Impaired expression and insulin-stimulated phosphorylation of Akt-2 in muscle of obese patients with atypical diabetes

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Submitted 27 October 2003; accepted in final form 27 February 2004

Gosmanov, Aidar R., Guillermo E. Umpierrez, Ana H. Karabell, Ruben Cuervo, and Donald B. Thomason. Impaired expression and insulin-stimulated phosphorylation of Akt-2 in muscle of obese patients with atypical diabetes. Am J Physiol Endocrinol Metab 287: E8–E15, 2004. First published March 9, 2004; 10.1152/ajpendo.00485.2003.—Although a pharmacological dose of insulin produces a dramatic increase in phosphorylation and activity of Akt isoforms 1 and 2 in mammalian skeletal muscle, few studies have examined the effect of physiological concentrations of insulin on the phosphorylation of Akt-1 and -2 in normal and diabetic tissue. This study examined the patterns of insulin-stimulated Akt isoform phosphorylation and protein expression in muscle biopsies obtained from obese patients with atypical diabetes immediately after presentation of severe hyperglycemia; Akt-1; insulin receptor; insulin receptor substrate; glucose transporter 4

IN SKELETAL MUSCLE, the acute action of insulin results in stimulation of glucose uptake and glycogen synthesis (4, 11, 18, 27, 32, 40). Insulin exerts its biological effects by sequential activation of a cascade of signaling molecules (22, 45). Insulin binds to its receptor, which in turn leads to receptor autophosphorylation and activation of several docking proteins containing a phosphotyrosine-binding domain, including insulin receptor substrates (IRS) 1–4. In mammalian skeletal muscle cells, it has been shown that IRS-1/2 proteins are indispensable for the insulin effects on glucose utilization (39, 48). Tyrosine phosphorylation of IRS-1/2 allows interaction with and activation of phosphatidylinositol 3-kinase (PI3K). Phosphatidylinositol 3,4,5-triphosphate (PIP3) production subsequently recruits the serine-threonine kinase Akt (protein kinase B) and its activating kinase 3-phosphoinositide-dependent protein kinase-1 (PDK-1) to the membrane to initiate activation of Akt (7). Full activation of Akt occurs only when Akt is dually phosphorylated on a threonine residue by PDK-1 and a serine residue by a putative PDK-2 (or kinase complex) that has yet to be fully characterized (2, 17, 42). Akt activation is required and may be sufficient for stimulation of glucose transport and glycogen accumulation in skeletal muscle cells (2, 4, 14, 47). In mammalian tissues, there are three Akt isoforms (Akt-1, -2, and -3), encoded by distinct genes. Insulin action in muscle predominantly involves Akt-1 and Akt-2 stimulation (25, 43, 46), and Akt-2 knockout mice have impaired glucose homeostasis, a diabetes-like syndrome (4, 5).

It is known that non-insulin-dependent diabetes mellitus (type 2 diabetes mellitus) is accompanied by a diminished ability of insulin to activate Akt (3, 29, 37), whereas insulin-sensitizing drugs improve insulin-stimulated Akt activity (24, 34). Hyperglycemia is now recognized as a deleterious factor that can lead to insulin resistance independent of genetic background (9, 28, 35, 45, 51). In the case of skeletal muscle, there is evidence that hyperglycemia by itself may decrease Akt activation (12, 30). Furthermore, experimental data demonstrate that hyperglycemia can cause alterations in the profile of cell protein expression such that insulin-counterregulatory mechanisms could prevail (9). Therefore, the correlative ties between muscle Akt signaling and patterns of protein expression during hyperglycemia are of great interest. One model in which to study the effects of hyperglycemia is a unique form of type 2 diabetes mellitus called atypical type 2 diabetes. This form of diabetes is a common clinical presentation involving ~50% of adult African Americans (and, increasingly, Hispanic and Native Americans) with obesity and newly diagnosed diabetes who present with ketosis and/or severe hyperglycemia. Although these patients are severely insulin resistant and insulinopenic on presentation, with intensive insulin therapy the majority of patients achieve near-normoglycemic remission within ~10–12 wk, become more insulin sensitive, and do not
require exogenous insulin (1, 33, 44). Therefore, an analysis of muscle Akt activation in these patients at presentation and remission provides an ideal opportunity to examine the association of hyperglycemia with Akt isoform activation.

In this study, we investigated the effect of insulin at physiological concentrations on Akt-1 and Akt-2 phosphorylation in muscle biopsies obtained from obese patients with atypical diabetes immediately following a hyperglycemic crisis and again following near-normoglycemic remission. Our experiments demonstrated that hyperglycemia is associated with a decrease in muscle Akt-2 expression and insulin-stimulated phosphorylation on the serine residue, without affecting threonine phosphorylation. Furthermore, data from antibody arrays comparing protein expression at patient presentation relative to near-normoglycemic remission indicated a significant correlation between Akt-2 expression and Ser476 phosphorylation with a number of proteins, including several that may counteract insulin action in mammalian tissues.

**MATERIALS AND METHODS**

**Materials.** Insulin was purchased from Sigma Chemical (St. Louis, MO). Phosphospecific antibodies to Akt on Thr178, Thr240/242, and Ser385, and an anti-total-Akt antibody were purchased from Cell Signaling Technology (Beverly, MA). Antibodies to phosphotyrosine (PY-99, sc-7020), insulin receptor β-subunit (sc-711), IRS-1 (sc-7200), IRS-2 (sc-8299), Akt-1 (sc-1618), Akt-2 (sc-5270), GLUT4 (sc-7938), extracellular signal-regulated kinase-1 (ERK1; sc-94-G), ERK2 (sc-153), and p38 (sc-353) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody Microarray 380 (catalog no. K1847–1) was purchased from BD Biosciences (Palo Alto, CA). All other chemicals were from Sigma Chemical.

**Human subjects and muscle biopsy.** The study group included four obese (2 males and 2 females) African American patients with new onset of diabetes mellitus who presented in hospital with severe hyperglycemia (glucose >400 mM). Obesity was defined as body mass index (BMI) >28 kg/m². The study protocol was approved by the Institutional Review Board of University of Tennessee Health Science Center and performed in the General Clinical Research Center. Written informed consent was obtained from all subjects before participation. Percutaneous needle biopsy of vastus lateralis muscle was performed on two occasions: 24–48 h after presentation with hyperglycemic crisis and 1 wk after discontinuation of insulin treatment during near-normoglycemic remission (~10 wk later). Biopsies were obtained from patients after a 12-h fast. To obtain a biopsy, the skin midway between the patella and the greater trochanter at the anterior border of the iliotibial band was shaved and cleaned with an antiseptic soap and an iodine swab. The skin over the muscle designated as the treatment control was similarly incubated but without insulin. Thereafter, the muscle was quickly washed, blotted, snap-frozen in liquid nitrogen, and stored at −80°C until further analysis. The muscle was homogenized in solubilization buffer at 4°C, as previously described (13), and centrifuged at 15,000 × g for 10 min. The protein concentration of the supernatant was measured in triplicate with the bicinchoninic acid assay (Pierce Biotechnology, Rockford, IL).

**Immunoprecipitation and Western blotting.** To measure tyrosine phosphorylation of the insulin receptor (InsR), 1,000 μg of protein from muscle lysates were first incubated with anti-InsR antibody for 2 h at 4°C and then subjected to overnight immunoprecipitation with protein A-agarose beads. The immune complexes were washed three times with lysis buffer, mixed with SDS denaturing buffer, warmed to 95°C for 5 min, electrophoresed on a 6% SDS-PAGE gel, and electroblotted onto polyvinylidene difluoride (PVDF) membranes. Tyrosine-phosphorylated proteins were visualized by incubation of membranes with phosphotyrosine PY-99 and horseradish peroxidase (HRP)-conjugated anti-mouse antibodies in blocking buffer (1.5 mM NaH2PO4, 8 mM Na2HPO4, 0.15 M NaCl, 0.3% Triton X-100, pH 7.4) containing 1% BSA at room temperature, followed by chemiluminescent exposure of X-ray film (ECL Plus). By reprobing of the membranes with anti-InsR, the antibody measured the total amount of corresponding protein. Bands were quantitated by video densitometry. Protein phosphorylation was calculated as the ratio of phosphorylated (phospho) to total protein expression, normalized to a mean basal value (no insulin stimulation) of 1.0.

To determine phosphorylation and abundance of Akt in whole muscle lysate, equal amounts of protein were resolved by 10% SDS-PAGE, transferred to PVDF membranes, and blocked overnight in 3% BSA blocking buffer. The membranes were incubated at room temperature with anti-phospho-Akt-Thr308/320 antibody and HRP-conjugated anti-rabbit IgG, followed by chemiluminescent exposure of X-ray film. The same membranes were then stripped two times to determine total Akt expression and phospho-Akt-Ser473/474 phosphorylation by use of anti-total-Akt and anti-phospho-Akt-Ser473/474 antibodies, respectively. The antibodies used in these experiments recognized the phosphorylation status and expression of all Akt isoforms. Bands were quantitated by video densitometry. Protein phosphorylation was calculated as the ratio of phosphorylated to total protein expression, normalized to a mean basal value of 1.0. GLUT4 abundance in whole muscle lysate was similarly determined by Western blot analysis.

To measure Akt-1 and Akt-2 phosphorylation, muscle lysates were immunoprecipitated with Akt-1 or Akt-2 isoform-specific antibodies, respectively, for 2 h at 4°C, followed by overnight incubation with protein A-agarose beads. After being washed, immune complexes were resolved on 10% SDS-PAGE and electroblotted onto PVDF membranes. Akt-1 and Akt-2 phosphorylation on threonine (Thr308 for Akt-1 and Thr320 for Akt-2) or serine (Ser473 for Akt-1 and Ser474 for Akt-2) was determined, as described above. Specificity of the Akt-1 and the Akt-2 immunoprecipitation was determined by testing for cross-reactivity of the immunoprecipitates. No cross-reactivity was detected.

**Antibody array analysis.** Antibody arrays from BD Biosciences (lot no. 2090032) were prepared according to product instructions. Briefly, 1,000-μg aliquots of protein lysate from muscle biopsies taken at presentation and at remission were each labeled with Cy3 and Cy5 dyes (Amersham Biosciences, Piscataway, NJ). Unbound dye was removed from the samples by filtration through PD-10 columns (Amersham Biosciences). Labeling efficiency was estimated from dye absorbance and was typically 2.5–3 molecules of dye per protein molecule. Cy3 dye-labeled protein (100 μg) from the presentation sample was mixed with 100 μg of Cy5 dye-labeled protein from the
remission sample. Similarly, 100 μg of Cy5 dye-labeled protein from the presentation sample was mixed with 100 μg of Cy3 dye-labeled protein from the remission sample. Each of these two mixes was incubated separately with antibody microarray slides for 30 min and washed. The slides were dried and analyzed immediately in a Scan-Array 5000XL confocal laser scanner. To correct for differences in dye labeling efficiency, we calculated for each sample an internally normalized ratio [the square root of the ratio of the Cy5 (presentation)/Cy3 (remission) fluorescence from one slide and Cy5 (presentation)/Cy3 (presentation) fluorescence from the other slide] for each of the duplicate antibody-antigen spots on each array. The internally normalized ratio is the expression of the antigen at presentation relative to remission. Data are deposited in the Gene Expression Omnibus repository at http://www.ncbi.nlm.nih.gov/geo, series record GSE634.

Statistics. Comparisons within and among groups for insulin-stimulated phosphorylation and expression were made by analysis of variance. Differences were considered significant at P < 0.05. Data are reported as means ± SE. Standard errors that were too small to be distinguished from the data points were not plotted. For differences that were not significant, the power of these tests was typically >0.85. Overrepresentation of molecular function gene ontologies was calculated using the EASE program (16).

RESULTS

Insulin-stimulated phosphorylation of total Akt, Akt-1, and Akt-2 in muscle biopsies of patients with severe hyperglycemia.

The clinical characteristics and selected metabolic parameters of study participants are shown in Table 1. Middle aged, obese (BMI > 28 kg/m²) subjects presenting with severe hyperglycemia were involved in the study (mean fasting plasma glucose 30.5 ± 4.8 mM or 550 ± 86.5 mg/dl). After ~10 wk of treatment with subcutaneous insulin and ≥1 wk of discontinuation of insulin therapy, fasting blood glucose significantly decreased to near-normoglycemic values. BMI and plasma insulin did not change during treatment.

Muscle biopsies obtained at presentation with severe hyperglycemia and following near-normoglycemic remission were incubated in the absence or presence of 100 μU/ml insulin. To ensure that the insulin effect on Akt signaling was associated with canonical activation of insulin receptor tyrosine phosphorylation, muscle lysates of three patients were immunoprecipitated with InsR antibody. We found a similar threefold stimulation of InsR tyrosine phosphorylation by insulin at presentation and near-normoglycemic remission (Fig. 1). Regardless of glycemic status, insulin increased Thr308/309 phosphorylation of total Akt 2.2- to 2.5-fold (Fig. 2). Consistent with these results, insulin increased Akt-1 and Akt-2 phosphorylation on Thr308/309 at presentation and remission (Fig. 2). In contrast, insulin did not stimulate Ser474 phosphorylation of total Akt at presentation, although insulin-stimulated phosphorylation tended to be greater at remission (Fig. 2). It is possible to compare the presentation values with the remission values only for a particular residue of an individual isoform, because different combinations of antibodies (which have different avidities of their immune complexes) were used to detect the Akt isoforms and their phosphorylated residues. Assessing the contribution of the Akt isoforms to total Akt phosphorylation on serine, we found that Akt-1 phosphorylation on Ser473 was stimulated by insulin during the hyperglycemic period and remission. In contrast, insulin was not able to increase Akt-2 phosphorylation on Ser474 at presentation with hyperglycemia. However, in near-normoglycemic remission, insulin caused a marked elevation of Akt-2 phosphorylation (Fig. 2). In addition, Akt expression was 69% higher at remission (P < 0.05; Fig. 2). This elevation in Akt expression at remission appeared to be largely due to a 94% increase in Akt-2 abundance (Fig. 2). Because of the improved Akt expression at remission, we asked whether there was a pattern of signal transduction protein expression associated with the severe hyperglycemia.

Pattern of protein expression in muscle biopsies of patients with severe hyperglycemia.

We used antibody arrays to measure the expression of 380 proteins in muscle biopsies taken at presentation and remission. For selected proteins (InsR, GLUT4, ERK1/2, p38 MAPK), simultaneous Western blot analysis was used to confirm the antibody array data and to provide additional data; there was significant correspondence between data obtained by Western blot and antibody array (P < 0.001, contingency coefficient = 0.775). The expression of each protein at presentation was normalized to its expression
at remission (a value of <1 represents decreased expression at presentation). There were no differences between muscle samples obtained at presentation and remission in the expression of InsR, IRS-1, and PI3K (Fig. 3). GLUT4 abundance trended lower (P = 0.06) at presentation (Fig. 3) but not to the same extent as the decreased Akt-2 expression. The expression of a significant number of proteins was correlated among patients, with a distinct trend toward decreased expression at presentation (shown for 2 patients in Fig. 3). A subset of 64 proteins was identified whose expression was decreased at presentation by ≥10% and whose range of relative expression (presentation/remission) among the patients was <15% (Fig. 3). Among these proteins that had decreased expression at presentation, molecular function gene ontologies comprising intracellular signaling were overrepresented (on the basis of their expected frequency of occurrence within the entire array and their observed occurrence within the subset of proteins; Fig. 4). The decreased expression of specific proteins at presentation was apparently not a generalized catabolism of muscle protein,
because the expression of many proteins was unchanged (as exemplified by the expression of InsR, IRS-1, and PI3K) or increased (Fig. 3); the protein mass profiles (determined by surface-enhanced laser desorption/ionization, or SELDI) were also not different between presentation and remission (not shown). To determine which proteins were associated with the decreased Akt-2 expression and activation at presentation, we calculated the correlation coefficient for the relative expression of specific proteins (presentation/remission) vs. relative Akt-2 expression or insulin-stimulated Akt-2 Ser474 phosphorylation (presentation/remission) (Table 2). Consistent with the over-representation of signal transduction functional ontologies among the proteins whose expression was decreased at presentation, there was significant correlation between Akt-2 expression and insulin-stimulated Ser474 phosphorylation at presentation and the expression of many signal transduction proteins associated with insulin signaling $(P < 0.05)$. At this level of significance, only 19 proteins would have been expected to correlate with Akt-2 expression or insulin-stimulated Ser474 phosphorylation through random variation.

**DISCUSSION**

A hallmark of insulin action in skeletal muscle is stimulation of Akt phosphorylation and activity. Akt activation appears to be necessary for many of insulin’s biological effects (reviewed in Refs. 2, 15, 22, 31). Three Akt proteins, Akt-1, -2, and -3, have been identified in mammalian cells, including muscle cells (46). An emerging body of evidence demonstrates that Akt isoforms are differentially activated by insulin (25, 43, 46). Furthermore, it has been shown that the absence of Akt-2 in skeletal muscle of mice leads to impaired insulin-mediated glucose uptake, thereby contributing to the development of a diabetes-like syndrome (4, 5). Recently, Jiang et al. (21) demonstrated that partially depleting Akt-2 in 3T3-L1 adipocytes with small interfering RNA significantly diminishes insulin-stimulated hexose transport and glycogen synthase kinase-3 phosphorylation; almost complete Akt-1 depletion had much less effect (21), indicating a pivotal role for Akt-2 in insulin responsiveness. The data presented here show that both Akt-1 and Akt-2 can be fully phosphorylated by a physiological dose of insulin in the muscle of normoglycemic humans. However, diminished Akt-2 expression and insulin-stimulated phosphorylation of Akt-2 on Ser474 is associated with hyperglycemia in diabetic humans.

It is well documented that a physiological dose of insulin (100 $\mu$U/ml, or 0.6 nM) stimulates glucose uptake in human and rodent skeletal muscle ex vivo (4, 11, 18, 27, 32, 40). We verified that in human muscle this concentration of insulin activated the proximal element of insulin signaling, InsR (Fig. 1). The recruitment and subsequent activation of PI3K by IRS-1 and -2 result in generation of PIP3, which, in turn, leads to translocation of Akt from the cytosol to the plasma membrane and activation of PDK-1 (7). Akt is fully active only when it is dually phosphorylated on the Thr308 residues by PDK-1 and on Ser473/474. The kinase responsible for Ser473/474 phosphorylation is still elusive. Although there is evidence arguing for either PDK-1 or autophosphorylation (2), recent data indicate the existence of a distinct PDK-2 activity that appears to be associated with the cytoskeleton and to involve the integrin-linked kinase (17, 42). In previous studies, we found that 100 $\mu$U/ml insulin is effective in Akt phosphorylation in rat skeletal muscle (13). However, Akt phosphorylation by physiological insulin concentration was very different in muscle from diabetic patients with severe hyperglycemia (Fig. 2).

Hyperglycemia may contribute to the development and progression of diabetes mellitus by directly diminishing the insulin responsiveness of skeletal muscle (45, 51). Indeed, correction of hyperglycemia in diabetic rodents and humans improves skeletal muscle insulin sensitivity in vivo and in vitro (10, 38, 49, 50). Diminished Akt activation has been proposed as a probable mechanism for hyperglycemia-induced insulin resistance in skeletal muscle (29, 41, 51). Hyperglycemia in concentrations up to 30 mM can directly decrease insulin-induced Akt phosphorylation on Ser473/474 in rat skeletal muscle (30, 35, 37). Previously, there was no evidence for an effect of hyperglycemia on Akt isoform phosphorylation in muscle of patients with diabetes mellitus. Atypical type 2 diabetes are an ideal population to test for an effect of hyperglycemia on muscle Akt activation, because the majority of newly diagnosed patients achieve near-normoglycemic remission (1, 33, 44). Because of their clinical course, the presence of pancreatic insulin reserve at presentation and remission, and the absence of autoimmune markers, such patients are recognized as having an atypical type 2 diabetes. Importantly, in these patients hyperglycemic crisis is associated with severe insulin resistance, whereas restoration of blood glucose level increases peripheral insulin sensitivity. Here, we show that overt hyperglycemia is associated with decreased stimulation of Akt serine phosphorylation by a physiological concentration of insulin without changes in Akt threonine phosphorylation (Fig. 2). Intriguingly, only Akt-2-Ser474 phosphorylation was impaired by the hyperglycemia, whereas physiological insulin was able to stimulate Akt-1 phosphorylation regardless of the patient’s glycemic status (Fig. 2). Although the data were obtained from four patients, the latter result is consistent with the reports that Akt-1 activation by a physiological concentration of insulin is
not impaired in the muscle of mildly hyperglycemic diabetic patients and diabetic rats (29, 40). In both of these studies, insulin-stimulated glucose transport by muscle was reduced despite normal Akt-1 activation (29, 40), in accord with the previous reports (30, 37, 40). Instead, our data indicate that Akt-2 phosphorylation on Ser 474 was impaired during hyperglycemia is not clear. Glucose, its metabolite glucosamine, and oxidative byproducts are powerful modulators of signal transduction and gene expression (6, 26, 36). Our data indicate that alterations in upstream insulin signaling are not involved, because InsR phosphorylation and expression of InsR, IRS-1/2, and PI3K were unchanged (Fig. 3), consistent with previous reports (30, 37, 40). Instead, our findings demonstrate that Akt-2 phosphorylation on Ser 474 was impaired during the hyperglycemic episode (Fig. 2). The impaired phosphorylation of Akt-2 on Ser 474 was associated with changes in the expression of specific proteins (Figs. 3 and 4; Table 2), including decreased expression of Akt-2 during hyperglycemia (Table 2). Prominent among these were PKC-ε, PKC-θ, and the NF-κB-inhibitory kinase IKKα. Hyperglycemia and free fatty acids (FFAs) can increase synthesis of diacylglycerol, a cofactor for activation of conventional and novel PKCs (19). Treatment of skeletal muscle strips from obese, insulin-resistant patients with pharmacological inhibitors of conventional and novel PKCs causes significant augmentation of insulin-

### Table 2. Relative expression of specific proteins in muscle samples (presentation/remission) correlated with relative Akt-2 expression and insulin-stimulated Akt-2 Ser474 phosphorylation (presentation/remission)

<table>
<thead>
<tr>
<th>Positive</th>
<th>Correlation with Akt-2 Expression</th>
<th>Correlation with Insulin-Stimulated Akt-2 Ser474 Phosphorylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldehyde dehydrogenase 1A1</td>
<td>0.964</td>
<td>Actin filament-associated protein</td>
</tr>
<tr>
<td>Amphiphysin</td>
<td>0.980</td>
<td>AMP kinase-β</td>
</tr>
<tr>
<td>DNA polymerase e</td>
<td>0.980</td>
<td>Ankyrin B</td>
</tr>
<tr>
<td>erbB-2</td>
<td>0.958</td>
<td>Bridging integrator 1</td>
</tr>
<tr>
<td>Xeroderma pigmentosum D</td>
<td>0.982</td>
<td>Breast carcinoma amplified protein 1</td>
</tr>
<tr>
<td>Calcinurin</td>
<td>0.963</td>
<td>Calcinexin</td>
</tr>
<tr>
<td>Akt-2</td>
<td>0.994</td>
<td>CD3 antigen-ζ</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Negative</th>
<th>Correlation with Akt-2 Expression</th>
<th>Correlation with Insulin-Stimulated Akt-2 Ser474 Phosphorylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adapin g</td>
<td>0.959</td>
<td>c-myc</td>
</tr>
<tr>
<td>Annexin XI</td>
<td>0.993</td>
<td>DNA fragmentation factor 45 (ICAD)</td>
</tr>
<tr>
<td>Retinoblastoma binding protein 9</td>
<td>0.978</td>
<td>DnaJ homolog A1</td>
</tr>
<tr>
<td>Calretinin</td>
<td>0.967</td>
<td>Eg5</td>
</tr>
<tr>
<td>c-src Tyrosine kinase</td>
<td>0.964</td>
<td>elf-5</td>
</tr>
<tr>
<td>elf-4E</td>
<td>0.991</td>
<td>Endoglin</td>
</tr>
<tr>
<td>Heme oxygenase 1</td>
<td>0.994</td>
<td>MAPK1</td>
</tr>
<tr>
<td>IKKα/IKKβ</td>
<td>0.994</td>
<td>MAPK3</td>
</tr>
<tr>
<td>MAP3K</td>
<td>0.984</td>
<td>MAP3K8</td>
</tr>
<tr>
<td>M33 (chronobox homolog 2)</td>
<td>0.975</td>
<td>GABA br2 (G protein-coupled receptor 51)</td>
</tr>
<tr>
<td>Multiple PDZ domain protein 1</td>
<td>0.999</td>
<td>Neurexin 1</td>
</tr>
<tr>
<td>Peroxisome biogenesis factor 1</td>
<td>0.969</td>
<td>PIP5K type 1γ</td>
</tr>
<tr>
<td>PKC-θ</td>
<td>0.990</td>
<td>Annexin II</td>
</tr>
<tr>
<td>Synaptonemal complex protein 3</td>
<td>0.973</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>Serotonin receptor (5-HT2AR)</td>
<td>0.981</td>
<td>Cytokine-inducible kinase (Fnk)</td>
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<tr>
<td>Ser-Arg protein kinase 1</td>
<td>0.993</td>
<td>LIM domain binding 3</td>
</tr>
<tr>
<td>Stat6</td>
<td>0.994</td>
<td>PKC-ε</td>
</tr>
<tr>
<td>Tat (HIV)-specific factor</td>
<td>0.971</td>
<td>Polo-like kinase 1</td>
</tr>
<tr>
<td>Zinc finger protein 148</td>
<td>0.953</td>
<td>Transcription elongation factor</td>
</tr>
</tbody>
</table>

Only significant correlations are shown. Significance of the correlation was tested at $P < 0.05$ for 380 proteins.
mediated glucose uptake (8). FFA infusion can also induce insulin resistance, apparently through activation of inhibitory kinases of NF-κB (20). In mice lacking the IKKβ isoform, FFA infusion does not induce insulin resistance (23). Together, these data indicate that impaired glucose utilization by patients with atypical diabetes (44) is associated with a combination of factors that could contribute to insulin resistance: decreased Akt-2 expression, decreased Akt-2 phosphorylation, decreased GLUT4 expression, and expression of mechanisms counterregulatory to insulin action. The interdependencies among all of these factors will have to be determined by future studies.

In summary, we found that there was no association between glycemic status and Akt-1 expression or insulin-stimulated Akt-1 phosphorylation in muscle biopsies obtained from diabetic patients during hyperglycemic crisis and upon near-normoglycemic remission. However, a physiological concentration of insulin was unable to induce Akt-2 phosphorylation on Ser473 during the hyperglycemic period. In addition, the hyperglycemia was associated with decreased Akt-2 protein expression and increased expression of proteins with specific or potential counterregulatory roles for insulin signaling. Together, these data indicate that correction of hyperglycemia is associated with increased responsiveness of Akt-2 to physiological concentrations of insulin.

ACKNOWLEDGMENTS

We are grateful to L. A. Malinick for assistance with publication graphics. The assistance of National Eye Institute Vision Core (PHS 3P03 EY-13080) for antibody array scanning is greatly appreciated.

GRANTS

The research was supported, in part, by General Clinical Research Center Grant M01-RR-00211 and the American Diabetes Association (to G. E. Umpierrez).

REFERENCES