Insulin Resistance, Defective Insulin Receptor Substrate 2–Associated Phosphatidylinositol-3′ Kinase Activation, and Impaired Atypical Protein Kinase C (ζ/λ) Activation in Myotubes From Obese Patients With Impaired Glucose Tolerance

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Impaired glucose tolerance (IGT) is characterized by insulin resistance. Recently, defects in the insulin-signaling cascade have been implicated in the pathogenesis of insulin resistance. To study insulin signaling in IGT, we used human skeletal muscle cells in primary culture from patients with IGT and control subjects. In these cultured myotubes, we assessed insulin-induced 2-deoxyglucose uptake and early steps of the metabolic insulin-signaling cascade. Myotubes in culture from patients with IGT had insulin-induced glucose uptake that was roughly 30–50% less than that from control subjects. This insulin resistance was associated with impaired insulin receptor substrate (IRS)-2–associated phosphatidylinositol 3′ (PI3) kinase activation and IRS-2 tyrosine phosphorylation as well as significantly decreased protein kinase C (PKC)–ζ/λ activation in response to insulin. IRS-1–associated PI3 kinase activation and insulin receptor autophosphorylation were comparable in the two groups. Protein expression levels for the insulin receptor, IRS-1, IRS-2, the p85 regulatory subunit of PI3 kinase, Akt, PKC–ζ/λ, GLUT1, and GLUT4 were also similar in the two groups. In conclusion, myotubes from patients with IGT have impaired insulin-induced glucose uptake. This is associated with impaired IRS-2–associated PI3 kinase activation and PKC–ζ/λ activation. Our results suggest that these defects may contribute to insulin resistance in IGT patients. 

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Insulin resistance is a key feature of impaired glucose tolerance (IGT) and type 2 diabetes (1). It is characterized by a diminished ability of insulin-sensitive tissues to take up and metabolize glucose in response to insulin.

Skeletal muscle is the primary site of insulin-mediated glucose disposal and contributes significantly to decreased glucose uptake, as seen in states of insulin resistance (1). Defects in the early insulin-signaling cascade leading to glucose uptake have been shown to play a key role in the pathogenesis of insulin resistance (2).

In target tissues, such as skeletal muscle, insulin promotes glucose uptake through the translocation of the GLUT4 glucose transporter from an intracellular vesicular pool to the plasma membrane (3). Insulin binding to the extracellular α-subunit of its receptor results in autophosphorylation of tyrosine residues in the receptor β-subunit and activation of a tyrosine kinase intrinsic to the β-subunit. This leads to the recruitment and tyrosine phosphorylation of intracellular substrates such as insulin receptor substrates (IRSs) 1–4. Phosphotyrosines on the IRS proteins bind the p85 regulatory subunit of phosphatidylinositol 3′ (PI3) kinase. PI3 kinase is a heterodimer of a regulatory subunit (p85) and a catalytic subunit (p110), and its activation in response to insulin results primarily through its association with the IRS proteins (4). PI3 kinase activation is required for GLUT4 translocation and subsequent glucose uptake. Current data indicate that PI3 kinase activates the serine/threonine kinase Akt/PKB, which may be involved in insulin-induced GLUT4 translocation (5), even though a definitive role has not been proven (6). Indeed, in rat adipocytes, only a relatively small fraction of insulin-stimulated GLUT4 translocation is inhibited by a kinase inactive form of Akt (7). Furthermore, in 3T3-L1 adipocytes, studies with a dominant-negative mutant of Akt suggest that Akt is not required for insulin-stimulated GLUT4 translocation and glucose transport (8). In adipocytes and muscle tissue, insulin also activates, in a PI3 kinase–dependent manner, atypical forms of protein kinase C (PKC) (λ and ζ), which have also been suggested to mediate glucose transport (6,9,10).

IRS-1 and IRS-2 are important in transmitting the metabolic actions of insulin. They are strongly expressed in insulin-sensitive tissues (4). Mice with targeted disruption of the IRS-1 or IRS-2 gene show either insulin resistance (11,12) or type 2 diabetes (13), respectively, whereas IRS-3 and IRS-4 knockout mice have only minimal metabolic abnormalities (14,15).

There is increasing evidence that defects in the insulin-signaling pathway contribute to insulin resistance in humans and in tissues like the skeletal muscle. For example,
in patients with type 2 diabetes or morbid obesity, several investigators have shown, in muscle biopsies, decreased autophosphorylation of the insulin receptor (16,17), decreased IRS-1 tyrosine phosphorylation (18), and decreased PI3 kinase activation (18,19), which could all contribute to the observed insulin resistance. But patients with type 2 diabetes often have complex metabolic abnormalities such as hyperinsulinemia, hyperglycemia, and dyslipidemia, which can induce insulin resistance per se (20) and introduce confounding factors in the study of potential signaling defects.

Therefore, we chose to study patients with IGT. Patients with IGT are insulin resistant (1) and at increased risk to develop type 2 diabetes (21,22). They do not have fasting hyperglycemia. To minimize the effects of environmental factors, we used human skeletal muscle cells in primary culture. Human skeletal muscle cells in primary culture display numerous features of mature skeletal muscle (23–25), and muscle cells from different individuals can be studied under strictly controlled experimental conditions. Therefore, these cells are a useful tool to search for primary defects in skeletal muscle. This system has been used previously to study muscle metabolism in patients with type 2 diabetes or in Pima Indians (23,26–28).

Here, we show that muscle cells in culture from obese patients with IGT have decreased insulin-mediated glucose uptake. Furthermore, we demonstrate that IRS-2–associated PI3 kinase activation and PKC-ζ/λ activation in response to insulin are decreased. This result suggests that these defects might be involved in the pathogenesis of insulin resistance in skeletal muscle.

**RESEARCH DESIGN AND METHODS**

**Materials.** Culture growth media (SkGM Bulletkit) was obtained from Clonetics (San Diego, CA). α-Modified Eagle’s medium (MEM) was purchased from Irvine Scientific (Santa Ana, CA). FCS was from Life Technologies (Rockville, MD). Anti-IRS-1, anti-IRS-2, anti-p85, anti-insulin receptor, and β-subunit were from Upstate Biotechnology (Lake Placid, NY); anti-phospho-tyrosine antibody (PY20) was from Transduction Laboratories (Lexington, KY); anti-Akt 1/2 and polyclonal anti–PKC-ζ/λ were from Santa Cruz Biotechnology (Santa Cruz, CA); anti-phospho-Akt was from New England Biolabs (Beverly, MA); and anti-GLUT1 and anti-GLUT4 were from Chemicon International (Temecula, CA). 125I-Ser-PKC-ε (amino acids 153–164)–NH2 substrate was from Biosource International (Camarillo, CA). [2-3H]deoxyglucose and L-[1-3H(N)]glucose were from DuPont-NEN (Boston, MA), and [γ-32P]ATP was from Amersham Pharmacia (Piscataway, NJ). All other reagents or chemicals were purchased from Sigma (St. Louis, MO).

**Human subjects.** Seven healthy control subjects and six patients with IGT participated in these studies. All the subjects underwent total hip or knee joint replacement during which muscle tissue was taken either from the gluteus maximus or the vastus lateralis. All experimental protocols were approved by the local Ethics Committee of the University of Lausanne. Informed written consent was obtained from all subjects after explanation of the protocol. No subject was taking pharmacological agents known to affect carbohydrate metabolism. All subjects underwent, before surgery, an oral glucose tolerance test (75 g glucose) after an overnight fast, and blood glucose was determined at basal and after 2 h. Percentage of body fat was determined with the skinfold density of 6,000 cells/well, and for other studies, 15,000–20,000 cells were plated on 100-mm dishes and grown to 80% confluence. They were then fused for 4 days in α-MEM containing 2% FCS, penicillin (100 units/ml), and streptomycin (100 μg/ml). All studies were carried out on cultures after first passage.

**2-Deoxyglucose transport.** The procedure for glucose uptake has been described previously (23). Cells were incubated in serum-free media containing increasing concentrations of insulin (basal, 0.17 nmol/l [1 ng/ml]; 1.7 nmol/l [100 pmol/l]), and 17 nmol/l [1 μmol/l] for 60 min at 37°C in an incubator containing 95% O2/5% CO2. Glucose uptake was determined in triplicate at each point after the addition of 10 μl substrate ([2-3H]deoxyglucose/[L-1-3H(N)]glucose, 0.1 mCi, to a final concentration of 0.01 nmol/l) to provide a concentration at which cell membrane transport is rate limiting. The value for L-glucose uptake was subtracted to correct each sample for the contributions of diffusion and trapping. Protein content was determined using a Bradford method.

**Immunoblotting analysis.**

**Sample preparation.** All samples for Western blotting were prepared from confluent monolayers of fused myoblasts. After insulin stimulation (at the indicated time and concentration), cells were washed with ice-cold PBS buffer, and lysis buffer (50 mmol/l HEPES, 150 mmol/l NaCl, 1% Triton X-100, 4 mmol/l sodium orthovanadate, 20 mmol/l sodium pyrophosphate, 200 mmol/l sodium fluoride, 10 mmol/l EDTA, 2 mmol/l phenylmethylsulfonyl fluoride, and 10% glycerol) was added to the culture and the cells scraped into a rubber policeman into Eppendorf tubes. After centrifugation at 20,000g for 10 min, supernatants were collected, and protein content was determined using a Bradford method with BSA as a standard.

**SDS-PAGE.** SDS-PAGE was carried out as previously described. For GLUT1, GLUT4, and insulin receptor, samples were not boiled. To compare and quantify expression levels between the different subjects, in all the experiments, an internal standard consisting of the same cell lysate of a control subject was run in parallel. After electrophoresis to either nitrocellulose or PVDF (polyvinylidene fluoride), membranes were blocked with 5% (wt/vol) nonfat dry milk or 2.5% BSA. After blocking, membranes were blotted with specific monoclonal or polyclonal antibodies, washed, and incubated with anti-rabbit or anti-mouse IgG conjugated with horseradish peroxidase. Immune complexes were detected using enhanced chemiluminescence.

**Insulin receptor autophosphorylation.** Cells were starved overnight, stimulated with insulin (17 mmol/l) for 30 min, and lysed. Insulin receptor was immunoprecipitated with a specific antibody against the β-subunit of the receptor and then separated by SDS-PAGE. After transfer, the membranes were blotted first with a specific anti-phosphotyrosine antibody. The same membrane was then stripped and blotted with an insulin receptor β-subunit antibody to check for equal loading.

**IRS-1 and IRS-2 tyrosine phosphorylation.** Myotubes were starved overnight and stimulated with 17 mmol/l insulin for 3, 10, 15, 30, and 60 min. Cells were lysed, and IRS-1 and IRS-2 were immunoprecipitated with specific antibodies and then separated by SDS-PAGE. After transfer, membranes were first probed with an anti-phosphotyrosine antibody and then stripped and probed with either an anti–IRS-1 or –IRS-2 antibody to check for equal loading. Specificity of the IRS-1 and IRS-2 antibodies was confirmed in immunoprecipitation experiments after transient overexpression of tagged full-length IRS-1 and IRS-2 proteins (data not shown).

**Quantification.** Quantification was performed using scanning densitometry with a Fluor-S Imager from Biorad (Hercules, CA) with Quantity One software (Biorad). Protein expression levels are expressed as arbitrary units.

**P3 kinase activity.** After stimulation with insulin for the indicated time periods, skeletal muscle cells were lysed at 4°C in a lysis buffer containing 50 mmol/l HEPES, 150 mmol/l NaCl, 1% Triton X-100, 4 mmol/l sodium orthovanadate, 20 mmol/l sodium pyrophosphate, 200 mmol/l sodium fluoride, 10 mmol/l EDTA, 2 mmol/l phenylmethylsulfonyl fluoride, and 10% glycerol, pH 7.4. Lysates were centrifuged at 20,000g for 10 min at 4°C. Supernatants (50 μg total protein) were then incubated in anti–IRS-1, anti–IRS-2, or anti-phosphotyrosine antibodies and recombinant protein A-agarose overnight at 4°C. Beads were pelleted by a 10-min spin at 20,000g and washed three times with buffer A (Tris-buffered saline pH 7.5, 1% NP-40, and 100 μmol/l Na3VO4). buffer B (100 mmol/l Tris, pH 7.5, 500 mmol/l LiCl, and 100 μmol/l Na3VO4), and buffer C (10 mmol/l Tris, pH 7.5, 100 mmol/l NaCl, 1 mmol/l EDTA, and 100 μmol/l Na3VO4). Pellets were then resuspended in 50 μl buffer C without Na3VO4. As previously described, P3 kinase activity was assessed by the phosphorylation of phosphatidylidylinositol in the presence of 20 μCi [γ-32P]ATP. After the separation of lipids by thin-layer chromatography using a chloroform/methanol buffer, radiolabeled phosphoinositides were visualized and quantified using an FX Personal Imager phosphorimager (Biorad).

**Akt activation.** After insulin stimulation for the indicated time periods, Akt activity in immunoprecipitates was measured using a kit from Upstate Biotechnology.
When comparing protein expression between the muscle biopsy and the cells cultured from patients with IGT, differences in expression between control subjects and patients with IGT were observed. These same proteins directly in the muscle biopsy material and found no statistically significant differences. In the cultured cells, GLUT1 expression levels were roughly two-fold higher in the control cells compared to patients with IGT. Western blots were then quantified for GLUT1 expression and found no significant differences between the two groups.

Subjects.

Summary data for the subjects are given in Table 1. The subjects were matched for age. Patients with IGT had a significantly higher BMI and body fat than control subjects. Basal blood glucose values were comparable in both groups. Myotubes from control subjects displayed insulin-mediated glucose uptake, which is comparable with published data. Myotubes from patients with IGT also displayed increased insulin-mediated glucose uptake, but this was not statistically significant (Fig. 5). Alternatively, insulin-stimulated 2-deoxyglucose uptake was significantly impaired in myotubes from patients with IGT, as shown in Fig. 5. This result was statistically significant after 3 and 10 min of insulin stimulation.

PI3 kinase activity.

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Statistical analysis.

All results are given as means ± SE. Differences between the control and IGT groups were determined using an unpaired Student’s t-test, and changes over time were compared with ANOVA. Statistical significance was determined with a P-value < 0.05.

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control and IGT subjects. In contrast, PI3 kinase activation associated with IRS-2 was significantly impaired in patients with IGT (Fig. 6C). IRS-2–associated PI3 kinase activation increased after 3 min of insulin stimulation and decreased more rapidly over time than IRS-1–associated PI3 kinase activity.

Akt activation. Akt has been suggested to play a role in insulin-induced glucose uptake and also GLUT4 translocation. Insulin stimulation of Akt, assessed either by an in vitro kinase assay or by phosphorylation at serine 473, was comparable in both groups (Fig. 7).

PKC-β/α activation. PKC-β/α activation has been suggested to play a role in insulin-mediated glucose uptake and GLUT4 translocation. In the basal state, immunoprecipitable PKC-β/α activity was comparable in the two groups. Insulin-mediated PKC-β/α activation was decreased by ~40% in patients with IGT (P < 0.05) (Fig. 8).

**DISCUSSION**

Defects in the insulin-signaling cascade leading to GLUT4 translocation and glucose uptake play an important role in the pathogenesis of insulin resistance in skeletal muscle (2,31). Most of the data have been gathered in patients with full-blown type 2 diabetes. However, little is known regarding signaling defects in prediabetic stages such as IGT. The study of insulin signaling in patients with IGT could give insight into the early defects involved in the pathogenesis of insulin resistance.

To study insulin action in a relevant target tissue, we used an in vitro model of skeletal muscle cells in primary culture from patients with IGT. Myotubes in culture express proteins characteristic of differentiated muscle cells (23) and are insulin responsive, with expression of molecules involved in the insulin-signaling pathway (23,26,32). Cells from different patients can be studied under strictly controlled conditions, minimizing the influence of environmental factors such as hyperinsulinemia and hyperglycemia, which could have unforeseen effects on skeletal muscle during in vivo experiments.

The main finding of this study was that insulin-mediated glucose uptake in cultured myotubes from patients with IGT was roughly 30–50% less than that in myotubes obtained from control subjects. This defect in glucose uptake was associated with impaired IRS-2–associated PI3 kinase activation and PKC-β/α activation in response to insulin.

To study underlying defects that may lead to the observed insulin resistance in vitro, we first analyzed expression levels of relevant proteins of the insulin-signaling pathways. In the Zucker fatty rat model, decreased levels of IRS proteins are thought to contribute to insulin resistance (33), and in adipose tissue of patients with type 2 diabetes, decreased levels of IRS-1 have been found (34). In the present studies, expression levels of insulin receptor, IRS-1, IRS-2, p85, Akt, PKC-β/α, GLUT1, and GLUT4 were comparable in both groups.

We next turned our attention to insulin-signaling steps. The major findings were that IRS-2–associated activation of PI3 kinase and PKC-β/α activation were impaired in myotubes of patients with IGT. This impairment was not related to a decreased insulin receptor β-subunit autophosphorylation in response to insulin stimulation but was associated with decreased IRS-2 tyrosine phosphorylation. PI3 kinase activation in response to insulin is key to induce the translocation of the GLUT4 glucose transporter.
from an intracellular pool to the cell membrane leading to increased glucose uptake (35,36). Even though IRS-1 and IRS-2 are homologous, it has become clear that they have distinct functions, but their specific role in transmitting the insulin signal is not completely understood. IRS-2 has been shown to mediate metabolic responses and GLUT4 translocation in response to insulin (37–39). In addition, it was recently shown that in muscle biopsies from patients with type 2 diabetes, there was a decreased IRS-2–associated PI3 kinase activation in addition to defects of signaling through IRS-1 (19). In the IRS-1 knockout mouse, which is insulin resistant, IRS-2 expression is not increased, but there is increased activation of PI3 kinase associated with IRS-2, which could explain why these mice are only mildly insulin resistant (12). IRS-2 knockout mice display a phenotype that resembles type 2 diabetes in humans; this is due to the simultaneous presence of insulin resistance in skeletal muscle liver and adipose tissue (40) and the decreased insulin secretion related to lower β-cell mass in the pancreas (13). In contrast to our findings in humans, Higaki et al. (41) found normal glucose uptake in response to insulin in isolated muscles of IRS-2 knockout mice. One potential explanation for this discrepancy could be that in the absence of IRS-2, compensatory mechanisms may be involved. Thus, whereas the exact role of IRS-2 in metabolic insulin signaling in skeletal muscle is not fully understood, our data could be consistent with the concept that defects in signaling through IRS-2 may contribute to insulin resistance.

In the present study, the decreased IRS-2–associated PI3 kinase activation was associated with a decreased IRS-2 tyrosine phosphorylation in myotubes in response to insulin. It is well established that tyrosine phosphorylation of IRS proteins by the insulin receptor is key for their association with and activation of PI3 kinase. In the present experiments, however, the ability of the insulin receptor to be autophosphorylated was comparable in both groups. There could be at least two potential explanations for the impaired IRS-2 tyrosine phosphorylation. First, IRS-2 in myotubes from IGT patients could be phosphorylated on serine of threonine residues. Increased serine/threonine phosphorylation of IRS-1 is known to impair its tyrosine phosphorylation in response to insulin and to hamper intracellular signaling (42). Second, IRS-2 could be more rapidly dephosphorylated in myotubes from patients with IGT. For example, overexpression of protein tyrosine phosphatase (PTPase) 1 in L6 cells negatively regulates insulin signaling by decreasing IRS-1 tyrosine phosphorylation (43). Consistent with this hypothesis, PTPase activity is augmented in skeletal muscle and adipose tissue of obese subjects (44,45).

![Image 1](image1.png)

**FIG. 4.** Insulin receptor autophosphorylation. Starved myotubes from control subjects or IGT patients were either treated without (basal) or with 17 nmol/l insulin for 30 min. The insulin receptor β-subunit was then immunoprecipitated from equal amounts of total proteins. Autophosphorylation was determined with anti-phosphotyrosine antibodies. Representative Western blots in basal conditions (B) or after insulin stimulation (I) are shown for one subject of each group. The bar graphs represent summary data (means ± SE) for each group. There was no difference in the basal or insulin-induced autophosphorylation of the insulin receptor between the two groups. *P < 0.05.

![Image 2](image2.png)

**FIG. 5.** IRS-1 and IRS-2 tyrosine phosphorylation. Myotubes were starved overnight and then stimulated with insulin (17 nmol/l) for the indicated times. Cells were lysed and IRS-1 and IRS-2 immunoprecipitated from an equal amount of total proteins and separated by SDS-PAGE. Tyrosine phosphorylation was determined with anti-phosphotyrosine antibodies. Line graphs represent summary data (means ± SE) at each time for control subjects (■) and IGT patients (○). Representative Western blots are shown for a control subject and an IGT subject. IRS-2 tyrosine phosphorylation was decreased in IGT patients. *P < 0.05. IP, immunoprecipitation; WB, Western blot.
To further analyze potential insulin-signaling defects downstream of PI3 kinase, we assessed Akt and PKC-ζ/λ activation. The main finding was that in myotubes of obese patients with IGT, PKC-ζ/λ activation in response to insulin is markedly impaired, whereas Akt activation is comparable in the two groups. Atypical PKCs (ζ/λ) are activated by insulin in a PI3 kinase–dependent manner and have been suggested to be involved in insulin-mediated glucose uptake (6,10,46). Indeed, in L6 myotubes, overexpression of a kinase-defective PKC-ζ mutant almost completely blocked insulin-mediated glucose uptake (10). Furthermore, in vivo adenoviral delivery of recombinant PKC-ζ stimulated glucose transport activity in rat skeletal muscle (47). These data would suggest that impaired PKC-ζ/λ activation in myotubes of our IGT patients may be involved in the observed insulin resistance. The defect in PKC-ζ/λ activation is unlikely to be related to decreased PI3 kinase activity associated with tyrosine phosphoproteins or IRS-1 (because they were comparable in the two groups) but rather with the decreased IRS-2–associated PI3 kinase activation. Consistent with this possibility, Oriente et al. (48) showed that in L6 myotubes, overexpression of the kinase regulatory loop binding domain of IRS-2, which decreased IRS-2 but not IRS-1, tyrosine phosphorylation, and PI3 kinase activation, inhibited PKC-ζ/λ activation in response to insulin. These findings may also suggest that IRS-2–associated PI3 kinase activation may occur in a different intracellular compartment from where IRS-1–associated PI3 kinase is activated. Consistent with this possibility, tyrosine-phosphorylated IRS-1 and IRS-2 are differentially located inside the cell (49,50).

FIG. 6. PI3 kinase activation in response to insulin. Myotubes were starved overnight and then stimulated with insulin (17 nmol/l) for the indicated times. Cells were lysed and tyrosine phosphoprotein (A), IRS-1 (B), and IRS-2 (C) immunoprecipitated with specific antibodies from equal amount of proteins, and PI3 kinase activity was determined in the pellets by an in vitro kinase assay. Line graphs show summary data (means ± SE) of increased activation over basal for control subjects (■) and IGT patients (○). Inserts show characteristic PI3 kinase assays for a control subject (C) and an IGT patient at basal and 30 min of insulin stimulation. PI3 kinase activity associated with IRS-2 was significantly decreased in patients with IGT. *P < 0.005. IP, immunoprecipitation.

FIG. 7. Akt activation in response to insulin. Myotubes were starved overnight and stimulated with insulin (17 nmol/l) for the indicated times. Phospho-Akt was determined in cell lysates with specific phospho-Akt antibodies (ser 473) (A), and Akt activity was determined in immunoprecipitates using an in vitro kinase assay (B). Representative Western blots are shown for a control subject and an IGT patient. Line graphs show summary data (means ± SE) for control subjects (■) and IGT patients (○). Activation and phosphorylation of Akt were similar in both groups.

FIG. 8. PKC-ζ/λ activation in response to insulin. Myotubes were starved overnight and treated without or with insulin (17 nmol/l) for 10 min. After incubation, PKC-ζ/λ was immunoprecipitated with anti-COOH-terminal antiserum and assayed. Bar graphs are summary data (means ± SE) for control subjects (■) and IGT patients (○). PKC-ζ/λ activation in patients with IGT was significantly decreased (*P < 0.05).
In vitro, Akt has been suggested to participate in insulin-induced GLUT4 translocation. Akt activation, assessed by an in vitro kinase assay and phosphorylation of serine 473, was comparable in the two groups. These results suggest that Akt activation is not a key step downstream of PI3 kinase that has to be activated in response to insulin for glucose uptake. These findings can be related to a recent report in which Akt activation measured in muscle biopsies during a euglycemic-hyperinsulinemic clamp in patients with type 2 diabetes and control subjects was comparable (19). Finally, we cannot exclude that a defect in Akt activation in a specific subcellular compartment associated with decreased IRS-2–associated PI3 kinase activity may contribute to the observed insulin resistance in myotubes from patients with IGT.

Our results show for the first time that myotubes from obese patients with IGT are insulin resistant in vitro. It has previously been reported that myotubes from patients with type 2 diabetes are insulin resistant in vitro (23,28). In these studies, the defect observed in vitro was well correlated with in vivo measurements of insulin sensitivity. In another report, Krizfeldt et al. (32) measured insulin sensitivity of myotubes from normoglycemic first-degree relatives of patients with type 2 diabetes and found no difference in glucose metabolism compared with control subjects. In their study, insulin-resistant subjects were not glucose intolerant.

In contrast to earlier reports, in our system, we did not observe decreased IRS-1 tyrosine phosphorylation and IRS-1–associated PI3 kinase activation (18,19,51). There could be several explanations for this. First, most of these studies were performed in muscle biopsies from patients with type 2 diabetes, whereas we studied patients with IGT. Some of the studies using muscle biopsies were performed in subjects who were hyperglycemic, which is known to induce insulin resistance per se (glucotoxicity). In the study by Goodyear et al. (18), the subjects were morbidly obese (BMI >50 kg/m²), whereas we studied patients with a BMI of 34 kg/m². Insulin signaling was studied in muscle strips where there also could still have been the influence of environmental factors such as increased free fatty acids. In the myotube culture system, glycemia and insulinoemia were well controlled.

In conclusion, myotubes from patients with IGT display insulin resistance in vitro, and this defect is associated with impaired IRS-2–associated PI3 kinase activation and PKC-ζ/δ activation in response to insulin. Finally, although our patients clearly had IGT, they were also obese, and we cannot exclude that some of the observed effects may be due to obesity per se.

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