Fatty Acid Infusion Selectively Impairs Insulin Action on Akt1 and Protein Kinase C λ/γ but Not on Glycogen Synthase Kinase-3*

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To determine the mechanism(s) for insulin resistance induced by fatty acids, we measured the ability of insulin to activate phosphoinositide 3-kinase (PI3K) and multiple distal pathways in rats. Following a 5-h infusion of lipid or glycerol (control), rats underwent a euglycemic hyperinsulinemic clamp. Insulin stimulated IRS-1-associated PI3K activity in muscle of glycerol-infused rats 2.4-fold but had no effect in lipid-infused rats. IRS-2- and phosphoryrin-associated PI3K activity were increased 3.5- and 4.8-fold, respectively, by insulin in glycerol-infused rats but only 1.6- and 2.3-fold in lipid-infused rats. Insulin increased Akt1 activity 3.9-fold in glycerol-infused rats, and this was impaired 41% in lipid-infused rats. Insulin action on Akt1 and insulin-stimulated glucose transport is still controversial (14–20).

Three Akt isoforms have been cloned (21, 22). Akt1 knockout mice do not have insulin resistance, and in type 2 diabetes (37, 38). Insulin stimulates glycogen synthase kinase-3 (GSK-3) results in activation of PI3K, which is necessary for insulin action on glucose transport (9–13). The extent to which downstream effectors, Akt/protein kinase B, and PKCA/γ, mediate insulin action on glucose transport is still controversial (14–20).

Studies suggest that activation of the atypical PKC isoforms λ and γ is required for insulin stimulation of glucose uptake and Glut4 translocation (18–20). Overexpression of a dominant negative mutant of PKCα or PKCγ abrogated insulin-stimulated glucose transport and Glut4 translocation in adipose (18, 30) and muscle cells (19, 31). Overexpression of constitutively activated PKCα in adipocytes (18) or wild type PKCγ in muscle in vivo (32) enhanced both basal and insulin-stimulated glucose transport. In addition, insulin-stimulated PKCα/γ activity was impaired in the skeletal muscle and adipose tissue of diabetic rats (33, 34), in the muscle of obese type 2 diabetic humans (35), and in myotubes of obese humans (36). It is therefore possible that PKCα/γ could play an important role in insulin resistance in skeletal muscle and adipose tissue.

Glucose transport is the rate-controlling step for insulin-stimulated muscle glycogen synthesis under normal conditions and in type 2 diabetes (37, 38). Insulin stimulates glycogen synthase by both activating protein phosphatase-1 (PP-1) and inhibiting glycogen synthase kinase 3 (GSK-3) (39). Two iso-

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1 The abbreviations used are: IRS, insulin receptor substrate; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PP-1, protein phosphatase-1; GSK-3, glycogen synthase kinase-3; MAPK, mitogen-activated protein kinase.
forms of GSK-3 have been identified: GSK-3α and GSK-3β (40). GSK-3 is a serine/threonine kinase that inhibits glycogen synthase activity by phosphorylating glycogen synthase (39). Insulin inactivates GSK-3 in skeletal muscle of rats and humans (41–43), which leads to activation of glycogen synthase and therefore increased glycogen synthesis (39). The upstream signaling pathways necessary for insulin activation of glycogen synthase include PI3K (44, 45). Activation of PI3K by insulin is impaired in the muscle of humans with type 2 diabetes (25), and both basal and insulin-stimulated glycogen synthase activity are reduced (25), but the important intermediary pathways are still unknown.

Plasma fatty acids are often elevated with obesity and type 2 diabetes (3, 46) and most likely contribute to the insulin resistance in these states. We previously demonstrated that fatty acid infusion decreases whole body glucose uptake and glycogen synthesis and that this is associated with a defect in insulin-stimulated PI3K activity associated with IRS-1 in muscle (47, 48). In this study we sought to determine the mechanisms(s) for the fatty acid-induced impairment in insulin action by measuring key steps downstream of PI3K leading to GSK-3 inhibition in skeletal muscle. We found differential regulation of Akt isoforms and discordance between insulin action on Akt and GSK-3 activity was determined as described previously (23).

**Determination of PKCα/ζ Activity**—Muscle lysates (500 µg of protein) were subjected to immunoprecipitation for 4 h at 4 °C with 2 µg of a polyclonal PKCα/ζ antibody (Santa Cruz Biotechnology, Santa Cruz, CA) that recognizes PKCα and PKCζ. The immune complexes were washed, and the PKCα/ζ activity was determined as described previously (33).

**Determination of GSK-3 Activity**—Muscle lysates (500 µg of protein) were subjected to immunoprecipitation for 4 h at 4 °C with either 2 µg of an anti-phospho-Thr21/27 (Ser9) GSK-3 antibody (4100 dilution; gift from Dr. Hagit Eldar-Finkelman, Tel-Aviv University, Israel), coupled to protein A-Sepharose or protein G-Sepharose beads. The immune complexes were washed twice with 50 mM Tris, pH 7.4, 500 mM LiCl, and 1 mM dithiothreitol and twice with 50 mM Tris, pH 7.4. The kinase assay was performed on the immunoprecipitates in reaction mixtures including 50 mM Tris, 10 mM MgCl₂, 0.5 mM bovine serum albumin, 100 µM ATP (0.25 mM/C), and 100 µM phosphoglycerate kinase-1 peptide substrate, and incubation was carried out for 20 min at 30 °C. The reaction mixtures were spotted on PS1 phosphocellulose paper, washed with 75 mM phosphoric acid, and counted for radioactivity (49).

**Determination of Glycerogen Synthesis Activity**—20 mg of muscle were homogenized using a polytron at half-maximum speed for 1 min on ice in 0.5 ml of extraction buffer (50 mM Hepes, 10 mM EDTA, 100 mM NaF, 5 mM dithiothreitol, 1 µM leupeptin, 1 µM pepstatin, and 200 µM phenylmethylsulfonyl fluoride, pH 7.5). Homogenates were used to measure glycogen synthesis activity as described previously (50). The glycogen synthesis activity was determined at a physiologic concentration of substrate (0.3 mM UDP-glucose), calculated as nanomoles of UDP-glucose incorporated into glycogen/min/milligram of total protein and expressed as the ratio of activity assayed at 0 mM glucose-6-phosphate divided by the activity at 7.2 mM glucose-6-phosphate. This is an indicator of the change in the phosphorylation state of glycogen synthase in response to insulin (51).

**Determination of MAPK Activity**—The muscle lyses (500 µg of protein) were subjected to immunoprecipitation for 4 h at 4 °C with 1 µl of a MAPK (Erk1 and Erk2) antibody (1:500 dilution; gift from Dr. John Blenis, Harvard Medical School) coupled to protein A-Sepharose beads. The immune complexes were washed three times with Buffer A and twice with 20 mM Tris, pH 7.4, 20 mM MgCl₂, 1 mM dithiothreitol, and 0.2% β-mercaptoethanol, 2 mM/ml glycogen, pH 7.4) containing 0.1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, and 10 mM/ml aprotinin. Muscle homogenates (10 µg) were preincubated with MAPK homogenization buffer containing 4.5 mM okadaic acid for 2 min at 37 °C. The reaction was initiated by adding 15 µg of 32P-labeled phosphorylase a in the presence of 1 mM okadaic acid and 5 mM caffeine. The phosphate release was determined as described previously (52).

**Determination of PP-1 Activity**—20 mg of muscle were homogenized using a polytron at half-maximum speed for 1 min on ice in 0.5 ml of PP-1 homogenization buffer (50 mM Tris, 2 mM EDTA, 0.2% β-mercaptoethanol, 2 mM/ml glycogen, pH 7.4) containing 0.1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, and 10 mM/ml aprotinin. Muscle homogenates (10 µg) were preincubated with PP-1 homogenization buffer containing 4.5 mM okadaic acid for 2 min at 37 °C. The reaction was initiated by adding 15 µg of 32P-labeled phosphorylase a in the presence of 1 mM okadaic acid and 5 mM caffeine. The phosphate release was determined as described previously (52).

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**Determination of p70S6 Kinase Mobility Shift and Amounts of Signaling Proteins**—10–100 µg of tissue lysates protein/lane were resolved by SDS-PAGE (8 and 10% gel) and transferred to nitrocellulose membranes (Schleicher & Schuell). The membranes were blocked with 5% nonfat dry milk for 1 h at room temperature and incubated with the following antibodies: Akt1- or Akt2-specific polyclonal antibody gift from T. Franke, Columbia University, or Akt1 antibody from Santa Cruz and Akt2 antibody from Upstate Biotechnology, which gave similar results to our antibodies), a polyclonal antibody of PKCα/ζ (Santa Cruz Biotechnology), a monoclonal antibody for GSK-3 (Upstate Biotechnology) that recognizes both GSK-3 α and GSK-3 β, a polyclonal antibody against the p85α subunit (Upstate Biotechnology) that recognizes all isoforms of the regulatory subunit of PI3K or p110α subunit of PI3K, a polyclonal antibody against PKA (gift from H. Haspel, Henry Ford Hospital, Detroit, MI), a polyclonal PP-1 antibody (Upstate Biotechnology), a polyclonal glycogen synthase antibody (gift from J. Lawrence, University of Virginia), or a polyclonal IRS-1 or IRS-2 antibody (gift from M. White, Joslin Diabetes Center) in 1% nonfat dry milk overnight at 4 °C. The membranes were washed, and the bands were visualized as described previously (23).
PI3K Activity—After 30 min of insulin stimulation, IRS-1-associated PI3K activity in skeletal muscle of glycerol-infused rats was stimulated 2.4-fold above basal levels in saline-infused rats (Fig. 1A). In contrast, in lipid-infused rats, IRS-1-associated PI3K activity did not respond to insulin and was similar to that in saline control rats. Insulin also stimulated IRS-2-associated PI3K activity 3.5-fold in muscle of glycerol-infused rats but only 1.6-fold in lipid-infused rats (Fig. 1B). Because of the possibility that PI3K activity associated with other phosphoproteins could contribute to the effect of insulin on glucose uptake, we also measured antiphosphotyrosine-associated PI3K (Fig. 1C). Insulin increased antiphosphotyrosine-associated PI3K activity 4.8-fold in glycerol-infused rats but only 2.3-fold in lipid-infused rats.

Akt Isoforms Activity and Protein Level—Insulin-resistant states are associated with differential effects on Akt1, Akt2, and Akt3 isoforms (23). Fig. 2 shows that insulin stimulated Akt1 activity in skeletal muscle 3.9-fold in glycerol-infused rats and 2.3-fold in lipid-infused rats compared with saline-infused control rats. The insulin-stimulated increment in Akt1 activity above the saline value is reduced 55% in lipid-infused rats compared with glycerol-infused rats (p < 0.05) (Fig. 2A). Insulin also increased Akt2 activity 1.8-fold in glycerol-infused rat and 2.3-fold in lipid-infused rats compared with control rats (Fig. 2B). However, Akt3 activity did not respond to insulin in either group (Fig. 2C). These results suggest that fatty acid infusion selectively impaired Akt1 isoform activity in skeletal muscle. Fig. 2D shows that the protein levels of Akt1 and Akt2 in skeletal muscle were not different among saline-infused, glycerol-infused, and lipid-infused rats.

PKCα/ζ Activity and Protein Level—To determine whether impaired activation of atypical PKC plays a role in fatty acid-induced insulin resistance in rats, we measured the activity and protein levels of PKCα/ζ in muscle. Fig. 3 shows that insulin stimulated PKCα/ζ activity in skeletal muscle 2.0-fold in glycerol-infused rats and 1.35-fold in lipid-infused rats compared with saline-infused rats (Fig. 3A). The increment in insulin-stimulated PKCα/ζ activity over saline was reduced 65% in lipid-infused rats compared with glycerol-infused rats (p < 0.05). Fig. 3B shows that the protein levels of PKCα/ζ in skeletal muscle were not different among saline-infused, glycerol-infused, or lipid-infused rats.

GSK-3 Isoform Activity and Protein Levels—To determine whether the insulin resistance induced by lipid infusion involves an impairment in the effect of insulin in inhibiting GSK-3 activity, we measured the activity of the two known GSK-3 isoforms, α and β. GSK-3α activity in skeletal muscle was reduced 30% in response to insulin in glycerol- and lipid-infused rats (p < 0.05) compared with saline-infused rats (Fig. 4A). In contrast, insulin inhibited GSK-3β activity 63% in muscle of glycerol-infused rats (p < 0.01 versus saline) and 68% in lipid-infused rats (p < 0.001) compared with non-insulin-infused control rats (Fig. 4B). The magnitude of decrease in GSK-3β activity was greater than the decrease in GSK-3α activity, suggesting greater susceptibility of GSK-3β to insulin regulation in rat skeletal muscle.

Glycogen Synthase Activity and Protein Level—to determine the effects of lipid infusion on glycogen synthase activity in rats, we measured glycogen synthase activity in skeletal muscle after a 30-min hyperinsulinemic euglycemic clamp following preinfusion with glycerol or lipid for 5 h. The ratio of glycogen synthase I form (without glucose-6-phosphate) over total activity (with glucose-6-phosphate) increased 2.0-fold in glycerol-infused rats but only 1.4-fold in lipid-infused rats compared with saline-infused rats (Fig. 5A). There is no significant difference in total glycogen synthase activity (with glucose-6-phosphate) in either group (data not shown).

Fig. 5B shows the protein levels of glycogen synthase in skeletal muscle of saline-infused, glycerol-infused, or lipid-infused rats. There were no significant differences in the amount of glycogen synthase protein in muscle among the three groups (Fig. 5B).

PP-1 Activity and Protein Level—to determine whether PP-1 activity is impaired in the skeletal muscle of lipid-induced insulin resistance in rats, we measured the activity and protein levels of PP-1 in muscle. Fig. 6A shows that insulin stimulated PP-1 activity in skeletal muscle 40% in glycerol-infused rats and 47% in lipid-infused rats compared with saline-infused rats (p < 0.05). Fig. 6B shows a representative blot indicating that the amount of PP-1 protein was unaltered in muscle of saline-infused, glycerol-infused, or lipid-infused rats.

MAPK Activity and p70S6 Kinase Gel Mobility Shift—to determine whether other downstream signaling steps are impaired by lipid infusion, we measured MAPK activity and p70S6 kinase phosphorylation in skeletal muscle using the same protocol of a 30-min insulin clamp following preinfusion with glycerol or lipid for 5 h. With this protocol, MAPK activity was not activated by insulin in the skeletal muscle of either glycerol-infused or lipid-infused rats (Fig. 7A). We saw a similar result in the phosphorylation of MAPK using a phosphospecific antibody (not shown). We routinely find that more extended periods of fasting are necessary to reduce basal MAPK activity in skeletal muscle of saline-infused rats enough to detect an insulin effect.

Fig. 7B shows the levels and insulin-stimulated hyperphos-
phorylation of p70S6 kinase in skeletal muscle of glycerol-infused and lipid-infused rats. In the basal state, we detect a single 66-kDa band for p70S6 kinase in muscle. After insulin stimulation of rats for 30 min, p70S6 kinase shifts to a hyperphosphorylated state in both glycerol-infused and lipid-infused rats. There is no significant change in the phosphorylation of p70S6 kinase in muscle of lipid-infused rats compared with glycerol-infused rats (Fig. 7B).

**DISCUSSION**

In insulin-resistant states such as obesity and type 2 diabetes, plasma fatty acids are elevated (3, 46). Lipid infusion in rats and humans results in reduced insulin-stimulated rates of muscle glycogen synthesis, whole body glucose oxidation, and glucose uptake (53–56). These changes are accompanied by a defect in insulin-stimulated PI3K activity associated with IRS-1 in skeletal muscle (47, 48). In the present study, we investigated the effects of lipid infusion on the downstream mediators of PI3K, with particular focus on whether impaired insulin action on Akt, PKCζ, GSK-3 isoforms, or PP-1 contributes to this insulin-resistant state. Here we show that 5 h of fatty acid infusion specifically impairs insulin-stimulated Akt1 activity in skeletal muscle, but insulin action on Akt2, GSK-3, and PP-1 is unaltered. Akt1 in rat skeletal muscle is highly responsive to insulin and the time course of its stimulation parallels that of inhibition of GSK-3 activity (24). Combined with in vitro data showing phosphorylation of GSK-3 by Akt1 (57), this makes it possible that Akt1 could be important for insulin action on glycogen synthesis. However, our study shows that Akt1 does not appear to be the major upstream regulator of GSK-3 activity in the insulin-resistant state induced by lipid infusion or that other Akt isoforms can substitute when Akt1 activation is defective. Furthermore, lipid infusion leads to impaired insulin-induced glycogen synthase activity but no defect in insulin action on GSK-3 or PP-1, indicating there is dominant regulation of glycogen synthase.
activity by non-GSK-3- and non-PP-1-mediated pathway(s) (58, 59). Finally, this insulin-resistant state also involves impaired insulin activation of PKCζ activity in skeletal muscle that could contribute to the metabolic defects.

There is increasing evidence that key steps in the insulin signaling pathway, including the insulin receptor, IRSs, and PI3K, are candidates for defects leading to insulin resistance in skeletal muscle of rodents and humans (23, 25, 60–62). Activation of PI3K is necessary for insulin-induced glucose transport and Glut4 translocation (9–13). In addition to the defect in insulin-stimulated IRS-1-associated PI3K activity in skeletal muscle of lipid-infused rats (47), we found that insulin-stimulated PI3K activity associated with IRS-2 or phosphotyrosine is also decreased by -50%. Similar results have been demonstrated in type 2 diabetic subjects as well as in obese nondiabetic subjects (25, 63) and a number of genetic models of diabetes and insulin resistance (23, 60, 61). In view of the evidence that lipid-induced insulin resistance involves a defect of glucose transport in skeletal muscle (37, 53), it is likely that the reduction of insulin-stimulated PI3K activity leads to an impairment of Glut4 translocation that plays a major role in the decreased glucose disposal.

The inhibition of Akt1 activation with lipid infusion contrasts with observations in insulin-resistant muscle from obese humans with type 2 diabetes (25) and glucosamine-infused rats, in which Akt activation was normal (64). However, insulin-stimulated Akt activity is diminished in some insulin-resistant states such as in nonobese type 2 diabetic humans (26) and Goto-Kakizaki rats (65). The difference may be due to differences in the metabolic disturbances, the degree of insulin resistance, or the dose, duration, or route of insulin treatment in these studies. It is not clear whether a defect in Akt1 activation in vivo could contribute to the development of fatty acid-induced insulin resistance. Mice with knockout of Akt1 are not insulin-resistant (28, 29). However, these mice are growth retarded, and developmental abnormalities or reduced fat mass could mask insulin resistance in muscle.

Akt is required for the effect of insulin to inhibit GSK-3 in cell culture (57). However, we find that insulin-induced inactivation of GSK-3 isoforms is normal despite decreased activation of Akt1 in skeletal muscle of lipid-infused rats. We see a similar discrepancy in skeletal muscle of lactate-infused rats (66) and in Zucker fa/fa obese rats.2 Taken together, these data suggest that either maximal Akt1 activation is not required for maximal inhibition of GSK-3 or that Akt1 is not a major upstream regulator of GSK-3 in this insulin-resistant state in vivo. This raises the possibility of other Akt isoforms or other pathways downstream of PI3K playing a role in insulin action on glycogen synthesis.

The atypical PKC isoforms, PKCδ and ζ, are also downstream targets of PI3K (see Introduction) (18–20). Here we show that insulin-stimulated PKCζ activity is impaired in skeletal muscle of lipid-infused rats compared with glycerol-infused rats. Consistent with our current results are data from several insulin-resistant models including Goto-Kakizaki diabetic rats (33), high fat fed rats (67), and obese Zucker rats.2 as well as obese nondiabetic and obese diabetic humans (35), all of

2 Y.-B. Kim and B. B. Kahn, unpublished data.
which have decreased activation of PI3K and PKCζ/ε. This raises the possibility that defective activation of atypical PKCs in muscle could contribute to the decreased glucose uptake and metabolism in those states.

A recent study demonstrates that PKCζ enhances Akt-mediated GSK-3 phosphorylation in skeletal muscle cells in vitro (68), indicating that both PKCζ and Akt are required for the potent inhibition of GSK-3 activation. In our study, lipid infusion compromises insulin-stimulated PKCζ/ε activity, but GSK-3 inhibition is normal. Because the impairment of insulin-stimulated PKCζ/ε activity is partial, the remaining PKCζ/ε activity may be sufficient to enable GSK-3 phosphorylation in vivo. Because insulin action on Akt1, but not Akt2 or Akt3, is selectively impaired in skeletal muscle of lipid-infused rats, other Akt isoforms may also contribute to GSK-3 phosphorylation and inhibition in this model.

Another discrepancy revealed by the lipid-infused model is the effect of insulin on GSK-3 and glycogen synthase activity. GSK-3 is thought to be a major regulator of glycogen synthase activity. Inhibition of GSK-3 blocks the ability of insulin to inactivate GSK-3 and increases glycogen synthase activity in cultured muscle cells (69, 70) and adipocytes (71). Additionally, overexpression of GSK-3ζ in 293 cells causes a reduction in basal glycogen synthase activity (72). However, lipid infusion results in a reduction of glycogen synthase activity in skeletal muscle despite the fact that insulin-stimulated GSK-3ζ activation is normal, suggesting that glycogen synthase activity can be regulated independent of GSK-3 in vivo. Support for this comes from studies showing that GSK-3 is not essential for glycogen synthase activation by insulin in adipocytes (58), rat-1 fibroblasts (58), and hepatocytes (59). Here we demonstrate that two other possible pathways, MAPK and p70S6 kinase, are not involved in the modulation of glycogen synthase in this insulin-resistant state.

Glycogen synthase has nine phosphorylation sites, which are

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**Fig. 6.** PP-1 activity (A) and protein level (B) in muscle of rats after hyperinsulinemic euglycemic clamp following preinfusion with either glycerol or intralipid for 5 h. The control rats were infused only with saline for 5.5 h. The PP-1 activity in muscle homogenates (10 μg) was measured using 32P-labeled phosphorylase a as substrate. B, proteins in muscle lysates (50 μg) were separated by SDS/PAGE on 8% gels and transferred to nitrocellulose membrane. PP-1 was visualized by immunoblotting with a PP-1 antibody. The results are the means ± S.E. for three rats for saline and for six or seven rats for glycerol or lipid infusion. *p < 0.05 versus saline.

**Fig. 7.** MAPK activity (A) and p70S6 kinase mobility shift (B) in muscle of rats after hyperinsulinemic euglycemic clamp following preinfusion with either glycerol or intralipid for 5 h. The control rats were infused only with saline for 5.5 h. A, muscle lysates (500 μg) were subjected to immunoprecipitation with MAPK antibody. The immune pellets were assayed for kinase activity using myelin basic protein as substrate. The results are the means ± S.E. for three rats for saline and for seven or eight rats for glycerol or lipid. B, proteins in muscle lysates (50 μg) were separated by SDS/PAGE on 8% gels and transferred to nitrocellulose membranes. p70S6 kinase was visualized by immunoblotting with a p70S6 kinase antibody. This blot is representative of three blots on three to eight rats/group.

**Fig. 8.** Signaling protein levels in muscle of rats after hyperinsulinemic euglycemic clamp following preinfusion with either glycerol or intralipid. The control rats were infused only with saline for 5.5 h. The proteins in muscle lysates (40–100 μg) were separated by SDS/PAGE on 8 or 10% gels and transferred to nitrocellulose membranes. p85, p55, p50, p110, IRS-1, IRS-2, and Glut4 were visualized by immunoblotting with specific antibodies. This blot is representative of three blots on three to ten rats/group.
located at both NH$_2$ and COOH termini (73). GS-3 phosphorylates sites 4, 3c, 3b, and 3a (39), but sites 3a and 3b can also be phosphorylated by currently unidentified protein kinases or unknown factors (73, 74). These two sites are the most important for regulation of glycogen synthase (73). It is therefore conceivable that lipid infusion increases phosphorylation of sites 3a and 3b directly or through unidentified protein kinases, leading to a decrease in glycogen synthase activity. Alternatively, altered PP-1 activity could be involved. However, our results demonstrate that PP-1 activation and expression is normal in the lipid-infused insulin-resistant state.

In conclusion, the impaired insulin-stimulated glucose disposal in skeletal muscle induced by lipid infusion is associated with a decrease in insulin-stimulated PI3K activation. Several observations challenge the recent linear concept of the insulin signaling cascade regulating glycogen synthesis. For example, lipid infusion impairs insulin action on Akt1 in skeletal muscle, but action on GS-3 is preserved. This may be due to the fact that other Akt isoforms can regulate GS-3 activity or that only submaximal activation of Akt1 is required to fully inhibit GS-3. Interestingly, Akt isoforms are differentially affected in this insulin-resistant state. Despite a marked impairment in insulin action on PI3K, Akt2 activation is normal, suggesting possible alternative upstream pathways for Akt2 activation. Furthermore, insulin action on glycogen synthase is impaired with no defect in action on GS-3 inhibition or PP1-activation, also suggesting alternative pathways.

Lipid infusion associates insulin resistance by impairing insulin signaling in a manner that selectively affects specific downstream mediators of the PI3K pathway. Understanding the molecular mechanisms for this selectivity and the potential alternative pathways for regulating glucose metabolism could lead to new therapeutic targets for obesity and type 2 diabetes.

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Fatty Acid Infusion Impairs Akt1 and PKCζ Activation

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