

Decreased Insulin Receptor Tyrosine Kinase Activity and Plasma Cell Membrane Glycoprotein-1 Overexpression in Skeletal Muscle From Obese Women With Gestational Diabetes Mellitus (GDM) Evidence for Increased Serine/Threonine Phosphorylation in Pregnancy and GDM

Jianhua Shao, Patrick M. Catalano, Hiroshi Yamashita, Irene Ruyter, Steven Smith, Jack Youngren, and Jacob E. Friedman

The cellular mechanisms for the insulin resistance of pregnancy and gestational diabetes mellitus (GDM) are unknown. The membrane protein plasma cell membrane glycoprotein-1 (PC-1) has been identified as an inhibitor of insulin receptor tyrosine kinase (IRTK) activity. We investigated insulin receptor function and PC-1 levels in muscle from three groups of obese subjects: women with GDM, pregnant women with normal glucose tolerance, and nonpregnant control subjects. Subjects ($n = 6$ for each group) were similar in age and degree of obesity (body fat $>30\%$). IRTK activity, insulin receptor tyrosine phosphorylation, and protein levels of membrane glycoprotein PC-1 were determined in rectus abdominus muscle biopsies obtained at the time of either elective cesarean section or gynecological surgery. No significant differences were evident in basal insulin receptor tyrosine phosphorylation or IRTK activity in the three groups. After maximal insulin (10^{-7} mol/l) stimulation, IRTK activity measured with the artificial substrate poly(Glu,Tyr) increased in all subjects but was lower in women with GDM by 25% ($P < 0.05$) and 39% ($P < 0.001$) compared with pregnant and nonpregnant control subjects, respectively. Similarly, insulin receptor tyrosine phosphorylation was significantly decreased in subjects

with GDM ($P < 0.05$) compared with pregnant and nonpregnant control subjects. Treatment of the insulin receptors with alkaline phosphatase to dephosphorylate serine/threonine residues increased insulin-stimulated IRTK activity significantly in pregnant control and GDM subjects ($P < 0.05$), but these rates were still lower compared with nonpregnant control subjects ($P < 0.05$). PC-1 content in muscle from GDM subjects was increased by 63% compared with pregnant control subjects ($P < 0.05$) and by 206% compared with nonpregnant control subjects ($P < 0.001$). PC-1 content was negatively correlated with insulin receptor phosphorylation ($r = -0.55$, $P < 0.05$) and IRTK activity ($r = -0.66$, $P < 0.05$). These results indicate that pregnant control and GDM subjects had increased PC-1 content and suggest excessive phosphorylation of serine/threonine residues in muscle insulin receptors and that both may contribute to decreased IRTK activity. These changes worsen in women with GDM when controlling for obesity. These postreceptor defects in insulin signaling may contribute to the pathogenesis of GDM and the increased risk for type 2 diabetes later in life. *Diabetes* 49:603–610, 2000

From the Departments of Nutrition (J.S., H.Y., J.E.F.) and Reproductive Biology (P.M.C., J.E.F.), Case Western Reserve University School of Medicine, MetroHealth Medical Center, Cleveland, Ohio; the Pennington Biomedical Research Center (I.R., S.S.), Louisiana State University, Baton Rouge, Louisiana; and the Diabetes Research Laboratory (J.Y.), Mount Zion Hospital, University of San Francisco, San Francisco, California.

Address correspondence and reprint requests to Jacob E. Friedman, PhD, Department of Nutrition, Case Western Reserve University, School of Medicine, 10900 Euclid Ave., Cleveland, OH 44106-4935. E-mail: jef8@po.cwru.edu.

Received for publication 12 May 1999 and accepted in revised form 17 December 1999.

BCA, bicinchoninic acid; BSA, bovine serum albumin; ECL, enhanced chemiluminescence; ELAST, ELISA Amplification System; ELISA, enzyme-linked immunosorbent assay; GDM, gestational diabetes mellitus; HRP, horseradish peroxidase; IRS-1, insulin receptor substrate-1; IRTK, insulin receptor tyrosine kinase; OGTT, oral glucose tolerance test; PC-1, plasma cell membrane glycoprotein-1; PCOS, polycystic ovary syndrome; PMSF, phenylmethylsulfonyl fluoride; PVDF, polyvinylidene difluoride; TBST, Tris-buffered saline with Tween; TCA, trichloroacetic acid; TMB, tetra methyl benzidine; TNF- α , tumor necrosis factor- α .

During pregnancy, one of the most significant maternal metabolic adaptations is a decrease in insulin sensitivity. Studies with the hyperinsulinemic-euglycemic clamp technique indicate that insulin-mediated glucose disposal decreases as much as 40–60% from early to late pregnancy (1,2). This adaptation provides adequate glucose and other nutrients for the development of the fetus. However, in 3–5% of pregnant women, glucose intolerance develops. Gestational diabetes mellitus (GDM) is characterized by further decrements in insulin sensitivity and an inability to compensate with increased insulin secretion (3–6). Although pregnancy-induced insulin resistance and GDM are generally reversible after pregnancy, ~30–50% of women with a history of GDM go on to develop type 2 diabetes later in life, particularly if they are obese (7–9). In addition, the offspring of GDM patients have a

greater incidence of perinatal complications and a higher prevalence of obesity and diabetes (9).

GDM represents a combination of acquired and intrinsic abnormalities of insulin action. The cellular mechanisms of insulin resistance in GDM are unknown; however, the resistance to insulin-mediated glucose transport appears to be greater in skeletal muscle from GDM subjects than that in women who are pregnant but do not have GDM (10). A modest but significant decrease in maximal insulin receptor tyrosine phosphorylation is also evident in muscle from obese GDM subjects (10). With no change in insulin receptor binding affinity (11–14), the decreased insulin receptor tyrosine phosphorylation suggests that insulin resistance to glucose transport in pregnancy and GDM is very likely related to postreceptor binding events that change the activation of the insulin receptor. Insulin initiates its effects on cellular metabolism by binding to the α -subunit of the receptor, which causes a conformational change that activates the β -subunit to undergo autophosphorylation on at least six tyrosine residues (15). The β -subunit is a membrane protein containing a tyrosine-specific protein kinase activity on its cytosolic domain. The phosphorylation of tyrosine residues on the β -subunit activates the receptor tyrosine kinase (16,17). This insulin receptor tyrosine kinase (IRTK) activity catalyzes various cellular signaling proteins, including insulin receptor substrate-1 (IRS-1), to undergo tyrosine phosphorylation.

Recent studies have found that overexpression of plasma cell membrane glycoprotein-1 (PC-1), a glycoprotein, plays a role in insulin resistance in subjects with and without type 2 diabetes (18–23). PC-1 has been shown to inhibit the tyrosine kinase activity of the insulin receptor (21). PC-1 was initially reported in skin fibroblasts from certain insulin-resistant patients (18). Subsequently, PC-1 content in the muscle tissue of obese subjects was negatively correlated with insulin-stimulated glucose transport (22). Transfection of PC-1 into cultured cells has confirmed that overexpression of PC-1 not only reduces IRTK activity but also may influence insulin action at a postreceptor site (24).

IRTK activity is regulated by several mechanisms. Insulin stimulates tyrosine phosphorylation of the insulin receptor, and this enhances the kinase activity toward other signaling proteins. The insulin receptor is also phosphorylated on serine/threonine residues before and after insulin stimulation (25,26). Serine/threonine phosphorylation of the insulin receptor downregulates the tyrosine kinase activity (27). In the present study, we investigated IRTK activity and PC-1 content in skeletal muscle from nonpregnant women, pregnant women with normal glucose tolerance, and women with GDM. We also studied the effect of dephosphorylation of serine/threonine residues on IRTK activity. Our results suggest that increased insulin receptor serine/threonine phosphorylation and PC-1 may underlie the insulin resistance of pregnancy and contribute to the pathogenesis of GDM.

RESEARCH DESIGN AND METHODS

Materials. Microtiter plates were from Nunc (Maxisorp Immunoplates; Copenhagen, Denmark). Bovine serum albumin (BSA) was from Boehringer Mannheim (Indianapolis, IN). Human recombinant insulin was from Sigma (St. Louis, MO). Biotin-conjugated antiphosphotyrosine antibody was from UBI (Lake Placid, NY). Horseradish peroxidase (HRP)-conjugated streptavidin and the Sulfo-NHS-LC-Biotinylation Kit were from Pierce (Rockford, IL). The ELISA (enzyme-linked immunosorbent assay) Amplification System (ELAST) was from Du Pont-NEN (Boston, MA). The tetra methanol benzidine (TMB) reagent kit was from

Kirkegaard and Perry Laboratories (Gaithersburg, MD). Trichloroacetic acid (TCA) and H_2PO_4 were from Fisher (Fair Lawn, NJ). Mouse anti-insulin receptor antibody was from Lab Vision (Fremont, CA) and was biotinylated according to instructions of the Sulfo-NHS-LC-Biotinylation Kit. The anti-human insulin receptor monoclonal antibody MA-20 was prepared as previously described (28). Secondary conjugated antibody and chemiluminescence reagents (enhanced chemiluminescence [ECL] kit) were obtained from Amersham Life Science (Arlington, IL). Precast 4–12% gradient mini gels were from Novex (San Diego, CA), polyvinylidene difluoride (PVDF) membranes were from Boehringer Mannheim, and other gel and Western blotting reagents were from Bio-Rad (Hercules, CA) and Sigma. Bicinchoninic acid (BCA) protein assay reagents were purchased from Pierce (Rockford, IL). Leupeptin and pepstatin were from Calbiochem (La Jolla, CA), and phenylmethylsulfonyl fluoride (PMSF) was obtained from Sigma. Human subjects. We studied six obese nonpregnant subjects, six obese pregnant patients with normal glucose tolerance, and six obese pregnant patients diagnosed with GDM during the third trimester (GDM). Subjects were recruited as outpatient volunteers. Pregnant subjects scheduled for elective cesarean delivery were classified as normal or as having GDM after a 100-g oral glucose tolerance test (OGTT) according to the National Diabetes Data Group criteria (29). The OGTT was administered between 26 and 28 weeks of gestation. Subjects diagnosed with GDM were treated with diet therapy before delivery. None of the pregnant control subjects had a family history (first-degree relative) of diabetes. Nonpregnant women undergoing elective gynecological surgery were recruited as nonpregnant control subjects. None of the nonpregnant control subjects was classified as having type 2 diabetes based on a 75-g OGTT. The experimental protocol was approved by the Institutional Review Board at MetroHealth Medical Center and the Scientific Advisory Committee of the General Clinical Research Center at Case Western Reserve University. All subjects gave written informed consent before enrollment in the study. Pregnant subjects were studied between 36 and 40 weeks of gestation. Before delivery or surgery, subjects were admitted to the General Clinical Research Center for estimation of body composition by underwater weighing ($n = 6$) or anthropometric methods ($n = 10$) as described previously (30,31). Rectus abdominus muscle samples were obtained at the time of delivery or elective surgery and were frozen at -80°C .

Tissue preparation. Soluble extracts were prepared from frozen muscle tissue to measure insulin receptor content and IRTK activity. Approximately 150–200 mg of frozen muscle tissue was homogenized in 2 ml homogenization buffer (20 mmol/l Tris, 5 mmol/l $MgCl_2$, 1 mmol/l PMSF, 2 $\mu\text{mol/l}$ leupeptin, 2 $\mu\text{mol/l}$ pepstatin, pH 8.7) at 4°C by using a Polytron PTA 20S generator (Brinkman Instruments, Westbury, NY) at maximum speed for 30 s. Triton X-100 was added to a final concentration of 1%. The homogenates were allowed to sit on ice and solubilized for 60 min, followed by centrifugation at 100,000g for 60 min at 4°C . The supernatant was collected and stored at -70°C .

Measurement of insulin receptor content. Insulin receptor level was measured by ELISA as described previously (22). Microtiter 96-well plates were coated with 0.2 μg anti-insulin receptor monoclonal antibody MA-20 in 100 μl of coating buffer (20 mmol/l Na_2CO_3 , pH 9.6). After incubating for 4 h at 22°C , the plates were washed three times with Tris-buffered saline with Tween (TBST) (20 mmol/l Tris, 150 mmol/l NaCl, and 0.05% Tween 20) to remove unbound antibody. The wells were blocked with 150 μl of blocking buffer (TBST with 1% BSA) for 30 min at 56°C . The plates were washed three times with TBST, and then 50- μl muscle extracts or 10–100 pg of insulin receptor standard from human muscle extract (determined previously) were loaded with binding buffer (50 mmol/l HEPES, 150 mmol/l NaCl, 0.1% BSA, 0.1% Triton X-100, 1 mmol/l PMSF, 1 mg/ml bacitracin, 2 $\mu\text{mol/l}$ leupeptin, and 2 $\mu\text{mol/l}$ pepstatin, pH 7.6) with the final amount equal to 100 $\mu\text{l/well}$. After an overnight incubation at 4°C , the plates were washed five times with TBST, and then 30 ng/well of biotinylated anti-insulin receptor antibody in buffer A (50 mmol/l HEPES, 150 mmol/l NaCl, 0.1% BSA, 0.05% Tween 20, 1 mg/ml bacitracin, 1 mmol/l PMSF, 2 mmol/l Na_3VO_4 , pH 7.6) was added at 100 $\mu\text{l/well}$. The plates were incubated at 22°C for 2 h. After washing five times with TBST, the wells were loaded with 0.1 μg peroxidase-conjugated streptavidin in 100 μl of buffer B and were incubated for 30 min at 22°C . The plates were washed successively to remove unbound peroxidase-conjugated streptavidin, and 100 μl biotinyl-tyramide working solution (from the ELAST amplification kit) was added to each well and incubated for an additional 15 min at 22°C . After five washes with 100 μl of phosphate-buffered saline plus Tween 20 (137 mmol/l NaCl, 8 mmol/l Na_2HPO_4 , 2.7 mmol/l KCl, 1.15 mmol/l KH_2PO_4 , 1% BSA, 0.05% Tween 20), 0.2 μl of streptavidin-HRP solution was added to each well and incubated for 30 min at 22°C . After further washing with TBST, 100 μl TMB peroxidase substrate solution was added and incubated for 10–20 min at 22°C . The reaction was stopped by adding 100 $\mu\text{l/well}$ of 1 mol/l H_2PO_4 , and the absorption was measured at 450 nm with a microtiter plate reader. The concentration of insulin receptor from each patient was calculated from the standard curve by using 10–100 pg human insulin receptor.

IRTK activity assay. The ability of immunocaptured insulin receptors to phosphorylate exogenous substrates was determined as described by Youngren et al.

(32). Insulin receptors were immunocaptured on microtiter plates as described above for the receptor content assay, except that 2 ng of insulin receptor was added to each well. Insulin receptors were preincubated with 40 μ l of assay buffer (as above) for 60 min, and 100 nmol/l insulin was added or not 30 min before the start of reaction. The reaction was initiated by adding 10 μ l of 50 μ mol/l ATP in assay buffer with 50 μ g of substrate poly(Glu,Tyr) 1:4 and -0.2 μ Ci [γ - 32 P]ATP. The reaction was allowed to continue for 60 min at 22°C and was stopped by blotting 25 μ l of reaction mixture onto Whatman 3MM paper (Maidstone, U.K.). Filter papers were washed extensively with 10% TCA with 10 mmol/l $\text{Na}_4\text{P}_2\text{O}_7$ for 15 min at 4°C, for 15 min at 20°C, and for 2–5 min at boiling followed by a short rinse with acetone. Incorporation of 32 P was determined by liquid scintillation counting.

Insulin receptor dephosphorylation and kinase activity assay. Before testing the muscle samples with alkaline phosphatase, we analyzed the dose-response effect of alkaline phosphatase treatment on isolated insulin receptors from human placenta (a tissue with a high abundance of insulin receptors). Treatment with 20 U of alkaline phosphatase dephosphorylated the tyrosine residues as well as the serine/threonine residues remaining in the receptor, which resulted in a >80% reduction in basal IRTK activity (data not shown). The dephosphorylation and IRTK activity assay were performed as described above for the receptor kinase activity assay except that, before preincubation, the muscle receptors were dephosphorylated by incubating them with 20 U of calf intestine alkaline phosphatase (Stratagene, La Jolla, CA) at 25°C for 30 min (33).

Insulin receptor autophosphorylation assay. The autophosphorylation of muscle insulin receptor was measured by using the ELISA technique according to Youngren (32). A total of 60 pg of insulin receptors from each patient was added to the plates coated with anti-insulin receptor antibody. After overnight incubation, the receptors were preincubated with 80 μ l/well of assay buffer (50 mmol/l HEPES, 150 mmol/l NaCl, 0.05% BSA, 0.1% Triton X-100, 10 mmol/l MgCl_2 , 2 mmol/l MnCl_2 , pH 7.6) for 15 min at 22°C, with or without insulin (final concentration 10^{-7} mol/l). The reaction was initiated by the addition of 20 μ l ATP (50 μ mol/l, final concentration 10 μ mol/l) in assay buffer, and the reaction was allowed to continue for 60 min at 22°C. After washing, 30 ng of biotinylated antiphosphotyrosine antibody was added with 100 μ l of buffer B. The remaining steps were followed as described above.

PC-1 content measurement. PC-1 was measured by using Western blot analysis. Samples (30 μ g protein measured by the BCA method with BSA as the standard) were run on 4–12% gradient denaturing gel and transferred overnight to PVDF membranes. Membranes were blocked with 5% nonfat milk (Carnation; Nestlé Foods, Glendale, CA) in TBST, washed three times with TBST, and probed by using a polyclonal antibody against the final 18 amino acids of the COOH-terminus of the human PC-1 protein (antibody J14 1:1,000 dilution in TBST). After a 3-h incubation at room temperature, the membranes were washed as above and incubated with anti-rabbit IgG HRP (1:2,000 dilution in TBST) for 30 min. Membranes were again washed as above, and ECL detection reagents were added for 1 min and were immediately exposed to X-ray film. Quantification of the 110-kDa band was performed by using a Bio-Rad imaging densitometer with 8 μ g of whole cell lysate of the human hepatoma cell line HepG2 to control for gel-to-gel variation. Results are expressed as a ratio to the HepG2 control.

Statistical analyses. All data are means \pm SE for the indicated number of patients. Differences between groups were analyzed by two-way analysis of variance and post hoc testing. Linear regression was used for correlation analysis by using StatView II (Abacus Concepts, Berkeley, CA). $P < 0.05$ was considered to be statistically significant.

RESULTS

Subjects characteristics. Characteristics of the groups are summarized in Table 1. All subjects were considered obese because the average percentage of body fat for all three groups was >30%. GDM subjects had normal fasting glucose levels that were similar to the nonpregnant control subjects. Fasting glucose concentrations in pregnant control subjects were lower compared with nonpregnant control and GDM subjects ($P < 0.05$). Basal insulin levels were significantly higher in GDM patients compared with pregnant control and nonpregnant control subjects ($P < 0.05$).

Insulin receptor concentration and autophosphorylation. The insulin receptor protein concentration in skeletal muscle biopsies was similar in all three groups of patients (Table 2). We then measured basal and insulin-stimulated receptor autophosphorylation with ELISA by using an equal concentration of insulin receptor protein from each patient. Under

TABLE 1
Subject characteristics

	Nonpregnant control	Pregnant control	GDM
n	6	6	6
Age (years)	37.0 \pm 3.8	34.2 \pm 4.9	35.2 \pm 4.5
Body fat (%)	37.0 \pm 6.6	31.7 \pm 7.4	37.9 \pm 4.2
Fasting glucose (mg/dl)	88 \pm 16	76 \pm 8*	94 \pm 9
Fasting insulin (μ U/ml)	11.0 \pm 7.1	13.3 \pm 7.0	23.5 \pm 12.8†

Data are means \pm SD. * $P < 0.05$, significantly less than nonpregnant control and GDM subjects; † $P < 0.05$, significantly higher than nonpregnant control and pregnant control subjects.

basal conditions, the levels of insulin receptor tyrosine phosphorylation were not significantly different in muscles from all three groups. After 30 min of insulin (10^{-7} mol/l) stimulation, maximal insulin receptor tyrosine phosphorylation increased in all subjects. No significant difference was evident between nonpregnant control and pregnant control subjects. For GDM patients, the maximal insulin-stimulated receptor tyrosine phosphorylation was 19 and 22% lower compared with pregnant control and nonpregnant control groups, respectively ($P < 0.05$). These results of lower insulin-stimulated receptor phosphorylation in GDM subjects were similar to the in vivo results obtained previously by Western blotting in intact skeletal muscle strips (10). The insulin-stimulated increase of tyrosine phosphorylation over basal was significantly lower in pregnant control and GDM subjects compared with nonpregnant control subjects ($P < 0.05$).

IRTK activity and effect of alkaline phosphatase treatment. We next determined the IRTK activity toward an artificial substrate in vitro by using the ELISA procedure. In the absence of insulin, basal IRTK activities were not significantly different in muscle from the three groups (Fig. 1). After

TABLE 2
Insulin receptor protein levels and effect of insulin on receptor tyrosine phosphorylation in skeletal muscle in vitro

Subjects	Insulin receptor protein concentration (pg/ μ g total protein)	Insulin receptor tyrosine phosphorylation (arbitrary units)	
		- Insulin	+ Insulin
Nonpregnant control	10.45 \pm 2.85	6.70 \pm 0.83	15.00 \pm 0.74*
Pregnant control*	12.88 \pm 4.88	9.10 \pm 1.35	15.55 \pm 0.64*
GDM	10.89 \pm 4.07	6.70 \pm 0.44	12.60 \pm 0.51†

Data are means \pm SE (n = 6/group). Rectus abdominus muscle biopsies were obtained at the time of cesarean section delivery (pregnant control and GDM) or elective surgery (nonpregnant control). Soluble extracts were prepared from frozen muscle tissue to measure insulin receptor content and insulin receptor autophosphorylation by ELISA, as described in RESEARCH DESIGN AND METHODS and previous studies (22,23). * $P < 0.01$, significantly different vs. the - insulin value; † $P < 0.05$, significantly different vs. nonpregnant and pregnant control groups.

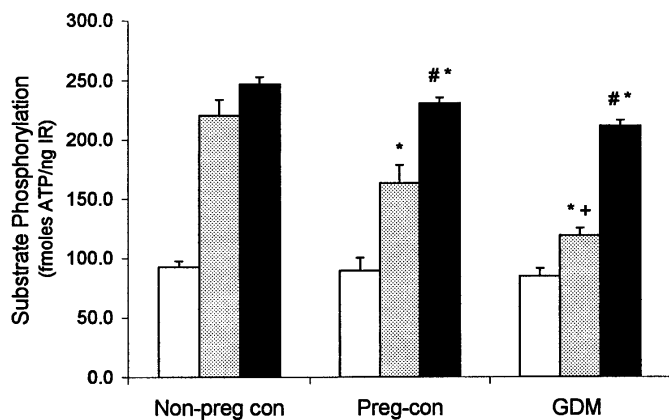


FIG. 1. Effect of prior alkaline phosphatase treatment on insulin-stimulated IRTK activity in skeletal muscle from nonpregnant control (Non-preg con), pregnant control (Preg-con), and GDM subjects. Rectus abdominus muscle biopsies were obtained during gynecological surgery or at the time of scheduled cesarean section delivery and were stored at -70°C . The muscle tissues were homogenized under denaturing conditions at 4°C , and the same amount of insulin receptors (determined previously) were immunocaptured on a 96-well plate precoated with anti-insulin receptor antibody as described in RESEARCH DESIGN AND METHODS. One aliquot of insulin receptors was treated with 20 U of alkaline phosphatase before assaying the IRTK activity as outlined in RESEARCH DESIGN AND METHODS. Before assaying the kinase activity, the receptors were incubated with 100 nmol/l of insulin at 22°C for 30 min and then incubated with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. Substrate phosphorylation was determined by adsorbing peptide substrate to filter paper and scintillation counting. IRTK activity is expressed as femtomoles of ATP incorporated into artificial substrate poly(Glu,Tyr) 1:4 per nanogram of insulin receptor. The data are means \pm SE for nonpregnant control ($n = 6$), pregnant control ($n = 6$), and GDM ($n = 6$) subjects. *Significantly lower than the nonpregnant control subjects ($P < 0.05$); +significantly lower than pregnant control subjects ($P < 0.05$); #significantly greater than the corresponding kinase activity in the same group without alkaline phosphatase treatment ($P < 0.05$). \square , insulin (-) and alkaline phosphatase (-); \square with diagonal lines, insulin (+) and alkaline phosphatase (-); \blacksquare , insulin (+) and alkaline phosphatase (+).

insulin stimulation, IRTK activity increased significantly in all samples. In muscle from GDM subjects, maximal IRTK activity was significantly lower than in pregnant control (25%, $P < 0.05$) and nonpregnant control (39%, $P < 0.001$) subjects (Fig. 1). IRTK activities of pregnant control subjects were 21% lower compared with nonpregnant control subjects ($P < 0.05$). No relationship was evident between IRTK activity and fasting glucose levels ($r = 0.38$, $P = 0.129$, $n = 16$).

After insulin stimulation, alkaline phosphatase-treated receptors showed significantly increased IRTK activity in GDM and pregnant control subjects. The percentage increase over basal did not change significantly in nonpregnant control subjects, whereas the insulin effect in pregnant control and GDM subjects increased by 48 and 80%, respectively. Alkaline phosphatase treatment increased the maximal IRTK activity in all three groups; however, IRTK activity for GDM and pregnant control subjects was still significantly lower than for nonpregnant control subjects.

Increased PC-1 content in pregnancy and GDM. The PC-1 content in muscle tissues was measured by using Western blot analysis. PC-1 levels in GDM subjects were the highest of all three groups (Fig. 2). PC-1 levels were 63% greater compared with pregnant control subjects and 206% greater compared with nonpregnant control subjects. Pregnant con-

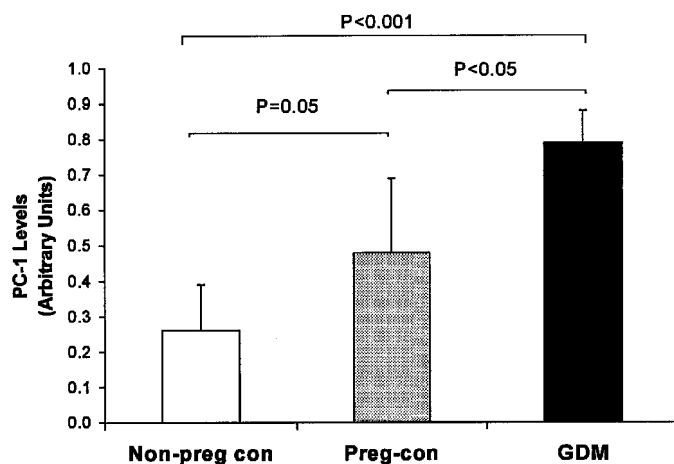


FIG. 2. PC-1 protein content in the skeletal muscle of nonpregnant control (Non-preg con), pregnant control (Preg-con), and GDM subjects. Frozen muscle tissues were homogenized, and the protein samples were run on a denatured gel system. After transfer to the membrane, the PC-1 was probed by polyclonal anti-PC-1 antibody. Quantification of the 110-kDa band was performed using a Bio-Rad imaging densitometer with 8 μg of whole cell lysate of the human hepatoma cell line HepG2 to control for gel-to-gel variation. Results are expressed as a ratio to the HepG2 control. Data are expressed in arbitrary units and as means \pm SE for six to eight patients per group.

rol subjects' PC-1 levels were higher compared with nonpregnant control subjects ($P = 0.05$). For all of the subjects, the PC-1 contents were positively correlated with basal plasma insulin concentrations ($r = 0.82$, $P < 0.05$) and were negatively correlated with insulin receptor phosphorylation ($r = -0.55$, $P < 0.05$) and IRTK activity ($r = -0.66$, $P < 0.05$) (Fig. 3). PC-1 content was also negatively correlated with IRTK activity after alkaline phosphatase treatment ($r = -0.79$, $P < 0.05$).

No significant correlation was evident between PC-1 and body fat mass ($r = 0.151$, $P = 0.584$) and between PC-1 and BMI ($r = 0.124$, $P = 0.65$) ($n = 16$). Although the GDM group had slightly higher fasting glucose levels, the correlation between PC-1 and glucose ($r = 0.109$, $P = 0.716$) was not significant. The relationship between tumor necrosis factor- α (TNF- α) and PC-1 levels ($r = 0.398$, $P = 0.182$) was also not significant, which suggests that TNF- α or glucose levels did not modulate PC-1 in our subjects. The relationship between IRTK and TNF- α levels was also not significant ($r = 0.215$, $P = 0.432$) ($n = 16$).

DISCUSSION

The purpose of the present study was to determine the mechanisms for insulin resistance in skeletal muscle during pregnancy in obese women with normal glucose tolerance and women with GDM. Specifically, we investigated the role of increased PC-1 and serine/threonine phosphorylation on insulin receptor function as two potential causal mechanisms for insulin resistance in obese GDM subjects. We elected to study a population of obese GDM subjects for clinical reasons. Obesity is a major risk factor for GDM, and most women who develop GDM are obese (33) as defined as a pregravid BMI of 27.3 kg/m^2 . Thus, understanding the mechanisms for most cases of GDM requires an obese population. To control for obesity as a potential confounding variable, we closely matched the pregnant control and GDM subjects for a similar degree of obesity based on percentage

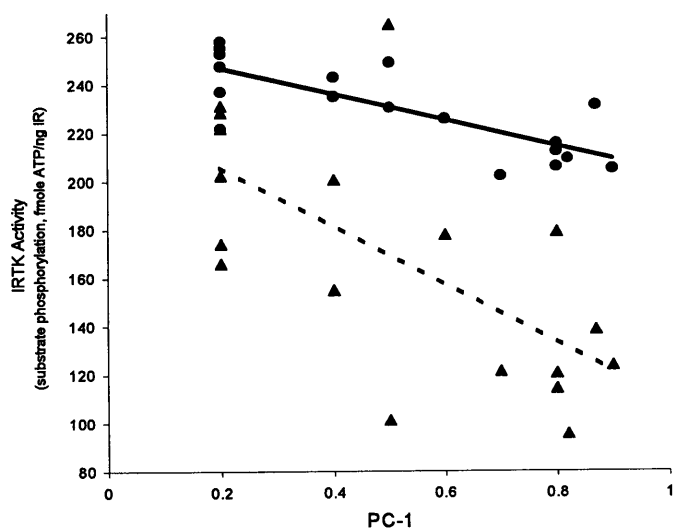


FIG. 3. Relationship between skeletal muscle PC-1 content and IRTK activity with and without alkaline phosphatase treatment. Equal amounts of insulin receptors were immunocaptured on a microtiter plate. The IRTK activity was assayed as outlined in Fig. 1. Substrate phosphorylation was determined by adsorbing peptide substrate to filter paper and scintillation counting. The muscle samples were divided into two aliquots; one sample of insulin receptors was treated with 20 U of alkaline phosphatase treatment before assaying the IRTK activity, and the other sample was not treated. The solid line indicates the correlation between IRTK and PC-1 content after alkaline phosphatase treatment. The broken line indicates the correlation between IRTK without alkaline phosphatase treatment. IR, insulin receptor. —, Alkaline phosphatase (+); ●, $r = -0.79$, $P < 0.05$. - - -, Alkaline phosphatase (-); ▲, $r = -0.66$, $P < 0.05$.

of body fat. Thus, the only difference between the GDM and pregnant control subjects was the diagnosis of GDM. The non-pregnant control subjects were also obese. This design allowed us to discriminate between the components of insulin resistance resulting from pregnancy alone versus those resulting from obesity or GDM.

We studied maximal insulin receptor autophosphorylation with a sensitive ELISA measurement by using the same concentration of muscle insulin receptor protein from each subject. We also formally tested the IRTK activity against an artificial substrate. After insulin stimulation *in vitro*, IRTK activity was significantly less in pregnant control and GDM subjects compared with nonpregnant control subjects with no change in basal IRTK activity. In addition, IRTK activity was significantly lower in GDM subjects compared with pregnant control subjects. In this study, all subjects studied were obese; thus, the decrease in IRTK activity in pregnant control subjects compared with nonpregnant control subjects is likely because of pregnancy and not because of obesity. The further decrease in IRTK activity in GDM may be because of an additional mechanism related to the intrinsically (genetically) greater insulin resistance in this population. The mechanisms for impaired IRTK activity have not been identified. Studies in rats have reported that pregnancy is associated with decreased IRTK activity in liver (34,35) but not in skeletal muscle (13). Damm et al. (14) reported no difference in IRTK in their population of lean pregnant women; however, their study protocol and methods cannot be compared with our own. Their samples were obtained 4–8 weeks before parturition from the vastus lateralis muscle. Because insulin sen-

sitivity declines most rapidly during the last trimester, this may be one factor for the lack of a change in IRTK found in their study. These investigators also relied on partial purification of isolated receptors by using wheat germ agglutinin before measuring IRTK activity. This procedure can disrupt the receptor environment necessary for maintaining the normal *in vivo* activity of the receptor. Thus, their data in lean pregnant subjects may not be comparable with our own data involving the insulin receptor ELISA. The different type of muscles sampled may also be a factor; however, this is speculative, and we have not compared the IRTK activity between vastus and rectus muscle. The decreased IRTK activity found in the present study in GDM subjects was also associated with decreased maximal receptor autophosphorylation (Table 2). These results *in vitro* correspond well with our former studies of insulin receptor and IRS-1 tyrosine phosphorylation measured in intact muscle fiber strips from these same subjects (10).

Most studies of human skeletal muscle have found that IRTK activity is impaired in type 2 diabetes and in subpopulations of insulin-resistant subjects (32,36,37). Phosphorylation of serine/threonine residues on the insulin receptor has been suggested as one cause of decreased insulin-stimulated IRTK activity in diabetes (27,38,39); however, this mechanism has not been demonstrated in humans with the disease (40). In the present study, we did not measure serine/threonine phosphorylation of the insulin receptors directly. However, after treatment with alkaline phosphatase to remove phosphorylation, the maximal insulin-stimulated IRTK activity increased significantly in receptors from pregnant control and GDM subjects. A slight increase was evident in non-pregnant control subjects, but it was not significant. The increase in IRTK activity in GDM patients was the highest of all three groups. These data suggest that insulin receptors from the muscles of pregnant control and GDM patients may be serine/threonine phosphorylated to a greater degree than insulin receptors from obese nonpregnant control subjects. Excessive serine phosphorylation has been demonstrated directly in fibroblasts and in skeletal muscle from certain women with severe insulin resistance resulting from polycystic ovary syndrome (PCOS) (41). Like diabetes, PCOS is a heterogeneous disorder. In general, women with PCOS are obese and have an increased leutinizing hormone/follicle-stimulation hormone ratio. Most are hyperinsulinemic, and some have demonstrated increased corticosterone production (42,43), which suggests some commonalities between the insulin resistance of PCOS and the insulin resistance of pregnancy. The reversibility of the IRTK activity by alkaline phosphatase suggests the involvement of posttranslational modification regarding the insulin receptor. In intact cells, insulin receptor serine/threonine phosphorylation can be stimulated by prolonged insulin treatment, phorbol esters, and cAMP analogs, presumably as a result of activation by protein kinase C (27). Increased protein kinase C has been suggested as a possible factor responsible for serine/threonine phosphorylation in type 2 diabetes and in certain kinds of insulin resistance (27,38,44). Expression of protein kinase C in skeletal muscle is higher in insulin-resistant and type 2 diabetic rats (45,46). In addition, the cytokine TNF- α has been shown to act as a serine/threonine kinase to inhibit IRS-1 and insulin receptor tyrosine phosphorylation (33,47). Circulating TNF- α levels increase during pregnancy and correlate well

with the extent of insulin resistance measured in humans during pregnancy (49). The relationship between IRTK and TNF- α levels, however, was not significant ($r = 0.215$, $P = 0.432$) ($n = 16$). Thus, variation in TNF- α levels does not appear to be associated with IRTK activity in these subjects. Evidence also exists that hyperglycemia induces serine phosphorylation and contributes to glucose-induced insulin resistance (37). The relationship between IRTK activity and fasting glucose levels in the present study was not significant ($r = 0.38$, $P = 0.129$). However, this is perhaps not surprising because none of the groups had fasting hyperglycemia, and the diagnosis of GDM was made on the basis of two or more elevated values from a 100-g 3-h OGTT.

Although a significant increase was evident in IRTK activities after alkaline phosphatase treatment in pregnant control and GDM subjects, the IRTK activities were still significantly lower compared with those of nonpregnant control subjects. The insulin receptors from muscle were dephosphorylated before insulin treatment by using 20 U of alkaline phosphatase, which is a concentration known to maximally dephosphorylate IRTK activity in human placenta (J.S., J.E.F., unpublished data). Although we did not test whether this concentration maximally dephosphorylated the muscle receptors, this would likely be underestimated in pregnancy because the basal IRTK activity was unchanged across different groups, and the amount of alkaline phosphatase used in the muscle samples (20 U) removed at least 80% of the IRTK activity in the placenta, which is a tissue with a high concentration of insulin receptors and high IRTK activity. We interpret the lower insulin-stimulated IRTK activity remaining in receptors from pregnant control and GDM subjects to suggest that excessive phosphorylation of serine/threonine residues is not the only mechanism for reducing IRTK activity in skeletal muscle from pregnant and GDM patients. Other factors inhibit the IRTK activity of skeletal muscle.

We found that PC-1 protein levels were elevated in muscle tissues from pregnant control subjects and more so in GDM subjects compared with nonpregnant control subjects. Moreover, a negative correlation existed between PC-1 content and both insulin receptor autophosphorylation and IRTK activity. No significant correlation was evident between PC-1 and body fat mass ($r = 0.151$, $P = 0.584$) or between PC-1 and BMI ($r = 0.124$, $P = 0.65$) ($n = 16$), which indicates that PC-1 is not modified by obesity per se. Our correlations suggest that a significant relationship exists between PC-1 and IRTK activity. The mechanisms for PC-1 inhibition of insulin signaling are currently under investigation. Reports have indicated that PC-1 may have threonine-specific protein kinase activity (50). However, the existence of this protein kinase activity has been questioned (51). The correlation coefficient between IRTK and PC-1 remained significant even after treatment with alkaline phosphatase. This seems to suggest that serine phosphorylation is not the mechanism of PC-1 effect and that multiple mechanisms exist for insulin resistance in this population, of which serine phosphorylation is only one. One factor that may account for the continued strong relationship between PC-1 levels and IRTK after dephosphorylation is that PC-1 binds directly to the insulin receptor α -subunit and interferes with activation of the receptor β -subunit by insulin (52,53). PC-1 and the insulin receptor appear to interact through amino acid residues 485–599 on the insulin receptor, thereby preventing the insulin-induced conformational

change (53). Overexpression of PC-1 inhibits IRTK activity but not insulin binding (54), and a significant negative relationship exists between increased PC-1 content and maximal 2-deoxyglucose transport in human skeletal muscle from obese subjects (22). In addition, muscle PC-1 content has been shown to correlate with IRTK activity in lean insulin-resistant subjects (55).

A recent article by Sakoda et al. (56) suggested that PC-1 overexpression does not inhibit IR function in mouse and human tissues. These investigators infected mouse 3T3-L1 cells with an adenovirus construct containing the human PC-1 coding sequence and used a high concentration of insulin to detect IR phosphorylation. Several explanations exist for these negative results. These investigators used a high concentration of insulin and used a nonspecific anti-phosphotyrosine antibody that could detect other tyrosine phosphorylated proteins such as the IGF-1 receptor that are not influenced by PC-1. They also did not measure PC-1 enzymatic activity to show that the PC-1 molecule was functional. Furthermore, a study recently showed that a common PC-1 polymorphism is associated with decreased IRTK activity and insulin resistance in nonobese patients with no change in PC-1 protein levels, which suggests more than one mechanism for PC-1 associated insulin resistance (57).

In conclusion, the present results suggest that increased PC-1 and impaired IRTK activity are not caused by obesity but rather may play an important role in the natural insulin resistance of pregnancy and may be the basis for the higher intrinsic insulin resistance of GDM by mechanisms that are not currently well understood. The findings suggest the possibility that the insulin receptor may be under tonic inhibition by serine phosphorylation and that the factors responsible for this may be upregulated during pregnancy. Further studies are underway to identify the specific sites of serine/threonine phosphorylation and to examine the causes for this in skeletal muscle biopsies obtained after pregnancy and GDM revert. The increase in PC-1 and decrease in IRTK activity, in addition to decreased IRS-1 expression/phosphorylation found previously (10), may underlie the insulin resistance of pregnancy and risk of progression to GDM.

ACKNOWLEDGMENTS

This research was supported by Perinatal Emphasis Research Center Grant NIH-HD11089 (J.E.F., P.M.C) and grant R01-DK5299 from the National Institutes of Health. In addition, J.F.Y. was supported by a grant from the Alexander M. and June L. Maisin Foundation of the Jewish Community Federation's Endowment Fund.

REFERENCES

1. Catalano PM, Tyzbir ED, Wolfe RR, Roman NM, Amini SB, Sims EA: Longitudinal changes in insulin release and insulin resistance in non-obese pregnant women. *Am J Obstet Gynecol* 165:1667–1672, 1991
2. Costrini NV, Kalkhoff RK: Relative effects of pregnancy, estradiol and progesterone on plasma insulin and pancreatic islet insulin secretion. *J Clin Invest* 50:922–929, 1971
3. Barden TP, Knowles HC: Diagnosis of diabetes in pregnancy. *Clin Obstet Gynecol* 24:3–19, 1981
4. Buchanan TA, Metzger BE, Freinkel N, Bergman RN: Insulin sensitivity and beta cell responsiveness to glucose during late pregnancy in lean and moderately obese women with normal glucose tolerance or mild gestational diabetes. *Am J Obstet Gynecol* 162:1008–1014, 1990
5. Kul C: Insulin secretion and insulin resistance in pregnancy and GDM. *Diabetes (Suppl. 1)* 2:18–24, 1991

6. Kautzky-Willer A, Prager R, Waldhausl W, Pacini G, Thomasset K, Wagner OF, Ulm M, Strelci C, Ludvik B: Pronounced insulin resistance and inadequate β -cell secretion characterize lean gestational diabetes during and after pregnancy. *Diabetes Care* 20:1717–1723, 1997
7. Kjos SL, Peter RK, Xiang A, Henry OA, Montoro M, Buchanan TA: Predicting future diabetes in Latino women with gestational diabetes: utility of early postpartum glucose tolerance testing. *Diabetes* 44:586–591, 1995
8. Hagbard L, Svanborg A: Prognosis of diabetes mellitus with onset during pregnancy: a clinical study of 71 cases. *Diabetes* 9:296–302, 1960
9. Pettitt DJ, Baird HR, Aleck KA, Bennett PH, Knowler WC: Excessive obesity in offspring of Pima Indian women with diabetes during pregnancy. *N Engl J Med* 308:242–245, 1983
10. Friedman JE, Ishizuka T, Huston L, Highman T, Shao JH, Catalano P: Impaired glucose transport and insulin receptor tyrosine phosphorylation in obese women with gestational diabetes mellitus. *Diabetes* 48:1807–1814, 1999
11. Moore P, Kolterman O, Weynat J, Olefsky JM: Insulin binding in human pregnancy: comparisons to the postpartum, luteal and follicular states. *J Clin Endocrinol Metab* 52:937–941, 1981
12. Puavilai G, Dorbnay EC, Domont LA: Insulin receptors and insulin resistance in human pregnancy: evidence for a postreceptor defect in insulin action. *J Clin Endocrinol Metab* 54:247–253, 1982
13. Camps M, Guma A, Testar X, Palacin M, Zorzano A: Insulin resistance of skeletal muscle during pregnancy is not a consequence of intrinsic modifications of insulin receptor binding or kinase activities. *Endocrinology* 127:2561–2570, 1990
14. Damm P, Handberg A, Kuhl C, Beck-Nielsen H, Molsted-Pedersen L: Insulin receptor binding and tyrosine kinase activity in skeletal muscle from normal pregnant women and women with gestational diabetes. *ObstetGynecol* 82:251–259, 1993
15. White MF, Shoelson SE, Keutmann H, Kahn CR: A cascade of tyrosine autophosphorylation in the B-subunit activates the insulin receptor. *J Biol Chem* 263:2969–2975, 1988
16. Wilden PA, Siddel K, Haring H, Backer JM, White MF, Kahn CR: The role of insulin receptor kinase domain autophosphorylation in receptor-mediated activities. *J Biol Chem* 267:13719–13726, 1992
17. White MF, Kahn CR: The insulin signaling system. *J Biol Chem* 269:1–4, 1994
18. Sbraccia P, Goodman PA, Muddux BA, Wong KY, Chen YD, Reaven GM, Goldfine ID: Production of an inhibitor of insulin receptor tyrosine kinase in fibroblasts from a patient with insulin resistance and NIDDM. *Diabetes* 40:295–299, 1991
19. Maddux BA, Sbraccia P, Reaven GM, Moller DE, Goldfine ID: Inhibitors of insulin receptor tyrosine kinase in fibroblasts from diverse patients with impaired insulin action: evidence for a novel mechanism of postreceptor insulin resistance. *J Clin Endocrinol Metab* 77:73–79, 1993
20. Maddux BA, Sbraccia P, Kumakura S, Sasson S, Youngren J, Fisher A, Spencer S, Grupe A, Henzel W, Stewart TA, Reaven GM, Goldfine ID: Membrane glycoprotein PC-1 and insulin resistance in non-insulin-dependent diabetes mellitus. *Nature* 373:448–451, 1995
21. Goldfine ID, Maddux BA, Youngren JF, Frittitta L, Trischitta V, Dohn GL: Membrane glycoprotein PC-1 and insulin resistance. *J Cell Biochem* 182:177–184, 1998
22. Youngren JF, Maddux BA, Sasson S, Sbraccia P, Tapscott EB, Swanson MS, Dohn GL, Goldfine ID: Skeletal muscle content of membrane glycoprotein PC-1 in obesity. *Diabetes* 45:1324–1328, 1996
23. Frittitta L, Sampinato D, Solini A, Nosadini R, Goldfine ID, Vigneri R, Trischitta V: Elevated PC-1 content in cultured skin fibroblasts correlates with decreased in vivo and in vitro insulin action in nondiabetic subjects: evidence that PC-1 may be an intrinsic factor in impaired insulin receptor signaling. *Diabetes* 47:1095–1100, 1998
24. Kumakura S, Maddux BA, Sung CK: Overexpression of membrane glycoprotein PC-1 can influence insulin action at a post-receptor site. *J Cell Biochem* 68:366–377, 1998
25. White MF, Takayama S, Kahn CR: Differences in the sites of phosphorylation of the insulin receptor in vivo and in vitro. *J Biol Chem* 260:9470–9478, 1985
26. Zick Y: The insulin receptor: structure and function. *Crit Rev Biochem Mol Biol* 24:217–269, 1989
27. Takatama S, White MF, Kahn CR: Phorbol ester-induced serine phosphorylation of the insulin receptor decreases its tyrosine kinase activity. *J Biol Chem* 263:3440–3447, 1988
28. Forsyth JR, Caro JF, Sinha MK, Maddux BA, Goldfine ID: Monoclonal antibodies to the human insulin receptor that activate glucose transport but not insulin receptor kinase activity. *Proc Natl Acad Sci U S A* 84:3448–3451, 1987
29. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose tolerance. *Diabetes* 28:1039–1057, 1979
30. Catalano PM, Wong WW, Drago NM, Amini SB: Estimating body composition in late gestation: a new hydration constant for body density and total body water. *Am J Physiol* 268:E153–E158, 1995
31. Drago NM, Wong W, Amini SB, Catalano PM: Anthropometric estimation of maternal body composition in late gestation (Abstract). *J Soc Gynecol Invest* 2:167, 1995
32. Youngren JF, Goldfine ID, Pratley RE: Decreased muscle insulin receptor kinase correlates with insulin resistance in normoglycemic Pima Indians. *Am J Physiol* 237:E1–E8, 1997
33. Berkowitz GS, Roman SH, Lapinski RH, Alvarez M: Maternal characteristics, neonatal outcome, and the time of diagnosis of gestational diabetes. *Am J ObstetGynecol* 167:976–982, 1992
34. Mouzoun S, Peraldi P, Alengrin F, Obberghen EV: Alteration of phosphotyrosine phosphatase activity in tissues from diabetic and pregnant rats. *Endocrinology* 132:67–74, 1992
35. Martinez C, Ruiz P, Andres A, Satrustegui J, Carrascosa JM: Tyrosine kinase activity of liver insulin receptor is inhibited in rats at term gestation. *Biochem J* 263:267–272, 1989
36. Nolan JJ, Freidenberg G, Henry R, Reichart D, Olefsky JM: Role of human skeletal muscle insulin receptor kinase in the in vivo insulin resistance of non-insulin-dependent diabetes mellitus and obesity. *J Clin Endocrinol Metab* 78:471–477, 1994
37. 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
38. Bollag GE, Roth RA, Beaudoin J, Mochly-Rosen D, Koshland DEJ: 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
39. Pillay TS, Xiao S, Olefsky JM: Glucose-induced phosphorylation of the insulin receptor. *J Clin Invest* 97:613–620, 1996
40. Kellerer M, Coghlan M, Capp E, Muhlofer A, Kroder G, Mosthaf L, Galante P, Siddle K, Haring H-U: Mechanism of insulin receptor kinase inhibition in non-insulin-dependent diabetes mellitus patients: phosphorylation of serine 1327 or threonine 1348 is unaltered. *J Clin Invest* 96:6–11, 1995
41. Dunaif A, Xia J, Book C-B, Schenker E, Tang Z: Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle: a potential mechanism for insulin resistance in the polycystic ovary syndrome. *J Clin Invest* 96:801–810, 1995
42. Raj SG, Thompson IE, Berger MJ, Taymor ML: Clinical aspects of the polycystic ovary syndrome. *ObstetGynecol* 49:552–556, 1977
43. Holte J: Polycystic ovary syndrome and insulin resistance: thrifty genes struggling with over-feeding and sedentary life style? *J Clin Endocrinol Invest* 21:589–601, 1998
44. Feener EP, Shiba T, Hu KQ, Wilden PA, White MF, King GL: Characterization of phorbol ester-stimulated serine phosphorylation of the human insulin receptor. *Biochem J* 303:43–50, 1994
45. Avignon A, Yamada K, Zhou X, Spencer B, Cardona O, Saba-Siddique S, Galloy L, Standaert ML, Farese RV: Chronic activation of protein kinase C in soleus muscles and other tissues of insulin-resistant type II diabetic Gokakazaki (GK), obese/aged, and obese/Zucker rats: a mechanism for inhibiting glycogen synthesis. *Diabetes* 45:1396–1404, 1996
46. Schmitz-Peiffer C, Browne CL, Oakes ND, Watkinson A, Chisholm DJ, Kraegen EW, Biden TJ: Alteration in the expression and cellular localization of protein kinase C isozymes epsilon and theta are associated with insulin resistance in skeletal muscle of the high-fat-fed rat. *Diabetes* 46:169–178, 1997
47. Paz K, Hemi R, LeRoith D, Karasik A, Elhanany E, Kanety H, Zick Y: A molecular basis for insulin resistance. *J Biol Chem* 272:29911–29918, 1997
48. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM: IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF- α and obesity-induced insulin resistance. *Science* 271:665–668, 1996
49. Catalano P, Highman T, Huston L, Friedman JE: Relationship between reproductive hormones/TNF- α and longitudinal changes in insulin sensitivity during gestation. *Diabetes* 45 (Suppl. 2):175, 1996
50. Oda Y, Kuo M-D, Huang SS, Huang JS: The plasma cell membrane glycoprotein, PC-1, is a threonine specific protein kinase stimulated by acidic fibroblast growth factor. *J Biol Chem* 266:16791–16795, 1991
51. Goding JW, Terkeltaub R, Maurice M, Deterre P, Sali A, Belli S: Ecto-phosphodiesterase/pyrophosphatase of lymphocytes and non-lymphoid cells: structure and function of the PC-1 family. *Immunol Rev* 161:11–26, 1998
52. Maddux BA, Goldfine ID: 2000 PC-1 inhibition of insulin receptor function occurs via direct interaction with the receptor alpha subunit. *Diabetes* 49:13–19, 2000
53. Belfiore A, Costantino A, Frasca F, Pandini G, Mineo R, Vigneri P, Maddux B, Goldfine ID, Vigneri R: Overexpression of membrane glycoprotein PC-1 in

- MDA-MB231 breast cancer cells is associated with inhibition of insulin receptor tyrosine kinase activity. *Mol Endocrinol* 10:1318–1326, 1996
54. Grupe A, Alleman J, Goldfine ID, Sadick M, Stewart TA: Inhibition of insulin receptor phosphorylation by PC-1 is not mediated by the hydrolysis of adenosine triphosphate or the generation of adenosine. *J Biol Chem* 270:22085–22088, 1995
55. Frittitta L, Youngren JF, Vigneri R, Maddux V, Trischitta V, Goldfine ID: PC-1 content in skeletal muscle of non-obese, non-diabetic subjects, relationship to insulin receptor tyrosine kinase and whole body insulin sensitivity. *Diabetologia* 39:1190–1195, 1996
56. Sakoda H, Ogihara T, Anai M, Funak M, Inukai K, Katagiri H, Fukushima Y, Onishi Y, Ono H, Yazaki Y, Kikuchi M, Oka Y, Asano T: No correlation of plasma cell 1 overexpression with insulin resistance in diabetic rats and 3T3-L1 adipocytes. *Diabetes* 48:1365–1371, 1999
57. Pizzuti A, Frittitta L, Argiolas A, Baratta A, Goldfine ID, Bozzali M, Ercolino T, Scarlato G, Iacoviello L, Vigneri R, Tassi V, Trischitta V: A polymorphism (K121Q) of the human glycoprotein PC-1 gene coding region is strongly associated with insulin resistance. *Diabetes* 48:1881–1884, 1999