The Role of Glycogen Synthase Kinase 3β in Insulin-stimulated Glucose Metabolism*

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To characterize the contribution of glycogen synthase kinase 3β (GSK3β) inactivation to insulin-stimulated glucose metabolism, wild-type (WT-GSK), catalytically inactive (KM-GSK), and uninhibitable (S9A-GSK) forms of GSK3β were expressed in insulin-responsive 3T3-L1 adipocytes using adenovirus technology. WT-GSK, but not KM-GSK, reduced basal and insulin-stimulated glycogen synthase activity without affecting the -fold stimulation of the enzyme by insulin. S9A-GSK similarly decreased cellular glycogen synthase activity, but also partially blocked insulin stimulation of the enzyme. S9A-GSK expression also markedly inhibited insulin stimulation of IRS-1-associated phosphatidylinositol 3-kinase activity, but only weakly inhibited insulin-stimulated Akt/PKB phosphorylation and glucose uptake, with no effect on GLUT4 translocation. To further evaluate the role of GSK3β in insulin signaling, the GSK3β inhibitor lithium was used to mimic the consequences of insulin-stimulated GSK3β inactivation. Although lithium stimulated the incorporation of glucose into glycogen and glycogen synthase enzyme activity, the inhibitor was without effect on GLUT4 translocation and pp70 S6 kinase. Lithium stimulation of glycogen synthesis was insensitive to wortmannin, which is consistent with its acting directly on GSK3β downsteam of phosphatidylinositol 3-kinase. These data support the hypothesis that GSK3β contributes to insulin regulation of glycogen synthesis, but is not responsible for the increase in glucose transport.

The peptide hormone insulin elicits a broad array of metabolic responses. In muscle and adipose tissue, insulin promotes the storage of sugar by coordinately accelerating both the rate of glucose entry into the cell and glycogen synthase activity. The former is mediated by the insulin-dependent redistribution of glucose transport proteins (GLUT4) from intracellular compartments to the plasma membrane (1), and the latter by multisite dephosphorylation of the enzyme coupled with activation by the soluble metabolite glucose 6-phosphate (2). Although much progress has been made characterizing the molecular signaling events emanating from the insulin receptor and progressing to these targets, the complete pathway has not yet been elucidated. The general purpose of this study was to evaluate the relative contribution of the serine/threonine kinase GSK3β to insulin regulation of these molecular events.

GSK3β was originally identified based on its kinase activity toward an in vitro substrate, glycogen synthase (3). Subsequent studies have described roles for the enzyme in many different cellular processes. For example, the potent GSK3β inhibitor lithium was used to identify roles for the enzyme in spore and stalk development in Dictyostelium (4, 5) and in expansion of dorsal mesoderm in Xenopus (4, 5). A critical role for GSK3β in pattern formation has been confirmed through investigation of the Drosophila ortholog shaggy/zeste-white 3 (6). Moreover, overexpression of GSK3β in cultured cells led to the proposal of a role for the enzyme in the initiation of apoptosis (7). Two types of studies support the view that GSK3β could contribute to insulin-stimulated anabolic metabolism. First, insulin inhibits GSK3β kinase activity in multiple insulin-responsive tissues (8–11), and a novel signaling pathway has been postulated whereby GSK3β inactivation follows the sequential activation of PI3K and Akt/PKB (12, 13). Second, GSK3β inhibits basal glycogen synthase activity when transfected into transformed cells (14). Since the effects of GSK3β on glycogen metabolism have only been evaluated in vitro or in non-insulin-responsive tissues, the relative contribution of GSK3β to regulation of these metabolic events by insulin remains unclear. Moreover, since Akt/PKB and PI3K have been implicated in insulin regulation of numerous other metabolic processes (15–18), the relative contribution of GSK3β to these events warrants evaluation.

The studies described herein evaluated the contribution of GSK3β to insulin-stimulated metabolism in 3T3-L1 adipocytes. These cells are a well recognized system for investigating insulin-stimulated glucose metabolism, as they respond to physiological doses of insulin with increases in glucose uptake, GLUT4 translocation, glycogen synthesis, and lipogenesis. Adenovirus-mediated overexpression of GSK3β mutants in these cells, as well as treatment with the GSK3β inhibitor lithium, was used to disrupt coupling of insulin regulation of the enzyme. Data support a model whereby GSK3β inactivation contributes to insulin regulation of glycogen synthesis, but not GLUT4 translocation. These results further indicate that GSK3β can inhibit insulin-stimulated IRS-1-associated PI3K activity, but has little effect on downstream signaling events.

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¶The abbreviations used are: GSK3β, glycogen synthase kinase 3β; PI3K, phosphatidylinositol 3-kinase; PAGE, polyacrylamide gel electrophoresis.
Regulation of Glucose Metabolism by GSK3β

Experimental Procedures

Materials—Crystalline porcine insulin was a gift of Lilly. Polyclonal anti-IRS-1 antibodies were a gift from Bayer (West Haven, CT). Polyclonal anti-pp70 S6 kinase antibodies were generously provided by Dr. Margaret Chou (University of Pennsylvania, Philadelphia, PA). Rabbit phospho-specific anti-S6 antibodies were raised against a peptide resembling the major phosphorylation site in ribosomal subunit protein S6 (CRKLRRSpLRSpyYSPRS) by Rockland Inc. (Gilbertsville, PA). Polyclonal rabbit anti-hemagglutinin antibodies were from Berkeley Antibody Co. (Berkeley, CA). Antibodies against GSK3β were purchased from Transduction Labs (Lexington, KY) or Quality Controlled Biochemicals (Hopkinton, MA). An antibody that recognizes the phosphorylated serine 9 regulatory site on human GSK3β and less well the mouse enzyme was also from Quality Controlled Biochemicals.

GSK3β Constructs, Adenovirus Infection, and Cell Culture—cDNAs encoding human wild-type GSK3β (WT-GSK) and catalytically inactive GSK3β (KM-GSK) were generously provided by Peter Klein (University of Pennsylvania). Additionally, the serine 9 regulatory site on WT-GSK was mutated to alanine (S9A-GSK) by polymerase chain reaction. The sequence of the latter was confirmed by DNA sequencing. The relative activities of all three constructs are described elsewhere (14). These constructs were subcloned into the vector pACCMV.pLpA and transferred to recombinant adenovirus by homologous recombination (19). A multiplicity of infection of 100 was used to infect 3T3-L1 adipocytes in Dulbecco’s modified Eagle’s medium containing 1% calf serum, with the virus being left on the cells for at least 18 h prior to its removal. Experiments were conducted 48 h after the initial addition of the virus. This method results in infection of >90% of the cells on the dish as assessed by expression of β-galactosidase.

3T3-L1 fibroblasts were differentiated into adipocytes 1 day post-confluence in Dulbecco’s modified Eagle’s/H-21 medium supplemented with 10% fetal bovine serum (Sigma), 1 μg/ml dexamethasone, and 112 μg/ml isobutylmethylxanthine. After 5 days, cells were maintained in Dulbecco’s modified Eagle’s/H-21 medium supplemented with 10% fetal bovine serum.

Glycogen Synthase Assay—3T3-L1 adipocytes differentiated in 6-well culture dishes were serum-deprived for 2 h in Leibovitz L-15 buffer supplemented with 0.2% bovine serum albumin. Cells were then incubated for 15 min with insulin and washed in glycogen synthase extraction buffer (100 mM NaF, 10 mM EDTA, 1 mM benzamidine, and 50 mM Tris-HCl (pH 7.8)). Adipocytes were disrupted using a Teflon/glass homogenizer, and the homogenate was centrifuged briefly at 2000 × g. The supernatant without the fat cake was assayed for enzyme activity as described previously (20), and glycogen synthase activity is the activity measured in the presence of low (0.1 mM) glucose 6-phosphate divided by the activity in the presence of high (10 mM) glucose 6-phosphate.

Incorporation of Glucose into Glycogen—The incorporation of glucose into a glycogen pellet was determined as described previously (21).

Preparation of Total Cell Extracts—Cells were serum-starved for 24 h in Dulbecco’s modified Eagle’s medium containing 0.5% bovine serum albumin and 10 mM HEPES (pH 7.5). Two hours prior to solubilization, cells were washed twice in phosphate-buffered saline and incubated at 37 °C in Leibovitz L-15 buffer containing 0.5% bovine serum albumin. Cells were washed again in phosphate-buffered saline and then incubated for 1 h at 37 °C in KRP buffer (136 mM NaCl, 4.7 mM KCl, 10 mM NaHPO4, pH 7.4, 0.9 mM MgSO4, and 0.9 mM CaCl2) containing 0.2% bovine serum albumin. For experiments using lithium, the indicated concentration of LiCl replaced an equal concentration of NaCl. Cells were solubilized in 66 mM Tris-HCl (pH 7.5) and 2% SDS additionally containing 1 mM vanadate, 100 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 2 μg/ml aprotinin. DNA was sheared by sonication, and insoluble material was pelleted for 10 min in a microcentrifuge.

Glucose Uptake and GLUT4 Translocation Assays—Methods for measuring glucose uptake rates and plasma membrane GLUT4 levels (using the plasma membrane “sheet” assay) have been described (16). For experiments involving lithium, cells were stepped down in KRP buffer with the indicated concentration of lithium replacing an equal concentration of NaCl.

PI3K Assays—PI3K assays were performed using methods previously described (22).

Results

Insulin inhibits GSK3β kinase activity in rat skeletal muscle and epididymal fat, mouse 3T3-L1 adipocytes, and human cultured myoblasts (8–11). Recent studies further indicate that Akt/PKB is required for insulin inactivation of GSK3β in NIH-3T3 cells overexpressing insulin receptors (12), and in vitro experiments suggest that this is the result of Akt-catalyzed phosphorylation of serine 9 (13). To investigate whether insulin and Akt/PKB stimulate phosphorylation of this regulatory residue in intact 3T3-L1 adipocytes, GSK3β was immunoprecipitated from cell lysates using a monoclonal anti-GSK3β antibody. Moreover, transfer to nitrocellulose membranes, GSK3β phosphorylation was detected using a phospho-specific antibody against the serine 9 portion of GSK3β. Insulin rapidly stimulated GSK3β phosphorylation in 3T3-L1 adipocytes infected with an empty vector (Fig. 1A). Moreover, stable expression of a constitutively active form of Akt/PKB (Myr-akt, described in Ref. 16) stimulated GSK3β phosphorylation in the absence of insulin (Fig. 1A). Immunoblotting with the monoclonal anti-GSK3β antibody confirmed that equal amounts of GSK3β were precipitated under all conditions (Fig. 1B). Mock lanes denote immunoprecipitations done in the absence of cell lysates to visualize the IgG bands.

To ascertain the role of GSK3β in insulin action, the effects of various overexpressed GSK3β constructs on glucose uptake and glycogen synthase enzyme activity were investigated. Using recombinant adenovirus, the wild-type (WT-GSK) and kinase-dead (KM-GSK) forms of GSK3β were expressed in 3T3-L1 adipocytes. In addition, a GSK3β construct with the regulatory serine residue changed to alanine (S9A-GSK) was also expressed. A previous study characterized the relative activity of all three constructs, including their effects on basal glycogen synthase activity, in non-insulin-responsive 293T cells. To evaluate the levels of expression, whole cell lysates were resolved by SDS-PAGE, transferred to nitrocellulose, and analyzed by immunoblotting. All three constructs were overexpressed roughly 3–5-fold (Fig. 2A) 48 h after infection with adenovirus. Moreover, immunofluorescent detection using an antibody against the hemagglutinin epitope revealed that 80–90% of the cells expressed S9A-GSK (Fig. 2B) 48 h after infection. This is comparable to the percentage of cells that expressed a β-galactosidase gene when a similar infection protocol was used with a control virus.

As shown in Fig. 3, overexpression of WT-GSK, but not KM-GSK, decreased glycogen synthase enzyme activity in 3T3-L1 adipocytes, with reductions apparent under both basal and insulin-stimulated conditions. Nonetheless, insulin still...
Regulation of Glucose Metabolism by GSK3β

**Fig. 2. Adenovirus-mediated GSK3β overexpression.** A. 3T3-L1 adipocytes were infected with recombinant adenoviruses encoding WT-GSK, KM-GSK, or S9A-GSK. Forty-eight hours after infection, cells were treated with or without insulin (100 nM, 15 min) and solubilized. Total cell extracts were harvested, resolved by SDS-PAGE, transferred to nitrocellulose, and immunoblotted. Monoclonal antibodies against full-length GSK3 (α-GSK) from Quality Controlled Biochemicals (QCB) or Transduction Labs (TL) were used. Data are representative of three independent experiments. B. 3T3-L1 adipocytes were infected with recombinant adenoviruses encoding S9A-GSK. Forty-eight hours after infection, cells were fixed and analyzed by immunofluorescence using Hoechst or anti-hemagglutinin (α-HA) antibodies.

**Fig. 3. Effect of GSK3β overexpression on glycogen synthase activity.** 3T3-L1 adipocytes were infected with adenovirus producing KM-GSK, WT-GSK, or S9A-GSK 48 h prior to stimulation with insulin (100 nM) for 10 min. Following stimulation, glycogen synthase assays were performed as described under “Experimental Procedures.” Data are presented as low Glu-6-P/G6P/high Glu-6-P ratios and means ± S.E. of three independent experiments. *, the value was statistically different from the control (+insulin) value at p ≤ 0.05; **, the value was statistically different from the WT-GSK (+insulin) value at p ≤ 0.05.

stimulated glycogen synthase activity ~2–2.5-fold over the new, lowered basal value, which was comparable to the stimulation induced in uninfectd or KM-GSK-infected cells. Overexpression of the uninhibitable S9A-GSK construct similarly reduced basal and insulin-stimulated glycogen synthase activity. More important, however, S9A-GSK also reduced the -fold stimulation of glycogen synthase activity by insulin (Fig. 3). This finding suggests a role for GSK3β phosphorylation in insulin regulation of glycogen synthase.

Akt/PKB has been implicated as a mediator of other metabolic responses to insulin, including glucose uptake and GLUT4 translocation (15–18). Since GSK3β is one of only a few identified substrates for Akt, we also sought evidence of the role of GSK3β in these insulin-regulated events. Inhibition of glycogen synthase activity by insulin (Fig. 3). This finding suggests a role for GSK3β phosphorylation in insulin regulation of glycogen synthase.

Akt/PKB has been implicated as a mediator of other metabolic responses to insulin, including glucose uptake and GLUT4 translocation (15–18). Since GSK3β is one of only a few identified substrates for Akt, we also sought evidence of the role of GSK3β in these insulin-regulated events. Insulin stimulated the uptake of non-metabolizable 2-deoxyglucose significantly in uninfected 3T3-L1 adipocytes. Overexpression of S9A-GSK slightly inhibited rates of 2-deoxyglucose uptake in both the presence and absence of insulin (Fig. 4). However, the dose-response curves for both infected and uninfected cells were nearly identical. The major effect of insulin on glucose uptake is due to the translocation of the GLUT4 isoform from intracellular storage sites to the plasma membrane (1). The commonly utilized GLUT4 sheet assay was used to measure plasma membrane levels of GLUT4 (16). Briefly, 3T3-L1 adipocytes differentiated on coverslips were sonified, liberating cellular structures from the coverslip, but leaving an intact sheet containing the plasma membrane with its cytosolic face exposed. Probing the sheet with antibodies against the carboxyl terminus of GLUT4 reflects the total amount of GLUT4 on the membrane. Expression of KM-GSK, WT-GSK, and S9A-GSK had no effect on plasma membrane GLUT4 levels (Fig. 5), demonstrating that GSK3β does not contribute to insulin stimulation of GLUT4 translocation. Collectively, these data indicate that GSK3β is unlikely to mediate insulin-stimulated glucose uptake.

To further investigate the consequences of GSK3β phosphorylation, experiments were conducted with the GSK3β inhibitor lithium. Lithium specifically inhibits GSK3β in the millimolar range, but has no effect on numerous other protein kinases, including cAMP-dependent protein kinase, mitogen-activated protein kinase, and casein kinase II (4). Lithium thus mimics the effects of physiological GSK3β inhibition, indicating which of insulin’s metabolic actions can be mediated solely by its effects on GSK3β. A 2-h treatment with 50 mM lithium stimulated glycogen synthesis 60–70% as well as insulin in control 3T3-L1 adipocytes (Fig. 6). Lithium-stimulated glycogen synthesis was not sensitive to wortmannin, which inhibited insulin-stimulated glycogen synthesis completely. Interestingly, lithium and insulin added simultaneously stimulated...
glycogen synthesis greater than either agent alone. Lithium also stimulated glycogen synthase enzyme activity (Fig. 7) and, once again, was partially additive with insulin. Under conditions in which lithium stimulated glycogen synthesis (Fig. 8A), it weakly stimulated glucose uptake (Fig. 8B). To determine whether lithium could mimic the effects of insulin on GLUT4 translocation, plasma membrane sheets were also determined using the aforementioned sheet assay. Lithium did not stimulate GLUT4 translocation in the absence of insulin (Fig. 8C), nor did it augment insulin-stimulated glucose uptake or GLUT4 translocation (data not shown).

Although GSK3β had no effect on glucose uptake or GLUT4 translocation, a prior report indicated that GSK3β is a negative regulator of IRS-1-mediated activation of PI3K in transformed cells. Consequently, GSK3β was proposed as a possible contributor to insulin resistance. To evaluate whether GSK3β also inhibited signaling in insulin-responsive 3T3-L1 adipocytes, IRS-1-associated PI3K activity was assayed in S9A-GSK-expressing cells (Fig. 9). S9A-GSK expression markedly inhibited insulin-stimulated activation of PI3K in anti-IRS-1 immunoprecipitates. However, S9A-GSK did not reduce insulin-stimulated phosphorylation of Akt, mitogen-activated protein kinase, and ribosomal S6 protein (Fig. 10). No effect was seen at submaximal doses of insulin either (data not shown). S9A-GSK expression did not affect the insulin-stimulated mobility shift for IRS-1 separated on SDS-polyacrylamide gel (Fig. 10). Thus, if GSK3 is inhibiting PI3K by direct phosphorylation of IRS-1, the kinases must be utilizing a subset of those sites phosphorylated in response to insulin or a distinct set of sites.

Lithium, under conditions in which glycogen synthase was activated (Fig. 8), did not affect insulin stimulation of PI3K, Akt, mitogen-activated protein kinase, and ribosomal S6 protein (Fig. 11). These latter studies also indicate that GSK3β is unlikely to mediate insulin and Akt stimulation of pp70 S6 kinase.

DISCUSSION

Insulin activates a number of pleiotropic metabolic responses that are governed by a similarly broad array of signaling events. Definitive assignment of particular metabolic functions to specific signaling cascades has been a recent challenge in insulin action research. Although insulin activates the Ras/Raf/mitogen-activated protein kinase cascade, which contributes to its regulation of DNA synthesis, studies from multiple laboratories have excluded these signaling molecules from insulin regulation of glucose uptake, GLUT4 translocation, or glycogen synthesis (21, 24–28). Similarly, insulin activates a protein phosphatase (SHPTP2) that contributes to its regulation of mitogenesis and GLUT1 expression, but is not required for acute stimulation of GLUT4 translocation (29). Instead, insulin stimulation of glucose uptake, GLUT4 translocation, and gly-
cogen synthesis requires a signaling cascade involving PI3K (30, 31) and possibly the downstream effector Akt/PKB (16–18, 32). The studies described herein demonstrate further divergence in insulin signaling downstream of PI3K and Akt. Although GSK3β is likely to contribute to insulin and Akt regulation of glycogen synthase, it apparently does not contribute to their activation of glucose transport, GLUT4 translocation, and pp70 S6 kinase.

Evidence supporting a role for GSK3β inactivation in insulin regulation of glycogen synthase includes the following. 1) Insulin and constitutively active forms of Akt/PKB stimulated phosphorylation of an inhibitory residue on GSK3β (Fig. 1). 2) Insulin and constitutively active forms of Akt/PKB inhibit the activity of GSK3β in intact cells (9–11, 32). 3) Expression of wild-type (but not catalytically inactive) GSK3β inhibited basal and insulin-stimulated glycogen synthase activity without affecting the -fold stimulation of the enzyme by insulin (Fig. 3). 4) Expression of a GSK3β mutant incapable of being phosphorylated on this inhibitory residue similarly decreased basal and insulin-stimulated glycogen synthase activity, but also partially blocked the -fold stimulation of the enzyme by insulin (Fig. 3). 5) Treatment with lithium, which mimics insulin's inhibitory effects on GSK3β, stimulated glycogen synthesis and glycogen synthase enzyme activity in the absence of increased GLUT4 translocation (Figs. 8–10). Since the phosphate groups on glycogen synthase turn over rapidly (33), inhibition of GSK3β could serve as a physiologically relevant mechanism for regulating glycogen synthase activity.

Previous studies suggest that GSK3β inhibition is not insulin’s only mechanism for stimulating glycogen accumulation. In a prior study in a different line of 3T3-L1 adipocytes, insulin was capable of stimulating glycogen synthase despite the fact that GSK3β was either not detectable or present in very low amounts (32). Similarly, another group reported that GSK3β expression decreases during 3T3-L1 differentiation, thus switching insulin’s primary mechanism for glycogen synthase activation from GSK3β inactivation to protein phosphatase activation (8). In contrast to these reports, GSK3β was clearly present in the 3T3-L1 adipocytes used in this study, and its expression was unaltered by adipogenesis (data not shown). Subtle differences in cell lines or differentiation conditions could account for discrepancies between these studies. Although the data presented above strongly support a role for GSK3β inhibition in glycogen synthesis, these studies also indicate the existence of an alternative pathway. First, insulin still stimulated glycogen synthase partially in cells infected with S9A-GSK, although its stimulation was significantly compromised. Second, lithium was partially additive with insulin.
in activating glycogen synthesis and glycogen synthase. The latter observation is particularly suggestive since lithium is likely to be a more effective inhibitor of GSK3β than insulin. Although these data could result from an effect of lithium independent of the inhibition of GSK3β, this is unlikely in view of the failure of other investigators to identify such a site of action (4). More probable is that the coupling of insulin stimulation of a protein phosphatase with its inhibition of GSK3β is the mechanism for its complete regulation of glycogen synthase.

A second role suggested by the literature for GSK3β upstream inhibitor Akt/PKB is implicated in insulin-stimulated glucose uptake and GLUT4 translocation (15–18), although this has been recently challenged (34), and GSK3β inactivation is implicated in other Akt-mediated events (7). GSK3β has a remarkably broad array of in vitro substrates and cellular functions (3, 35–38). Numerous sporadic reports over the last 30 years have attributed to lithium an “insulinomimetic” effect, i.e. the ability to stimulate both glucose uptake and glycogen synthesis in various systems (39–44). In some of these studies, the effect of lithium on glucose uptake was smaller than that observed on glycogen synthesis, whereas in others it was not. Nonetheless, the results presented above do not support the existence of a role for GSK3β in insulin-stimulated glucose uptake. Although in the experiments presented above, lithium did weakly stimulate glucose uptake, it did not stimulate GLUT4 translocation. This conclusion was further strengthened by the finding that WT-GSK and S9A-GSK expression did not alter insulin stimulation of glucose uptake or GLUT4 translocation.

A third role postulated for GSK3β is as a negative regulator of IRS-1-associated PI3K and as a contributor to insulin resistance (23). The results presented above confirm that GSK3β can inhibit insulin-stimulated PI3K activity in 3T3-L1 adipocytes, but suggest that this has little effect on insulin stimulation of glucose metabolism: under conditions in which S9A-GSK inhibited PI3K, it had no effect on glucose uptake or GLUT4 translocation; neither S9A-GSK nor lithium affected insulin stimulation of Akt/PKB or pp70 S6 kinase phosphorylation. Several mechanisms could account for the lack of correlation between IRS-1-associated PI3K activity and glucose uptake rates. First, only a small amount of PI3K activity might be required for full stimulation of glucose transport. Most studies evaluating the necessity of PI3K have utilized methods (such as potent PI3K inhibitors or dominant-negative antibodies) that completely eliminate PI3K activity. A partial inhibition of PI3K activity may not significantly affect insulin stimulation of downstream metabolic events. Alternatively, compensatory mechanisms could contribute to the requisite activation of PI3K. For example, in IRS-1 knockout mice, other members of the IRS family take over the responsibilities of IRS-1, stimulating PI3K to nearly normal levels (45). Moreover, recent work indicates that an IRS-independent, yet PI3K-dependent pathway contributes to insulin stimulation of GLUT4 translocation (46, 47). Such alternative mechanisms for activating PI3K could account for the nearly normal stimulation of glucose transport and downstream signaling events.

Although GSK3β was discovered over 15 years ago, its contribution to insulin signaling has not been fully addressed. The recent observation of molecular events linking activated insulin receptors to GSK3β rekindled interest in the enzyme. This identified signaling cascade, which includes PI3K and Akt/PKB, is postulated to regulate numerous metabolic responses. Although GSK3β is likely to contribute to insulin stimulation of glycogen synthesis, and not glucose uptake or GLUT4 translocation. Alternative signaling events must account for these important metabolic responses.

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