Involvement of Protein Kinase C in Human Skeletal Muscle Insulin Resistance and Obesity

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This study was conducted to investigate the possible involvement of protein kinase C (PKC) and serine/threonine phosphorylation of the insulin receptor in insulin resistance and/or obesity. Insulin receptor tyrosine kinase activity was depressed in muscle from obese insulin-resistant patients compared with lean insulin-responsive control subjects. Alkaline phosphatase treatment resulted in a significant 48% increase in in vitro insulin-stimulated receptor tyrosine kinase activity in obese but not lean muscle. To investigate the involvement of PKC in skeletal muscle insulin resistance and/or obesity, membrane-associated PKC activity and the protein content of various PKC isoforms were measured in human skeletal muscle from lean, insulin-responsive, and obese insulin-resistant patients. Membrane-associated PKC activity was not changed; however, PKC-β protein content, assayed by Western blot analysis, was significantly higher, whereas PKC-θ, -η, and -μ were significantly lower in muscle from obese patients compared with muscle from lean control subjects. Incubation of muscle strips with insulin significantly increased membrane-associated PKC activity in muscle from obese but not lean subjects. PKC-δ, -β, and -θ were translocated from the cytosol to the membrane fraction in response to insulin treatment. These results suggest that in skeletal muscle from insulin-resistant obese patients, insulin receptor tyrosine kinase activity was reduced because of hyperphosphorylation on serine/threonine residues. Membrane-associated PKC-β protein was elevated under basal conditions, and membrane-associated total PKC activity was increased under insulin-stimulated conditions in muscle from obese insulin-resistant patients. Thus, we postulate that the decreased tyrosine kinase activity of the insulin receptor may be caused by serine/threonine phosphorylation by PKC. *Diabetes* 49:1353–1358, 2000

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FFA, free fatty acid; IRS, insulin receptor substrate; PKC, protein kinase C.

Whereas insulin binding induces autophosphorylation of the insulin receptor on specific tyrosine residues, phosphorylation of the insulin receptor on serine/threonine residues reduces tyrosine kinase activity (1,2). In vitro purified protein kinase C (PKC) directly phosphorylated purified insulin receptors and decreased their tyrosine kinase activity (3). Furthermore, in tissue cultured cells overexpressing the insulin receptor, an increase in PKC activity was correlated to an increase in serine phosphorylation of the insulin receptor (4).

Evidence that increased activity of PKC may be associated with insulin resistance comes from many cell culture experiments (4–8). In addition, it was observed that membrane-bound PKC activity was increased in insulin-resistant rat fat cells, and insulin resistance was reversed with the addition of specific PKC inhibitors (9). Evidence from our laboratory (10) also suggests the involvement of PKC in insulin resistance. When insulin-resistant muscle was incubated with a PKC inhibitor, insulin action was restored. Incubation of insulin-sensitive muscle with a PKC activator reduced insulin action. This result is strong evidence to support the involvement of PKC in insulin resistance. A recent study demonstrated that an increase in free fatty acids (FFAs) may activate PKC-θ and induce skeletal muscle insulin resistance in rats (11). Because it was demonstrated that insulin receptor tyrosine kinase activity is impaired in skeletal muscle from insulin-resistant patients (12,13), PKC may phosphorylate the insulin receptor on serine residues and decrease the insulin receptor tyrosine kinase activity. Therefore, the purpose of this study was to investigate the involvement of PKC and excessive serine/threonine phosphorylation of the insulin receptor in insulin resistance in human rectus abdominus skeletal muscle.

RESEARCH DESIGN AND METHODS

Materials. 3-[^{125}I]iodotyrosyl-A_{14} human recombinant insulin (2,000 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). [γ-^{32}P]ATP (6,000 Ci/mmol) was purchased from NEN Life Science Products (Boston, MA). Monoclonal anti–insulin receptor antibody was purchased from Calbiochem (San Diego, CA). The PKC assay system was purchased from Amersham. PKC-θ and -μ monoclonal antibodies were purchased from Transduction Laboratories. PKC-η polyclonal antibody was purchased from Calbiochem. All remaining PKC antibodies (polyclonal) were purchased from Santa Cruz (Santa Cruz, CA). Unless otherwise specified, all other reagents were purchased from either Sigma (St. Louis, MO) or Fisher Scientific (Springfield, NJ).

Tyrosine kinase assay. The insulin receptor tyrosine kinase activity and insulin receptor content were measured as described by Zhou et al. (14). Briefly, 0.4 μg anti–insulin receptor antibody was used to coat a 96-well microplate. Muscle (100 mg) was homogenized in 1 ml homogenization buffer (50 mmol/l HEPES, pH 7.5, 150 mmol/l NaCl, 10 mmol/l Na pyrophosphate, 2 mmol/l Na_3VO_4, 5 mmol/l $MgCl_2$, 1 mmol/l $CaCl_2$, 10 mmol/l NaF, 2 mmol/l EDTA, 2 mmol/l phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 0.2 μmol/l

aprotinin, and 1% Triton X-100). There was 500 µg of protein extract added to each well of the microplate. Wells were incubated either with 100 µl of 0.1 mol/l Tris (pH 8.5) or alkaline phosphatase (30 U/well) (type VII N from bovine intestinal mucosa) in 0.1 mol/l Tris (pH 8.5). To measure the tyrosine kinase activity of the bound insulin receptors, 80 µl assay buffer (50 mmol/l HEPES, 0.05% bovine serum albumin, 150 mmol/l NaCl, 0.1% Triton X-100, 20 mmol/l $MgCl_2$, and 2 mmol/l $MnCl_2$) and 20 µl reaction mixture (30 nmol/l ATP, 4 µCi [γ-^{32}P]ATP, and 3 mg/ml Poly Glu:Tyr) were added. A Beckman LS 6500 Multi-purpose Scintillation Counter (Beckman Instruments, Fullerton, CA) was used to determine incorporation of [γ-^{32}P]ATP.

In vitro human skeletal muscle incubations. Lean insulin-responsive patients and obese insulin-resistant patients undergoing elective surgeries (primarily hysterectomies for lean patients and gastric bypass for obese patients) served as subjects. The experimental protocol for obtaining muscle biopsies was explained to all patients, and informed consent was obtained from each subject. This procedure was approved by the East Carolina University Policy and Review Committee on Human Research. Of the patients in each group, 9 out of 12 were taking medication at the time of surgery. Medications that were taken by 3–5 patients included the following: droperidol, ondansetron HCl, cefazolin sodium, midazolam HCl, metoclopramide HCl, promethazine HCl, meperidine HCl, furosemide, heparin sodium, and morphine sulfate. There was no discernible difference in medication use among groups, and medication did not appear to alter results within a group. When surgery was performed on these patients, a muscle biopsy held by a clamp was obtained from the rectus abdominus muscle and placed in a container with oxygenated Krebs-Henseleit buffer for transport to the laboratory. Muscle fiber strips were "teased" from the muscle sample, clamped in Lucite clips, and placed into separate wells containing Krebs-Henseleit buffer (plus 1% bovine serum albumin and 1.0 mmol/l pyruvate) at 29°C under normoxic conditions (95% O_2, 5% CO_2).

Measurement of glucose transport into human muscle in our laboratory has been described elsewhere (15). In this study, preincubation experiments (lasting 60 min) for PKC activity and Western blot analysis were conducted under the following conditions: basal (no insulin added) and porcine insulin added (10^{-7} mol/l; last 10 min). Muscle fiber strips were frozen in liquid nitrogen and stored at –80°C until further use.

Western blot analysis. Western blot analysis was performed as described elsewhere (16). Frozen rectus abdominus skeletal muscle was homogenized. The protein content was determined using Bio-Rad protein assay (catalog #500-0002, Bio-Rad, Hercules, CA). Samples were separated using 8% SDS-PAGE electrophoresis and electrotransferred to a polyvinylidene fluoride microporous membrane. Isoform-specific PKC antibodies at a dilution factor of 1:1,000 (unless otherwise stated) in 5% nonfat milk were added to the membranes. Secondary antibody conjugated to horseradish peroxidase was added at a dilution of 1:5,000, and membranes were subjected to enhanced chemiluminescence (Western blot chemiluminescence reagent; Dupont). Autoradiographs were quantitated on a Hewlett Packard Scan Jet IIcx/T using Imagequant software (Molecular Dynamics). Because our immunoblots showed a wide range of signal intensities, we produced multiple exposures and attempted to optimize linearity of the signal.

PKC enzyme assay. PKC activity was determined using the PKC enzyme assay system (Amersham). Rectus abdominus skeletal muscle was homogenized and assayed as described in the Amersham protocol kit. The protein content was determined using a Bio-Rad protein assay. The phosphorylated peptides were separated using binding paper discs and counted in 5 ml scintillant using a Beckman LS 6500 Multi-purpose Scintillation Counter.

Statistical analysis. The insulin receptor tyrosine kinase activity data were analyzed using 2-way analysis of variance with repeated measures (alkaline phosphatase and insulin treatments as repeated measures). Western blot analysis data of various PKC isoforms' protein content, insulin-stimulated glucose transport, and PKC activity data were analyzed with an unpaired *t* test.

RESULTS

Although muscle from an obese group of individuals is more insulin resistant than that from a lean group (15), we occasionally study lean patients that do not demonstrate insulin stimulation of muscle glucose transport and obese patients that demonstrate insulin stimulation. This heterogeneity has also been observed in adipocytes from obese patients (17,18). Therefore, to study the mechanisms of insulin resistance in human rectus abdominus skeletal muscle, we chose to study only muscle samples from lean insulin-responsive patients (insulin stimulation of glucose transport >1.5-fold) and obese insulin-resistant patients (insulin stimulation of glucose transport <1.5-fold). Patient characteristics are summarized in Table 1, and the glucose transport data are shown in Fig. 1.

TABLE 1
Patient characteristics

	Lean	Obese
Age (years)	43 ± 1 (12)	41 ± 3 (12)
Sex (M:F)	0:12	1:11
BMI (kg/m^2)	25 ± 1 (12)	57 ± 6 (12)*
Glucose (mg/dl)	90 ± 5 (12)	103 ± 9 (12)
Insulin (µU/ml)	3.0 ± 1 (12)	12.0 ± 3 (12)*
FFAs (mmol/l)	0.40 ± 0.05 (6)	0.67 ± 0.10 (6)*

Data are means ± SE (number of observations), unless otherwise indicated. *Significantly different from the corresponding lean value ($P < 0.05$).

Using an insulin receptor capture assay, we found that the in vitro insulin-stimulated insulin receptor tyrosine kinase activity was significantly decreased in skeletal muscle from obese insulin-resistant patients (Table 2). To investigate whether the decreased tyrosine kinase activity was due to increased serine/threonine phosphorylation of the insulin receptor, insulin receptors from lean insulin-responsive and obese insulin-resistant skeletal muscle were isolated and treated with alkaline phosphatase to dephosphorylate the insulin receptor before insulin treatment. In vitro insulin-stimulated receptor tyrosine kinase activity was increased by 48% in obese insulin-resistant human skeletal muscle but was not significantly changed in muscle from lean insulin-reponsive patients after alkaline phosphatase treatment (Table 2). This result suggests that the increased serine/threonine phosphorylation of the insulin receptor may in part be associated with reduced insulin receptor tyrosine kinase activity in obese insulin-resistant skeletal muscle.

Although the kinase responsible for the excessive serine/threonine phosphorylation of the insulin receptor is not yet known, data from the literature support the involvement

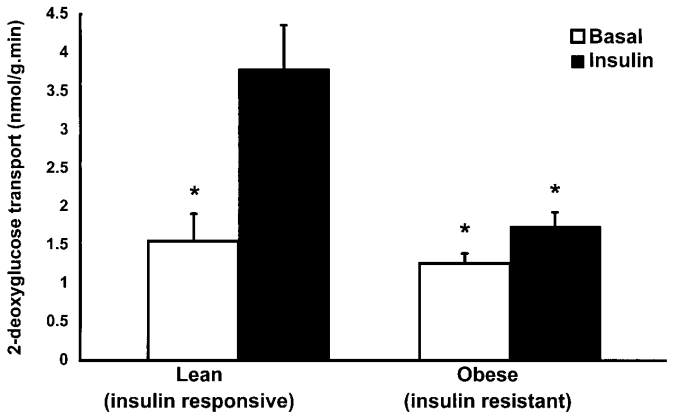

FIG. 1. Effect of insulin on glucose transport. Rectus abdominus skeletal muscle samples were obtained from lean insulin-responsive and obese insulin-resistant subjects. Muscle fiber strips were treated with 10^{-7} mol/l insulin during the last 10 min or without (basal) insulin. 2-Deoxyglucose transport was measured as described in RESEARCH DESIGN AND METHODS. *Significantly different from the corresponding insulin-treated lean value ($P < 0.05$). $n = 12$.

TABLE 2
The effect of alkaline phosphatase treatment on insulin-stimulated receptor tyrosine kinase activity in human rectus abdominus skeletal muscle

Observations (*n*)	Without phosphatase (pmol · 5,000 cpm^{-1} insulin receptor · min^{-1})	With phosphatase (pmol · 5,000 cpm^{-1} insulin receptor · min^{-1})	Increase (%)
Obese patients (6)	1.36 ± 0.10	2.01 ± 0.18*	48.1 ± 5.1
Lean patients (6)	2.69 ± 0.14†	2.94 ± 0.16†	10.6 ± 7.8†

Data are means ± SE. *Significantly different from the corresponding value without alkaline phosphatase treatment ($P < 0.05$). †Significantly different from the corresponding obese value ($P < 0.05$).

of PKC. To investigate the possible involvement of PKC in skeletal muscle insulin resistance, Western blot analysis was used as a technique to measure the protein content of different PKC isoforms in muscle from lean insulin-responsive and obese insulin-resistant patients. Western blots of membrane fractions were probed with PKC antibodies for the different PKC isoforms. The membrane fraction contained sarcolemma, t-tubules, sarcoplasmic reticulum, nuclei, and mitochondria (19). The protein content of the conventional PKC isoform β was significantly higher ($P < 0.05$) in the membrane fraction of skeletal muscle from obese patients compared with the lean control subjects. In contrast, the protein content of the novel PKC isoforms (η, θ, and μ) were significantly lower ($P < 0.05$) in the membrane fraction of skeletal muscle from obese patients compared with the lean control subjects (Table 3, Fig. 2). No significant difference in the protein content of various PKC isoforms was observed in the cytosolic fraction in muscle from obese patients compared with the control subjects (Table 4, Fig. 3).

Membrane-associated PKC activity was measured in human rectus abdominus muscle from lean insulin-responsive and obese insulin-resistant patients. There was no significant change in membrane-associated PKC activity in muscle from obese patients (lean 292 ± 42 pmol · min^{-1} · mg^{-1} protein; obese 255 ± 30 pmol · min^{-1} · mg^{-1} protein).

TABLE 3
Particulate (membrane) PKC content in skeletal muscle from lean insulin-responsive versus obese insulin-resistant subjects

PKC isoform	Lean subjects	Obese subjects
θ	33,020 ± 45,334	18,420 ± 3,505*
ε	3,458 ± 968	4,344 ± 521
μ	2,546 ± 316	1,630 ± 233*
γ	2,422 ± 320	3,398 ± 1,062
δ	500 ± 58	573 ± 78
η	23,270 ± 1,640	11,350 ± 1,643*
β	923 ± 165	2,477 ± 544*

Data (arbitrary units) are means ± SE. The autoradiographs were quantitated by densitometric analysis. $n = 6$ for all observations, except for PKC-β; $n = 5$ for muscle from lean subjects. *Significantly different from the corresponding lean value ($P < 0.05$). Patient characteristics for the above lean subjects were as follows: age 42 ± 1 years, sex (M:F) 0:6, BMI 24 ± 0.8 kg/m^2, glucose 84 ± 3.7 mg/dl, and insulin 3.2 ± 0.9 μU/ml. Patient characteristics for the above obese subjects were as follows: age 41 ± 2 years, sex (M:F) 1:5, BMI 46 ± 5.7 kg/m^2, glucose 103 ± 17 mg/dl, and insulin 8 ± 0.5 μU/ml.

To investigate whether insulin treatment in vitro is associated with increased PKC activity in muscle, muscle fiber strips from lean insulin-responsive and obese insulin-resistant subjects were incubated with and without insulin, and membrane-associated PKC activity was measured. Insulin stimulation of PKC activity in the membrane fraction was significantly greater ($P < 0.05$) in muscle from obese insulin-resistant patients than lean insulin-responsive control subjects (Fig. 4).

Which PKC isoform(s) translocates to the membrane fraction in response to insulin was investigated in rectus abdominus muscle fiber strips from obese patients incubated with and without insulin. Western blot analysis using antibodies for different PKC isoforms was used to determine the protein content of various PKC isoforms in the membrane fraction. PKC-β, -δ, and -θ showed a significant increase in membrane-associated protein in response to insulin treatment. In con-

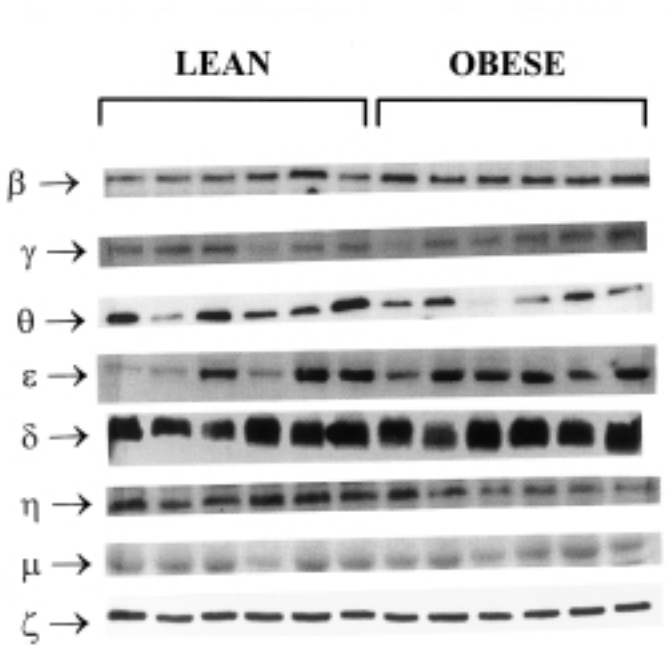

FIG. 2. Effect of insulin resistance on isoform-specific PKC content in the membrane fraction. Rectus abdominus skeletal muscle samples were obtained from lean insulin-responsive and obese insulin-resistant subjects. The particulate (membrane) proteins (100 μg) were separated by SDS-PAGE electrophoresis, transferred to an Immobilon-P transfer membrane, and probed with various PKC isoform antibodies. A representative Western blot of the various PKC isoforms was investigated, with $n = 6$ for all observations. The value for the fifth lane from the left (lean) for PKC-B was not included in Table 3. The individual values for the 6 lean samples are as follows: 876, 747, 1,066, 1,456, 11,486, and 471.

TABLE 4
Cytosolic PKC content in skeletal muscle from lean insulin-responsive versus obese insulin-resistant subjects

PKC isoform	Lean subjects	Obese subjects
θ	1,844 ± 548	2,315 ± 1,241
ε	3,805 ± 291	3,302 ± 1,634
μ	5,510 ± 751	4,805 ± 401
γ	1,016 ± 152	1,086 ± 100
δ	8,383 ± 1,809	9,302 ± 1,658
η	12,500 ± 1,798	12,600 ± 1,893
β	16,120 ± 1,290	15,000 ± 2,051

Data (arbitrary units) are means ± SE. The autoradiographs were quantitated by densitometric analysis. $n = 6$ for all observations.

trast, PKC-ζ, -ε, and -μ did not show any significant change in translocation in response to insulin (Fig. 5).

DISCUSSION

In skeletal muscle from women with polycystic ovary syndrome, excessive serine/threonine phosphorylation of the insulin receptor has been suggested as a potential mechanism for insulin resistance (20). In skeletal muscle from obese Zucker rats, insulin-stimulated tyrosine kinase activity in obese rats was reduced by 39%. Alkaline phosphatase treatment of receptors from obese muscle increased insulin-stimulated receptor tyrosine kinase activity 3 times more than that of lean control muscle (14). This result suggests that excessive serine/threonine phosphorylation of the insulin receptor may be a cause of insulin resistance in skeletal muscle of obese Zucker rats.

In the present study, we investigated whether insulin receptors from obese insulin-resistant human rectus abdominus muscle were excessively phosphorylated at serine/threonine residues and whether relieving this excessive phosphorylation may restore insulin action. To this end, insulin receptors from obese insulin-resistant and lean insulin-responsive control subjects were treated with alkaline phosphatase to remove both serine/threonine and tyrosine phosphates. We were able to increase the insulin-stimulated receptor tyrosine kinase activity in muscle from obese patients by 48%, thus partially restoring insulin action. There was no significant increase in the insulin-stimulated tyrosine kinase activity in muscle from the lean control subjects. This result suggests that excessive serine/threonine phosphorylation may play a role in the decreased insulin-stimulated receptor tyrosine kinase activity seen in muscle from obese insulin-resistant patients. As mentioned above, the insulin-stimulated tyrosine kinase activity of insulin receptors from obese muscle was partially (not completely) restored compared with the lean control subjects. One explanation is that our treatment of the insulin receptors with alkaline phosphatase resulted in the removal of some but not all of the phosphates. Complete dephosphorylation of the insulin receptors may require prolonged treatment with alkaline phosphatase, which leads to loss of tyrosine kinase activity (data not shown).

A recent study from our laboratory (10) demonstrated that the PKC inhibitor GF 109203X restores insulin stimulation of glucose transport in insulin-resistant muscles. In addition, incubation of insulin-responsive muscles with 12-deoxyphorbol

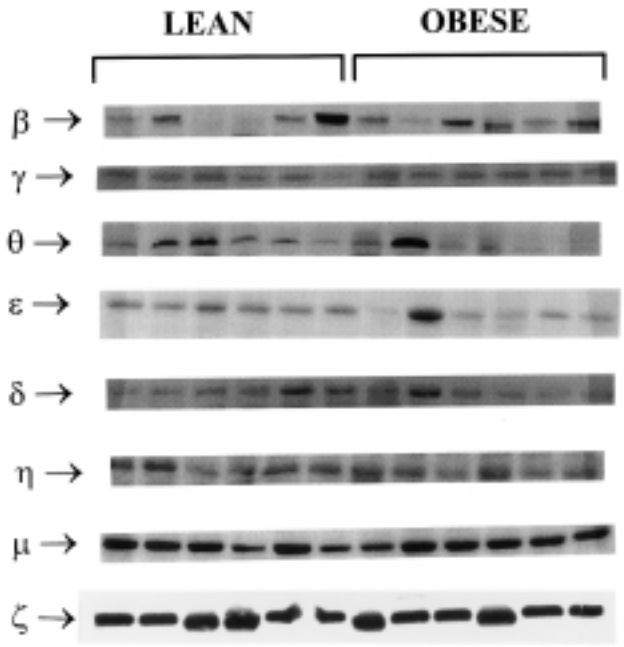

FIG. 3. Effect of insulin resistance on isoform-specific PKC content in the cytosolic fraction. Rectus abdominus skeletal muscle samples were obtained from lean insulin-responsive and obese insulin-resistant subjects. The cytosolic proteins (100 μg) were separated by SDS-PAGE electrophoresis, transferred to an Immobilon-P transfer membrane, and probed with various PKC isoform antibodies. A representative gel of the various PKC isoforms was investigated, with $n = 6$ for all observations.

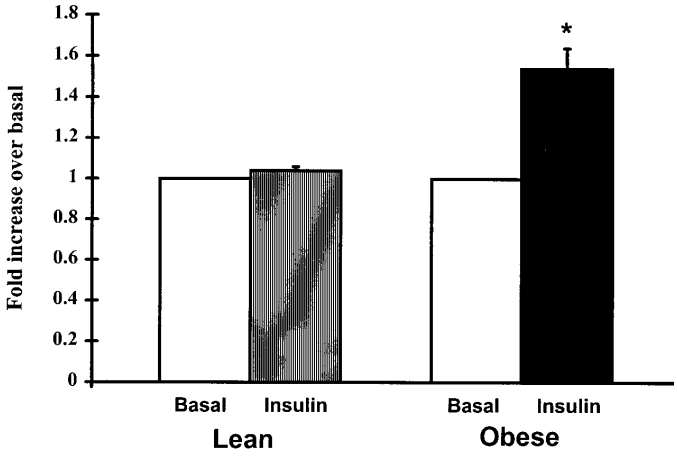

FIG. 4. Effect of insulin treatment on membrane-associated PKC activity. Rectus abdominus skeletal muscle fiber strips from lean insulin-responsive and obese insulin-resistant subjects were incubated with (insulin-treated) and without (basal) 100 nmol/l insulin. Insulin-stimulated PKC activity was measured in the membrane fractions. Data were calculated as picomoles of incorporated ^{32}P per minute per milligram protein and expressed as the fold-increase over basal (insulin-treated/basal), with $n = 5$ for lean subjects and $n = 5$ for obese subjects. (The basal activities were 194 ± 29 pmol · min^{-1} · mg^{-1} protein for the lean subjects and 170 ± 29 pmol · min^{-1} · mg^{-1} protein for the obese subjects.) *Significantly different from the corresponding lean value ($P < 0.05$).

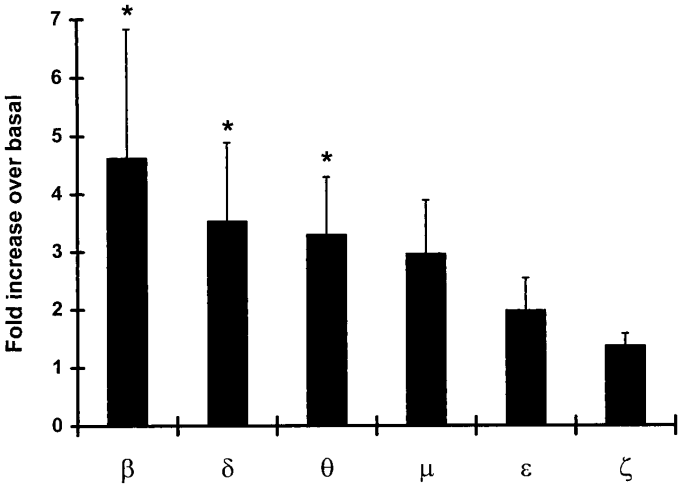

FIG. 5. Translocation of PKC to the membrane fraction in response to insulin. Rectus abdominus skeletal muscle fiber strips from obese subjects were incubated with (insulin-treated) and without (basal) 100 nmol/l insulin. Proteins (100 μg) of the particulate fraction (membrane) were separated by SDS-PAGE electrophoresis, transferred to an Immobilon-P transfer membrane, and probed with various PKC isoform antibodies. Data were analyzed by densitometric analysis and expressed as the fold-increase over basal (insulin-treated/basal), with the number of observations in parentheses. For PKC-β, -δ, -θ, and -ε, *n* = 10. For PKC-ζ and -μ, *n* = 9 and 7, respectively. *Significantly different from the corresponding basal value ($P < 0.05$).

13-phenylacetate 20 acetate (dPPA), a PKC activator, causes insulin resistance. These results provide strong support for the hypothesis that PKC is involved in causing insulin resistance.

There are a number of studies that have shown that PKC isoforms are elevated in insulin-resistant tissue. Liver PKC-α, -ε, and -ζ were increased in type 2 diabetic patients and may contribute to the insulin resistance seen in type 2 diabetes (21). In the soleus muscle of insulin-resistant type 2 diabetic Goto-Kakizaki rats, PKC-α, -β, -ε, and -δ protein content and activity were increased in the membrane fraction and decreased in the cytosolic fraction. In addition, PKC-θ protein content was reduced in both the membrane and cytosolic fractions, whereas PKC-ζ was not changed (22). In the skeletal muscle of high fat–fed rats, changes in the expression and localization of PKC-ε and -θ could be associated with skeletal muscle insulin resistance (23). In a recent study (11), infusion of lipids raised plasma FFA concentration in rats and resulted in skeletal muscle insulin resistance. This result is consistent with a decrease in insulin-stimulated insulin receptor substrate (IRS)-1 tyrosine phosphorylation, reduced insulin-stimulated IRS-1–associated phosphatidylinositol 3-kinase activity, and activation of PKC-θ in the membrane fraction. This finding suggests the possibility that in rat skeletal muscle, an increase in FFA causes insulin resistance mediated by activation of PKC-θ, which may increase serine phosphorylation of IRS-1 leading to reduced association of IRS-1 with phosphatidylinositol 3-kinase and reduced glucose uptake.

In this study, we investigated various PKC isoforms in human rectus abdominus skeletal muscle from obese insulin-resistant patients and lean insulin-responsive control subjects. In skeletal muscle from obese insulin-resistant patients, PKC-β protein content in the membrane fraction was significantly higher compared with that of the lean control subjects. In measuring the membrane-associated PKC activity, we found no significant difference in muscle from obese and lean control subjects. This finding could be because PKC-β protein content was significantly higher in muscle from obese patients, whereas PKC-η, -θ, and -μ protein contents were lower.

Studies have shown that insulin treatment causes activation and translocation of PKC-α, -β, and -ζ in 3T3/L1 cells (24) and PKC-α, -β, -θ, and -ε in rat skeletal muscle (25). We wondered if insulin might be increasing PKC activity to cause insulin resistance in obese subjects. Muscle fiber strips from lean insulin-responsive and obese insulin-resistant patients were incubated with and without insulin, and membrane-associated PKC activity was measured. Insulin-stimulated PKC activity in the membrane fraction was significantly increased in muscle from obese insulin-resistant patients compared with the lean insulin-responsive control subjects. In addition, the membrane-to-cytosol ratio further points toward translocation of PKC to the membrane and its activation in muscle from obese patients (data not shown). This result suggests that PKC in muscle from obese patients is more sensitive to translocation and activation upon insulin treatment. To determine which PKC isoforms in muscle from obese patients translocate to the membrane fraction in response to insulin, we incubated muscle fiber strips with insulin and measured the protein content of various PKC isoforms. PKC-β, -δ, and -θ translocated to the membrane fraction in response to insulin, which may explain the increased insulin-stimulated PKC activity seen in the membrane fraction of muscle from obese subjects.

In summary, these results suggest that in skeletal muscle from insulin-resistant obese patients, insulin receptor tyrosine kinase activity was reduced because of hyperphosphorylation on serine/threonine residues. Membrane-associated PKC-β protein was elevated under basal conditions, and membrane-associated total PKC activity was increased under insulin-stimulated conditions in muscle from obese insulin-resistant patients. Thus, we postulate that the decreased tyrosine kinase activity of the insulin receptor may be caused by serine/threonine phosphorylation by PKC.

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REFERENCES

1. Lee J, Pilch PF: The insulin receptor: structure, function, and signaling. *Am J Physiol* 266:C319–C334, 1994
2. Takayama S, White MF, Khan CR: Phorbol ester-induced serine phosphorylation of the insulin receptor decreases its tyrosine kinase activity. *J Biol Chem* 263:3440–3447, 1988
3. Bollag GE, Roth RA, Beaudoin J, Mochly-Rosen D, Koshland DE Jr: Protein kinase C directly phosphorylates the insulin receptor *in vitro* and reduces its protein-tyrosine kinase activity. *Proc Natl Acad Sci U S A* 83:5822–5824, 1986
4. Chin JE, Dickens M, Tavare JM, Roth RA: Overexpression of protein kinase C isoenzymes α, βI, γ, and ε in cells overexpressing the insulin receptor: effects on receptor phosphorylation and signaling. *J Biol Chem* 268:6338–6347, 1993
5. Berti L, Mosthaf L, Kroder G, Kellerer M, Tippmer S, Mushack J, Seffer E, Seedorf K, Häring H: Glucose-induced translocation of protein kinase C isoforms in rat-1 fibroblasts is paralleled by inhibition of the insulin receptor tyrosine kinase. *J Biol Chem* 269:3381–3386, 1994

6. Anderson CM, Olefsky JM: Phorbol ester-mediated protein kinase C interaction with wild-type and COOH-terminal truncated insulin receptors. *J Biol Chem* 266:21760–21764, 1991
7. Chin JE, Liu F, Roth RA: Activation of protein kinase C inhibits insulin-stimulated tyrosine phosphorylation of insulin receptor substrate-1. *Mol Endocrinol* 8:51–58, 1994
8. Bossenmaier B, Mosthaf L, Mischak H, Ullrich A, Häring HU: Protein kinase C isoforms βI and βII inhibit the tyrosine kinase activity of the insulin receptor. *Diabetologia* 40:863–866, 1997
9. Muller HK, Kellerer M, Ermel B, Mühlhöfer A, Obermaier-Kusser B, Vogt B, Haring HU: Prevention by protein kinase C inhibitors of glucose-induced insulin receptor tyrosine kinase resistance in rat fat cells. *Diabetes* 40:1440–1448, 1991
10. Cortright RN, Azevedo JL, Zhou Q, Sinha M, Pories WJ, Itani SI, Dohm GL: Protein kinase C modulates insulin action in human skeletal muscle. *Am J Physiol* 278:E553–E562, 2000
11. Griffin ME, Marcucci MJ, Cline GW, Bell K, Barucci N, Lee D, Goodyear LJ, Kraegen EW, White MF, Shulman GI: Free fatty acid–induced insulin resistance is associated with activation of protein kinase C θ and alterations in the insulin signaling cascade. *Diabetes* 48:1270–1274, 1999
12. Caro JF, Sinha MK, Raju SM, Ittoop O, Pories WJ, Flickinger EG, Meelheim D, Dohm GL: Insulin receptor kinase in human skeletal muscle from obese subjects with and without noninsulin dependent diabetes. *J Clin Invest* 79: 1330–1337, 1987
13. Maegawa H, Shigeta Y, Egawa K, Kobayashi M: Impaired autophosphorylation of insulin receptors from abdominal skeletal muscles in non-obese subjects with NIDDM. *Diabetes* 40:815–819, 1991
14. Zhou Q, Dolan PL, Dohm GL: Dephosphorylation increases insulin-stimulated receptor kinase activity in skeletal muscle of obese Zucker rats. *Mol Cell Biochem* 194:209–216, 1999
15. Dohm GL, Tapscott EB, Pories WJ, Dabbs DJ, Flickinger EG, Meelheim D, Fushiki T, Atkinson SM, Elton CW, Caro JF: An *in vitro* human muscle preparation suitable for metabolic studies: decreased insulin stimulation of glucose transport in muscle from morbidly obese and diabetic subjects. *J Clin Invest* 82:486–494, 1988
16. Ways DK, Cook P, Webster C, Parker PJ: Effect of phorbol ester on PKC-ζ. *J Biol Chem* 267:4799–4805, 1992
17. Olefsky JM, Ciaraldi TP, Kolterman OG: Mechanisms of insulin resistance in non-insulin-dependent (type II) diabetes. *Am J Med* 79 (Suppl. 3B):12–22, 1985
18. Kolterman OG, Insel J, Saekáw M, Olefsky JM: Mechanism of insulin resistance in human obesity: evidence for receptor and post-receptor defects. *J Clin Invest* 65:1272–1284, 1980
19. Fushiki T, Wells JA, Tapscott EB, Dohm GL: Changes in glucose transporters in muscle in response to exercise. *Am J Physiol* 19:E580–E587, 1989
20. Dunaif A, Xia J, Book CB, Schenker E, Tang Z: Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle. *J Clin Invest* 96:801–810, 1995
21. Considine RV, Nyce MR, Allen LE, Morales LM, Triester S, Serrano J, Colberg J, Lanza-Jacoby S, Caro JF: Protein kinase C is increased in the liver of humans and rats with non-insulin-dependent diabetes mellitus: an alteration not due to hyperglycemia. *J Clin Invest* 95:2938–2944, 1995
22. Avignon A, Yamada K, Zhou X, Spencer B, Cardona O, Saba-Siddique S, Galloway L, Standaert ML, Farese RV: Chronic activation of protein kinase C in soleus muscle and other tissues if insulin-resistant type II diabetic Goto-Kakizaki (GK), obese/aged, and obese/Zucker rats. *Diabetes* 45:1396–1404, 1996
23. Schmitz-Peiffer C, Browne CL, Oakes ND, Watkinson A, Chisholm DJ, Kraegen EW, Biden TJ: Alterations in the expression and cellular localization of protein kinase C isozymes ε and θ are associated with insulin resistance in skeletal muscle of high-fat-fed rat. *Diabetes* 46:169–178, 1997
24. Bandyopadhyay G, Standaert ML, Zhao L, Yu B, Avignon A, Galloway L, Karnam P, Moscat J, Farese RV: Activation of protein kinase C (α, β, and ζ) by insulin in 3T3/L1 cells. *J Biol Chem* 272:2551–2558, 1997
25. Yamada K, Avignon A, Standaert ML, Cooper DR, Spencer B, Farese RV: Effects of insulin on the translocation of protein linase C-θ and other protein kinase C isoforms in rat skeletal muscles. *Biochem J* 308:177–180, 1995