Decreased Akt kinase activity and insulin resistance in C57BL/KsJ-Lepr<sup>db/db</sup> mice

J Shao, H Yamashita, L Qiao and J E Friedman

Department of Nutrition, Case Western Reserve University School of Medicine, 10900 Euclid Avenue, Cleveland, Ohio 44106, USA
(Requests for offprints should be addressed to J E Friedman; Email: jed.friedman@uchsc.edu)

Abstract

Recent studies suggest that the serine/threonine kinase protein kinase B (PKB or Akt) is involved in the pathway for insulin-stimulated glucose transporter 4 (GLUT4) translocation and glucose uptake. In this study we examined the components of the Akt signaling pathway in skeletal muscle and adipose tissue in vivo from C57BL/KsJ-Lepr<sup>db/db</sup> mice (db/db), a model of obesity, insulin resistance, and type II diabetes. There were no changes in the protein levels of GLUT4, p85α, or Akt in tissues from db/db mice compared with non-diabetic littermate controls (+/+). In response to acute insulin administration, GLUT4 recruitment to the plasma membrane increased twofold in muscle and adipose tissue from +/+ mice, but was significantly reduced by 42–43% (P<0·05) in both tissues from db/db mice. Insulin increased Akt-Ser<sup>473</sup> phosphorylation by two- to fivefold in muscle and adipose tissue from all mice. However, in db/db mice, maximal Akt-Ser<sup>473</sup> phosphorylation was decreased by 32% (P<0·05) and 69% (P<0·05) in muscle and adipose tissue respectively. This decreased phosphorylation in db/db mice corresponded with a significant decrease in maximal Akt kinase activity using a glycogen synthase kinase-3 fusion protein as a substrate (P<0·05). The level of insulin-stimulated tyrosine phosphorylation of p85α from phosphatidylinositol 3 (PI 3)-kinase, which is upstream of Akt, was also reduced in muscle and adipose tissue from db/db mice (P<0·05); however, there was no change in extracellular signal-regulated kinase-1 or -2 phosphorylation. These data implicate decreased insulin-stimulated Akt kinase activity as an important component underlying impaired GLUT4 translocation and insulin resistance in tissues from db/db mice. However, impaired insulin signal transduction appears to be specific for the PI 3-kinase pathway of insulin signaling, while the MAP kinase pathway remained intact.


Introduction

Type II diabetes is characterized by hyperglycemia and insulin resistance to glucose uptake in peripheral tissues, especially skeletal muscle and adipose tissue (Kahn 1998). The mechanisms underlying insulin resistance in type II diabetes are not well understood. Normally, insulin stimulates glucose uptake by recruitment of insulin-sensitive glucose transporters, particularly glucose transporter 4 (GLUT4), from intracellular vesicular compartments to the plasma membrane. A cascade of signaling events is required for this process (Czech & Corvera 1999). There is a body of research indicating that the phosphatidylinositol 3 (PI 3)-kinase is necessary for insulin-stimulated glucose transport as well as for glycogen synthesis and protein synthesis (White & Kahn 1994, Shepherd et al. 1998). However, the signaling pathways downstream from PI 3-kinase leading to GLUT4 recruitment are just beginning to be understood.

Recently, protein kinase B (PKB or Akt) has been shown to function in the insulin-signaling cascade (Burgering & Coifer 1995, Franke et al. 1995, Datta et al. 1996). In vitro studies indicate that Akt can bind to GLUT4-containing vesicles and mediate insulin-stimulated glucose transport (Kohn et al. 1996a, Cong et al. 1997, Tanti et al. 1997, Mónica et al. 1998, Kupriyanova & Kandror 1999). In adipose and muscle cells, Akt over-expression results in increased GLUT4 translocation and glucose uptake (Kohn et al. 1996b, Cong et al. 1997, Wang et al. 1999). Likewise, expression of constitutively active forms of PKB in adipocytes and muscle cells induced 2-deoxy-d-glucose uptake even in the absence of insulin (Ueki et al. 1998). Conversely, microinjection of Akt substrate or anti-Akt antibody into cells reduced insulin-stimulated GLUT4 translocation to the plasma membrane by 66 or 55% (Hill et al. 1999). These studies support a role for Akt in insulin-stimulated glucose transport in adipose tissue and muscle cells in culture.

Akt is a serine/threonine kinase and has three isoforms. Akt-1 and -2 are activated by insulin and growth factors, and the activation by insulin is prevented by inhibitors of
PI 3-kinase (Franke et al. 1995, 1997, Kohn et al. 1996a). Akt-1 and Akt-2 are the main isoforms of Akt activated by insulin in muscle, hepatocytes, and adipocytes (Mónica et al. 1998, Walker et al. 1998). Akt-3 is not activated by insulin in muscle and adipose tissue. Wortmannin, a PI 3-kinase inhibitor, not only prevents the activation of Akt but also prevents its translocation (Wijkander et al. 1997). These data suggest that PI 3-kinase transduces the insulin signal to Akt, which stimulates glucose uptake. The full activation of Akt requires phosphorylation of Thr308 and Ser473 (Kohn et al. 1996a). Akt is a serine kinase, but cannot auto-phosphorylate itself (Alessi et al. 1996).

Whether impaired Akt protein expression or insulin-stimulated activation contributes to insulin resistance in vivo in type II diabetes is still not clear. Krook et al. (1998) reported that insulin-stimulated maximal Akt activity was reduced by 66% in muscle from type II diabetic subjects, but the total Akt protein levels were unchanged. Adipocytes from obese patients with type II diabetes demonstrated both impaired sensitivity and reduced total serine phosphorylation and PKB activation in response to insulin (Rondinone et al. 1999). Recently, however, Kim et al. (1999b) reported no impairment of insulin-stimulated Akt activation in intact skeletal muscle from type II diabetic humans, compared with lean and obese subjects. Although they found that the total Akt protein levels were similar among all the subjects, Akt activation was correlated with glucose uptake in the lean control group. In rat skeletal muscle, insulin resistance induced by hyperglycemia has been associated with decreased Akt kinase activity (Krook et al. 1997, Kim et al. 1999a, Kurowski et al. 1999, Song et al. 1999, Storz et al. 1999). However, the role of Akt in hyperinsulinemic, hyperglycemic obese animals has not been investigated previously.

The db/db mouse is a well-established genetic model of obesity and type II diabetes (Hummel et al. 1966). db/db mice have characteristics similar to human type II diabetes including obesity, hyperglycemia, and extreme insulin resistance. The mice are obese and hyperinsulinemic up to 1 month of age, then insulin resistance worsens with the appearance of hyperglycemia (Kodama et al. 1994). The insulin resistance in mature db/db mice is a manifestation of hyperglycemia and remaining hyperinsulinemia that occurs between 2 and 3 months of age (Kodama et al. 1994). The purpose of the present study was to determine whether insulin signal transduction through Akt signaling is altered in this model of diabetes, and whether changes in Akt expression or activity are related to impaired GLUT4 translocation and the mechanism for insulin resistance. Additionally, we examined the expression and phosphorylation of extracellular signal-regulated kinase-1 (ERK-1) and ERK-2, signaling proteins in the mitogen-activated protein kinase (MAPK) pathway, to test whether insulin resistance was specific to the metabolic and/or mitogenic pathway.

### Materials and Methods

#### Materials

The insulin radioimmunoassay kit was from Linco Research (St Louis, MO, USA). The protease inhibitors, aprotinin and leupeptin, were purchased from Boehringer Mannheim Corp. (Indianapolis, IN, USA). Glucose assay kit and all other reagent grade chemicals were from Sigma (St Louis, MO, USA). GLUT4 antibody was obtained from Chemicon International, Inc. (Temecula, CA, USA). Anti-total Akt and Phosph-Akt (Ser473) antibodies were from New England Biokabs (Beverly, MA, USA). Anti-ERK1/2 and active MAPK antibodies were from Promega Corporation (Madison, WI, USA).

#### Experimental animals

Female C57BL/KsJ-Leprdb/db mice weighing 39–41 g and their homozygote lean littermates (+/+ or −) were received from the Jackson Laboratory (Bar Harbor, ME, USA) at 8 weeks of age. They were maintained on a 12 h light:12 h darkness cycle in a temperature-controlled room and given free access to commercial mouse chow and water.

#### Acute insulin stimulation in vivo and tissue collection

At 9 weeks of age, the mice were fasted overnight and blood was obtained from the tails for measurement of insulin and glucose concentration. The mice were anesthetized with ketamine (150 mg/kg) and acepromazine (5 mg/kg), and abdominal cavities were opened and the portal veins exposed. Adipose tissue adjacent to one side of the urinary bladder and ~300 mg gastrocnemius muscle from one hindlimb were rapidly removed and frozen immediately in liquid nitrogen. A maximal bolus of insulin (10 U/kg body weight) was then injected into the portal vein. This produced a maximal sustained increase in IR, IRS-1, and p85α phosphorylation within 5 min (Ishizuka et al. 1999). The plasma insulin levels achieved were >100 ng/ml in control animals. At 5 min after injection, adipose tissue and gastrocnemius muscle from the opposite limb were excised and frozen immediately. The samples were stored at −80 °C until analysis.

#### Total and plasma membrane GLUT4 immunoblotting

Muscle (300 mg) or adipose tissue (200 mg) were homogenized in 2 ml ice-cold lysis buffer (50 mM Tris–HCl, pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 5 μg/ml leupeptin, 1 mM Na3VO4, and 1 mM NaF) using a Polytron PTA 20S generator at maximum speed for 30 s. The homogenates were allowed to sit on ice and solubilized for 30 min, followed by centrifugation at 350 000 g for 60 min at 4 °C. The supernatant was collected and stored at −20 °C as
total protein samples. For the preparation of plasma membranes, ~200 mg frozen hindlimb muscle or adipose tissue was used, as described previously (Brozinick et al. 1996). Briefly, muscle was homogenized in a buffer consisting of 255 mM sucrose, 100 mM Tris–HCl, pH 7·6, and 0·2 mM EDTA, then centrifuged at 3400 g for 20 min. The pellets were resuspended and separated into total plasma membrane (PM) by centrifugation at 68 000 g for 16 h with sucrose gradient.

Phosphorylation and Western blotting of Akt, p85α, and ERK1/2

To determine the level of insulin-stimulated Akt phosphorylation, 50 µg protein (using bovine serum albumin (BSA) as a standard) from pre- and post-insulin-stimulated samples were subjected to 7% SDS-PAGE. After transferring and blocking, the membrane was incubated with Phospho-Akt (Ser473) antibody (1:1000 in Tris-buffered saline with Tween (TBS-T) with 1% BSA; New England Biolabs) overnight at 4 °C. The membrane was detected with enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech, Arlington Heights, IL, USA) according to the manufacturer’s instructions. For tyrosine phosphorylation of p85α, 500 µg protein of crude homogenate was immunoprecipitated with 5 µg anti-phosphotyrosine antibody (PY20; Transduction Laboratories, Lexington, KY, USA) overnight at 4 °C. The membrane was washed with 20 µl protein A-Sepharose (50% slurry; Pharmacia Biotech, Uppsala, Sweden) for 2 h at 4 °C. The immunoprecipitates were washed four times with 1 ml TBS, then resuspended in 40 µl Laemmli sample buffer, and heated for 5 min. Samples were resolved by SDS-PAGE on a 7% gel and transferred to polyvinylidene difluoride membrane using a mini trans-blot transfer cell (Bio-Rad, Hercules, CA, USA). The membrane was blocked with 5% non-fat milk in TBS-T overnight at 4 °C. After washing, the membrane was washed three times in TBS-T and incubated with anti-mouse IgG-horseradish peroxidase (HRP) (1:2000 dilution in TBS-T) or HRP-conjugated anti-rabbit IgG secondary antibody for 1 h at room temperature. Membranes were reimmunoblotted with supernatant for 5 min. Twenty microliters of the boiled sample was incubated with 20 µl immobilized anti-Akt antibody, gently rocking for 2 h at 4 °C. The immune pellets were washed twice with 500 µl lysis buffer, and twice with 500 µl kinase buffer (25 mM Tris, pH 7·5, 5 mM β-glycerolphosphate, 2 mM dithiothreitol, 0·1 mM Na3VO4, and 10 mM MgCl2). The samples were kept on ice during the wash. The pellets were resuspended in 40 µl kinase buffer supplemented with 200 µM ATP and 1 µg GSK-3 fusion protein and incubated at 30 °C for 30 min. The reaction was terminated by adding 20 µl 3 × Laemmli sample buffer and centrifuged for 2 min, then boiling the supernatant for 5 min. Twenty microliters of the boiled supernatant were subjected to 15% SDS-PAGE gel. The resolved proteins were transferred to nitrocellulose membranes. The remaining procedures were the same as Western blotting, as above, except that the first antibody Phospho-GSK-3α/β (Ser21/9) antibody was used. The bands were visualized with ECL and quantified by densitometry. The results are presented as arbitrary units.

Statistical analysis

Data are expressed as means ± s.e. Statistical differences were determined by analysis of variance or by Student’s t-test. P<0·05 was considered statistically significant.

Results

Concentration of glucose and insulin in db/db mice

The characteristics of db/db mice are shown in Table 1. The db/db mice used in this study were 210% heavier...
Table 1  Body weight, fasting glucose and insulin in C57BL/KsJ-Lepr<sup>db/db</sup> and their lean (+/+ ) littermates at 9 weeks of age. Values are means ± S.E.M. (n=6)

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<tr>
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<th>+/+</th>
<th>db/db</th>
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<tr>
<td>Body weight (g)</td>
<td>19.0 ± 0.57</td>
<td>40.15 ± 0.22*</td>
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<tr>
<td>Fasting glucose (mg/dl)</td>
<td>87.0 ± 0.6</td>
<td>430 ± 8.6*</td>
</tr>
<tr>
<td>Fasting insulin (ng/ml)</td>
<td>0.20 ± 0.02</td>
<td>8.21 ± 0.51*</td>
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*P<0.001 vs +/+ control littermates.

(P<0.01) than their lean +/- littermates. Fasting plasma glucose and insulin were significantly elevated (P<0.01) by fivefold and 40-fold in db/db mice compared with their lean littermates, indicative of severe insulin resistance.

**GLUT4 protein and translocation**

There was no significant difference in total muscle or adipose tissue GLUT4 protein content between db/db and +/- mice (Fig. 1). In +/- mice after insulin stimulation, the plasma membrane GLUT4 content increased more than twofold in adipose and muscle tissues (P<0.01). However, in db/db mice, the plasma membrane GLUT4 level increased slightly, but not significantly, above non-stimulated levels. After insulin injection, the levels of GLUT4 in skeletal muscle and adipose tissue plasma membranes were significantly lower in db/db mice by 42–43% (P<0.05) compared with +/- mice.

**Insulin-stimulated Akt-Ser<sup>473</sup> phosphorylation** In order to determine whether impaired GLUT4 translocation in db/db mice is related to changes in the abundance of Akt and its phosphorylation, we measured these in skeletal muscle and adipose tissues (Fig. 2). There was no difference in Akt protein content between db/db and +/- mice in either skeletal muscle or adipose tissue (P>0.05). Insulin activation of Akt involves phosphorylation of Ser<sup>473</sup> and Thr<sup>308</sup>. Despite significant hyperinsulinemia in db/db mice, the basal levels of Akt-Ser<sup>473</sup> phosphorylation in skeletal muscle and adipose tissues from db/db mice were similar compared with +/- mice. After insulin injection, the level of Akt-Ser<sup>473</sup> phosphorylation increased two- to fivefold in both muscle and adipose tissue from db/db and +/- mice (all P<0.001). In skeletal muscle from db/db mice, insulin-stimulated Ser<sup>473</sup> phosphorylation of Akt was decreased by 32% compared with +/- mice (P<0.05, Fig. 2A). For adipose tissue, this difference was 69% compared with +/- mice (P<0.05, Fig. 2B).

**Insulin-stimulated Akt kinase activity** We measured the basal and insulin-stimulated maximal Akt kinase activity in muscle and adipose tissue using GSK-3 fusion protein as substrate (Fig. 3). We immunoprecipitated total Akt by agarose-conjugated anti-Akt antibody and incubated the immune pellets with GSK-3 fusion protein, followed by detecting the phosphorylation of Ser<sup>21/9</sup> on the substrate. The level of Ser<sup>21/9</sup> phosphorylation is representative of the Akt kinase activity. Basal Akt kinase activities in muscle and adipose were slightly lower in db/db mice, but there was no statistical difference (P>0.05). The maximal insulin-stimulated Akt kinase activities in muscle and adipose tissues were 10% and 13% lower in db/db mice compared with +/- mice respectively (P<0.05).

**Expression and phosphorylation of p85α and ERK1/2** PI 3-kinase is upstream of Akt in the insulin-signaling cascade and is associated with Akt activation (Kohn et al. 1996, Wijkander et al. 1997). In order to see if the impaired metabolic action of insulin on Akt activity and GLUT4 translocation is related to decreased expression or phosphorylation of p85α, we immunoprecipitated with an anti-phosphotyrosine antibody, and probed the membrane with anti-p85α antibody. As shown in Fig. 4, in db/db mice, insulin-stimulated tyrosine phosphorylation of p85α was reduced by 40% and 44% respectively in muscle and adipose tissue compared with +/- mice (all
The levels of p85α were not changed in db/db mice relative to +/+ mice. In order to see whether the block in insulin signaling extends to the mitogenic aspects of insulin signaling, we analyzed the effects of insulin on the insulin-signaling proteins ERK-1 and ERK-2. The ERKs are part of the MAPK signaling cascade and mediate important aspects of MAPK signaling to affect mitogenesis (Seger & Krebs 1995). There was no difference in the levels of total protein and insulin-stimulated phosphorylation of ERK-1 or ERK-2 in muscle and adipose (Fig. 5).

**Discussion**

The main goal of this study was to investigate whether the insulin resistance in genetically obese diabetic db/db mice is related to impaired Akt level or kinase activity. Akt protein expression in skeletal muscle and adipose tissues were unchanged. However, insulin-stimulated Akt-Ser⁴⁷³ phosphorylation and Akt kinase activity were both decreased in skeletal muscle and adipose tissue. These results suggest that reduced Akt kinase activity may...
Insulin resistance and Akt in db/db mice

Figure 4 Insulin-stimulated tyrosine phosphorylation of p85α (Phospho p85α) in (A) muscle and (B) adipose tissue from db/db and +/+ mice. The in vivo insulin stimulation and tissue preparation were identical to those described in the legend for Fig. 2. Fifty micrograms of total protein were used for measurement of p85α protein levels. Five hundred micrograms of the protein of the homogenate were immunoprecipitated with anti-phosphotyrosine antibody (PY20). The immunoprecipitates were resolved by SDS-PAGE gel. The transferred membranes were detected with anti-p85 antibody. There is a representative blot for muscle and adipose tissue in each panel. The values are the mean ± S.E. of the densitometry values expressed in arbitrary units as compared with values obtained in the internal control. *P<0.05 versus insulin-stimulated +/+ mice.

contribute to the cellular mechanism for decreased GLUT4 translocation and insulin resistance in this animal model.

It is well established that insulin, acting through PI 3-kinase, stimulates Akt kinase, leading to increased glucose transport in vitro (Shepherd et al. 1998, Czech & Corvera 1999). We found that Akt protein levels were unchanged in db/db mice. These results are similar in Krook et al. (1997), who measured Akt protein expression in skeletal muscle from Goto–Kakizaki diabetic rats and found no difference. The same group of investigators also showed that Akt protein level in skeletal muscle from type II diabetic human subjects was similar to healthy controls (Krook et al. 1998). Rondinone et al. (1999) measured Akt β in cultured adipocytes from type II diabetic subjects, and found that Akt 2 protein levels were similar in type II diabetic and control groups. In animal models that have created insulin resistance, the expression levels of Akt protein did not change (Kurowski et al. 1999, Storz et al. 1999). Thus it seems clear that, at least in skeletal muscle, Akt protein, like GLUT4, is unchanged in obesity and diabetes, and does not explain insulin resistance to glucose transport.

The level of Akt-Ser<sup>473</sup> phosphorylation closely follows Akt kinase activity and is required for full activation. Our results demonstrate that, in skeletal muscle and adipose tissue, Akt-Ser<sup>473</sup> phosphorylation in vivo increases more than tenfold in response to insulin, even in hyperinsulinemic obese db/db mice. In adipose tissue, the increase was approximately fivefold. No significant differences were found in the basal Akt-Ser<sup>473</sup> phosphorylation between db/db and +/+ mice. However, insulin-stimulated Akt-Ser<sup>473</sup> phosphorylation in skeletal muscle and adipose tissue from db/db mice was decreased by 32% and 69% respectively, compared with control animals. These results differ from those of Kim et al. (1999b), who reported that insulin-stimulated Akt-serine phosphorylation was similar between lean non-diabetic, obese non-diabetic, and obese type II diabetic subjects in human skeletal muscle in vivo. These investigators infused insulin for 3 h prior to measuring Akt-serine phosphorylation in muscle biopsies by gel shift mobility. Although they described no difference in serine phosphorylation of Akt between 15 min and 3 h of insulin stimulation, suggesting steady-state conditions, our experiments were conducted to measure maximal insulin stimulation after only 5 min. Our results are consistent with previous observations showing reduced maximal insulin-stimulated Akt activity in adipose tissue and skeletal muscle in vitro from type II diabetic patients, and in animal models (Krook et al. 1997, 1998, Kurowski et al. 1999, Rondinone et al. 1999, Storz et al. 1999). Animal studies have shown that insulin-stimulated Akt kinase activity in skeletal muscle from Goto–Kakizaki diabetic rats is markedly reduced. However, after 4 weeks of normalization of blood glucose concentration with phlorizin, maximal insulin-stimulated Akt kinase activity was restored to normal (Krook et al. 1997). In animals infused with high concentrations of glucose or with free fatty acid-induced insulin resistance, the insulin-stimulated activation of Akt kinase is inhibited (Kurowski et al. 1999, Storz et al. 1999). These results, combined with the present observations of decreased GLUT4 translocation in db/db diabetic mice, suggest that hyperglycemia or free fatty acids can impair insulin-stimulated Akt kinase activity and may inhibit insulin signaling to recruit GLUT4 to the plasma membrane. Our results also show that although the decrease in the absolute amount of insulin-stimulated Akt kinase activity is modest (10–15%), it may have a significantly greater effect downstream on GLUT4 recruitment to the plasma membrane.
Akt is a serine/threonine kinase, activated by insulin and other growth factors (Alessi & Cohen 1998). The exact mechanism of activation has not been fully elucidated. Akt has a pleckstrin homology domain at its NH2 terminal, which has been implicated in interactions with the products of PI 3-kinase, PI 3,4-bisphosphate (PIP2) and PI 3,4,5-trisphosphate (PIP3) in vitro (Franke et al. 1995, 1997). After insulin stimulation, PIP2 and PIP3 bind with Akt and translocate to the plasma membrane where Akt is phosphorylated by phosphatidylinositol-dependent kinase (PDK1) and PDK2 on Thr308 and Ser473 respectively (Stokoe et al. 1995, Alessi & Cohen 1998, Stephens et al. 1998, Andjelkovic et al. 1999). The activation of PI 3-kinase by insulin requires phosphorylation of tyrosine residues on both p85α and p110 (Hayashi et al. 1992, Ruiz-Larrea et al. 1993). Former studies showed that activation of PI 3-kinase not only increases serine phosphorylation and activates Akt kinase but also induces the translocation of Akt to the plasma membrane, where Akt is activated (Wijkander et al. 1997, Goransson et al. 1998). Studies have also found that inhibition of p85α completely blocks insulin- or growth factor-stimulated activation of Akt kinase and insulin-stimulated glucose transport (Franke et al. 1995, Kohn et al. 1996, Wijkander et al. 1997, Goransson et al. 1998). The tyrosine phosphorylation of p85α has been demonstrated to closely follow the activation of PI 3-kinase. We found there were no differences in p85α expression levels in db/db and +/+ mice. After maximal insulin stimulation, insulin-stimulated phosphorylation of p85α in skeletal muscle and adipose tissue from db/db mice was significantly lower than in +/+ mice. We speculate that the decreased insulin-stimulated phosphorylation of p85α in muscle and adipose tissue may be responsible for decreased Akt kinase activity.

Figure 5 Protein abundance and insulin-stimulated phosphorylation of ERK1/2 in the muscle from db/db mice. The muscle was stimulated by injection of insulin (10 IU/kg body weight) through a portal vein for 5 min. The homogenate protein was subjected to SDS-PAGE and detected by anti-total ERK1/2 and active MAPK (Phospho ERK1/2) antibodies. Data are means ± s.e. of the densitometry values expressed in arbitrary units.
and GLUT4 translocation. However, further studies are needed to elucidate the relationship between the activation of PI 3-kinase and Akt kinase, and which of them is the critical impairment under different conditions of insulin resistance. At least one study has suggested that protein kinase C, rather than Akt, is more critical to insulin-stimulated glucose transport (Kotanik et al. 1998).

Recent studies indicate that the insulin-stimulated glucose uptake is regulated by a multi-pathway signaling system (Czech & Corvera 1999). Although PI 3-kinase is a main signaling protein in the insulin-signaling system, there are other factors that stimulate the translocation of glucose transporter(s) and glucose uptake without activation of PI 3-kinase. For example, Kurowski et al. (1999) reported that, in hyperglycemia-induced insulin-resistant rats, the activation of PI 3-kinase was unaffected but the reduced glucose uptake and metabolism were related to inhibition of Akt activation. Therefore, reduced PI 3-kinase activity may be one reason for impaired activation of Akt in db/db diabetic mice, but we cannot rule out other factors in the present study.

Several lines of experimental evidence suggest that resistance to insulin-stimulated glucose transport may not be accompanied by resistance to insulin signaling at the mitogenic branches of insulin signaling. Recently, Draznin et al. (2000) have found that the typical ‘metabolic’ insulin resistance (diminished glucose uptake) in chronically hyperinsulinemic animals and humans co-existed with normal to increased effects of insulin on famesylated p21 ras. The Ras pathway participates in diverse processes as cell proliferation, differentiation, transformation, and apoptosis. Insulin regulates Ras through the nucleotide exchange factor son-of-sevenless protein that can load Ras with GTP. The GTP-bound form of Ras signals by its preferential binding to several effector molecules, and initiates a kinase cascade through MAPK or ERK kinase, a dual-specificity protein kinase, which in turn phosphorylates ERK, another serine/threonine kinase. Our current observations strongly suggest that the hyperinsulinemia that develops as a compensatory feature of the ‘metabolic’ insulin resistance in obesity and impaired glucose metabolism has no dampening effect on the expression and phosphorylation of ERK-1 or ERK-2 in response to insulin in db/db mice. The significance of this observation may lie in the ability of hyperinsulinemia, along with other growth factors, to stimulate increased proliferation of adipose tissue and other cell types that contribute to diabetic complications.

In summary, we have shown that in the skeletal muscle and adipose tissue from db/db mice there is no difference in protein abundance and phosphorylation of Akt and p85α in the basal state. After insulin stimulation in vivo, Akt–Ser473 phosphorylation and tyrosine p85α phosphorylation are decreased. The insulin-stimulated maximal Akt kinase activity is reduced in both muscle and adipose tissue from db/db mice. However, the ability of insulin to stimulate the MAPK pathway, via ERK-1 and ERK-2, are normal. Thus, the results suggest that the reduction of Akt kinase activity may be linked to decreased GLUT4 translocation and impaired insulin-stimulated glucose uptake in tissues from db/db mice, while insulin signaling via the mitogenic pathway is intact.

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