Mechanism and Management of AKT Inhibitor-Induced Hyperglycemia

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Abstract

Purpose: Insulin-like growth factor-I receptor and phosphoinositide 3-kinase/AKT/mammalian target of rapamycin pathways are among the most active areas of drug discovery in cancer research. However, due to their integral roles in insulin signaling, inhibitors targeting these pathways often lead to hyperglycemia and hyperinsulinemia. We investigated the mechanism of hyperglycemia induced by GSK690693, a pan-AKT kinase inhibitor in clinical development, as well as methods to ameliorate these side effects.

Experimental Design: The effect of GSK690693 on blood glucose, insulin, and glucagon levels was characterized in mice. We then evaluated the effects of commonly prescribed antidiabetic agents on GSK690693-induced hyperglycemia. The mechanism of blood glucose increase was evaluated using fasting and tracer uptake studies and by measuring liver glycogen levels. Finally, approaches to manage AKT inhibitor-induced hyperglycemia were designed using fasting and low carbohydrate diet.

Results: We report that treatment with antidiabetic agents does not significantly affect GSK690693-induced hyperglycemia in rodents. However, administration of GSK690693 in mice significantly reduces liver glycogen (~90%), suggesting that GSK690693 may inhibit glycogen synthesis and/or activate glycogenolysis. Consistent with this observation, fasting before drug administration reduces baseline liver glycogen levels and attenuates hyperglycemia. Further, GSK690693 also inhibits peripheral glucose uptake and introduction of a low-carbohydrate (7%) or 0% carbohydrate diet after GSK690693 administration effectively reduces diet-induced hyperglycemia in mice.

Conclusions: The mechanism of GSK690693-induced hyperglycemia is related to peripheral insulin resistance, increased gluconeogenesis, and/or hepatic glycogenolysis. A combination of fasting and low carbohydrate diet can reduce the magnitude of hyperglycemia induced by an AKT inhibitor.

Dysregulation of insulin-like growth factor-I receptor (IGF-IR) signaling and the phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway has been implicated in many human cancers (1, 2). Inhibition of these signaling kinases through various approaches including antisense, antibodies, small-molecule kinase inhibitors, small interfering RNA, etc., decreases proliferation and survival of tumor cells *in vitro* and *in vivo* and thus validates these kinases as important therapeutic targets for anticancer therapy (3–5). Currently, a large number of pharmaceutical and biotechnology companies as well as academic research centers have active drug development programs in these areas, and several novel agents have advanced into human clinical trials.

The IGF-IR and insulin receptor (IR) share ~70% homology in their amino acid sequence (6). IGF-IR plays a critical role in cell proliferation, survival, and metastasis of cancer cells (7). On ligand stimulation, both IR and IGF-IR activate the IR substrate and signal through PI3K. PI3K converts $PI(4,5)P_2$ to $PI(3,4,5)P_3$, which recruits protein kinase B (AKT) and phosphoinositide-dependent protein kinase-1 to the plasma membrane where phosphoinositide-dependent protein kinase-1 and -2 (mTORC2) activate AKT. Activated AKT then phosphorylates >15 known substrates including glycogen synthase kinase-3 (GSK-3) and forkhead transcription factors (FOXO), thus transmitting the functional outputs of IGF-IR and/or IR activation.

AKT, GSK-3, FOXO, and mTOR are considered essential components of the insulin signaling pathway (8–10). For instance, insulin promotes glycogen storage through a coordinated effort of glucose uptake and glycogen synthesis. On insulin stimulation, there is an activation of intracellular signaling cascades that ultimately result in the translocation of the GLUT4 transporter to the plasma membrane to facilitate glucose uptake. Simultaneously, activated AKT also

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Translational Relevance

Hyperglycemia has often been reported as an on-target side effect of agents targeting IGF-IR, PI3K, AKT, and mTOR signaling. Using rodent models, we show that administration of GSK690693, a pan-AKT kinase inhibitor, resulted in an increase in blood glucose, which is associated with altered hepatic glycogen metabolism and blockage of peripheral glucose uptake. The drug-induced hyperglycemia can be attenuated by decreasing the liver glycogen store using fasting combined with a low-carbohydrate diet. To our knowledge, this is first report elucidating the possible mechanisms and the methods of intervention for the AKT inhibitor-induced hyperglycemia. Our findings have significant implications in the exploration of inhibitors of this pathway for their full therapeutic potential.

phosphorylates GSK-3, which inactivates its ability to inhibit glycogen synthase, thereby enhancing overall glycogen synthesis (11). Insulin also suppresses glucose production in the liver and kidney by inhibiting the expression of two rate-limiting enzymes in gluconeogenesis and glycogenolysis, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (12). FOXO1 influences this insulin-mediated process by modulating glucose-6-phosphatase expression in a dex/cyclic AMP-dependent manner (13). Besides glucose metabolism, insulin also regulates protein synthesis by activating the translational machinery, including eukaryotic initiation factors and eukaryotic elongation factors. This again is mediated through the canonical PI3K/AKT pathway. In this case, AKT phosphorylates the TSC1-TSC2 complex (tuberous sclerosis complex) and relieves its inhibitory effect on mTOR. mTOR then activates the translation initiation and elongation process through eIF4E and eEF2, respectively (14).

Due to the integral roles of IGF-IR, PI3K, AKT, and mTOR in insulin signaling, it is likely that inhibition of their kinase activities through antibodies, small-molecule inhibitors, small interfering RNA, or dominant-negative mutants can lead to abrogated insulin function. Experimental evidence from many preclinical models suggests that the loss of insulin signaling in the peripheral tissues and in pancreatic β cells through the blockade of IGF-IR, IR substrate, PI3K, AKT, or mTOR results in hyperglycemia and diabetes (15). For example, IRS1-knockout mice exhibit impaired glucose tolerance and hyperglycemia with peripheral insulin resistance (16). AKT2-null mice show insulin resistance, hyperglycemia, hyperinsulinemia, and glucose intolerance (17). Recently, it was found that treatment with rapamycin results in mTOR inhibition and causes exacerbation of diabetes (18).

Adverse effects associated with novel therapeutic agents can greatly affect their clinical development. Administration of a small-molecule IGF-IR inhibitor, BMS-554417, induces hyperglycemia presumably through blockade of the IR (19, 20). Similarly, hyperglycemia and hyperinsulinemia were also noted as the most common on-target toxicities with inhibitors targeting PI3K, AKT, and mTOR (21–24). Interestingly, the mechanism of IGF-IR inhibitor-induced hyperglycemia is through blockade of IR function and can be managed by metformin (20). However, treatment with metformin showed no effect on PX-866 (PI3K inhibitor)-associated hyperglycemia, indicating that the mechanism of PX-866-induced hyperglycemia differs from that of IGF-IR inhibition (21). Furthermore, although the decreased glucose tolerance associated with PX-866 can be reduced by insulin infusion and pioglitazone, the mechanism of hyperglycemia is still not fully understood (21). Thus far, methods for management of the hyperglycemia associated with agents targeting AKT kinases have not been identified. In the present study, we used preclinical models to explore the mechanism of hyperglycemia associated with GSK690693, a pan-AKT kinase inhibitor (24), and identified potential strategies to circumvent increases in blood glucose.

Materials and Methods

Drugs and materials. GSK690693, rosiglitazone maleate, and vildagliptin were synthesized at GlaxoSmithKline. Metformin was purchased as prescription drug Riomet (NDC63304-206-02) from Ranbaxy Laboratories. GSK690693 was formulated in 5% mannitol, except for studies in combination with exendin-4 and vildagliptin, where it was formulated in 4% DMSO/40% hydroxypropyl-β-cyclodextrin in water (pH 6.0). Rosiglitazone maleate was prepared as a suspension in 0.5% hydroxypropylmethyl cellulose and 0.1% Tween 80. Metformin was formulated in sterile water. Insulin (Novolin) was purchased from Novo Nordisk. Exendin-4 peptide was purchased from American Peptide and diluted in saline for *in vivo* use. Diets containing varied carbohydrate content were prepared by Research Diets.

Animals. Female CB-17 severe combined immunodeficient mice and male Sprague-Dawley rats were obtained from Charles River Laboratories. All animal studies were done in compliance with federal requirements, GlaxoSmithKline policy on the Care and Use of Animals, and related codes of practice. Blood glucose levels in mice were measured from tail vein nicks using an Accu-Chek Compact glucometer (Roche Diagnostics). Circulating insulin levels were measured in plasma from mice using a rodent insulin ELISA kit (Crystal Chem). Plasma glucagon levels were measured using a rodent glucagon ELISA kit (Wako). For the rat study, 1 week before the experiment, rats were anesthetized with isoflurane by inhalation and catheters were surgically implanted in jugular vein and carotid artery using aseptic techniques. After a recovery period, rats were placed in customized cages designed to facilitate frequent sampling over 24 to 36 h with minimal stress on the animals. Arterial blood samples were taken at desired time points for serum glucose analysis using an Olympus Au640 clinical chemistry analyzer (Olympus America).

Tissue protein extraction and immunoblotting. Livers from mice treated with vehicle or GSK690693 were weighed and homogenized in 10× (w/v) ice-cold radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors. The insoluble nuclei/debris was removed by centrifugation at 8,000 × *g* for 5 min at 4°C. The lysates were diluted with sample loading buffer (Invitrogen), and 100 μg total protein was resolved on 4% to 20% Tris-glycine SDS-PAGE. Proteins were then transferred to ploy vinylidene difluoride membranes and probed with the antibodies for phospho-GSK-3β (Ser^{9}), total GSK-3β, phospho-IR (Tyr^{1150}/Tyr^{1151}), total IR, and α-tubulin at 1:1,000 dilution. IRDye-680- or IRDye-800CW-conjugated secondary antibodies (Li-Cor Biosciences) were used at 1:10,000 dilutions. All primary antibodies were obtained from Cell Signaling Technology, except phospho-GSK-3β (R&D Systems) and anti-tubulin (Sigma) antibodies. All blots were analyzed on Li-Cor Infrared Imaging analyzer (Li-Cor Biosciences).

Liver glycogen extraction and glucose conversion. Liver tissue was homogenized in 10× (w/v) 0.2 mol/L sodium acetate buffer (pH 4.8) to extract glycogen. The homogenate (1 mL) was boiled for 5 min and

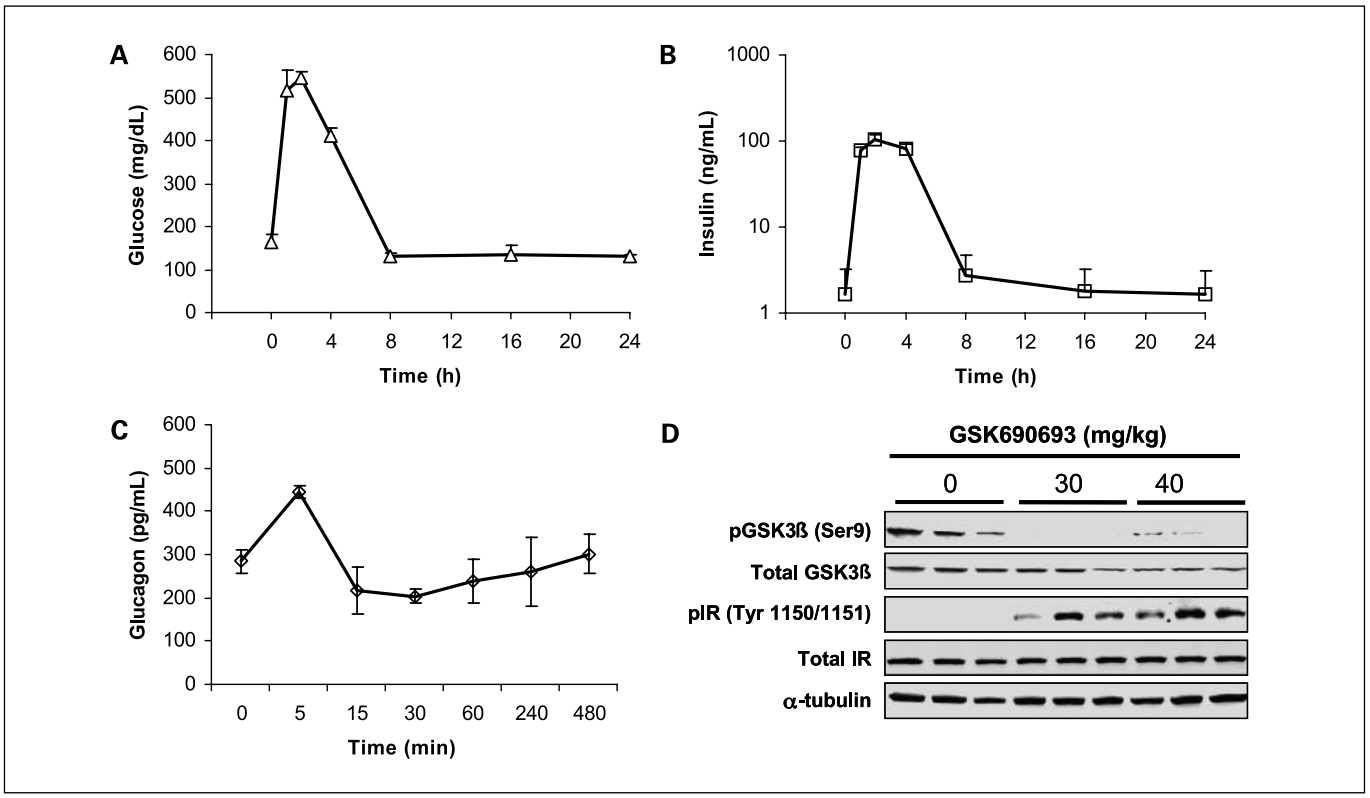

Fig. 1. GSK690693 induces hyperglycemia and hyperinsulinemia *in vivo*. Time course of blood glucose (*A*) and insulin (*B*) levels after GSK690693 administration (30 mg/kg intraperitoneally) in mice. Mean ± SD for three mice in each group. *C,* time course of plasma glucagon level in mice treated with GSK690693 (30 mg/kg intraperitoneally). Mean ± SD for three mice in each group. *D,* GSK690693 inhibits AKT activity in liver. Liver extracts were prepared from mice 2 h after dosing with GSK690693 (0, 30, or 40 mg/kg intraperitoneally) and the lysates were analyzed for various phosphorylated or total proteins by immunoblotting.

centrifuged at 10,000 × *g* for 15 min, and glycogen was precipitated from the supernatant using 2× (v/v) 100% ethanol. After centrifugation at 10,000 × *g* for 20 min, the pellet was reconstituted in 0.2 mol/L sodium acetate buffer (pH 4.8) and converted to glucose by incubating with amylglucosidase (Sigma) at 40°C for 2 h. The resulting glucose concentration was determined using Amplex-Red glucose assay (Invitrogen) following the manufacturer's instruction.

2-Deoxy-2-[^{18}F]fluoro-D-glucose positron emission tomography imaging. Micro-positron emission tomography imaging was done as described before with minor modifications (25). 2-Deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]DG) was purchased from PETNET Solutions. Severe combined immunodeficient mice bearing subcutaneous BT474 tumor xenografts (human breast carcinoma) were fasted for 16 h before a basal [^{18}F]DG positron emission tomography image was obtained on day -1. On day -4, mice fasted for 16 h were treated with GSK690693 (30 mg/kg intraperitoneally) and anesthetized 2 h later with 2% isoflurane in air and injected with ~250 μCi [^{18}F]DG. Ten minutes after tracer injection, mice were positioned in a positron emission tomography scanner (Siemens Preclinical) and a dynamic emission scan was obtained. Maximum [^{18}F]DG uptake values, during 55 to 60 min post-tracer injection, in the tissues were further quantified by applying corrections for body weight and injected activity and expressed as standardized uptake value = %ID/g tissue × weight (g)/100.

Results

GSK690693 inhibits AKT activity* in vivo *and induces hyperglycemia. GSK690693 is an ATP-competitive, novel pan-AKT kinase inhibitor, with apparent K_i^* values of 1, 4, and 12 nmol/L against full-length AKT1, AKT2, and AKT3, respectively (24). It exhibits antiproliferative activity *in vitro* and *in vivo* and is currently being evaluated in human clinical trials. However, in preclinical studies, it was noted that GSK690693 induced transient hyperglycemia as well as hyperinsulinemia in animals. Administration of GSK690693 at 30 mg/kg intraperitoneally to mice (fed *ad libitum*) resulted in transient elevation of both glucose and insulin (Fig. 1A and B). The glucose elevation induced by GSK690693 reaches its peak within 1 h following compound administration, and the effect lasts for ≥4 h. Under normal conditions, insulin is released as a result of an increase in glucose levels and promotes peripheral glucose uptake followed by glycolysis and glucose oxidation as well as glycogen storage in metabolically relevant tissues. In the GSK690693-treated mice, concomitant with elevated blood glucose, plasma insulin was significantly increased following similar temporal dynamics as glucose elevation. Contrary to insulin, glucagon secretion is normally a critical counterregulation of insulin for glucose homeostasis. Unexpectedly, mice treated with GSK690693 showed slight and transient glucagon elevation reaching a peak at 5 min and then returning to pretreatment levels by 15 min (Fig. 1C). This result suggests that a defect of glucagon secretion may occur in the presence of blockade of IR signaling by GSK690693. Corresponding to the increased insulin level, an increase in the phosphorylation of IR (Tyr^{1150}/Tyr^{1151}) was observed in liver lysates of GSK690693-treated mice 2 h after compound administration (Fig. 1D). However, animals treated with GSK690693 showed a reduction in phospho-GSK-3β

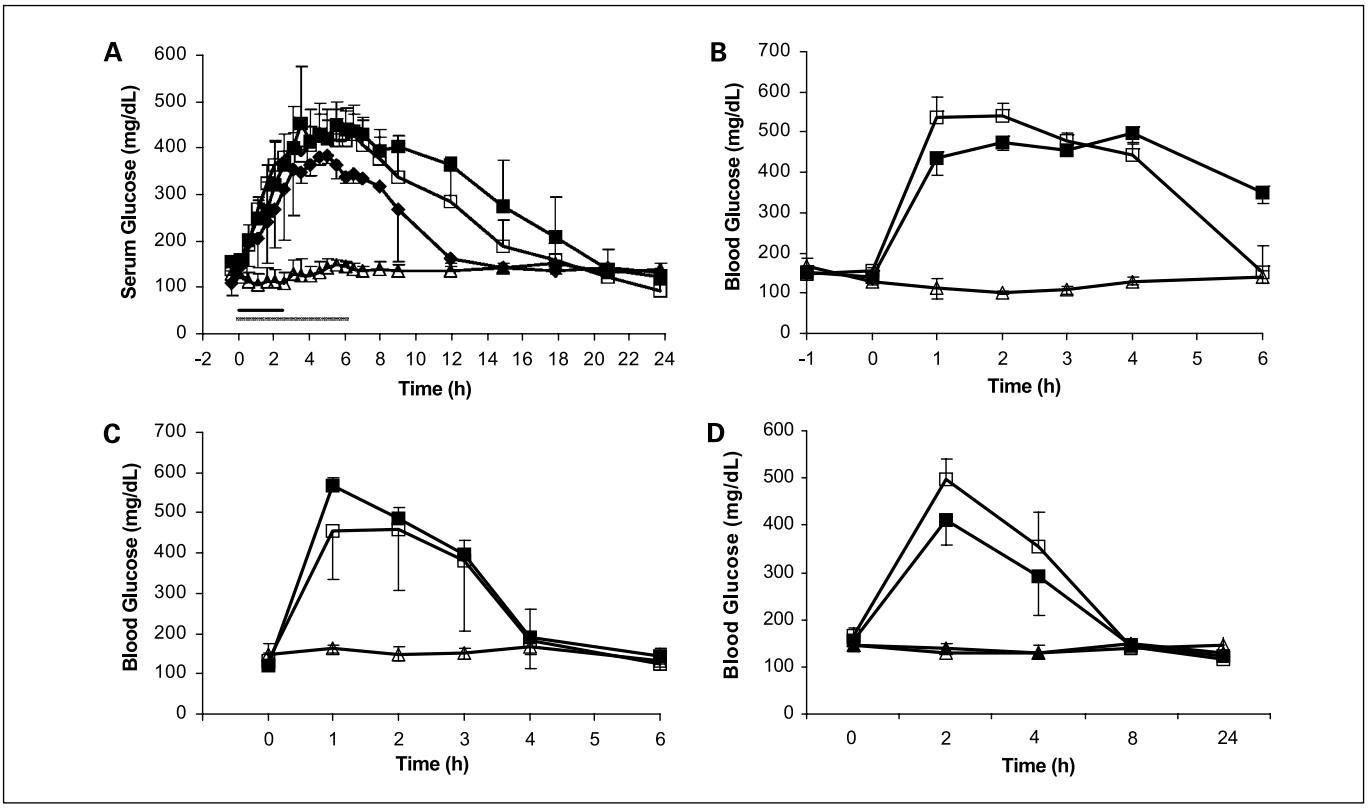

Fig. 2. Approved antidiabetic agents have minimal effect on AKT inhibitor-induced hyperglycemia. *A,* Sprague-Dawley rats were pretreated with rosiglitazone (5 mg/kg orally twice daily × 7 d) or with no pretreatment and subsequently treated with GSK690693 (31 mg/kg, 2.5 h infusion) and concurrent insulin (100 mU/min/kg, 6 h infusion). Serum glucose was measured during the 24 h period from start of infusion (n = 3 rats per group). *Open triangle,* vehicle; *open square,* GSK690693; *filled diamond,* GSK690693 + rosiglitazone + insulin; *filled square,* GSK690693 + rosiglitazone; *solid line,* infusion duration for GSK690693; *checkered line,* duration of insulin infusion. *B,* mice were dosed with GSK690693 (20 mg/kg intraperitoneally) alone or with exendin-4 (10 μg/mouse, 1 h before GSK690693) and the blood glucose was measured using tail vein nicks (n = 4 mice per group). *Open square,* GSK690693; *open triangle,* exendin-4; *closed square,* GSK690693 + exendin-4. *C,* mice were dosed with GSK690693 (5 mg/kg intraperitoneally) alone or with vildagliptin (10 mg/kg intraperitoneally) and the blood glucose was measured for 6 h (n = 4 mice per group). *Open square,* GSK690693; *open triangle,* vildagliptin; *closed square,* GSK690693 + vildagliptin. Mean ± SD. *D,* mice were dosed with GSK690693 (20 mg/kg intraperitoneally) alone or with metformin (250 mg/kg, PO × 8 d) and the blood glucose was measured for 24 h (n = 3 mice per group). *Open triangle,* vehicle; *closed triangle,* metformin; *open square,* GSK690693; *closed square,* GSK690693 + metformin. Mean ± SD.

(Ser^9), indicative of inhibition of AKT kinase activity by the compound, despite increased IR activation (Fig. 1D).

Antidiabetic agents do not restore the insulin function abrogated by GSK690693. Clinically, hyperglycemia is often regarded as a consequence of the relative or absolute deficiency in insulin signaling. Because insulin infusion as well as some antidiabetic agents such as pioglitazone and metformin were shown to be effective in reducing the hyperglycemia associated with the IGF-IR inhibitor, BMS-554417, and the PI3K inhibitor, PX-866, it is conceivable that an agent that improves insulin sensitivity could limit the degree of hyperglycemia. Pretreatment with rosiglitazone, an insulin sensitizer (26), for 7 days at the maximal antidiabetic dose (5 mg/kg twice daily) did not attenuate the magnitude or duration of the hyperglycemia induced by GSK690693 (Fig. 2A). Further, pretreatment with rosiglitazone, along with aggressive insulin therapy (insulin infusion initiated 30 min before the start of the GSK690693 infusion and then rapidly increased to a rate of 100 mU/kg/min and maintained for 6 h), had only a modest effect on attenuating the magnitude and duration of hyperglycemia (Fig. 2A). Exendin-4 is a 39-amino acid peptide related to the human glucagon-like peptide 1, which works by potentiating glucose-induced insulin secretion, decreasing glucagon release, and slowing down gastric emptying, was recently approved for the treatment of type 2 diabetes (27). However, female severe combined immunodeficient mice treated with exendin-4 in combination with GSK690693 showed no significant reduction in the blood glucose levels compared with mice treated with GSK690693 alone (Fig. 2B). Vildagliptin, a dipeptidyl peptidase-4 inhibitor, inhibits the degradation of endogenous glucagon-like peptide 1 leading to potentiation of glucose-induced secretion of insulin and a suppression of glucagon release by the pancreas (28). Similar to the results with exendin-4, vildagliptin had no significant effect on GSK690693-induced glucose elevation in mice (Fig. 2C). Finally, pretreatment with metformin (250 mg/kg/d for 8 days), an orally available treatment for type 2 diabetes mellitus, which reduces hepatic gluconeogenesis and increases peripheral glucose uptake (29), did not attenuate the extent and duration of GSK690693-induced hyperglycemia in female severe combined immunodeficient mice (Fig. 2D).

Fasting before drug administration reduces liver glycogen and attenuates GSK690693-induced hyperglycemia. Insulin and glucagon are two key hormones responsible for regulating the concentration of glucose in the blood. These hormones target the liver, where readily available glucose is stored in the form of glycogen, and by coordinate regulation of *de novo* hepatic glucose production as well as glycogenolysis are key

contributors to whole-body glucose homeostasis. Because treatment with GSK690693 induces a rapid increase in blood glucose, we hypothesized that the primary mechanism of glucose elevation could be associated with glycogenolysis in the liver. Hepatic glycogen stores are normally regulated by the action of two enzymes, glycogen synthase and glycogen phosphorylase. AKT regulates glycogen phosphorylase activity as well as glycogen synthase through GSK-3, which phosphorylates glycogen synthase at multiple serine residues (30). Such reciprocal regulation of paired targets prevents the increase of glucose levels during glycogenolysis from triggering the resynthesis of glycogen (31). Following the decrease in phospho-GSK-3β (Fig. 1D), concomitant with elevated blood glucose, liver glycogen content was significantly decreased in mice (~90%; $P < 0.001$, t test) 4 h after GSK690693 administration (Fig. 3A). These observations support our hypothesis that inhibition of AKT can result in GSK-3 activation and lead to inhibition of glycogen synthesis as well as activation of glycogenolysis. Because liver glycogen is the primary source of glucose supply during periods of fasting, we subsequently investigated the effect of fasting on liver glycogen stores. Mice fasted for 16 to 20 h show a >90% decrease in liver glycogen (Fig. 3A). Treatment of fasted mice with GSK690693 resulted in minimal increases in blood glucose levels compared with nonfasted mice (Fig. 3B), suggesting that liver glycogen is the primary source of blood glucose elevation observed post-GSK690693 treatment. Notably, although the hyperglycemic effect is minimized by fasting, hyperinsulinemia was still observed in both fasted and nonfasted mice despite the observed differences in glucose levels (Fig. 3C).

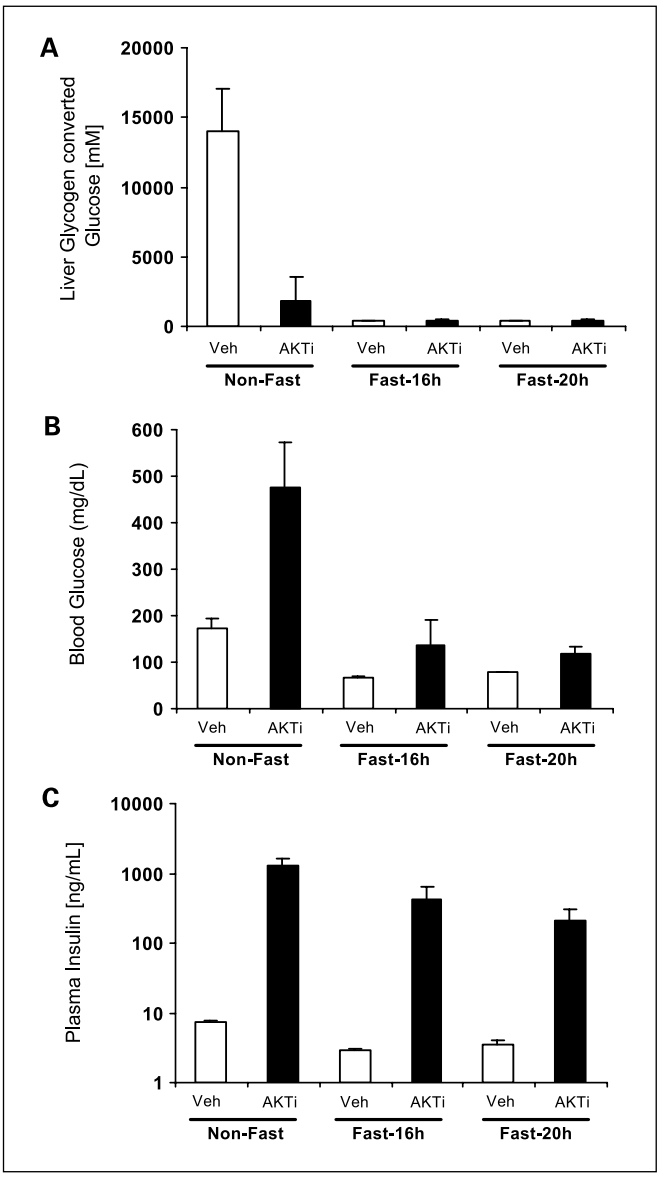

Fig. 3. AKT inhibitor-induced hyperglycemia is associated with induction of liver glycogenolysis as well as reduction in peripheral glucose uptake. *A* to *C*, GSK690693-induced hyperglycemia is associated with decrease in liver glycogen content. Liver glycogen levels 4 h after treatment with vehicle or GSK690693 (30 mg/kg intraperitoneally) in mice fed *ad libitum* or fasted for 16 or 20 h (*A*). Liver glycogen was measured by converting it to glucose with amylglucosidase and then measuring glucose levels using Amplex-Red kit. Blood glucose (*B*) and plasma insulin (*C*) concentration in fed and fasted mice after treatment with GSK690693.

Introduction of a low-carbohydrate diet after GSK690693 administration effectively reduces the diet-induced hyperglycemia. As described earlier, insulin promotes glucose uptake and glycogen synthesis, and AKT is a key mediator of insulin-mediated glucose transport. Therefore, we evaluated the effect of feeding and glucose challenge on blood glucose levels in fasted animals treated with GSK690693. Severe combined immunodeficient mice were fasted for 16 h and then dosed with vehicle or GSK690693 at 30 mg/kg intraperitoneally. Four hours after GSK690693 administration, mice were fed with normal mice chow or challenged with glucose (2 g/kg intraperitoneally). In nonfasted mice, administration of GSK690693 led to blood glucose elevations from 2 to 4 h post-compound administration (Fig. 4A). In fasted mice, basal glucose levels were lower than those observed in fed mice, and treatment with GSK690693 resulted in modest increases in blood glucose levels (Fig. 4B). Feeding normal mice chow to fasted mice led to blood glucose elevations in both vehicle and GSK690693-treated mice. In fasted mice treated with vehicle, administration of bolus glucose with food led to a spike in blood glucose levels, which returned to the levels similar to those observed with food alone within 2 h (Fig. 4B). However, in fasted mice treated with GSK690693, administration of a normal diet and a bolus of glucose resulted in significant and prolonged elevation of blood glucose, suggesting that GSK690693 impaired peripheral glucose uptake in mice (Fig. 4B). To further substantiate whether GSK690693 impaired peripheral glucose uptake, we employed the [^{18}F]DG imaging to monitor the effect of systemic [^{18}F]DG uptake following GSK690693 treatment. Mice administered with [^{18}F]DG tracer showed basal tissue uptake of [^{18}F]DG, whereas the same mice injected with GSK690693 (30 mg/kg intraperitoneally) then administered with tracer showed reduced peripheral [^{18}F]DG uptake in tumor xenograft, kidney, and muscle (Fig. 4C) but not in the liver. This result suggested that the diet-induced hyperglycemia following GSK690693 treatment is likely resulting from the impairment of peripheral glucose disposal induced by the compound.

Because we found that GSK690693 can impair peripheral glucose uptake, this suggested that the amount of carbohydrate in the diet of mice treated with GSK690693 may affect their blood glucose levels. The rate of glucose production from food is faster for carbohydrate followed by protein and fat;

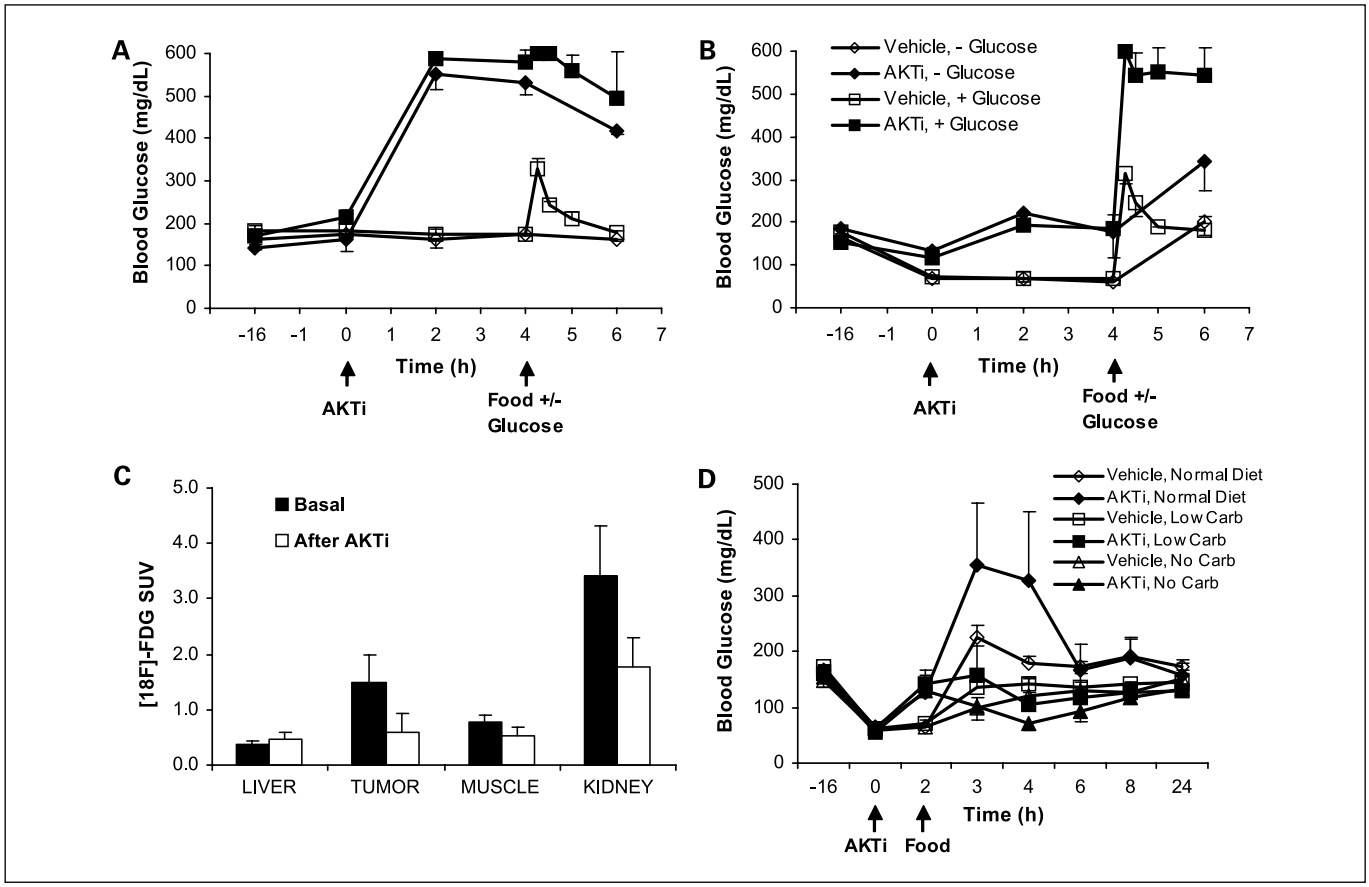

Fig. 4. GSK690693 decreases peripheral glucose uptake. Mice were fed *ad libitum* (*A*) or fasted for 16 h (*B*) and then treated with GSK690693 (30 mg/kg intraperitoneally). Four hours later, food was provided with and without Intraperitoneal Glucose Tolerance Tests (2 g/kg intraperitoneally). Blood glucose was monitored for 7 h post-GSK690693 administration. *Open symbols,* vehicle; *filled symbols,* GSK690693; *diamonds,* food without glucose; *squares,* food with glucose challenge. *C,* [^{18}F]DG uptake in mice after treatment with GSK690693. Mice fasted for 16 h were administered with [^{18}F]DG and scanned on day -1 for basal [^{18}F]DG uptake. Mice were resumed to fed *ad libitum* for 2 d of recovery. The same mice were fasted 16 h again on day -4 and then dosed with GSK690693 (30 mg/kg intraperitoneally). Two hours after compound administration, the [^{18}F]DG tracer was injected and the mice were scanned for [^{18}F]DG uptake in the tissues. Mean ± SD for three animals. *D,* fasting and low-carbohydrate diet minimizes AKT inhibitor-induced hyperglycemia. Mice fasted for 16 h were administered with vehicle (*open symbols*) or GSK690693 (30 mg/kg; *closed symbols*). Two hours later, mice were fed *ad libitum* with sterilized experimental diets containing 67% (normal diet; *diamonds*), 7% (low-carbohydrate diet; *squares*), and 0% (no carbohydrate diet; *triangles*) carbohydrates. Blood glucose was measured from tail vein nicks. Mean ± SD for three mice in each group.

therefore, a diet containing a lower carbohydrate content along with higher fat and moderate protein content is likely to produce a steady but lower level of glucose in the plasma. We thus designed a low-carbohydrate (7%) and a 0% carbohydrate mouse diet to test whether implementing such a diet can minimize the glucose elevation caused by GSK690693. Mice fasted for 16 h and then treated with vehicle show a modest glucose elevation at 2 h after they received normal chow (67% carbohydrate) or chow containing 7% or 0% carbohydrate. Mice pretreated with GSK690693 and receiving normal chow show a significant blood glucose elevation after 1 to 2 h of feeding (Fig. 4D). However, GSK690693-treated mice fed with a 7% or 0% carbohydrate diet showed no significant elevation in blood glucose after feeding compared with vehicle-treated mice (Fig. 4D).

Discussion

The PI3K/AKT pathway is constitutively active in many human malignancies. Signaling through this pathway plays a critical role in promoting cancer cell proliferation, survival, and chemoresistance; thus, intense efforts are under way to develop inhibitors of AKT as cancer therapy. The novel pan-AKT kinase inhibitor, GSK690693, is currently being evaluated in a phase I clinical trial for patients with solid tumors or lymphoma. The potential side effects of GSK690693 include transient hyperglycemia and hyperinsulinemia as observed in preclinical animal models (Figs. 1 and 2). As described previously, daily administration of GSK690693 (30 mg/kg intraperitoneally) for 21 days was well tolerated in mice (24) and there was no significant difference in the blood glucose elevation between days 1 and 21, suggesting that GSK690693-induced hyperglycemic effect remains transient with repeat dosing. Hyperglycemia was also observed in patients treated with perifosine and triciribine phosphate, both of which inhibit AKT activation (32, 33). Because hyperglycemia could be dose-limiting in human clinical trials, it is important to assess the mechanisms as well as methods of intervention for the hyperglycemia, so that the therapeutic potentials of these promising AKT inhibitors can be fully realized.

Treatment with GSK690693 in mice resulted in reduction of phospho-GSK-3β (Ser^{9}), a well-characterized substrate of AKT,

indicative of AKT kinase inhibition (Fig. 1D). However, the increase in phosphorylated IR observed in the treated animals suggests that the blockade of AKT activity by GSK690693 may lead to a systemic insensitivity to insulin, thereby inducing the positive feedback of insulin secretion as well as IR activation. Consistent with this observation, fasting animals for 16 to 20 h ameliorated GSK690693-induced hyperglycemia but did not abrogate increases in circulating insulin levels (Fig. 3C). These results may imply a systemic inadequacy of insulin signaling due to AKT inhibition, so that the hyperinsulinemia was induced as a compensatory mechanism to maintain relative euglycemia. Conversely, transgenic mice expressing a kinase-dead mutant of AKT1 exhibited defective insulin secretion in the β cells (34), whereas hyperinsulinemia was observed in AKT2-null mice, suggesting that hyperinsulinemia may be associated with inhibition of a specific isoform of AKT (17). Further investigation will be required to elucidate the underlying mechanism of hyperinsulinemia induced by AKT inhibition.

The inability of antidiabetic agents to alter GSK690693-induced hyperglycemia suggests that the mechanism of glucose elevation induced by GSK690693 is likely distinct from that of IGF-IR and PI3K inhibitors. It is possible that AKT may function as the gatekeeper of insulin signaling, such that reactivation or potentiation of insulin signaling at the receptor level using antidiabetic agents cannot fully restore insulin function in the presence of an AKT inhibitor. Because AKT can regulate the glycogen metabolism through GSK-3, we also investigated the role of GSK-3 inhibitor on GSK690693-induced hyperglycemia. Treatment with lithium chloride, a low millimolar GSK-3 inhibitor, showed little or no effect on GSK690693-induced hyperglycemia (data not shown), suggesting that inhibition of GSK-3 alone cannot shut off the GSK690693-induced glycogenolysis owing to the fact that protein kinase A and C and many other enzymes were also shown to be involved in glycogen metabolism (35). In addition, despite the ability of lithium to restore insulin sensitivity in skeletal muscle (36), lithium can also reciprocally suppress plasma insulin and elevate glucagon secretion (37). As such, lithium exerts its effect through a combination of several biochemical properties, some of which are likely antagonized by the AKT inhibitor.

Under normal physiologic conditions, circulating blood glucose stimulates insulin secretion and suppresses glucagon release. However, suppression of glucagon release may require the combination of insulin as well as hyperglycemia (38). Particularly, if there is no appropriate IR signaling in the α cells, the defective regulation of glucagon secretion may occur. The transient elevation of plasma glucagon observed following treatment (Fig. 1C) suggests the inhibition of AKT not only perturbs insulin signaling and cause hyperinsulinemia but also causes inadequate suppression of glucagon in the presence of insulin and hyperglycemia. However, over the prolonged course of hyperinsulinemia and hyperglycemia, circulating levels of the glucagon decreased (Fig. 1A-C).

As described earlier, insulin promotes glucose uptake and increases hepatic glycogen store. AKT is the key mediator of insulin signaling; conceivably, blockade of AKT activity perturbs insulin functions. This perturbation of insulin functions is evident in the compound-induced hepatic glycogenolysis (Fig. 3A and B) as well as the reduced [^{18}F]DG uptake in peripheral tissues (Fig. 4C) and the diet-induced hyperglycemia (Fig. 4A and B). Particularly, treatment with GSK690693 in fasted mice resulted in decreased [^{18}F]DG uptake in the nonhepatic tissues, and a diet-induced hyperglycemia indicates that the inhibition of AKT impaired peripheral glucose uptake. Whether GSK690693 induces full scope of insulin resistance still remains to be determined. However, the animal data suggest that fasting and diet modification provide an effective strategy for the management of AKT inhibitor-induced hyperglycemia and may overcome the treatment-related impairment in suppression of hepatic glucose output and the reduced peripheral glucose uptake.

Recent knockout mice studies have shown that AKT1-null mice do not display a diabetic phenotype, whereas AKT2-deficient mice exhibit insulin resistance, hyperglycemia, hyperinsulinemia, and glucose intolerance (39, 40). These findings not only provide evidence that the functions of individual isoforms may be very different but also reveal potential on-target toxicity for each AKT isoform. The hyperglycemia induced by GSK690693 is consistent yet distinct from the phenotype observed in AKT2 knockout mice. Specifically, treatment with GSK690693 and deletion of the AKT2 gene both result in hyperglycemia, hyperinsulinemia, insulin resistance, and glucose intolerance in mice. However, GSK690693 induces acute elevation of glucose and insulin as well as potentially an increased rate of glucose production and a decreased rate of peripheral glucose disposal. In contrast, a delayed progression of the diabetic phenotype was observed in AKT2-null mice. This is likely due to the compensation of AKT1 and AKT3 for the AKT2 functions during development in the AKT2-null mice. In addition, GSK690693 potently inhibits all three AKT kinases, which may be associated with the pronounced hyperglycemic effects, although the off-target inhibition on protein kinase A and C and AMPK by this compound may also contribute to the severity of observed hyperglycemia and hyperinsulinemia (24).

The range of metabolic alterations observed with other inhibitors of PI3K, AKT, mTOR, and IGF-IR differs, further suggesting that kinase selectivity among various inhibitors may in part be responsible for the different levels of glucose and insulin elevation observed with these agents (18, 19, 21–24, 41–47). Whether the hyperglycemia and hyperinsulinemia are associated with inhibition of AKT2 or simultaneous inhibition of all three AKT isoforms is currently unknown. Future investigations using AKT isoform-specific inhibitors with different kinase profiles may further elucidate the molecular mechanisms of hyperglycemia and hyperinsulinemia induced by AKT inhibition. Nevertheless, the underlying mechanism(s) of hyperglycemia associated with each particular agent will greatly influence which strategy (antidiabetic agents, fasting, or low-carbohydrate diet) should be implemented. For example, our data suggest that an AKT inhibitor, such as GSK690693, can induce glycogenolysis in the liver as well as inhibit peripheral glucose uptake, resulting in significant hyperglycemia. Therefore, a strategy, combining fasting before drug administration (to reduce liver glycogen) and a low-carbohydrate diet post-drug administration (to reduce availability of circulating glucose from diet), may assist in the clinical management of GSK690693-induced hyperglycemia. Similarly, hyperglycemia associated with UCN-01 (a protein kinase C inhibitor), which is caused by insulin insensitivity in peripheral tissues (47) and may require prolonged insulin infusions in patients (48), could

also be influenced by a low-carbohydrate diet post-drug treatment.

One caveat about the generalizability of the preclinical models evaluated in these experiments to the human experience is that the stores and metabolism of glycogen in patients with advanced cancer may not be equivalent to those of healthy mice. For instance, hepatic glycogen can be significantly depleted (~90%) after 16 to 20 h of fasting in mice (Fig. 3), whereas overnight fasting, in general, does not deplete liver glycogen in healthy individuals, and the reduced mobility in advanced cancer patients is likely to decrease the glycogen depletion process even further. In addition, a low-carbohydrate/high-fat/high-protein diet may shift energy metabolism to ketone production, which may not be appropriate in patients with advanced cancer whose oral intake may be reduced.

An intravenous formulation of GSK690693 is currently being evaluated in a dose escalation phase I clinical trial in patients with advanced solid tumors or lymphoma. GSK690693 has been administered to 14 patients who have received weekly doses of 25 to 115 mg with patients remaining on study from 4 to >30 weeks (49). Thus far, two patients have shown transient, drug-related increases in blood glucose levels above 250 mg/dL (49), an effect consistent with our preclinical observations. As dose escalation of GSK690693 continues, implementation of pre-dose fasting requirements and low-carbohydrate meals in the trial may reduce the absolute glucose and insulin levels observed in the patients after dosing. Modulation of the glucose and insulin levels may allow evaluation of higher doses of GSK690693 than would be allowed without these dietary modifications.

In summary, our results highlight the importance of AKT in glucose homeostasis. Not only did we elucidate the specific mechanisms associated with GSK690693-induced hyperglycemia, but we also explored how specific dietary modifications and various medications, including intravenous insulin, rosiglitazone, exendin-4, vildagliptin, and metformin, could influence this process. Overall, our investigation of GSK690693-induced hyperglycemia has yielded data that can serve as a guide for future investigations of hyperglycemia associated with novel agents targeting IGF-IR, PI3K, AKT, and mTOR, thereby allowing their full therapeutic potential to be evaluated.

Disclosure of Potential Conflicts of Interest

All authors are current or former employees of GlaxoSmithKline.

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