Chronic selective glycogen synthase kinase-3 inhibition enhances glucose disposal and muscle insulin action in prediabetic obese Zucker rats

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Dokken, Betsy B. and Erik J. Henriksen. Chronic selective glycogen synthase kinase-3 inhibition enhances glucose disposal and muscle insulin action in prediabetic obese Zucker rats. Am J Physiol Endocrinol Metab 291: E207–E213, 2006. First published February 14, 2006; doi:10.1152/ajpendo.00628.2005.—Increasing evidence supports a negative role of glycogen synthase kinase-3 (GSK-3) in regulation of skeletal muscle glucose transport. We assessed the effects of chronic treatment of insulin-resistant, prediabetic obese Zucker (fa/fa) rats with a highly selective GSK-3 inhibitor (CT118637) on glucose tolerance, whole body insulin sensitivity, plasma lipids, skeletal muscle insulin signaling, and in vitro skeletal muscle glucose transport activity. Obese Zucker rats were treated with either vehicle or CT118637 (30 mg/kg body wt) twice per day for 10 days. Fasting plasma insulin and free fatty acid levels were reduced by 14 and 23% (P < 0.05), respectively, in GSK-3 inhibitor-treated animals compared with vehicle-treated controls. The glucose response during an oral glucose tolerance test was reduced by 18% (P < 0.05), and whole body insulin sensitivity was increased by 28% (P < 0.05). In vivo insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation (50%) and IRS-1-associated phosphatidylinositol-3 kinase (79%) relative to fasting plasma insulin levels were significantly elevated (P < 0.05) in plantaris muscles of GSK-3 inhibitor-treated animals. Whereas basal glucose transport in isolated soleus and epitrochlearis muscles was unaffected by chronic GSK-3 treatments, insulin stimulation of glucose transport above basal was significantly enhanced (32–60%, P < 0.05). In summary, chronic treatment of insulin-resistant, prediabetic obese Zucker rats with a specific GSK-3 inhibitor enhances oral glucose tolerance and whole body insulin sensitivity and is associated with an amelioration of dyslipidemia and an improvement in IRS-1-dependent insulin signaling in skeletal muscle. These results provide further evidence that selective targeting of GSK-3 in muscle may be an effective intervention for the treatment of obesity-associated insulin resistance.

glucose tolerance; insulin receptor substrate-1; phosphatidylinositol 3’-kinase; glucose transport

INSULIN RESISTANCE of skeletal muscle glucose transport and metabolism is a hallmark of both the prediabetic state and overt type 2 diabetes (17, 42). The etiology of skeletal muscle insulin resistance is clearly multifactorial and can involve defective expression and functionality of multiple elements in the insulin-signaling cascade that regulates the glucose transport process (see reviews in Refs. 17, 42). Both in animal models of insulin resistance, such as the obese Zucker rat, and in humans with prediabetes or overt type 2 diabetes, there is diminished insulin-stimulated GLUT4 protein translocation (13, 24, 41) and glucose transport activity (7, 13, 20, 41) in skeletal muscle. This insulin resistance of muscle glucose transport is associated with well-defined defects in insulin signaling, including tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) and IRS-1 association with the p85 regulatory subunit of phosphatidylinositol 3’-kinase (PI 3-kinase) (1, 3, 14, 22, 34).

Glycogen synthase kinase-3 (GSK-3), a serine/threonine kinase consisting of highly homologous α- and β-isforms (38), can phosphorylate and inactivate glycogen synthase (29, 32, 40). GSK-3 is constitutively active and is acutely deactivated by insulin signaling through the sequential activation of IRS-1, PI 3-kinase, and, ultimately, via the action of Akt to phosphorylate specific serine residues on the enzyme (8). GSK-3 can phosphorylate IRS-1 on serine and threonine residues, causing impairment of insulin signaling (11). These observations support the hypothesis that GSK-3 can serve as a negative modulator of insulin action on glycogen synthase and, potentially, on glucose transport activity.

GSK-3 is overexpressed and is overactive in diabetic human and diabetic and prediabetic rodent skeletal muscle (9, 28), and this inadequate inhibitory control of GSK-3 has been linked to impaired insulin action (Refs. 12, 28; reviewed in Refs. 10, 18, 37). Acute pharmacological inhibition of GSK-3 with substituted aminopyrimidine molecules increases insulin action on glycogen synthase and glucose transport in isolated muscle cells and tissues (9, 21, 27, 31), likely due to enhanced IRS-1-dependent insulin signaling (9). Acute administration of these substituted aminopyrimidine GSK-3 inhibitors causes a significant lowering of blood glucose, dramatically improves whole body glucose tolerance and insulin sensitivity, and enhances insulin-stimulated skeletal muscle glucose transport activity in a variety of mouse and rat models of obesity-associated insulin resistance (18, 21, 31, 37). Additional acute oral treatment with this type of GSK-3 inhibitor improves oral glucose tolerance in mildly diabetic obese rhesus monkeys (37). However, no other studies to date have directly evaluated the effect of chronic inhibition of GSK-3 activity with these aminopyrimidine-based GSK-3 inhibitors on in vivo insulin action in mammalian skeletal muscle.

The present investigation was designed to test the overall hypothesis that chronic administration of a selective organic GSK-3 inhibitor would enhance insulin action in an animal model of obesity-associated insulin resistance. Therefore, the specific purposes of this study were 1) to determine the whole body response of insulin sensitivity to chronic treatment with a selective GSK-3 inhibitor (CT118637) in insulin-resistant, prediabetic obese Zucker (fa/fa) rats; 2) to evaluate the effect of chronic selective GSK-3 inhibition on in vivo IRS-1-dependent insulin signaling in skeletal muscle of the obese Zucker rat; and 3) to assess the effect of chronic GSK-3 inhibition in obese

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Zucker rats on insulin-stimulated glucose transport activity in isolated skeletal muscle.

METHODS

Animals, treatments, and oral glucose tolerance tests. Female obese Zucker (fa/fa) rats were purchased from Harlan (Indianapolis, IN) at the age of 8–9 wk, and treatments were commenced at 10 wk of age. All animals were housed in a temperature-controlled room (20–22°C) with a 12:12-h light-dark cycle (lights on from 7:00 AM to 7:00 PM) at the Central Animal Facility of the University of Arizona. The animals had free access to chow (Teklad, Madison, WI) and water, and all procedures were approved by the University of Arizona Animal Care and Use Committee.

The GSK-3 inhibitor CT118637 (kindly provided by Dr. Steve Harrison, Chiron, Emeryville, CA) is structurally very similar to and has identical pharmacokinetic properties to selective GSK-3 inhibitors used previously by our research group (Refs. 18, 21, 31; reviewed in Ref. 37). It inhibits both GSK-3α and GSK-3β in vitro with Ki values <10 nM in an ATP-competitive manner (9, 37). Obese Zucker rats were treated by gavage with either vehicle or CT118637 (30 mg/kg: dissolved in 100 mM Tris buffer, pH 7.4) twice daily (8:00 AM and 5:00 PM) for 10 days and were studied 15–18 h after the final treatments. This dose of GSK-3 inhibitor is based on its effectiveness to induce a significant effect on glucose tolerance and insulin sensitivity when administered acutely (up to 20 h) (see Refs. 4, 21, 31, 37). The acute administration of this class of GSK-3 inhibitor results in elevated levels of the inhibitor in plasma and skeletal muscle for at least 4 h (data not shown). Additional information on the pharmacokinetic properties of this class of GSK-3 inhibitor can be found in Refs. 4 and 37.

On the 8th day, after animals had been food restricted (4 g of chow given at 5:00 PM the previous evening), at 8:00 AM they underwent an oral glucose tolerance test (OGTT) consisting of 1 g/kg body wt glucose feeding by gavage. Blood (0.2 ml) was collected from a small cut at the tip of the tail immediately before and at 15, 30, 60, and 120 min after the glucose feeding. Whole blood was mixed thoroughly with EDTA (18 mM final concentration) and centrifuged at 13,000 g to isolate the plasma. The plasma was stored at −80°C and subsequently assayed for glucose (Sigma Chemical, St. Louis, MO), insulin (Linco Research, St. Charles, MO), and free fatty acids (FFAs; Wako Chemicals, Richmond, VA). Fasting whole body insulin sensitivity was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR) by using the formula [fasting plasma glucose (mg/dl) × fasting plasma insulin (µU/ml)]/405 (26). Immediately after completion of the OGTT, all animals received a 2.5-ml subcutaneous injection of 0.9% sterile saline to compensate for plasma loss, and treatments continued for two more days.

In vitro treatments of skeletal muscle. On the 11th day, after an overnight food restriction (chow was restricted to 4 g at 5:00 PM and was consumed immediately), animals were deeply anesthetized at 8:00 AM with an intraperitoneal injection of pentobarbital sodium (50 mg/kg), and intact epitrochlearis muscles and strips of soleus muscles were removed, trimmed of excess fat and connective tissue, quickly frozen between aluminum blocks cooled with liquid nitrogen, and weighed. These muscle were dissolved in 0.5 ml of 0.5 N NaOH, 5 ml of scintillation cocktail were added, and the specific intracellular accumulation of 2-DG was determined as described previously (19). This method for assessing glucose transport activity in isolated muscle has been validated (15).

Measurement of tissue glycogen concentration and glycogen synthase activity. For determination of glycogen (16), muscle was dissolved in 1 ml of 5 N KOH. Glycogen was purified by ethanol precipitation and then hydrolyzed to glucose by heating for 3 h at 100°C in 2 N HCl. After cooling, the sample was neutralized to pH 6–8 with 4 N NaOH, 0.1 M triethanolamine-HCl, and assayed spectrophotometrically for glucose (2). Glycogen synthase activity in plantaris was assessed as the activity ratio (activity in the absence of glucose 6-phosphate divided by activity in the presence of 5 mM glucose 6-phosphate) using the filter paper assay of Thomas et al. (35).

Assessment of insulin signaling factor protein expression and functionality. Frozen tissues were homogenized in 8 vol of ice-cold lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 20 mM Na-pyrophosphate, 20 mM β-glycerophosphate, 10 mM NaF, 2 mM Na3VO4, 2 mM EDTA, 1% Triton X-100, 10% glycerol, 1 mM MgCl2, 1 mM CaCl2, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 0.5 µg/ml pepstatin, and 2 mM PMSF). Homogenates were incubated on ice for 20 min and then centrifuged at 13,000 g for 20 min at 4°C. Total protein concentration was determined using the bicinchoninic acid method (BCA; Sigma Chemical). Insulin-signaling proteins were separated by heating in 7.5 or 12% polyacrylamide gels (Bio-Rad Laboratories, Hercules, CA) and transferred to nitrocellulose membranes. To determine protein expression of insulin-signaling factors, we incubated the blots with commercially available antibodies against insulin receptor β-subunit (IRb), IRS-1 and IRS-2, the p85 regulatory subunit of PI 3-kinase, GSK-3α/β (Upstate Biotechnology, Lake Placid, NY), and Akt1/2 (Cell Signaling Technology, Beverly, MA). Blots from skeletal muscle were also incubated with antibody against the GLUT4 glucose transporter isoform (Biogenesis, Brentwood, NH). Membranes were then incubated with secondary goat anti-rabbit antibody conjugated with horseradish peroxidase (HRP; Chemicon, Temecula, CA). The proteins were visualized on Kodak X-Omat AR film (Kodak, Rochester, NY) with the use of an enhanced chemiluminescence detection system (ECL; Amersham Pharmacia, Piscataway, NJ). The band intensities on the autoradiographs were quantified on a scanning densitometer (Bio-Rad model GS-800) using Quantity One software (Bio-Rad).

For analysis of tyrosine-phosphorylated IRS-1 (IRS-1/pY), 0.5 µl of diluted homogenate (500 µg total protein) were immunoprecipitated with 25 µl of agarose-conjugated anti-IRS-1 antibody (Upstate Biotechnology). For analysis of IRS-1-associated p85 (IRS-1/p85), 0.5 µl of diluted homogenate were immunoprecipitated with 25 µl of agarose-conjugated anti-p85 antibody (Upstate Biotechnology). After an incubation period of 4 h for IRS-1/p85 and overnight incubation at 4°C for IRS-1/pY, samples were centrifuged, and the supernatant was removed. The beads were washed three times with ice-cold PBS, mixed with SDS sample buffer, and boiled for 5 min. Proteins were separated by SDS-PAGE on 7.5% polyacrylamide gels (Bio-Rad Laboratories) and transferred to nitrocellulose membranes. For anal-
ysis of IRS-1/pY, the nitrocellulose membrane was incubated in anti-phosphotyrosine antibody (PY99; Santa Cruz Biotechnology, Santa Cruz, CA). Immunoblotting for detection of IRS-1/pY85 was completed as described above with the use of an antibody against IRS-1 (Upstate Biotechnology). Thereafter, the membranes were incubated with secondary goat anti-mouse antibody conjugated with HRP (Santa Cruz Biotechnology). Protein bands of interest were exposed, visualized, and quantified as described above.

For determination of Akt and GSK-3 serine phosphorylation, samples containing equal amounts of total protein were separated by SDS-PAGE on 12% polyacrylamide gels (Bio-Rad Laboratories) and transferred to nitrocellulose membranes. Membranes were incubated with antibodies against phospho-Akt Ser473 and phospho-GSK-3α/β Ser21/9 (Cell Signaling Technology) overnight. In our hands, although the GSK-3α protein can be detected, Ser21 phosphorylation of GSK-3α in muscle from the obese Zucker rat is very low (Dokken BB, Sloniger JA, and Henriksen EJ, unpublished data), and therefore all GSK-3 data in this study are restricted to GSK-3β Ser9 phosphorylation. Subsequently, membranes were incubated with secondary goat anti-rabbit antibody conjugated with HRP. Protein bands of interest were exposed to film, visualized, and quantified as described above.

Statistical analysis. All values are expressed as means ± SE. Differences between two groups were determined using an unpaired Student’s t-test. A level of P < 0.05 was set for statistical significance.

RESULTS

Effect of chronic selective GSK-3 inhibition on whole body glucose tolerance and insulin sensitivity. The final average body weight of the obese Zucker rats treated chronically with the selective GSK-3 inhibitor was not different from that of the vehicle-treated animals (269 ± 8 g in vehicle-treated animals vs. 280 ± 7 g in CT118637-treated animals; 5 animals/group). Whereas fasting plasma glucose was not affected by the chronic GSK-3 inhibitor treatment, fasting plasma insulin (14%) and FFA (23%) levels were significantly (P < 0.05) lower in the obese animals treated with CT118637 compared with the vehicle-treated obese rats (Table 1). The HOMA-IR value, inversely related to insulin sensitivity in the fasting state (26), was significantly reduced in the GSK-3 inhibitor-treated group (Table 1), indicating that the chronic GSK-3 inhibition enhanced fasting whole body insulin sensitivity.

There was no long-lasting effect of the GSK-3 inhibition on fasting glycogen concentrations in liver (37.5 ± 2.7 vs. 39.1 ± 3.2 nmol/mg) and plantaris muscle (15.4 ± 1.5 vs. 15.3 ± 2.7 nmol/mg), and there was no effect of the GSK-3 inhibitor treatment on total activity of glycogen synthase in this skeletal muscle (1.28 ± 0.17 vs. 1.24 ± 0.19 nmol·mg muscle⁻¹·min⁻¹). Although the absolute glycogen synthase activity ratio in plantaris muscle (0.376 ± 0.039 vs. 0.379 ± 0.052) did not differ between the vehicle-treated group and the GSK-3 inhibitor-treated group, when expressed relative to the prevailing plasma insulin level, the glycogen synthase activity ratio was significantly greater (P < 0.05) in the GSK-3 inhibitor-treated group (3.33 ± 0.18 × 10⁻³ ml/µU) compared with the vehicle-treated control group (2.80 ± 0.15 × 10⁻³ ml/µU).

The glucose and insulin responses during the oral glucose tolerance test are shown in Fig. 1, top. The glucose response to the oral glucose load was markedly reduced (P < 0.05) in the chronic GSK-3 inhibitor-treated group at the 15- (23%) and 30-min (15%) time points and remained reduced up to 120 min. The insulin response was likewise blunted in the chronic GSK-3 inhibitor-treated group, with a 25% reduction (P < 0.05) at the 15-min time point. The total area under the glucose curve (AUCg) was significantly reduced (18%, P < 0.05) by the chronic GSK-3 inhibitor treatment (Fig. 1, bottom left). The total insulin area under the curve (AUCi) was also slightly reduced in this group compared with the vehicle-treated control group (Fig. 1, bottom middle), but this difference did not reach statistical significance.

Whole body insulin sensitivity was also assessed from the OGTT data by determining the reciprocal of the glucose-insulin index, defined as the product of the AUCg and the AUCi, and inversely related to whole body insulin action (5). This whole body insulin sensitivity index was enhanced by 28% (P < 0.05) in the chronic GSK-3 inhibitor-treated group (Fig. 1, bottom right).

Effect of chronic selective GSK-3 inhibition on in vivo muscle insulin signaling. The chronic treatment of the obese Zucker rats with the selective GSK-3 inhibitor did not alter the protein expression of IRβ, IRS-1, IRS-2, the p85 subunit of PI 3-kinase, Akt, GSK-3β, or the GLUT4 glucose transporter in soleus and plantaris muscles (data not shown). In addition, protein expression of these various insulin signaling factors was not altered in liver of these GSK-3 inhibitor-treated animals (data not shown).

The in vivo functional states of IRS-1 and PI 3-kinase were assessed in skeletal muscle of the vehicle-treated and chronic GSK-3 inhibitor-treated obese animals (Figs. 2 and 3). In the plantaris, IRS-1 tyrosine phosphorylation (79%) and IRS-1 associated p85 (50%), a surrogate measure of PI 3-kinase activity, were both significantly (P < 0.05) greater in the GSK-3 inhibitor-treated group when expressed either in absolute terms (data not shown) or relative to the fasting plasma insulin concentration (Fig. 2). In the soleus, IRS-1 association with the p85 subunit of PI 3-kinase was significantly elevated (250%, P < 0.05) when expressed relative to the prevailing in vivo plasma insulin level (Fig. 3). No differences in the phosphorylation states of Akt or GSK-3β between the vehicle-treated and GSK-3 inhibitor-treated groups were detected in these muscles, regardless of whether these variables were

Table 1. Effects of chronic GSK-3 inhibitor treatment on fasting plasma glucose, insulin, free fatty acids, and whole body insulin sensitivity in obese Zucker rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma Glucose, mg/dl</th>
<th>Plasma Insulin, µU/ml</th>
<th>Plasma Free Fatty Acids, mM</th>
<th>HOMA-IR, mg/dl⁻¹·µU/ml⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese vehicle treated</td>
<td>116±7</td>
<td>134±3</td>
<td>1.46±0.08</td>
<td>38.3±2.4</td>
</tr>
<tr>
<td>Obese GSK-3 inhibitor treated</td>
<td>116±3</td>
<td>115±5*</td>
<td>1.13±0.10*</td>
<td>32.0±1.6*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 animals per group. GSK-3, glycogen synthase kinase-3; HOMA-IR, homeostasis model assessment of insulin resistance. *P < 0.05 vs. obese vehicle-treated controls.
normalized to total protein or to the expression of the specific protein (data not shown).

Effect of chronic selective GSK-3 inhibition on in vitro muscle glucose transport activity. Like the plantaris, the epitrochlearis is composed of primarily type II fibers (33). Basal and insulin-stimulated glucose transport activities were assessed in isolated type II epitrochlearis and type I soleus muscles from the vehicle-treated and chronic GSK-3 inhibitor-treated obese Zucker rats. As shown in Fig. 4, there was no difference between treatment groups for basal glucose transport activity in either muscle type. In contrast, the effect of a maximally effective concentration of insulin to stimulate glucose transport activity above basal was significantly (*P < 0.05) enhanced in both soleus (32%, Fig. 4A) and epitrochlearis (60%, Fig. 4B) muscles of the chronic GSK-3 inhibitor-treated animals.

DISCUSSION

In the present investigation, we have made the novel finding that chronic oral treatment of prediabetic, insulin-resistant obese Zucker rats with a selective organic inhibitor of GSK-3 improves glucose tolerance and whole body insulin sensitivity (Fig. 1 and Table 1). Previous investigations using the Zucker diabetic fatty (ZDF) rat, a related model of overt type 2 diabetes, had demonstrated that acute (4 h) oral treatment with (21, 31) or short-term (20 h) infusion (4) of these substituted aminopyrimidine-based GSK-3 inhibitors enhanced oral glucose tolerance and whole body insulin sensitivity. The findings in the present investigation now indicate that longer term beneficial effects of GSK-3 inhibition on glucoregulation can be realized in a rat model of severe insulin resistance, even 15–18 h after the last oral administration of the GSK-3 inhibitor.

The enhancement of whole body insulin sensitivity in the fasting state elicited by the chronic GSK-3 inhibitor treatment in the obese Zucker rats (i.e., reduced HOMA-IR; Table 1) was associated with significant improvements in the functional status of key elements of the insulin-signaling cascade in muscle, namely, tyrosine phosphorylation of IRS-1 and IRS-1 association with the p85 subunit of PI 3-kinase (Figs. 2 and 3).
Moreover, the maximal capacity for insulin stimulation of glucose transport activity was also upregulated in type I soleus and type II epitrochlearis muscles of the chronic GSK-3 inhibitor-treated obese animals (Fig. 4). These alterations in IRS-1-dependent signaling and glucose transport activity are similar to those observed by our group following acute in vitro treatment of skeletal muscle from obese Zucker rats with this same GSK-3 inhibitor (9). However, it is unlikely that these enhancements in insulin action on the skeletal muscle glucose transport system were induced by the last administration of GSK-3 inhibitor, because the acute effects of these substituted aminopyrimidine compounds are largely gone 4 h after the final gavage treatment (21), at least in ZDF rats. Indeed, at the time that muscle glucose transport activity was assessed, muscle and liver glycogen levels were the same as in the vehicle-treated obese animals.

The chronic oral treatments with the selective GSK-3 inhibitor caused a significant decrease in plasma FFA levels (Table 1). It is possible that this improvement in the lipemic state of the obese Zucker rats is mechanistically associated with the enhanced whole body insulin sensitivity. It is known that FFAs or their derivatives can directly and indirectly inhibit the functionality of IRS-1-dependent insulin signaling in skeletal muscle (39). Therefore, a decrease in the plasma levels of these inhibitory lipids following chronic GSK-3 inhibitor treatment...
could allow for greater signaling via IRS-1-dependent steps, such as PI 3-kinase (Fig. 3), and a similar enhancement of muscle glucose disposal.

The chronic GSK-3 inhibition upregulated the functionality of IRS-1 and PI 3-kinase in muscle in the absence any significant alterations in the protein expression of a wide variety of insulin signaling factors and GLUT4. These data are in contrast to the findings of Nikouлина et al. (27), who showed that long-term exposure of cultured human myocytes to a similar substituted aminopyrimidine-base GSK-3 inhibitor caused a downregulation of GSK-3 protein expression and an upregulation of IRS-1 protein expression. One important difference between these two studies is that the obese Zucker rats in the present study were exposed twice daily to a large dose of GSK-3 inhibitor, whereas the cultured myocytes were exposed continually to a constant concentration of the GSK-3 inhibitor. It is clear that intermittent exposure to the GSK-3 inhibitor in vivo is insufficient to elicit long-lived changes in protein expression of these insulin-signaling factors and GLUT4 in tissues of these prediabetic rats.

To our knowledge, this is the only investigation to date that has addressed the chronic effects of GSK-3 inhibition in a rat model of prediabetes. However, in a recent study (23), Kaidanovich-Beilin and Eldar-Finkelman investigated the consequences of the chronic intraperitoneal administration of a novel competitive peptide inhibitor of GSK-3 in ob/ob mice. In agreement with the present study, the chronic GSK-3 inhibition in the ob/ob mice caused a significant improvement of glucose tolerance. However, these investigators also reported increased hepatic IRS-2 protein expression and glycogen concentration, whereas in skeletal muscle, GLUT4 protein expression was enhanced and glycogen levels were slightly elevated (23), findings that were not corroborated in the present study using obese Zucker rats. There are clearly some different chronic adaptive responses to GSK-3 inhibition in rat and mouse models of glucose dysregulation.

Related to these investigations are the results from a recent investigation utilizing muscle-specific GSK-3 transgenic mice (30). Selective overexpression of GSK-3β in skeletal muscle of male mice was associated with an increase in fat mass, a decrease in muscle IRS-1 protein expression, and decreased glycogen synthase activity and glycogen levels in muscle. These muscle-specific GSK-3β transgenic mice also were characterized by marked glucose intolerance and hyperinsulinemia, consistent with reduced whole body insulin sensitivity, and by elevated plasma FFA and triglycerides (30). These findings are consistent with the interpretation that overactivity of GSK-3 specifically in skeletal muscle is associated with whole body insulin resistance, hyperinsulinemia, and dyslipidemia, similar to the defects observed in the obese Zucker rat.

Although the present investigation has focused primarily on the consequences of chronic GSK-3 inhibitor administration on the skeletal muscle glucose transport system, the potential contribution of altered hepatic glucose production to the improvement of glucose regulation following GSK-3 inhibition also must be discussed. Short-term infusion of these aminopyrimidine-based GSK-3 inhibitors in the type 2 diabetic ZDF rats significantly reduced hepatic glucose production (4). Importantly, chronic administration of a peptide-based competitive inhibitor of GSK-3 in insulin-resistant ob/ob mice reduced hepatic mRNA levels of phosphoenolpyruvate carboxykinase (PEPCK), an important enzyme involved in hepatic gluconeogenesis, possibly due to a diminution of cAMP-responsive element binding protein, a transcription factor critical in the regulation of PEPCK gene expression (23). This finding is consistent with cell-based studies demonstrating downregulation of PEPCK gene expression after in vitro exposure to maleimide-based GSK-3 inhibitors (6, 25). These data indicate that in insulin-resistant states, an additional important action of GSK-3 inhibitors is a reduction of hepatic glucose production, likely mediated by downregulation of genes associated with gluconeogenesis.

In conclusion, chronic administration of a selective organic inhibitor of GSK-3 to the obese Zucker rat, a model of insulin resistance and prediabetes, improves oral glucose tolerance and ameliorates whole body insulin resistance. These metabolic improvements are associated with a diminution of dyslipidemia, an enhancement of insulin-stimulated glucose transport in skeletal muscle, and increases in IRS-1-dependent insulin signaling in skeletal muscle. These results provide further evidence that selective targeting of GSK-3 in muscle may be an effective intervention in obesity-associated insulin resistance.

### GRANTS

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