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Original Studies

Selective Insulin Resistance in the Polycystic Ovary Syndrome[1]

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Abstract

References

Polycystic ovary syndrome (PCOS) is characterized by hyperandrogenemia that is amplified by insulin in the presence of resistance to insulin's action to stimulate glucose uptake in muscle and fat. To explore the mechanisms for this paradox, we examined the metabolic and mitogenic actions of insulin and insulin-like growth factor I (IGF-I) in cultured skin fibroblasts from PCOS (n = 16) and control (n = 11) women. There were no significant decreases in the number or affinity of insulin- or IGF-I-binding sites in PCOS compared to control fibroblasts. Basal rates were similar, but there were significant decreases in insulin-stimulated (control, 51.8 ± 7.0; PCOS, 29.5 ± 2.9 nmol/10^6 cells·2 h at 1,000,000 pmol/L; $P < 0.005$) and IGF-I-stimulated (control, 48.9 ± 6.7; PCOS, 33.0 ± 3.2 PCOS nmol/10^6 cells·2 h at 100,000 pmol/L IGF-I; $P < 0.05$) glucose incorporation into glycogen in PCOS fibroblasts, a metabolic action of insulin. Stimulation of thymidine incorporation, a mitogenic action of insulin, was similar in PCOS and control fibroblasts in response to both insulin and IGF-I. There were also no significant differences in insulin- or IGF-I-stimulated insulin receptor substrate-1-associated phosphatidylinositol-3-kinase activity in PCOS compared to control fibroblast cells. We conclude that 1) there is a selective defect in insulin action in PCOS fibroblasts that affects metabolic, but not mitogenic, signaling pathways; 2) there is a similar defect in IGF-I action, suggesting that insulin and IGF-I stimulate glycogen synthesis by the same postreceptor pathways; and 3) insulin receptor substrate-1-associated phosphatidylinositol 3-kinase activation by insulin and IGF-I is similar to the control value, suggesting that the metabolic signaling defect is in another pathway or downstream of this signaling step in PCOS fibroblasts.

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POLYCYSTIC ovary syndrome (PCOS) is among the most common endocrinopathies of premenopausal women (1, 2). The disorder is characterized by hyperandrogenism, chronic anovulation, and hyperinsulinemia (1). Its etiology remains unknown, but it is clear that hyperinsulinemia secondary to insulin resistance plays an important role in the pathogenesis of reproductive abnormalities by stimulating ovarian androgen production (1, 3, 4). It has long been proposed that this insulin action on steroidogenesis is mediated by spillover occupancy of the type 1 insulin-like growth factor (IGF) receptor (IGF1R) (1, 5, 6). Circulating insulin levels in PCOS, however, are usually not high enough to activate the IGF1R in nonovarian tissues (7, 8). Recent studies have supported this observation by demonstrating that insulin is acting through its cognate receptor to modulate steroidogenesis in normal and in PCOS ovaries (9, 10). It is not

known how this insulin action on steroidogenesis is preserved when there is profound resistance to insulin action on both carbohydrate (1) and lipid metabolism (11) in PCOS.

Insulin acts via its cell surface receptor, which is a ligand-activated protein tyrosine kinase (1, 12, 13). After tyrosine autophosphorylation, the activated receptor phosphorylates endogenous substrates, such as insulin receptor substrate-1 and -2 (IRS-1 and IRS-2) (12, 13). Phosphorylated tyrosine residues on these substrates then bind to a variety of molecules to activate signaling pathways, such as those mediating carbohydrate metabolism, lipogenesis, protein synthesis, gene transcription, and cell growth/differentiation (12, 13). The IGF1R shares substantial sequence and structural homology with the insulin receptor and uses many of the same signaling pathways (13, 14). The mechanisms of insulin receptor signal specificity are the subject of intense investigation (12, 13, 14). Current evidence suggests that IRS-1/2-mediated activation of phosphatidylinositol 3-kinase (PI3-kinase) controls insulin-mediated glucose transport and carbohydrate metabolism (15, 16, 17, 18, 19). Separate pathways regulated through mitogen-activated protein kinase activation appear to control mitogenesis (12, 13, 14, 19, 20). Activation of PI3-kinase may also be involved in these pathways (13, 17).

It is possible to experimentally block certain insulin receptor signaling pathways while others are preserved, producing selective insulin resistance (18, 20). Clinical examples of selective insulin resistance have been reported in pseudoacromegaly (21) and in diabetic patients with a strong family history of type 2 diabetes (22). Resistance to insulin action on carbohydrate metabolism with preserved insulin stimulation of mitogenesis has been demonstrated in cultured skin fibroblasts from such patients (21, 22). We performed this study to determine whether a similar selective defect in insulin action was present that might explain the persistent insulin-mediated changes in reproductive function despite profound resistance to insulin effects on carbohydrate metabolism in PCOS. We also examined the actions of IGF-I on these metabolic and mitogenic pathways to determine whether analogous defects existed. We used cultured skin fibroblasts that had been removed from the *in vivo* environment for generations so that we could examine intrinsic, rather than acquired, defects in insulin action (13, 21, 22, 23, 24, 25, 26, 27, 28, 29). We report for the first time that there is a selective defect in hormone action on carbohydrate metabolism in PCOS that affects pathways used by both insulin and IGF-I.

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Subjects

Sixteen women with PCOS and 11 reproductively normal control women were studied. All women were between the ages of 18–43 yr, in good health, and taking no medications known to alter sex hormone or carbohydrate metabolism for at least 1 month before the study, except for contraceptive agents that were stopped 3 months before the study. The studies were approved by the institutional review boards of the Mt. Sinai School of Medicine (Mt. Sinai) and the Pennsylvania State University College of Medicine (Penn State); all women gave written informed consent before the study. PCOS was diagnosed by the combination of 6 or fewer menses/yr and an elevation of total and/or nonsex hormone-binding globulin-bound testosterone levels greater than 2 SD above the control range established in women aged 18–40 yr with 27- to- 35-day regular menstrual cycles and no hirsutism or acne (28, 29, 30, 31, 32, 33, 34). Disorders of the pituitary, adrenals, and ovaries were excluded by appropriate tests before the diagnosis of PCOS was made (30). No PCOS women had diabetes (35). Control women had 27- to 35-day regular menstrual cycles, no hirsutism or acne, no personal history of diabetes or hypertension, and no first degree relative with diabetes (28, 29, 32, 33, 34, 36, 37). Total testosterone, unbound testosterone, and dehydroepiandrosterone sulfate levels were within the normal range in control women.

A 75-g glucose load oral glucose tolerance test was performed in all women after a 3-day 300-g carbohydrate/day diet and a 10-h or longer overnight fast (35). Blood for glucose and insulin levels was obtained every 30 min for 2 h, and glucose tolerance was assessed according to WHO criteria (35). All subjects had participated in our studies of insulin action in PCOS (28, 29, 31, 32, 33, 34, 36, 37), and their androgen levels, glucose tolerance, and insulin sensitivity data have been previously reported. Glucose tolerance data, fasting insulin levels, and insulin sensitivity (SI), as determined by modified, frequently sampled, iv glucose tolerance (4, 34), are summarized with clinical features in Table 1. The methodology for the insulin assay was not the same as that for Mt. Sinai (30) and Penn State (4) subjects; thus, the fasting insulin and SI results were not directly comparable. However, the PCOS women were not selected for this study on the basis of their individual insulin sensitivity measurements. Normal ranges for insulin and SI measurements for each site are given in Table 1. All subjects had a skin biopsy performed under sterile conditions after local anesthesia with 1% lidocaine with epinephrine (28, 29). A 2-mm punch biopsy was taken from the volar aspect of the forearm. Table 2 summarizes the studies in which each subject's fibroblast cell lines were used.

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Table 1. Clinical features

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Table 2. Studies

Materials

DMEM (4 g/L glucose), low glucose (1 g/L) DMEM, penicillin-streptomycin solution, trypsin, ethylenediamine tetraacetate (EDTA), and silica gel 60 thin layer chromatography (TLC) plates were obtained from Fisher Scientific (Pittsburgh, PA). FBS was purchased from Life Technologies, Inc./BRL (Gaithersburg, MD). Isoton II was obtained from Coulter Corp. (Miami, FL). HEPES, Triton X-100, and dithiothreitol were purchased from Roche Molecular Biochemicals (Indianapolis, IN). Protein A-Sepharose CL-4B was obtained from Pharmacia LKB (Piscataway, NJ). Human insulin was purchased from Eli Lilly & Co. (Indianapolis, IN), and IGF-I was purchased from Bachman (Torrance, CA). A-14-tyrosine-[^{125}I]insulin, [*methyl*-^{3}H]thymidine, and D-[U-^{14}C]glucose were purchased from DuPont (Wilmington, DE). [γ-^{32}P]ATP was obtained from ICN Biochemicals, Inc. (Costa Mesa, CA), and [^{125}I]IGF-I was purchased from Amersham Pharmacia Biotech (Arlington Heights, IL). The anti-IRS-1 antibody (JD66) was a gift from Dr. Morris White of the Joslin Diabetes Center (Boston, MA). The remaining reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

Skin fibroblast cultures

Skin was cultured in DMEM (4 g/L glucose) supplemented with 10% FBS and 1% penicillin-streptomycin solution in a humid 37 C incubator with 5% CO_2 (28, 29). In 7–14 days, fibroblast outgrowth from the primary biopsy was evident and was allowed to grow until near confluence before further subculture. Cells were used for study between the 5th and 15th passage.

Cells were counted with each of the assays performed. Because these assays required slightly different culture conditions, each cell line was counted after exposure to the various conditions. All cells were rinsed twice with ice-cold PBS (pH 7.4), solubilized in trypsin, suspended in Isoton II, and immediately counted with a Coulter counter (Coulter Electronics, Inc., Hialea, FL). The mean value of triplicate wells was used to normalize results from different fibroblast cultures. Cells from a control and a PCOS woman were always run together in each assay; hence, assays were repeated in several control subjects because we had fewer well characterized control than PCOS cell lines.

Insulin and IGF-I binding

The numbers and affinities of insulin and IGF-I receptors in each fibroblast cell line were determined as described previously (28, 29). Cultured skin fibroblasts from control and PCOS women were grown to confluence in six-well trays before incubation for 48 h in serum-free DMEM containing 0.1% BSA. Cells were incubated for 4 h at room temperature with 0.1 μCi ^{125}I-labeled insulin or IGF-I, increasing concentrations (1,000,000 pmol/L) of unlabeled ligands, and binding buffer (50 mmol/L HEPES, 50 mmol/L Tris, 10 mmol/L $MgCl_2$, 2 mmol/L EDTA, 10 mmol/L dextrose, 50 mmol/L NaCl, 5 mmol/L KCl, 10 mmol/L $CaCl_2$, and 1% BSA, pH 7.4). Reactions were terminated by washing the cells with ice-cold PBS (pH 7.4), solubilizing them in 0.2 N NaOH, and counting the amount of ^{125}I-labeled ligand in a γ-counter. Receptor number

and affinity were determined per 10^4 cells by Scatchard analysis using the Ligand computer program (28).

Thymidine incorporation

Mitogenic effects of insulin in fibroblasts were assessed by the cellular incorporation of [*methyl*-^{3}H]thymidine (38, 39). Cultured skin fibroblasts from control and PCOS women were grown to approximately 80% confluence in six-well trays before incubation for 48 h in serum-free DMEM containing 0.1% BSA. Cells then were incubated overnight at 37 C with DMEM containing 0.3% FBS and increasing doses of insulin (1,000,000 pmol/L) or IGF-I (100,000 pmol/L). Radioactive thymidine (1.5 μCi) was added to each well for an additional 4 h at 37 C. Incorporation of [*methyl*-^{3}H]thymidine was terminated by aspirating media and rapidly washing the cells sequentially with ice-cold solutions of PBS (pH 7.4), 10% trichloroacetic acid, and 80% ethanol. Cells were solubilized with 1 N NaOH, neutralized with 1 N HCl, and quantitated in duplicate by liquid scintillation counting. The percentage of [*methyl*-^{3}H]thymidine incorporated at each dose of insulin or IGF-I was reported per million cells.

Glucose incorporation into glycogen

Metabolic effects were estimated by the incorporation of glucose into glycogen in fibroblasts (40). Cultured skin fibroblasts from control and PCOS women were grown to confluence in six-well trays using low glucose DMEM with 10% FBS before incubation overnight at 37 C in serum-free low-glucose DMEM containing 0.5% BSA. Cells then were incubated in low glucose DMEM containing 1% BSA, increasing doses of insulin (1,000,000 pmol/L) or IGF-I (100,000 pmol/L), and D-[U-^{14}C]glucose (0.4 μCi). After 2 h at 37 C, cells were washed with ice-cold PBS (pH 7.4) and solubilized in 2 N NaOH. Glycogen was extracted by repeated ethanol precipitations, and the amount of incorporated D-[U-^{14}C]glucose was determined in duplicate by liquid scintillation counting. Results were expressed as nanomoles of radioactive glucose incorporated into glycogen per million cells.

PI3-kinase activity

Insulin and IGF-I signaling were assessed in PCOS fibroblasts by PI3-kinase activity associated with IRS-1 (41, 42). Cultured skin fibroblasts from control and PCOS women were grown to confluence in 150-mm dishes before overnight incubation in serum-free DMEM with 0.5% BSA. Cells were treated with 0–100,000 pmol/L doses of insulin or 0–10,000 pmol/L doses of IGF-I for 10 min at 37 C, rinsed twice in ice-cold PBS (pH 7.4) to terminate the reaction, and solubilized in 20 mmol/L Tris-HCl (pH 8.0) containing 137 mmol/L NaCl, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, 10% glycerol, 1% Nonidet P-40, 150 μmol/L sodium vanadate, 1 mmol/L phenylmethylsulfonylfluoride, and 10 μg/mL leupeptin. Equal protein concentrations of clarified lysates (1 mg) were immunoprecipitated with anti-IRS-1 antibody overnight at 4 C. Antibody-antigen complexes were incubated for 2 h at 4 C with protein A-Sepharose beads, and the beads were washed sequentially in PBS (pH 7.4) with 1% Nonidet P-40 (three times); 100 mmol/L Tris-HCl (pH 7.6) with 500 mmol/L LiCl (twice); 10 mmol/L Tris-HCl (pH 7.4), 100 mmol/L NaCl, and 1 mmol/L EDTA (once); and finally once in PI3-kinase assay buffer (20 nmol/L Tris, pH 7.4, containing 100 mmol/L NaCl, 10 mmol/L $MgCl_2$, 0.5 mmol/L ethyleneglycol-bis-(ß-aminoethyl ether)-*N*,*N*,*N*',*N*'-tetraacetic acid, and 120 μmol/L adenosine). Washed beads were preincubated for 10 min at 30 C in 40 μL PI3-kinase assay buffer and 5 μL lipid mixture containing phosphatidylserine and

phosphatidylinositol in 20 mmol/L HEPES and 1 mmol/L EDTA (pH 7.4). Kinase reactions were started by the addition of 100 μmol/L ATP containing 15 μCi [γ-^{32}P]ATP. After 10 min at 30 C, reactions were terminated by the addition of 1 N HCl, and phosphorylated lipids were extracted twice in chloroform-methanol (1:1). Products of PI3-kinase (15 μL) were spotted onto 1,2-cyclohexanediaminetetraacetic acid-treated silica gel 60 TLC plates and were resolved using the chromatography system of Walsh *et al.* (43). Labeled PI-3-phosphate was detected by autoradiography, scraped from the TLC plates, and quantified by Cherenkov scintillation counting. Results were expressed as counts per min of ^{32}P/mg protein.

Data analysis

Because considerable evidence suggests that defects in target tissue insulin action secondary to obesity, hyperinsulinemia, and/or hyperglycemia are acquired rather than intrinsic in both normal and PCOS individuals (1, 13, 24, 25, 26, 27, 28, 29, 44, 45, 46, 47), we pooled the data from obese and nonobese subjects. However, we also analyzed the data in a homogeneous subgroup of obese non-Hispanic white PCOS (n = 10) and control (n = 7) women. Simple linear correlations were assessed between parameters of fibroblast insulin/IGF-I action and body mass index (BMI), glucose tolerance, glucose/insulin ratio (G:I) (36), and insulin sensitivity. As different insulin assays were used at Mt. Sinai and at Penn State, the correlations between fibroblast insulin/IGF-I action and fasting insulin levels, G:I, or SI were examined only in the Penn State subjects who had the larger dataset. Statistical analyses were performed using SAS software (SAS Institute, Inc., Cary, NC), and all data are reported as the mean ± SE, except for reference data for insulin levels and SI in Table 1, which are reported as the mean ± SD.

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Clinical features

There were no significant differences in age between the PCOS and control subjects (data not shown). There were no significant differences in BMI in the obese non-Hispanic white PCOS compared to the obese non-Hispanic white control women (data not shown). Five of 16 PCOS women had impaired glucose tolerance, but none had diabetes mellitus by WHO criteria (Table 1) (35). There was a broad range of SI values in the PCOS women, with some values overlapping the normal range, as the PCOS subjects were not selected on the basis of insulin resistance.

Binding

There were no significant differences in insulin binding (control, 0.07 ± 0.04%; PCOS, 0.03 ± 0.01% bound/total/10^4 cells; P = NS) or binding affinity (data not shown) between control and PCOS fibroblasts, consistent with previous studies (28, 29, 48). The amount of IGF-I binding in both control and PCOS fibroblasts was approximately 10-fold higher than the amount of insulin binding. There was no significant difference in IGF-I binding (control, 0.91 ± 0.30%; PCOS, 0.97 ± 0.30% bound/total/10^4 cells; P = NS) or binding affinity (data not shown) between control and PCOS cells, similar to a previous report (48).

Insulin and IGF-I action

Basal rates of glycogen synthesis and thymidine incorporation did not differ significantly in PCOS compared to control cells (Figs. 1 and 2). Maximal rates of insulin-stimulated glucose incorporation into glycogen were significantly decreased in PCOS compared to control cells ($P < 0.005$; Fig. 1). These results remained significant in the subgroup analysis of obese non-Hispanic white PCOS *vs.* control women ($P < 0.05$; data not shown). Fold stimulation by insulin of glucose incorporation into glycogen was 4.2 ± 0.4-fold in control cells and 2.7 ± 0.4-fold in PCOS cells ($P < 0.05$). The insulin dose-response curves in PCOS women were so flat that the ED_{50} could not be reliably estimated. The ED_{50} insulin in control women for glycogen synthesis was 1565 ± 440 pmol/L. There were no significant differences in maximal rates of insulin-stimulated thymidine incorporation in PCOS compared to control cells in the entire sample (Fig. 2) or in the obese non-Hispanic white subgroup. The ED_{50} insulin for thymidine incorporation was 4018 ± 841 pmol/L in controls *vs.* 5,122 ± 2,065 pmol/L in PCOS women (P = NS).

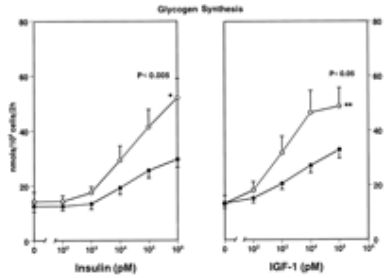

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Figure 1. Insulin (PCOS, n = 12; control, n = 7) and IGF-I (PCOS, n = 11; control, n = 7) stimulation of glucose incorporation into glycogen in fibroblasts. Basal rates of glycogen synthesis are similar in PCOS and control cells. Stimulated responses are significantly decreased for both insulin at 1,000,000 pmol/L (*, $P < 0.005$) and IGF-I at 100,000 pmol/L (**, $P < 0.05$) in PCOS compared to control fibroblasts.

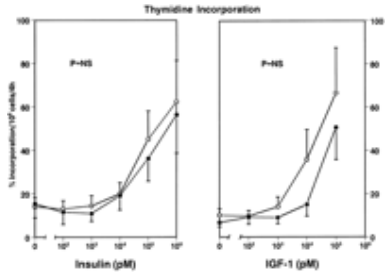

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Figure 2. Insulin and IGF-I stimulation of thymidine incorporation in PCOS (n = 10) and control (n = 6) fibroblasts. There are no significant differences noted in basal rates or insulin/IGF-I-stimulated rates of thymidine incorporation in PCOS compared to control fibroblasts.

IGF-I action was examined over physiological concentrations of this hormone (49). IGF-I-stimulated glycogen synthesis was significantly decreased ($P < 0.05$) in PCOS compared to control cells at 100,000 pmol/L (Fig. 1). The results remained similar in the obese non-Hispanic white subgroup, but the difference no longer achieved statistical significance. IGF-I-stimulated thymidine incorporation was similar in PCOS and control cells (Fig. 2). These results did not change in the obese non-Hispanic white subgroup. As maximally stimulating concentrations of IGF-I were not examined, the ED_{50} for IGF-I action could not be calculated. The dose-response curves for insulin and IGF-I action on glycogen synthesis and thymidine incorporation indicated that stimulation of glycogen synthesis and thymidine incorporation occurred at physiological concentrations of these hormones (22, 28, 29, 31, 32, 33, 49, 50). This is consistent with each ligand acting through its cognate receptor (8).

Basal and insulin-stimulated IRS-1-associated PI3-kinase did not differ in PCOS and control cells at any insulin concentration (Fig. 3). Consistent with the approximately 10-fold greater number of IGF1Rs compared to insulin receptors, IGF-I-stimulated, IRS-1-associated PI3-kinase was about 10-fold greater than insulin-stimulated, IRS-1-associated PI3-kinase activity, but did not differ at 0–10,000 pmol/L IGF-I in PCOS compared to control cells (Fig. 3). These results did not differ in the obese non-Hispanic white subgroup. The anticipated significant inverse correlations between BMI and SI or G:I were present (data not shown) (34, 36). There were no significant correlations, however, between fibroblast insulin/IGF-I action and BMI, glucose levels, insulin levels, G:I, or SI.

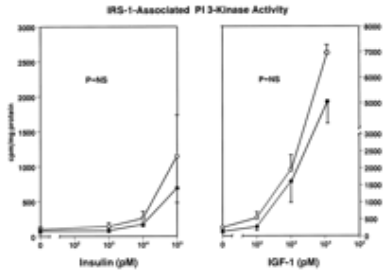

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Figure 3. Insulin (PCOS, n = 10; control, n = 9) and IGF-I (PCOS, n = 5; control, n = 5) stimulation of IRS-1-associated PI3-kinase activation. There are no significant differences noted in basal or insulin/IGF-I-stimulated IRS-1-associated PI3-kinase activity in PCOS compared to control fibroblasts. However, there is an approximately 10-fold greater IGF-I-stimulated than insulin-stimulated IRS-1-associated PI3-kinase activity, consistent with the approximately 10-fold greater amount of IGF-I- compared to insulin-binding sites.

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Fibroblast cell lines from women with PCOS have significantly decreased insulin-stimulated glucose incorporation into glycogen, but similar insulin-stimulated thymidine incorporation, compared to cell lines from reproductively normal control women. This suggests that there is a selective defect in insulin action in PCOS that affects the metabolic, but not the mitogenic, actions of insulin (21, 22). Consistent with previous studies in adipocytes (32) and fibroblasts (28, 29, 48), this is a postreceptor defect, as there were no significant decreases in the amount or the affinity of insulin-binding sites on PCOS compared to control cells. There was an analogous defect in IGF-I-stimulated glycogen synthesis observed at physiological concentrations of this hormone, suggesting that it was acting through its cognate receptor (8). This suggests that the same postreceptor signaling pathway was used for the action of both of these hormones on glycogen synthesis in human fibroblasts. This is consistent with studies in rats, in which glucosamine induces resistance to both insulin and IGF-I stimulation of glycogen synthesis (51). IGF-I-stimulated thymidine incorporation was similar in PCOS and control cells, consistent with normal mitogenic pathways (13, 21, 22, 48).

We have previously shown reduced insulin-stimulated receptor autophosphorylation and substrate phosphorylation in receptors isolated from PCOS skin fibroblasts (29). This appeared to be secondary to constitutive serine phosphorylation of the receptor, which inhibits its tyrosine kinase activity. This decrease in receptor tyrosine kinase activity would be predicted to result in diminished phosphorylation of endogenous receptor substrates, such as IRS-1, which would result in decrease activation of PI3-kinase (1, 12, 13). We have shown decreased insulin-stimulated PI3-kinase activation in association with decreased glucose uptake in PCOS skeletal muscle (52). In the present study, however, there were no significant decreases in PI3-kinase activation at physiological insulin doses in PCOS compared to control fibroblasts despite significant decreases in insulin-stimulated glycogen synthesis. This suggests that this signaling pathway was not impaired by the defect in insulin receptor phosphorylation that we have previously reported (29), and that the defect impairing glycogen synthesis is in another signaling pathway or at a locus downstream of IRS-1-mediated PI3-kinase activation.

Fibroblasts do not contain the insulin-stimulated glucose transporter isoform, GLUT4, and manifest minimal insulin-stimulated glucose transport (21). It is thus possible that tissue differences explain our failure to detect decreases in IRS-1-associated PI3-kinase activation in fibroblasts, whereas such abnormalities are present in PCOS skeletal muscle (52). Indeed, recent studies suggest that IRS-2 mediates insulin's metabolic effects in many tissues (53), and IRS-2 messenger ribonucleic acid is present in human fibroblasts (**Dunaif**, A., unpublished observations). As IRS-2-associated PI3-kinase activation was not examined in the present study, we were unable to address this possibility. A recent report in patients with pseudoacromegaly found decreased stimulation of IRS-1-associated PI3-kinase activity in cultured fibroblasts at submaximal (1,000 pmol/L), but not maximal (100,000 pmol/L), insulin doses (50). However, insulin stimulation of glycogen synthesis was normal in these patients, supporting the hypothesis that IRS-1-associated PI3-kinase activation is not involved in the regulation of glycogen synthesis in human fibroblasts. Further, in our PCOS fibroblasts there were no significant decreases in PI3-kinase activation at any insulin dose, suggesting that the mechanisms of insulin resistance differ from those in pseudoacromegaly (50) despite the fact that PCOS women occasionally have acromegaloid features (54).

Ciaraldi and colleagues failed to find decreases in insulin-stimulated glycogen synthesis in fibroblasts from insulin-resistant PCOS compared to those from control women (48). Otherwise, their results were similar to ours. They studied a smaller sample of PCOS (n = 6) and control (n = 5) women than we did, but we still found significant decreases in insulin-stimulated glycogen synthesis in our subgroup analysis of obese non-Hispanic white PCOS and control women with a similar sample size to that in their study (48). Their subjects had very low mean SI measurements, whereas some of our PCOS women had values within the normal range. However, there were no significant correlations between fibroblast insulin/IGF-I action and BMI, glucose levels, insulin levels, or insulin sensitivity in our study. Our subjects were recruited from different geographic regions compared to those of Ciaraldi *et al.* (48), so it remains possible that more subtle ethnic differences explain the discrepant findings between our studies.

The failure to detect a relationship between insulin action in the cultured fibroblasts and adiposity or glycemia is compatible with evidence indicating that these factors produce reversible changes in insulin sensitivity (13, 24, 26, 44, 45, 46, 47). The failure to detect a relationship between *in vivo* insulin (fasting insulin levels or SI) and fibroblast

(glycogen synthesis) parameters of insulin action may reflect the fact that fibroblasts are not target tissues for insulin-mediated glucose uptake (55). We also failed to find a significant relationship between fibroblast insulin receptor phosphorylation and *in vivo* insulin action in our previous study (29). Insulin action *in vivo* has been significantly correlated with glycogen synthesis in cultured muscle cells, the major target tissue for insulin-mediated glucose uptake (56). Alternatively, other factors, such as a relatively small sample size or a more complex relationship between insulin action *in vivo* and in fibroblasts, may have accounted for our failure to find significant associations. It is also possible, as suggested by Ciaraldi *et al.* (48), that there are tissue differences in insulin action in PCOS.

The persistence of defects in insulin action in cultured cells that have been removed from the *in vivo* environment for generations is consistent with a genetic defect. In our previous report (29), abnormalities in insulin receptor phosphorylation were also found in cultured cells, suggesting a genetically programed abnormality. Most recently, we found familial clustering of hyperandrogenemia in PCOS sisters (57). Taken together, these observations provide further support for our hypothesis that both the insulin resistance and the reproductive abnormalities of PCOS have a genetic component (1).

There is now considerable evidence to indicate that hyperinsulinemia amplifies hyperandrogenemia in PCOS by stimulating ovarian and adrenal androgen production (1, 3, 4, 6, 10) as well as pituitary LH release (1, 4). It had been suggested that this insulin action was mediated via spillover occupancy of the IGF1R (1, 6). Studies in normal and PCOS ovarian cells (9, 10), however, strongly suggest that insulin is acting through its own receptor to stimulate steroidogenesis. The current study provides a mechanism by which there could be resistance to insulin's action on carbohydrate metabolism, but preserved insulin action on steroidogenesis in PCOS: a selective defect affecting metabolic, but not mitogenic, signaling pathways. This hypothesis will require investigation in steroidogenic tissues, such as thecal cells, from PCOS women.

In summary, we have shown that cultured PCOS fibroblasts are insulin resistant. Moreover, this resistance affects the metabolic, but not the mitogenic, actions of insulin. IGF-I action is similarly impaired, suggesting that the same postreceptor signaling pathways are used for insulin and IGF-I action on glycogen synthesis in human fibroblasts. Activation of IRS-1-associated PI3-kinase does not appear to be impaired. This observation suggests that other signaling pathways or pathways downstream of IRS-1-mediated PI3-kinase activation are defective and that the abnormality we have reported in PCOS fibroblast insulin receptor phosphorylation does not impair IRS-1 activation of PI3-kinase in this cell type. PCOS will provide an excellent model in which to further investigate the mechanisms of insulin receptor signal specificity.

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Footnotes

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