). Akt apparently controls SREBP-1c expression through TORC1 and S6 kinase [17]. How aPKC regulates SREBP-1c is uncertain.

More recently, PKCβ has been proposed to act like or in concert with aPKC in mediating insulin action on SREBP-1c expression, and therefore promote lipogenesis; presumably this effect is mediated by MEK and Sp1 sites of the SREBP-1c promoter [18].

aPKCs also regulate hepatic cytokine production by phosphorylation/activation of IKKβ, which phosphorylates Inhibitor of nuclear factor kappa-B (NFκB), IκB, thereby releasing NFκB for nuclear uptake and transactivation of genes encoding proinflammatory cytokines, e.g., TNF-α and IL-1β. Interestingly, both exogenous insulin and simple feeding (presumably via endogenous insulin) provoke increases in aPKC-dependent IKKβ and NFκB activity and proinflammatory cytokine expression in rodent liver [7,8]. Whether Akt

and DAG-sensitive PKCs are similarly involved is uncertain. Although proinflammatory cytokines promote systemic insulin resistance and atherogenesis, it is uncertain if liver-derived, insulin-stimulated cytokines are contributory. It is also uncertain if insulin activates aPKCs and proinflammatory pathways in macrophages that impact cardiovascular events.

Opposite to increasing expression of lipogenic and proinflammatory factors downstream of SREBP-1c and NF κ B, insulin diminishes expression of hepatic gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase). This inhibitory effect of insulin appears to be largely mediated by Akt [15], which phosphorylates and thereby causes nuclear exclusion/inactivation of FoxO1, which otherwise enhances PEPCK/G6Pase expression.

Although Akt is thought to be the major factor that mediates inhibitory effects of insulin on hepatic PEPCK/G6Pase expression, some studies suggest that aPKCs may contribute [15,19], perhaps by phosphorylating CBP, i.e., CAMP response-element (CREB) binding protein, and subsequent dissolution of a CREB/CBP/TORC2 complex that drives expression of peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α)-dependent and subsequent increases in gluconeogenic gene transcription [19].

On the other hand, *inhibition* of hepatic aPKC in obese/T2DM mice diminishes *fasting*-dependent increases in PEPCK/G6Pase expression [8]. Stated differently, hepatic aPKC inhibition has insulin-like effects on gluconeogenic enzyme expression. In this regard, aPKC inhibition produces insulin/Akt-like increases in FoxO1 phosphorylation in mouse liver [20] and isolated human hepatocytes, [21] suggesting that aPKC tonically restrains hepatic Akt actions on FoxO1 and PEPCK/G6Pase expression. Whatever the explanation, it is fortuitous that aPKC inhibition diminishes PEPCK/G6Pase expression, as this insulin-like effect would be expected to diminish hepatic glucose output and improve glucose intolerance. This potential benefit adds to that of decreasing lipogenic and proinflammatory pathways during aPKC inhibition.

Insulin Action in Muscle in Obesity and T2DM

Insulin signaling to aPKC and glucose transport in muscle is almost invariably diminished in obese and T2DM rodents and humans [1]. This defect partly reflects diminished IRS-1/PI3K activity, which impairs both aPKC and Akt. However, in human T2DM muscle, whereas Akt impairment is best seen at submaximal rather than supra-maximal insulin levels (probably reflecting sparseness of insulin receptors), aPKC impairment is evident at both submaximal and maximal insulin levels [22] and probably reflects more distal “post-receptor” abnormalities, viz., diminished aPKC responsiveness to PIP₃ [22,23] and decreased aPKC levels [23,24]. More recently, mRNA, as well as protein levels of PKC- ι , the major aPKC in human muscle, was found to be diminished in muscles of T2DM humans [21].

It is of interest that muscle-specific knockout of PKC- λ (98% homologous to human PKC- ι), diminishes glucose transport in muscle, and this isolated defect, particularly in heterozygous mice, causes glucose intolerance, insulin resistance and hyperinsulinemia, which inordinately activates hepatic aPKC and provokes increases in expression/activity of SREBP-1c and NF κ B [8,9]. Thereupon, SREBP-1c promotes hepatic lipogenesis and development of hepatosteatosis, hypertriglyceridemia, hyperfattyacidemia, and hypercholesterolemia (increased LDL/decreased HDL), and, over time, downregulation of insulin signaling in various tissues and fasting hyperglycemia, i.e., T2DM [8,9]. Also note that appetite is mildly increased in heterozygous muscle-specific PKC- λ knockout (Het-M λ KO) mice, and coupled with increased hepatic lipogenesis, leads to abdominal obesity [9].

It is of further interest to note that muscle aPKC levels are diminished by approximately 40% in both Het-M λ KO mice [9], and T2DM humans [23,24]. In contrast, aPKC levels are not diminished in human obesity or rodent forms of T2DM [8]. In this regard, the Het-M λ KO mouse may be a unique model for studies of human T2DM.

Differences between human and rodent aPKC levels in both muscle and liver (see below) probably reflects the fact that insulin drives expression of the human aPKC isoform, PKC- ι , but not PKC- ζ or mouse PKC- λ , by an aPKC-dependent mechanism, i.e., it is autocatalytic [21]. Thus, in muscle, where IRS-1/PI3K controls PKC- ι activity, PKC- ι expression is diminished, and, in liver, where IRS-2/PI3K controls PKC- ι activity, PKC- ι expression is increased. This provides an explanation for observed increases in hepatic PKC- ι and decreases in muscle PKC- ι in human forms of T2DM [21].

There may of course be additional reasons why muscle aPKC activity is decreased in human forms of obesity, and muscle aPKC levels, as well as activity, are diminished in human forms of T2DM. For example, in both conditions, increases in circulating lipids and cytokines may alter activities of IRS-1 and aPKC. Also, micro-RNAs may participate in regulating PKC- ι transcription.

Insulin Action in Liver in Obesity and T2DM

High-fat-feeding (HFF) is a commonly-used experimental surrogate for diet-induced obesity in rodents. In our experience, short-term (2–4 weeks) feeding of a Western (40% milk fat) diet to mice provokes decreases in insulin signaling to IRS-1/PI3K, Akt and aPKC, and decreases in glucose transport in muscle; however, in liver, this diet does not diminish insulin effects on activities of IRS-1/PI3K, IRS-2/PI3K, Akt or aPKC [6,8]. Moreover, hyperinsulinemia in this obesity model provokes inordinate increases in hepatic insulin signaling to aPKC, SREBP-1c and NF κ B [8]; as a consequence, hepatic aPKC-dependent lipogenic and proinflammatory pathways are up-regulated, but Akt-dependent gluconeogenic pathways remains suppressible by insulin. However, over time, overt T2DM can ensue as hepatic IRS-1/PI3K/Akt activation is compromised and gluconeogenesis is increased in HFF mice.

As alluded to above, in rodent models of T2DM, whereas insulin activation of IRS-1/PI3K and Akt is impaired in muscle and liver, and, whereas insulin activation of aPKC is impaired in muscle, insulin activation of hepatic IRS2/PI3K and aPKC is conserved or heightened by hyperinsulinemia [6,7]. A similar pattern of Akt hypoactivity owing to diminished levels and activity of IRS-1, and aPKC hyperactivity owing to both conserved IRS-2 levels and actual increases in protein and mRNA levels of PKC- ι , is seen in isolated hepatocytes obtained from T2DM humans [21]. As discussed above, increased expression of hepatic PKC- ι in livers of T2DM humans apparently reflects increases in activation of IRS-2 and PKC- ι , which in turn increases PKC- ι self-expression [21]. As may be surmised, this feed-forward, positive-feedback mechanism culminates in a vicious cycle that remains active as long as it is driven by hyperinsulinemia. In this regard, note that sufficiently long removal of insulin, as well as use of PKC- ι inhibitors, abrogates this cycle and reverses aberrant increases in expression of lipogenic, proinflammatory and gluconeogenic pathways in hepatocytes of T2DM humans [21].

Imbalance Between Muscle and Liver aPKC in T2DM

To summarize, in T2DM humans, PKC- ι mRNA and protein levels are diminished in muscle and elevated in liver. As discussed, this imbalance in PKC- ι expression probably reflects that: (a) expression of PKC- ι (but not PKC- ζ or PKC- λ) is stimulated in human hepatocytes by insulin through a mechanism mediated by IRS-2 and PKC- ι itself; and (b)

muscle PKC- α is activated and expressed through IRS-1/PI3K, which is downregulated in T2DM. In addition, since hepatic aPKC overactivity diminishes insulin signaling and actions in muscle, and, *vice versa*, since muscle aPKC underactivity, via hyperinsulinemia, provokes increases in hepatic aPKC activity/expression, this itself is a multi-organ PKC- α vicious cycle that is superimposed upon the intra-hepatic PKC- α vicious cycle.

Inhibition of Hepatic aPKC in Obesity and T2DM

Fortunately, the above-described multi-organ and intra-hepatic vicious cycles of PKC- α activation that exist in obesity and T2DM can be broken by selective inhibition of hepatic aPKC. In rodent forms of obesity (HFF and Het-M λ KO mice) and T2DM (Goto-Kakizaki rats, ob/ob mice), 5 days after intravenous injection of adenovirus expressing kinase-inactive aPKC, aPKC activation was selectively diminished in liver, and this was accompanied by decreases in hepatic expression/activation of SREBP-1c and NF κ B, and remarkable improvements of hepatosteatosis, hypertriglyceridemia, hyperfattyacidemia, hypercholesterolemia and hyperinsulinemia [7,8]. Additionally, insulin signaling in muscle and liver (save for hepatic aPKC which was inhibited by expressed kinase-inactive aPKC) improved, and fasting levels of hepatic PEPCK/G6Pase diminished, presumably leading to further improvements in glucose homeostasis.

As followup to the adenoviral approach, two small-molecule chemical inhibitors of PKC- α/λ , have been developed and used, first, in hepatocytes of T2DM humans to verify that these inhibitors do in fact diminish overactive lipogenic, proinflammatory and gluconeogenic pathways, and, second, to treat heterozygous muscle-specific PKC- λ knockout mice that have abdominal obesity, MetSyn and T2DM. Importantly, both inhibitors selectively inhibited hepatic aPKC and exaggerated increases in expression/activities of SREBP-1c, NF κ B, and PEPCK/G6Pase. Moreover, within 7 days of PKC- α/λ inhibitor treatment, there were dramatic reversals of abdominal obesity, hepatosteatosis, hypertriglyceridemia, hyperfattyacidemia, hypercholesterolemia, hyperinsulinemia and hyperglycemia, and insulin signaling was improved in muscle, adipose and liver tissues [20].

Apparent Lack of a Requirement for Akt in Hepatic Lipogenesis in T2DM

Excessive SREBP-1c activity/expression in livers of T2DM rodents and hepatocytes of T2DM humans in the face of diminished Akt activation (functionally confirmed by increases in PEPCK/G6Pase expression) presents an unresolved conundrum: if Akt is required for activation/expression of hepatic SREBP-1c, and, if Akt activation is markedly diminished in diabetic liver, how is this requirement met or bypassed in T2DM liver? Stated differently, how can lipogenic and gluconeogenic pathways be simultaneously increased in T2DM?

To understand how Akt controls lipogenic and gluconeogenic pathways, several inhibitors were recently used in isolated hepatocytes [17]. First, a presumably specific Akt inhibitor was found to block insulin-induced increases in SREBP-1c/FAS and decreases in PEPCK/G6Pase expression in rat hepatocytes; thus, verifying the importance of Akt in these actions. However, signaling to these lipogenic and gluconeogenic pathways appeared to split downstream of Akt, as insulin effects on SREBP-1c/FAS, but not PEPCK/G6Pase, required mTOR1c, as determined by rapamycin sensitivity. It was therefore surmised that, in normal conditions, Akt uses mTOR1c to activate the lipogenic pathway, and FoxO1 phosphorylation to inhibit the glucogenic pathway.

However, left unresolved was the question of how insulin effects on the lipogenic pathway is maintained or heightened in livers of T2DM rodents and hepatocytes of T2DM humans, but, on the other hand, insulin effects on the gluconeogenic pathway are inhibited. Whether a low activation level of overall Akt activation, or an uninhibited subgroup of Akt serves to

maintain SREBP-1c activation in T2DM liver/hepaocytes, or whether another signaling factor obviates a need for Akt in T2DM hepatocytes, is unclear. Militating against the former possibility, effects of Akt inhibitor on lipogenesis occur at much lower doses than effects on glucogenesis [17].

As to the possibility that a non-Akt factor may substitute for Akt in T2DM liver, as discussed above, PKC- β is proposed to function along with aPKC and Akt in mediating insulin effects on hepatic lipogenesis [18]. Moreover, note that: PKC- β is activated in livers of T2DM humans, and this effect of insulin on a cPKC seems likely to reflect increases in de novo production of DAG, rather than hyperglycemia-induced increases in DAG [26]; and PKCs like Akt, may activate mTOR1c and S6-kinase. Accordingly, it is possible that PKC- β and/or other DAG-sensitive c/nPKCs that is/are activated by insulin itself or other factors in diabetic liver may obviate the need for Akt in insulin-stimulated lipogenesis in diabetic liver. Needless to say, the roles of specific cPKCs and nPKCs in explaining paradoxical increases in lipogenesis and gluconeogenesis in T2DM liver needs further study.

Metabolic Effects of Metformin

Metformin, commonly used to treat insulin resistance and hyperglycemia in T2DM, also modestly improves obesity and hyperlipidemia. Improved glucose homeostasis largely reflects insulin-like decreases in expression of PEPCK/G6Pase. In addition, metformin increases glucose transport in rodent muscle by an aPKC-dependent mechanism [10], and, in T2DM humans, improves muscle aPKC responsiveness to insulin in vivo and PIP₃ in vitro, and overall insulin signaling in muscle [25,27].

Metformin is thought to control metabolic processes largely through activation of AMP-activated protein kinase (AMPK). Stimulation of glucose transport by AMPK activators, viz., metformin and 5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranoside (AICAR), in rat muscle, rat-derived L6 myotubes and muscles of intact mice is mediated, as with insulin, through aPKC activation [10,12]. Indeed, PKC- λ knockout in intact mice, and inhibition or knockdown of aPKC in isolated L6 myotubes, block the activation of glucose transport by metformin and AICAR, as well as insulin [10]. However, unlike insulin, AMPK apparently uses the MEK1/ERK/phospholipase D/phosphatidic acid, rather than the PI3K/PIP₃, to activate aPKC [10,12]; thus, AMPK activation can bypass impairments in IRS-1/PI3K signaling in insulin-resistant states.

In some tissues, aPKC functions upstream of AMPK, e.g., in endothelial cells, aPKC phosphorylates LKB1, a protein-threonine-kinase that phosphorylates/activates AMPK [28]. Whether aPKC simultaneously functions downstream and upstream of AMPK in these tissues is unknown.

In liver, like insulin, metformin diminishes fasting-dependent increases in expression of PEPCK/G6Pase. A recent report suggests that the effects of both metformin and insulin on PEPCK/G6Pase in mouse liver are mediated through aPKC-dependent phosphorylation of CREB binding protein (CBP), causing disruption of a CREB/CBP/TORC2 complex needed for PGC-1 α expression and gluconeogenic gene transcription [19]. However, insulin also uses PI3K/PIP₃/Akt to phosphorylate FoxO1 and diminish PEPCK/G6Pase expression. Thus, relative contributions of aPKC/CBP-dependent vis-à-vis Akt/FoxO1-dependent mechanisms for control of gluconeogenesis during insulin action are uncertain. Also, note that, whereas metformin activated aPKC in isolated mouse hepatocytes, 30-min metformin treatment in vivo activated AMPK, but not aPKC, in rat and mouse liver [10]. These differences in aPKC activation require further study. Another difficulty with the idea that metformin activates hepatic aPKC, is that this would be expected to increase hepatic lipogenesis?

As to how metformin activates AMPK and how it regulates gluconeogenesis, it has been suggested that, in mice, LKB1 activates AMPK, which in turn phosphorylates TORC2, causing nuclear exclusion and diminished TORC2 availability for supporting PEPCK/G6Pase expression [29]. Presumably, aPKC would not be involved in this action of metformin. However, AMPK subunits in human liver reportedly differ from those in mouse liver, and it has been proposed that metformin activates AMPK in human liver by uncoupling of oxidative phosphorylation and generation of 5'-AMP [30]. Here again, more clarification of the mechanism of action of metformin is needed.

Conclusions and Future Directions

The evidence cited above strongly incriminates hyperactivity of hepatic aPKC as a key player for inordinate activation of lipogenic, proinflammatory and gluconeogenic pathways in livers of obese and T2DM rodents and humans. Accordingly, aPKCs appear to be particularly important in provoking or abetting the development of obesity, hepatosteatosis, hypertriglyceridemia, hyperfattyacidemia, and hypercholesterolemia, and, moreover, in contributing to further increases in systemic insulin resistance, hyperinsulinemia and glucose intolerance in clinical syndromes of obesity, MetSyn and T2DM. Thus, regardless of the initial cause(s) of insulin resistance (caloric excess, altered insulin signaling in muscle, liver or other tissues, partial deficiency in insulin secretion), hyperinsulinemia increases activity (and expression in humans) of hepatic aPKC (and possibly other c/nPKCs) and activities of hepatic lipogenic, proinflammatory and gluconeogenic pathways.

Accordingly, hepatic aPKC appears to be a unifying factor that underlies, or is critically required for pathogenesis of, many if not most clinical abnormalities that serve as cardiovascular risk factors; thus, hepatic aPKC is a unique therapeutic target. This idea is further strengthened by finding that selective inhibition of hepatic aPKC in rodent models of obesity and T2DM rapidly and dramatically improves these abnormalities.

Whether the approach of inhibiting hepatic aPKC will prove to be as effective for reversing metabolic abnormalities in humans as was found in initial studies of rodents remains for future studies. However, both newly-developed small molecule inhibitors of PKC- α are very effective in blocking lipogenic, inflammatory and gluconeogenic pathways, not only in livers of obesity/T2DM mice, but also in isolated hepatocytes of non-diabetic and T2DM humans. Of further importance, in the murine obesity/MetSyn/T2DM model that was used in initial studies, both PKC- α inhibitors, for uncertain reason, selectively diminished hepatic, rather than extra-hepatic, aPKC activity, and this was attended by improvements in insulin signaling in muscle, liver and adipose tissues, and notable reversals of clinical abnormalities, including, abdominal obesity, hepatosteatosis, hypertriglyceridemia, hypercholesterolemia, hyper-fattyacidemia, hyperinsulinemia and hyperglycemia. Clearly, there is an urgent need to obtain pharmacokinetic, pharmacodynamic and safety information, and more definitively assess the usefulness of these and other PKC- α inhibitors in rodents, subhuman primates and, ultimately, humans.

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- 18**. Yamamoto T, Watanabe K, Inoue N, Nakagawa Y, Ishigaki N, Matsuzaka T, Takeuchi Y, Kobayashi K, Yatoh S, Takahashi A, Suzuki H, Yahagi N, Gotoda T, Yamada N, Shimano H. Protein kinase C β mediates hepatic induction of sterol-regulatory element binding protein-1c by insulin. *J Lipid Res.* 2010; 51:1859–1870. In this paper, evidence from knockdown and inhibitor studies is used to suggest that DAG-sensitive PKC- β is required for insulin-induced increases in transcription of the hepatic SREBP-1c gene. Promoter studies in HepG2 cells further suggested that this effect is mediated by replacement of Sp3 by Sp1 on sites in the GC box of the SRE complex. Knockdown of PKC- β , but not PKC- ϵ , markedly diminished refeeding-induced (presumably insulin-mediated) increases in SREBP-1c and FAS mRNA, and plasma lipid and glucose levels. However, PKC- ϵ knockdown also elicited decreases in plasma lipid levels, despite increased expression of SREBP-1c and FAS. Also unexplained are increases in PEPCK expression with knockdown of hepatic PKC- β and PKC- ϵ in the face of decreases in plasma glucose levels. [PubMed: 20179320]
- 19**. He L, Sabet A, Djedjdos S, Miller R, Sun X, Hussain MA, Radovick S, Wondisford FE. Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. *Cell.* 2009; 15:635–646. In this paper, evidence is provided to suggest that both metformin and insulin use aPKC to phosphorylate CREB at serine 436 and thereby interfere with formation of a CREB/CPB/TORC2 complex needed for transcription of PGC-1 α and subsequent induction of genes encoding gluconeogenic enzymes. This aPKC-dependent mechanism is seen as being complementary to the PI3K/Akt/FoxO1 and other Akt-dependent mechanisms that diminish expression of gluconeogenic genes, PEPCK and G6Pase. In any case, the relative importance of this aPKC-dependent and Akt-dependent mechanisms for regulating hepatic gluconeogenesis during insulin action remains uncertain; also uncertain is how these pathways are altered in states of obesity and T2DM. In this regard, note that Akt seemed more important than aPKC for regulation of gluconeogenesis in the study of Taniguchi et al [reference 17]. Also note that: (a) liver-specific knockout of PKC-1 does not diminish insulin effects on gluconeogenic genes [14]; and (b) increases in hepatic aPKC activity were not observed after 30-min of metformin treatment of intact rodents, despite increasing hepatic AMPK activity [10]. Finally, note that AMPK, through which metformin is postulated to act, is different in mouse and human liver [30], and it is uncertain if metformin activates aPKC in human hepatocytes. Further work is

needed to determine if metformin is or is not an effective activator of hepatic aPKC in vivo (particularly in human hepatocytes), and if either insulin or metformin, uses aPKC to diminish gluconeogenesis. [PubMed: 19450513]

- 20 **. Sajjan MP, Nimal S, Mastorides S, Acevedo-Duncan M, Kahn CR, Fields AP, Braun U, Leitges M, Farese RV. Correction of Metabolic Abnormalities in a Rodent Model of Obesity, Metabolic Syndrome and Type 2 Diabetes by Inhibitors of Hepatic Protein Kinase C- ι . *Metabolism*. in press. In this paper, two newly developed, apparently specific inhibitors of PKC- ι/λ (major human and mouse aPKCs, respectively) were found to rapidly reverse aberrations in hepatic lipogenic, proinflammatory and gluconeogenic enzyme transcription, and markedly improve clinical abnormalities (abdominal obesity, hepatosteatosis, hypertriglyceridemia, hypercholesterolemia, hyperinsulinemia and glucose intolerance in a mouse model of obesity/MetSnn/T2DM. For uncertain reason, both inhibitors appeared to selectively target hepatic aPKC, and spare muscle and adipocyte aPKC when administered to intact mice.
- 21 **. Sajjan MP, Nimal S, Acevedo-Duncan M, Fields AP, Farese RV. Insulin Signalling in Hepatocytes of Type 2 Diabetic Humans. Excessive Expression and Activity of PKC- ι and Dependent Processes and Reversal by PKC- ι Inhibitors. *Diabetologia*. in press. In this paper, the expression, levels and activity of PKC- ι were found to be increased in hepatocytes of humans who have T2DM, relative to hepatocytes of non-diabetic humans; in contrast, Akt levels were normal and Akt activation by insulin was diminished in T2DM hepatocytes. The ability of insulin to increase aPKC activity in T2DM hepatocytes apparently reflected increases in insulin-stimulated activity of IRS-2/PI3K activity; in contrast, IRS-1 levels and insulin-stimulated IRS-1/PI3K activity were markedly diminished. Expression of PKC- ι (but not PKC- ζ) was increased by insulin and blocked by inhibitors of PKC- ι . This suggested that PKC- ι expression in T2DM hepatocytes is driven by insulin by a feed-forward positive feedback mechanism, which would essentially produce a vicious cycle in hyperinsulinemic states. Accompanying increases in hepatic aPKC activity, expression of lipogenic, proinflammatory and gluconeogenic genes were increased in T2DM hepatocytes. Of further note, two newly developed, apparently specific inhibitors of PKC- ι/λ (major human and mouse aPKCs, respectively) were found to reverse aberrations in hepatic lipogenic, proinflammatory and gluconeogenic enzyme transcription in T2DM hepatocytes. This hypo-reexpression of hepatic PKC- ι contrasts sharply with hypo-expression of muscle PKC- ι , which is controlled by IRS-1/PI3K (which is downregulated in T2DM muscle).
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