Tissue-specific expression and regulation of GSK-3 in human skeletal muscle and adipose tissue

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Glycogen synthase kinase-3 (GSK-3) is a ubiquitous kinase implicated in both insulin action and adipogenesis. To determine how these multiple roles may relate to insulin resistance, we studied the regulation of GSK-3 protein expression and phosphorylation in skeletal muscle and isolated adipocytes from nonobese healthy control (HC), obese control (OC), and obese type 2 diabetic (OT2D) subjects. At baseline there were no differences in the GSK-3 protein expression in adipocytes. OC subjects underwent a 6-mo caloric restriction resulting in a 7% decrease in body mass index (BMI) and a 21% improvement in insulin sensitivity. GSK-3 activity was increased in skeletal muscle of poorly controlled type 2 diabetic patients (26) as well as in animal models of insulin resistance (11, 12). In addition, inhibitors of GSK-3 have been shown to improve insulin action and glucose metabolism in human and rodent skeletal muscle (25, 11, 24) as well as in vivo in insulin-resistant rodents (6, 14, 18).

In addition to its role in glucose metabolism, GSK-3 is also a key regulator of multiple other processes, such as embryonic development, apoptosis, cytoskeletal organization, protein synthesis, and cell fate determination, including adipogenesis (10, 32). Indeed, treatment with GSK-3 inhibitors prevents the differentiation of 3T3-L1 preadipocytes (33). This finding raises the possibility that GSK-3-mediated adipogenesis may be involved in the development of obesity. Interestingly, tissue-selective overexpression of GSK-3β in skeletal muscle is associated with increased adipose tissue mass in male mice (27). Although GSK-3 is ubiquitously expressed in cells and tissues, tissue-specific effects of obesity on GSK-3 levels have been found. In C57BL/6J mice, which are diabetes and obesity prone, a high-fat diet resulted in a 30% increase in body weight and an increase in GSK-3 activity in epididymal fat tissue but not in muscle and liver (12). In contrast, A/J mice, which are resistant to diet-induced obesity and diabetes, had no change in GSK-3 activity after being fed a high-fat diet (12).

The common occurrence of obesity and insulin resistance and the possible role of GSK-3 in both of these events suggests that GSK-3 might represent a productive target for treating these disturbances. In this study, our goal was to examine the roles and regulation of GSK-3 in human skeletal muscle and adipose tissue, particularly in the development of obesity and insulin resistance. The approach was to first compare GSK-3 protein expression in subcutaneous abdominal adipose tissue from nonobese healthy control (HC), obese control (OC), and obese type 2 diabetic (OT2D) subjects, as we have previously reported (26) that skeletal muscle GSK-3 expression and activity were elevated in poorly controlled OT2D subjects relative to HC and OC. We also examined the effects of interventions having differing effects on adiposity and insulin sensitivity, weight loss, and treatment with the antidiabetic medications metformin and troglitazone on GSK-3 expression to determine whether there was tissue specificity for the regulation of GSK-3.

We found that there is tissue-specific regulation of GSK-3. Specifically, we show that in adipose tissue the expression of type 2 diabetes. For example, GSK-3 protein expression or activity is elevated in skeletal muscle of poorly controlled type 2 diabetic patients (26) and in animal models of insulin resistance (11, 12). In addition, inhibitors of GSK-3 have been shown to improve insulin action and glucose metabolism in human and rodent skeletal muscle (25, 11, 24) as well as in vivo in insulin-resistant rodents (6, 14, 18).

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GSK-3 is linked more with weight changes than with whole body glucose disposal (decreased after weight loss), whereas in skeletal muscle the expression or activity of GSK-3 is linked with insulin resistance (increased in type 2 diabetes and reduced after weight loss and thiazolidinedione treatment).

MATERIALS AND METHODS

Subjects. Adipose tissue and skeletal muscle biopsies were obtained from 9 nonobese HC, 14 OC, and 17 OT2D subjects. All control subjects had normal glucose tolerance as defined by American Diabetes Association criteria. Except for diabetes, the OT2D subjects were healthy and on no other medications known to influence glucose metabolism. The OC and OT2D groups included subjects who participated in the intervention studies described below.

Weight loss protocol. Tissue samples were obtained from six obese subjects (BMI 30–45 kg/m²) with normal glucose tolerance before and after weight loss. Patients were weight stable at their new level for 3 mo prior to the study. At the beginning of the study, subjects were admitted for baseline metabolic characterization, which included a 75-g oral glucose tolerance test, a hyperinsulinemic euglycemic clamp, fasting glucose, insulin, and free fatty acid levels, and muscle and fat biopsies. Subjects were then placed on a very low-calorie diet for 4–6 wk. Diet composition during the weight-stable and weight-maintenance stages was, as percentage of total calories: 55% carbohydrate, 30% fat, and 15% protein. The very low-calorie diet was 55%/3%/42%. Thus it is possible that diet and exercise alone could account for the differences observed.

Troglitazone/metformin treatment protocol. Tissue was obtained from a total of 12 poorly controlled [glycosylated hemoglobin (Hb A₁c) >8.5% and fasting plasma glucose >7.8 mmol/l] type 2 diabetic subjects divided equally between troglitazone and metformin arms. Subjects were between the ages of 30 and 70 yr and on at least half-maximal doses of any sulfonylurea agent. These subjects were part of a larger study group comparing the effects of troglitazone and metformin on the hyperglycemic clamp (2). After screening, their present sulfonylurea medication was discontinued, and all subjects were uniformly started on glyburide at 10 mg twice a day, for 2 wk. Baseline metabolic studies including fasting glucose, fasting insulin, hyperinsulinemic euglycemic clamp, and muscle and fat biopsies were then performed, after which subjects were randomized to the addition of either troglitazone or metformin to glyburide therapy. Subjects were counseled at 2-wk intervals to consume a weight-maintenance diet for the duration of the study. For 4–6 wk the troglitazone treatment was titrated ≤600 mg/day or metformin ≤2.550 mg/day as required to achieve glycemic goals. After 3–4 mo of this additional therapy, patients were readmitted for repeat metabolic studies. With regard to clinical parameters and response to treatment, the present subjects were indistinguishable from the larger study group (3).

All experimental protocols were approved by the Committee on Human Investigation of the University of California, San Diego, CA. Informed written consent was obtained after explanation of the protocol.

Hyperinsulinemic euglycemic clamp. Insulin action was determined by a 3-h hyperinsulinemic (300 mU·m⁻²·min⁻¹) euglycemic (5.0–5.5 mmol/l) clamp (36), where the glucose disposal rate (GDR) was determined during the last 30 min of the clamp.

Skeletal muscle tissue biopsy and protein extraction. Skeletal muscle was obtained by needle biopsy of the vastus lateralis skeletal muscle using a 5-mm side-cutting needle. Biopsy samples were immediately frozen in liquid nitrogen for storage until analysis. For analysis, a total of 50–100 mg of muscle were homogenized using a polytron at half-maximum speed for 1 min on ice in 500 μl of buffer A (20 mmol/l Tris, pH 7.5, 5 mmol/l EDTA, 10 mmol/l Na₃PO₄, 100 mmol/l NaF, and 2 mmol/l Na₃VO₄ containing 1% NP-40, 1 mmol/l phenylmethylsulfonyl fluoride, 10 mg/ml aprotinin, and 10 mg/ml leupentin. Tissue lysates were solubilized by continuous stirring for 1 h at 4°C and centrifuged for 10 min at 14,000 g. The supernatants were stored at −70°C until analysis.

Adipose tissue biopsy and preparation of human adipocytes. Adipose tissue was obtained by needle biopsy of the lower subcutaneous abdominal depot using a 5-mm side-cutting needle. Lidocaine (1%) was infiltrated in a square field fashion, and the biopsy was taken from the center of the field. Isolated adipocytes were prepared by a modification of the method of Rodbell (31). After digestion and filtration, the cells were washed twice in a buffer consisting of 150 mmol/l NaCl, 5 mmol/l KCl, 1.2 mmol/l MgSO₄, 1.2 mmol/l CaCl₂, 2.5 mmol/l NaH₂PO₄, 10 mmol/l HEPES, and 2 mmol/l pyruvate, pH 7.4, supplemented with 4% bovine serum albumin. The adipocytes were then washed twice in a buffer [HEPES washing salts (HWS)] consisting of 116 mmol/l NaCl, 5 mmol/l KCl, 0.5 mmol/l MgSO₄, 0.7 mmol/l CaCl₂, 25 mmol/l HEPES, 5 mmol/l NaCl, 2% BSA, pH 7.4, and two more times in BSA-free HWS before protein extraction.

Adipocyte extraction. A twice-concentrated solubilization buffer was added to the concentrated cells; final concentrations were ~20 mmol/l Tris·HCl, 145 mmol/l NaCl, 10% glycerol, 5 mmol/l EDTA, 1% Triton X-100, 0.5% NP-40, 200 μmol/l sodium orthovanadate, 200 μmol/l phenylmethylsulfonyl fluoride, 1 μmol/l leupentin, 1 μmol/l pepstatin, and 10 mg/ml aprotinin, pH 7.5. After lysis/extraction for 30 min at 4°C with repeated vortexing, nonsolubilized material was removed by centrifugation at 14,000 g (10 min, 4°C), and the total cell extracts were stored at −70°C before analysis.

Electrophoresis and Western blotting. The levels of GSK-3 isoforms (α and β) were determined by Western blotting using a monoclonal antibody raised against a peptide with a common sequence for GSK-3α and -β isoforms. Western blotting was performed by the method of Burnette (2). After SDS-PAGE on 10% acrylamide gels, proteins were transferred to nitrocellulose membranes. Nonspecific binding was reduced by incubation in a blocking solution containing Tris-buffered saline, 3% nonfat dry milk, and 0.05% Tween 20. Anti-mouse or anti-rabbit IgG conjugated with horseradish peroxidase was used as a secondary antibody. Proteins were visualized using the SuperSignal Chemiluminescent Substrate kit and exposed to specific supersensitive autoradiograph film (Hyperlamp; Amersham, Arlington Height, IL). Multiple exposures were performed to verify the linearity of the bands. The intensity of the bands was quantified by scanning densitometry using an Alpha Imnotech Multi-Image Light Cabinet and Chemilinger 4000 v.4.04 software (San Leandro, CA), and results were expressed in integrated optical density units [arbitrary units (AU)] per 10 μg total protein.

Changes in the phosphorylation state of GSK-3 were detected using a phosphospecific antibody that interacts with both phosphorylated isoforms of GSK-3. Proteins were visualized and quantified as described above. The proportion of proteins that were phosphorylated were calculated as the ratio of intensity of specific bands after being blotted with phosphospecific antibodies to the intensity of the bands with the same molecular weight after being blotted with an antibody that recognizes both phosphorylated and nonphosphorylated GSK-3. The same membrane was blotted with each of the different antibodies.

Materials. Collagenase was purchased from Worthington (Freehold, NJ). BSA (Cohn fraction V) was obtained from Roche (Indianapolis, IN). Horseradish peroxidase-conjugated anti-mouse and anti-rabbit IgGs were from Amersham, and SuperSignal enhanced chemiluminescence substrate was from Pierce (Rockford, IL). Electrophoresis...
reagents were purchased from Bio-Rad (Richmond, CA). All other chemicals were reagent grade and purchased from Sigma (St. Louis, MO).

Immunoaffinity purified polyclonal IgG against phosphor Ser317/Ser369 GSK-3α/β was obtained from Cell Signaling Technology (Danvers, MA), and anti-GSK-3/3Shaggy protein kinase family (51/46 kDa) mouse monoclonal IgG was purchased from Upstate Biotechnology (Charlottesville, VA).

Statistical analysis. Statistical analysis was performed using Prism 4 (GraphPad Software, San Diego, CA). Statistical significance was evaluated with Student’s t-test and repeated-measures analysis of variance. Paired t-test was employed for pre-/posttreatment comparisons. Data are presented as means ± SE. Significance was accepted at the P < 0.05 level. Because of limitations in tissue availability, not all determinations could be performed in all subjects. The number of subjects studied is given in the table and figure legends.

RESULTS

GSK-3 expression in human adipocytes. Subcutaneous abdominal adipose tissue from 40 subjects (9 HC, 14 OC, 17 OT2D) was studied. Clinical characteristics of these subjects are shown in Table 1. Defects in glycemic control in the OT2D group were demonstrated by increases in fasting glucose, insulin, and Hb A1c levels, and the presence of insulin resistance was established by the lower insulin-stimulated whole body glucose disposal (GDR). The OC group was matched to the OT2D subjects for BMI and was intermediate with regard to insulin action. Protein expression of both isoforms of GSK-3 was similar in adipocytes isolated from the subcutaneous abdominal depot of OT2D, OC, and HC (Fig. 1). This contrasts with our earlier reported observation (26) that both GSK-3α and -β protein were elevated in skeletal muscle from OT2D subjects, whereas HC and OC were similar. There were no correlations between adipocyte GSK-3α or -β protein expression and either GDR, fasting insulin level, or BMI for all of the subjects combined or for each of the groups analyzed separately (not shown). These observations differ from those in skeletal muscle, where there was a statistically significant inverse association between GSK-3 expression and insulin action (GDR) and a positive correlation with fasting insulin levels (26). Adipocyte GSK-3 expression was independent of age (not shown), reducing the impact of the difference in age between the OT2D subjects and the other groups (Table 1) on the end point of interest.

Effects of weight loss. Skeletal muscle and adipose tissue were obtained from six subjects in a weight reduction protocol. Clinical characteristics of these subjects before and after intervention are shown in Table 2. Aspects of this study have been reported earlier (20); the responses to treatment of this subset did not differ from that of the group as a whole. Subjects had a decrease in their BMI of 7% (P < 0.05). With this weight loss there was a significant improvement in insulin action (GDR), even with no significant change in indicators of glucose tolerance. There were tissue-specific differences in the response of GSK-3 to weight loss. In isolated adipocytes, both GSK-3α and -β protein expression were significantly decreased (P < 0.05) after weight loss (Fig. 2A). In contrast, in skeletal muscle there was an increase in both GSK-3α and -β protein expression after weight loss, although the change in GSK-3β did not attain statistical significance (P < 0.1; Fig. 2B). Interestingly, the greater the increase in skeletal muscle GSK-3α or -β protein expression, the smaller the improvement in GDR, a relationship that attained significance for GSK-3β (r = −0.75, P < 0.05).

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>OC</th>
<th>OT2D</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (F/M)</td>
<td>9 (5/4)</td>
<td>14 (6/8)</td>
<td>17 (4/13)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>49±3</td>
<td>44±4</td>
<td>55±2†</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.6±0.7</td>
<td>35.4±1.6†</td>
<td>36.3±2.4‡</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>4.6±0.1</td>
<td>5.1±0.1†</td>
<td>10.7±0.6*</td>
</tr>
<tr>
<td>Fasting insulin, pmol/l</td>
<td>63±15</td>
<td>100±13</td>
<td>210±30*</td>
</tr>
<tr>
<td>Hb A1c, %</td>
<td>5.5±0.2</td>
<td>5.5±0.2</td>
<td>9.1±0.3*</td>
</tr>
<tr>
<td>GDR, mg·m⁻³·min⁻¹</td>
<td>10.45±0.59</td>
<td>8.44±0.58†</td>
<td>6.00±0.63*</td>
</tr>
</tbody>
</table>

Values are means ± SE. HC, healthy control; OC, obese control; OT2D, obese type 2 diabetic; F/M, females/males; BMI, body mass index; Hb A1c, glycosylated hemoglobin; GDR, glucose disposal rate. †P < 0.05 vs. control groups; ‡P < 0.05 vs. HC.

Table 2. Clinical characteristics: effect of weight loss in OC subjects

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-Weight Loss</th>
<th>%Change</th>
</tr>
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<tbody>
<tr>
<td>n (F/M)</td>
<td>6 (3/3)</td>
<td>47±2</td>
<td>35.4±1.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>5.2±0.1</td>
<td>4.9±0.1</td>
<td>96±12</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>5.6±0.2</td>
<td>5.5±0.2</td>
<td>9.7±0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. baseline value for the same subject.
As mentioned earlier, serine phosphorylation of GSK-3 provides a means of acute control of enzyme activity, with this phosphorylation reducing GSK-3 activity (35). There was no change in the phosphorylation of either isoform of GSK-3 in adipocytes after weight loss (Fig. 3A), suggesting a normal specific activity combined with a reduction in enzyme protein. The relative increase in GSK-3 serine phosphorylation in muscle following weight loss (Fig. 3B), although not attaining statistical significance itself (\( P < 0.1 \)), exceeded the relative increase in protein expression for each individual (\( P < 0.05 \) for both \( \alpha \) - and \( \beta \)-isoforms), suggesting a net decrease in skeletal muscle GSK-3 activity.

**Antidiabetic treatment and regulation of GSK-3.** Skeletal muscle and subcutaneous abdominal adipose tissue were obtained from 12 subjects in the antidiabetic medication therapy (6 each on metformin or troglitazone) protocol. Other results from this study have been reported previously (5, 19). The responses to treatment of the present subset did not differ from that of the groups as a whole. Baseline and posttreatment characteristics of these subjects are shown in Table 3. At baseline there were no significant differences between the two groups in age, BMI, fasting glucose, fasting insulin, Hb A1c, or GDR (Table 3). The goal of the intervention was to match the extent of improvement in glycemic control, demonstrated by the fact that posttreatment Hb A1c levels were comparable in the two groups. The crucial differences between treatments are that, even with an improvement in glucose control, subjects treated with metformin did not have significant changes in either BMI or GDR, and the troglitazone treated subjects both gained a modest amount of weight and significantly improved their whole body insulin action (Table 3).

GSK-3 expression in adipocytes from these diabetic subjects prior to treatment (8.08 ± 1.35 and 8.85 ± 1.43 AU/10 μg protein, GSK-3\( \alpha \) , and -\( \beta \), respectively) was much lower than that in skeletal muscle (274 ± 32 and 458 ± 67 AU/10 μg protein).
protein, GSK-3α and -β, respectively) from the same individuals. GSK-3 protein expression in adipocytes was not significantly altered by troglitazone treatment (Fig. 4A). On the other hand, in skeletal muscle GSK-3α and -β protein expression were both lower after treatment with troglitazone (P < 0.05; Fig. 4B). In contrast, there were no significant changes in GSK-3α or -β protein expression in adipocytes (Fig. 4A) or skeletal muscle (Fig. 4B) after treatment with metformin.

Baseline serine phosphorylation of GSK-3 in adipocytes from diabetic subjects (pS-GSK-3/total GSK-3 protein = 2.12 ± 0.92 and 2.78 ± 1.18 for GSK-3α and -β, respectively) was somewhat higher than that in skeletal muscle (1.34 ± 0.27 and 1.00 ± 0.18). Although there was variability in the individual responses, metformin treatment had no significant effect on serine phosphorylation of GSK-3 in either adipocytes (Fig. 5A) or skeletal muscle (Fig. 5B). Troglitazone treatment also failed to significantly alter phosphorylation (Fig. 5) of either form in either tissue.

**DISCUSSION**

GSK-3 is a serine-directed kinase with a broad tissue distribution (13). An uncommon feature of GSK-3 is that it is constitutively active, with phosphorylation of Ser21/9 in the α- and β-isoforms, respectively, reducing activity (35). These sites are direct substrates for Akt, so it is by activation of this
kinase that insulin is able to reduce GSK-3 activity (9). Consistent with its characterization as a "multitasking" kinase (10), inhibition of GSK-3 is being studied as a potential therapeutic approach for type 2 diabetes, neurodegenerative diseases, cancer, and chronic inflammatory disease (8, 17).

Several lines of evidence support an involvement of GSK-3 in insulin resistance. GSK-3 activity has been shown to be elevated in the adipose tissue and skeletal muscle of insulin-resistant animals (11, 12). In obese Zucker rats this involves reduced phosphorylation of GSK-3β (11). Constitutive activation of GSK-3 in vitro (23) or muscle-specific overexpression of GSK-3β (27) resulted in insulin resistance for glucose metabolism. We previously found GSK-3 protein expression and activity to be elevated in the skeletal muscle of poorly controlled type 2 diabetic subjects (26). Other workers found skeletal muscle GSK-3 activity to be comparable in young and elderly twins (29) and normal and type 2 diabetic group (16). Given that we found an inverse correlation between skeletal muscle GSK-3 expression and whole body insulin action (GDR), elevated GSK-3 content may be a more general feature of severe insulin resistance and not exclusively limited to type 2 diabetes. This would be a quantitative difference, as all three reports in human muscle found a normal ability for insulin to acutely suppress GSK-3 activity (16, 26, 29). Perhaps the most consistent evidence for an involvement of GSK-3 with insulin resistance comes from studies with inhibitors of the enzyme. A number of chemically distinct compounds, including lithium, malemides, aminopyridine derivatives, and phosphopeptide pseudosubstrates, have been shown to be selective inhibitors of GSK-3 (reviewed in Refs. 8 and 38). There are numerous reports of both acute and chronic exposure to these agents resulting in stimulation of glycogen synthase and improving insulin action. Effects are seen both in vivo, in diabetic animal models (6, 18, 30), and in vitro (7, 14, 24, 28), including in human muscle cells (25).

Another feature commonly associated with insulin resistance is obesity, independent of the presence of diabetes, and it is interesting to note that GSK-3 plays an obligatory role in adipogenesis, because inhibition of GSK-3, either chemically (1, 33) or by constitutive activation of Wnt signaling (22), prevents adipocyte differentiation. As mentioned earlier, GSK-3 expression is elevated in the adipose tissue of obese, insulin-resistant animals (12). In addition, skeletal muscle-specific overexpression of GSK-3β results in animals with increased fat mass (27). Considered together, this information would suggest that augmented GSK-3 activity could contribute to obesity.

Given the potential for the involvement with obesity and insulin resistance, several key aspects of the metabolic syndrome, we have extended our studies of GSK-3 to human subcutaneous adipose tissue and found several differences between adipose tissue and skeletal muscle. Protein expression of GSK-3 in adipocytes is 2–5% of that in skeletal muscle. Unlike what we found in skeletal muscle, there were no differences in adipose tissue expression between groups differing in adiposity and insulin sensitivity. There were also no associations between GSK-3 in adipocytes with GDR (unlike in muscle) or BMI. The former is not surprising because the major portion of insulin-stimulated glucose disposal occurs in skeletal muscle.

To understand more about the potential involvement of GSK-3 in obesity and insulin resistance, we studied the effects of interventions with differing effects on body weight and insulin action. Between the low-calorie diet, metformin, and troglitazone treatments we would see a decrease, no change, and increase in body weight while having quantitatively different impacts on insulin action. This approach should aid us in trying to dissociate the two factors. The results in adipocytes indicated that, in at least one case, GSK-3 changed in the same direction as BMI, falling with weight loss. That the magnitude of the changes in GSK-3 were small could reflect the modest average changes in BMI in response to treatments, −7 to +4%.

Given the heterogeneity of adipose tissue from different depots (21), the present results are applicable for only the subcutaneous depot and not visceral adipose tissue. Interestingly, thiazolidinedione treatment has been associated with reductions in visceral adipose tissue mass and gains in the subcutaneous depot (15). Changes in adipocyte GSK-3 expression were independent of those in insulin sensitivity. Regulation of GSK-3 in the adipocyte appears to occur at the level of translation, as serine phosphorylation in the basal state was unaltered by any of the treatments.

Different mechanisms for the regulation of GSK-3 appear to function in skeletal muscle, where opposing changes in protein expression and phosphorylation would result in no change, or a small drop, in total GSK-3 activity with weight loss. Meanwhile, chronic troglitazone treatment worked on protein expression without changing phosphorylation. The consequence of both insulin-sensitizing treatments would be to reduce GSK-3 activity in skeletal muscle, but by different mechanisms. Meanwhile, these responses would be independent of the opposing changes in BMI to the two treatments. The lack of response to metformin for either protein expression or phosphorylation would be consistent with what we found previously concerning regulation of skeletal muscle phosphatidylinositol 3-kinase in these same subjects (19), further proof of differing mechanisms and sites of action for the two drugs.

The factors responsible for regulation of GSK-3 expression are uncertain. Although GSK-3 expression and activity in skeletal muscle are positively associated with fasting insulin levels (26), in the present study there were no correlations between changes in muscle GSK-3α or -β expression and changes in insulin levels. That may be due in part to the modest changes in fasting insulin levels with either troglitazone or metformin treatment. Information about the regulation of GSK-3 in humans is limited. Acute bouts of exercise have been shown to both increase (39) and decrease (34) GSK-3α activity in muscle. Differences in the duration and intensity of the exercise may be important variables. Prolonged (2 yr) treatment of obese impaired glucose tolerance subjects with a combined dietary/exercise regimen resulted in a reduction in skeletal muscle GSK-3 expression in a subgroup classified as having a higher proportion of myosin heavy-chain 1 isoform (37). Interestingly, that subgroup also had the greatest improvement in insulin resistance (37). The study design did not permit evaluation of the independent impacts of exercise and diet, but the results do support the concept that improvements in insulin action can be associated, at least in some instances, with reductions in GSK-3 expression and/or activity in muscle.

Although augmented serine phosphorylation of GSK-3 represents a mechanism for the acute control of GSK-3 activity in...
response to hormones such as insulin (9), as well as exercise (34), it does not appear to play a major role in the response to more chronic interventions. The one exception may be in skeletal muscle after weight loss, where the inactivating phosphorylation would counterbalance the increased protein expression. Rather, for the most part, it appears that changes in protein expression represent the primary means for chronic regulation of GSK-3.

The major finding of the present work is that there is tissue specificity in the regulation of GSK-3 expression in human tissues in response to in vivo interventions that may provide insight into the roles of GSK-3 in different tissues. Changes in GSK-3 in skeletal muscle occur in the opposite direction from those in insulin action on whole body glucose disposal; insulin action goes up as GSK-3 goes down. These changes are independent of changes in BMI. Conversely, alterations in GSK-3 in adipocytes have more in common with changes in BMI. These responses suggest that therapeutic interventions targeting GSK-3 could result in improvements in both skeletal muscle insulin action and reducing adiposity.

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