Lack of Insulin Resistance in Fibroblasts From Subjects With Polycystic Ovary Syndrome

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Insulin resistance in polycystic ovary syndrome (PCOS) is characterized by a novel defect in insulin signal transduction expressed in isolated human adipocytes as impaired insulin sensitivity for glucose transport and antilipolysis. To determine whether this is a generalized defect of a potentially genetic basis, or possibly a tissue-specific one, fibroblast cultures were established from age- and weight-matched obese normal cycling (NC; n = 5) and PCOS (n = 6) subjects. Adipocytes from the current PCOS subjects displayed impaired sensitivity for glucose transport stimulation (half-maximal effective concentration [EC_{50}], 317 ± 58 pmol/L in PCOS *v* 130 ± 40 in NC; $P < .025$). Specific insulin binding was similar in fibroblasts from NC (0.57% ± 0.10%/10^6 cells) and PCOS (0.45% ± 0.10%) subjects. Fibroblasts from NC (4.9- ± 0.5-fold stimulation) and PCOS (4.6- ± 0.3-fold) subjects were equally responsive to insulin for stimulation of glucose incorporation into glycogen. Insulin sensitivity for glycogen synthesis in fibroblasts did not differ between NC (EC_{50}, 9.6 ± 0.9 nmol/L) and PCOS (9.1 ± 0.9) cells. For thymidine incorporation into DNA, relative insulin responsiveness was similar in NC (2.3- ± 0.3-fold stimulation) and PCOS (2.1- ± 0.1-fold) fibroblasts. Insulin sensitivity for DNA synthesis was similar in NC (EC_{50}, 12.9 ± 2.4 nmol/L) and PCOS (7.6 ± 1.3) cells. In summary, (1) insulin receptor binding is normal in PCOS fibroblasts; and (2) PCOS fibroblasts have normal insulin sensitivity and responsiveness for metabolic and mitogenic responses. Impaired insulin signal transduction, while present in adipocytes from a group of PCOS subjects, is not found in fibroblasts from the same subjects. This defect is not generalized to all cell types, but may be limited to specific tissues and responses.

POLYCYSTIC OVARY SYNDROME (PCOS) is a common and highly complex endocrine disorder. Major features of the syndrome include hyperandrogenism, chronic anovulation of peripubertal onset, and hypersecretion of luteinizing hormone.[1,2] Obesity, hyperinsulinemia, and insulin resistance also occur in a large proportion of women with PCOS.[3,4] While insulin resistance and PCOS can exist together in lean women,[3,5] there appears to be a combination effect of obesity and PCOS on insulin resistance,[4,6] such that obese PCOS women are at greater risk for developing non–insulin-dependent diabetes mellitus (NIDDM).[4]

Even with this relationship between PCOS, obesity, and NIDDM, in vivo and in vitro studies indicate that the nature of insulin resistance differs between these states. In obesity and NIDDM, there are reductions in both insulin receptor binding and the activity of final effector systems such as glucose transport.[7] Yet, when corrected for insulin receptor number, insulin sensitivity is normal.[7] Recent investigations in isolated adipocytes from PCOS subjects have shown greatly impaired insulin sensitivity for glucose transport stimulation and antilipolysis, in the presence of normal insulin binding.[8-10] The function of the glucose transport system is normal[8] or slightly decreased,[11,12] indicating that insulin resistance in PCOS involves a novel defect in signal transduction.

PCOS patients are often both hyperinsulinemic and hyperandrogenic.[1] Insulin and androgens, especially testosterone, have been associated with impaired insulin action.[13-15] Since the behavior of freshly isolated cells, such as adipocytes, is reflective of the in vivo hormonal environment, it is possible that the defective signaling in PCOS adipocytes is influenced by these hormones. Studying cultured skin fibroblasts permits evaluation of cell function independent of the in vivo environment. Such an approach has shown that fibroblasts from NIDDM subjects express impaired insulin action on glycogen[16] and protein synthesis[17]; the first result is reflective of the behavior in diabetic skeletal muscle.[18] Thus, fibroblasts from diabetic subjects continue to express defects in insulin action. The current studies were initiated to determine if, in a similar manner, the defect in insulin action observed in PCOS adipocytes is present in other tissues when removed from the in vivo environment.

MATERIALS AND METHODS

PCOS Subjects

Six obese women (two white and four Hispanic) were studied. All were characterized by amenorrhea or persistent oligomenorrhea of perimenarchial onset, clinical or biochemical evidence of hyperandrogenism, and polycystic ovaries documented by ultrasonography. On the basis of clinical examination, there was no evidence of acanthosis nigricans. Clinical characteristics are summarized in Table 1.

Normal Subjects

Five normal cycling (NC) women (one white, two black, and two Hispanic) matched for age and weight (Table 1) served as controls. They were in general good health, with no clinical evidence of hyperandrogenism. All had 27- to 32-day cycles, and were studied during the early follicular phase (days 2 to 5) of the menstrual cycle. Neither PCOS nor NC subjects had used any hormonal preparation during the 3 months preceding the study.

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Submitted August 15, 1997; accepted February 16, 1998.

Supported by National Institutes of Health Grants No. DK33649, DK-33651, and HD-12303-19, by General Clinical Research Center Grant No. NIH M01RR00827, and in part by the Sankyo Diabetes Research Foundation and the Clayton Foundation for Research. S.S.C.Y. is a Clayton Foundation Investigator.

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0026-0495/98/4708-0009$03.00/0

Table 1. Subject Characteristics

Characteristic	NC	PCOS
No. of subjects	5	6
Age (yr)	31 ± 1	29 ± 3
BMI (kg/m²)	37.0 ± 3.4	40.5 ± 3.5
WHR	0.82 ± 0.03	0.82 ± 0.02
Fasting glucose (mmol/L)	5.0 ± 0.2	5.3 ± 0.4
Fasting insulin (pmol/L)	73 ± 25	328 ± 75†
S_I ($\times 10^{-4}$/min · μU/mL)	1.37 ± 0.25	0.72 ± 0.17*
Testosterone (nmol/L)	0.61 ± 0.07	1.72 ± 0.24†
LH (IU/L)	12.5 ± 1.3	18.7 ± 2.2*
FSH (IU/L)	10.9 ± 0.7	9.9 ± 1.3
LH/FSH ratio	1.21 ± 0.12	1.99 ± 0.26*

NOTE. Results are means ± SEM.

Abbreviations: BMI, body-mass index; WHR, waist/hip ratio; S_I, insulin sensitivity index determined from intravenous glucose tolerance test; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

*$P < .05$ *v* NC.

†$P < .01$.

Protocol

The study was approved by the Human Subjects Committee at the University of California, San Diego (UCSD), and informed written consent was obtained from each subject. After an overnight fast, subjects were admitted to the General Clinical Research Center at UCSD at 7 AM. A rapid-sampled intravenous glucose tolerance test was performed using previously described methods.[19] Analysis of the resulting data by the minimal model[20] provided values for the insulin sensitivity index (S_I), a measure of whole-body insulin action. Insulin was assayed by a double-antibody radioimmunoassay.[21] Glucose levels were measured by the glucose oxidase method using a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). On the following day (between 8 and 9 AM), all subjects had fat biopsies taken from the lower abdominal area.

Materials

Biosynthetic human insulin and insulin-like growth factor-1 (IGF-1) were kind gifts from Dr Ron Chance, Eli Lilly (Indianapolis, IN). A^{14}-^{125}I-insulin and ^{125}I-IGF-1 were supplied by Dr Bruce Frank, also of Eli Lilly. 3-*O*-Methyl-D-[1-^{14}C]glucose (30MG), ^{14}C-(U)-D-glucose and [2-^{3}H]thymidine were purchased from New England Nuclear (Boston, MA). Collagenase was obtained from Worthington Biochemical (Freehold, NJ); bovine serum albumin (BSA, fraction V) from Boehringer Manheim Biochemicals (Indianapolis, IN); phloretin from Biochemical Laboratories (Redondo Beach, CA); and silicone oil from Union Carbide (New York, NY). Cell culture materials, including Eagle's Minimal Essential Medium (MEM) with Earle's Balanced Salts, were purchased from Irvine Scientific (Irvine, CA). Fetal calf serum (FCS) was from Gemini Bioproducts (Calabasas, CA).

Preparation of Human Adipocytes

Adipose tissue was obtained by open biopsy of the lower abdominal wall by a previously described method.[22] Isolated adipocytes were prepared by a modification[22] of the method of Rodbell.[23] After digestion and filtration, the cells were washed four times in a buffer consisting of 150 mmol/L NaCl, 5 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L $CaCl_2$, 2.5 mmol/L NaH_2PO_4, 10 mmol/L Hepes, 2 mmol/L pyruvate, pH 7.4, supplemented with 4% BSA. Cells were then resuspended at approximately 5×10^5 cells/mL.

Cell counts were performed by a modification of method III of Hirsch and Gallian[24] in which cells were fixed in 2% osmium tetroxide and counted with a model ZB Coulter Counter (Hialeah, FL) using a 400-μm aperture tube.

Insulin Binding to Adipocytes

Isolated cells ($\sim 2 \times 10^5$ cells/mL) were combined with ^{125}I-insulin (33 pmol/L) and varying concentrations of unlabeled insulin (0 to 18 nmol/L). Binding was measured after incubation for 1 hour at 37°C using a previously described method.[25] Results are presented as the percentage of the tracer insulin (33 pmol/L) specifically bound to cells.

30MG Transport

Adipocytes ($\sim 5 \times 10^5$ cells/mL) were incubated at 37°C in the absence or presence of varying concentrations of insulin (60 minutes). Transport activity was assessed by measuring initial rates of uptake of tracer amounts (15 to 20 μmol/L) of 30MG, using a modification[22] of the method of Whitesell and Gliemann.[26]

Fibroblast Cell Culture

Fibroblast cultures were established from forearm punch biopsies of skin as described previously.[27,28] Cells were routinely grown in MEM-Earle's supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), and 20% FCS (vol/vol) in a 5% CO_2 humidified incubator. Media was changed every 2 days. Routine subculturing was performed at a 1:4 split on subconfluent cells (65% to 80% of confluency). All assays were performed on or before passage 10.

Insulin and IGF-1 Binding to Fibroblasts

Confluent cells were refed with growth media containing 10% FCS 24 hours before assay. Assay of hormone binding to fibroblasts used a modification of established methods.[27] Cells were washed in the same buffer as described earlier for adipocytes, except the BSA concentration was 1%. Fibroblasts were incubated with ^{125}I-insulin (83 pmol/L) or ^{125}I-IGF-1 (40 pmol/L) in the presence of unlabeled homologous hormone (0 to 5 nmol/L) for 2 hours at 24°C before determination of specific binding. Results are presented as the percentage of the tracer labeled hormone specifically bound to cells.

Glucose Incorporation Into Glycogen

Confluent cells were serum-starved for 16 to 18 hours before assay by refeeding with growth media containing 0.1% BSA instead of FCS. The media was replaced with fresh media and varying concentrations of insulin added together with ^{14}C-(U)-D-glucose (final [glucose], 5 mmol/L). Cells were incubated an additional 2 hours in the CO_2 incubator, repeatedly washed, and glycogen collected as described previously.[29] Results are presented as nanomoles of glucose incorporated into glycogen normalized to cell number. Cell counts were performed on replicate plates treated in parallel with those used for glycogen synthesis.

Thymidine Incorporation Into DNA

A modification of the method described by McClain et al[30] was used. Confluent cells were refed with serum-free medium for 24 hours. Cells were then treated with varying insulin concentrations, or FCS, for 16 hours. The media was replaced with Eagle's MEM, 0.1% BSA, pH 7.4, together with any treatments, and [^{3}H]thymidine (0.5 μCi) was added to each well. The cells were incubated for 2 hours at 37°C. Cells were then rinsed twice with 5 mL chilled phosphate-buffered saline (PBS), once with 1 mL methanol, twice with 5 mL of chilled 5% trichloracetic acid (wt/vol), followed by 5 mL of 4°C ethanol. The cells were then dissolved in 0.5 mL 1N NaOH and neutralized with an equal volume of 1N HCl. Aliquots were removed for liquid scintillation counting and protein determination. Results are presented as the percent of the total thymidine added incorporated into DNA, normalized to cell number.

Cell counts were performed on replicate plates treated in parallel with those used for thymidine uptake.

Data Analysis

Results are expressed as the mean ± SEM and compared by Student's *t* test, using the Statview SE + graphics program (Abacus Concepts, Berkely, CA). Significance was accepted at $P < .05$ (two-tailed *t* test). Half-maximal effective concentration (EC_{50}) values for responses were obtained by log-logit transformations of individual data.

RESULTS

Experimental Subjects

The NC and PCOS subjects were matched for age (Table 1). Since obesity can have dramatic effects on insulin action,[7] the subjects were matched for the extent of obesity so any differences observed would be due to the absence/presence of PCOS. As expected, the PCOS subjects had elevated testosterone levels, as well as an elevated luteinizing hormone/follicle-stimulating hormone ratio. Fasting glucose levels were normal in both groups, ruling out overt diabetes, but elevated insulin levels in PCOS subjects were suggestive of insulin resistance. The presence of whole-body insulin resistance in the PCOS group was clearly indicated by the response to an intravenous glucose challenge. The insulin sensitivity index, S_I, a measure of whole-body glucose disposal sensitivity to insulin,[20] was significantly lower in PCOS subjects compared with NC (Table 1). For reference purposes, S_I, values for normal and obese NIDDM subjects are 1.88 and 0.67, respectively.[20]

Insulin Action in Adipocytes

There were no differences between groups with regard to tracer insulin binding (0.73% ± 0.16% *v* 0.79% ± 0.09%/10^5 cells for NC and PCOS, respectively). Receptor affinities (50% inhibition of binding [EB_{50}], 0.50 ± 0.06 *v* 0.65 ± 0.22 nmol/L) were also comparable. There were no significant differences in absolute rates of glucose transport, in either the absence or presence of insulin (Fig 1). Maximal insulin responsiveness of glucose transport (fold-stimulation) was the same in both groups. However, adipocytes from PCOS subjects had greatly impaired insulin sensitivity (Fig 1) for the glucose transport response (EC_{50}, 130 ± 40 *v* 317 ± 58 pmol/L in NC and PCOS; $P < .025$). Thus, individually, and as a group, these PCOS subjects express both in vivo and in vitro insulin resistance.

Hormone Binding in Cultured Fibroblasts

Cultured fibroblasts have been shown to display numerous aspects of impaired insulin action from insulin-resistant donors, primarily diabetics.[17,31] To determine if there was a similar concordance in PCOS, explant cultures of skin fibroblasts were established. The initial event in insulin signaling, binding to its receptor, was measured first. Competition curves for labeled insulin binding are displayed in Fig 2. There was no significant difference in insulin binding between groups (Fig 2A). Binding affinities were also similar (EB_{50}, 0.87 ± 0.05 *v* 1.07 ± 0.28 nmol/L). Thus, insulin binding is normal in both adipocytes and fibroblasts from these PCOS subjects.

Type I IGF-1 receptors, which are present on fibroblasts, but not adipocytes,[32,33] can mediate metabolic responses to both

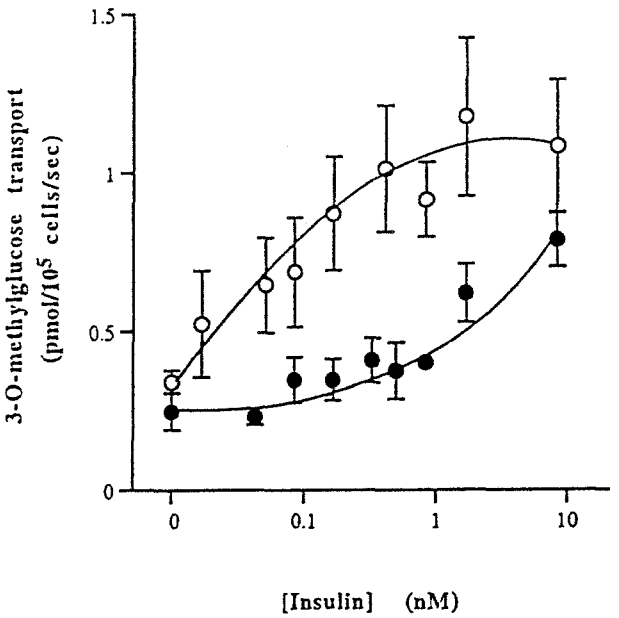


Fig 1. Insulin stimulation of glucose transport in isolated adipocytes from NC (○) and PCOS (●) subjects. Dose-response curves of 30MG transport were measured as described in the Methods. Results are the mean ± SEM; n = 5 for NC, n = 6 for PCOS.

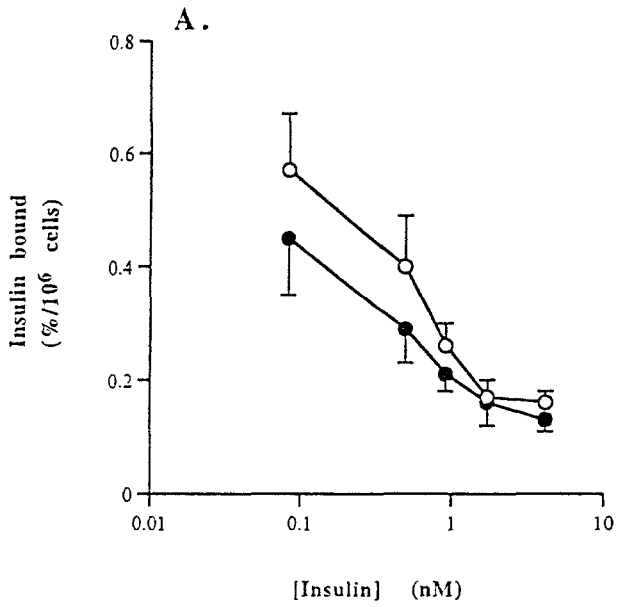
IGF-I and potentially insulin.[34] IGF-1 binding was far greater than insulin binding in the same cells (Fig 2B). Binding to NC cells did not differ significantly from that to PCOS cells. There was no difference in IGF-1 receptor affinity between groups.

Insulin Action on Glucose Metabolism

Insulin stimulation of glucose transport in adipocytes is primarily mediated by the GLUT4 transporter isoform.[35] As this isoform is absent in fibroblasts,[35] the transport response is not directly comparable between the two cell types. However, a major pathway by which insulin regulates glucose metabolism in both adipocytes and fibroblasts is through stimulation of glycogen synthesis, primarily by stimulation of glycogen synthase, which is present in both tissues. Glucose incorporation into glycogen measures the sum of these processes. Preliminary studies (not shown) established that overnight (16 to 18 hours) serum starvation showed the greatest extent of insulin responsiveness; this treatment was used for the following studies. Basal glucose incorporation into glycogen was similar in both groups (Fig 3). Insulin stimulated glucose incorporation in a dose-dependent manner (Fig 3). Maximal insulin responsiveness did not differ between NC (4.9- ± 0.6-fold stimulation) and PCOS (4.6- ± 0.3-fold) cells. Most importantly, insulin sensitivity for stimulation of net glycogen synthesis was the same in NC (EC_{50}, 9.6 ± 0.9 nmol/L) and PCOS fibroblasts (9.1 ± 0.9).

Insulin Action on DNA Synthesis

Unlike adipocytes, fibroblasts also display mitogenic responses to insulin. One measure of such responses is [^{3}H]thymidine incorporation into total DNA. Initial studies (not shown) showed that 24 hours of serum starvation, followed by 16 to 18 hours of treatment with insulin or serum, provided the optimal



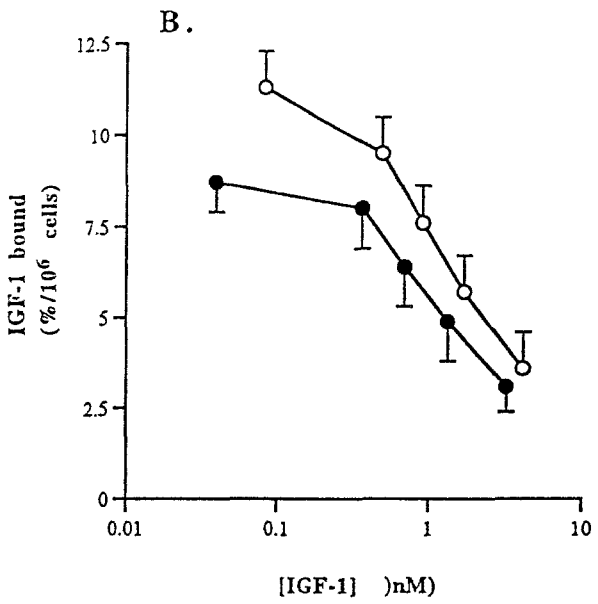


Fig 2. Competition curves of insulin and IGF-1 binding to cultured fibroblasts from NC (○) and PCOS (●) subjects. Specific binding was measured after 2 hours at 24°C as detailed in the Methods. Results are presented as the percentage of the tracer labeled hormone specifically bound to cells. (A) Insulin binding. (B) IGF-1 binding. Results are the mean ± SEM. Curves were repeated 3 to 6 times in each subject; n = 5 for NC, n = 6 for PCOS.

response to stimulation. Basal [^{3}H]thymidine incorporation was similar in NC and PCOS cells. [^{3}H]thymidine incorporation was stimulated by insulin in a dose-dependent manner (Fig 4). Maximal insulin responsiveness was the same in NC (2.3- ± 0.4-fold stimulation) and PCOS fibroblasts (2.1- ± 0.1-fold). Just like the situation for glycogen synthesis, and unlike that for

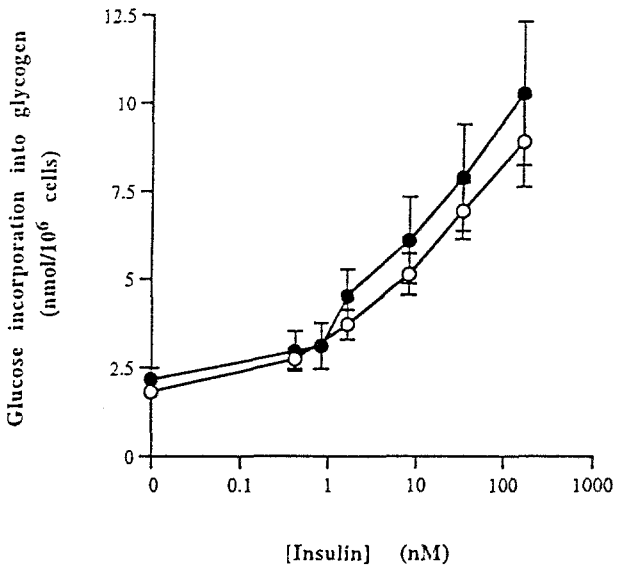


Fig 3. Insulin stimulation of glycogen synthesis in cultured fibroblasts from NC (○) and PCOS (●) subjects. Results are the mean ± SEM. Curves were repeated 3 to 4 times in each subject; n = 5 for NC, n = 6 for PCOS.

glucose transport in adipocytes, insulin sensitivity for DNA synthesis did not differ between NC (EC_{50}, 12.9 ± 2.4 nmol/L) and PCOS (EC_{50}, 7.6 ± 1.3) cells (Fig 4).

DISCUSSION

While the presence of insulin resistance has been established as a common, if not universal, characteristic of PCOS, the specific nature and mechanisms of such resistance are still unknown. Studies by several laboratories in freshly isolated adipocytes have shown that insulin resistance in PCOS involves impaired insulin sensitivity[8-10,12] that differs from insulin resis-

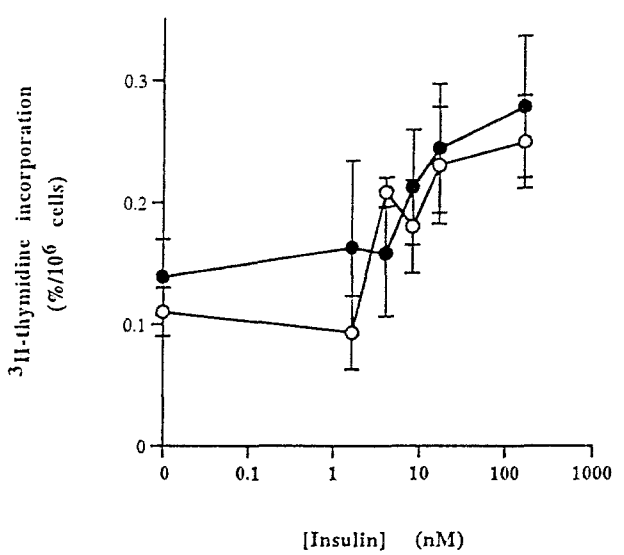


Fig 4. Insulin stimulation of DNA synthesis in cultured fibroblasts from NC (○) and PCOS (●) subjects. Results are the mean ± SEM, performed in triplicate. Curves were repeated 2 times in 3 subjects from each group.

tance in adipocytes from obese and NIDDM subjects.[7] This insulin resistance has been established for effects on glucose transport[8,9] and lipolysis,[10,12] two major insulin responses in these cells. Such studies give rise to a number of additional questions, one of which is: Is the insulin resistance seen in PCOS adipocytes a generalized defect or limited to certain cells or tissues? A second question about insulin resistance in PCOS is: If it is a primary defect due to genetic factors, secondary to the in vivo hormonal/metabolic environment, or a combination of the two?

The system we selected to investigate these questions was explant cultures of skin fibroblasts. Unlike the situation with freshly isolated adipocytes, repeated culture of fibroblasts would remove the influence of the in vivo environment and permit expression of genetic defects. Most importantly, fibroblasts have been show to reflect certain aspects of in vivo insulin action. For example, insulin stimulation of glycogen synthesis is impaired in fibroblasts of NIDDM subjects,[16] as it is in skeletal muscle. Thus, in diabetes, some defects in glucose metabolism are present in a wide array of tissues and are retained after removal from the in vivo metabolic melieu.

The PCOS subjects included in the present study were shown to express both in vivo (Table 1) and in vitro (Fig 1) insulin resistance. The impaired insulin sensitivity for adipocyte glucose transport stimulation is characteristic of obese PCOS individuals and confirms previous results.[8,9] In fibroblasts, both the number and affinity of insulin receptors were comparable in PCOS and NC subjects, matched for obesity (Fig 2A). One other report also found normal insulin binding in PCOS fibroblasts.[36] At this level, the results are in agreement with the finding in adipocytes, where binding is also not different from normal.[8,9] The general conclusion from our current and previous work is that insulin binding to noncirculating cells is essentially normal in PCOS. Several groups have also sequenced all[36] or a portion[37] of the insulin receptor from PCOS subjects and found no mutations that could account for insulin resistance. Thus, the initial event in insulin signaling appears to be intact in PCOS.

The current results also show that, at least for the responses of glycogen and DNA synthesis, fibroblasts from these PCOS subjects are normal with regard to insulin sensitivity and responsiveness and do not reflect the situation in vivo or in adipocytes from these same subjects. As fibroblasts from NIDDM subjects have impaired insulin action on glycogen synthesis,[16] this finding represents another difference between insulin resistance in PCOS and NIDDM. Insulin could also act through IGF-1 receptors, which are present in higher numbers. However, the affinity of insulin for the IGF-1 receptor is only 1% of that of the homologous hormone[32] and would occur only at high insulin concentrations, where there continue to be no differences between NC and PCOS cells. Impaired insulin action was found in activated T lymphocytes of obese PCOS subjects with acanthosis nigricans and severe hyperinsulinemia.[15] However, these subjects displayed characteristics of type I insulin resistance,[38] which is associated with insulin receptor mutations and functional impairments,[38] and, as discussed in an earlier report,[8] may represent a unique subset of PCOS patients. The normal response in the current group of PCOS fibroblasts could mean that insulin resistance may be limited to certain responses and/or tissues. Support for this possibility is provided by in vivo studies that showed a normal ability of insulin to suppress hepatic glucose output,[9] suggesting that insulin action in the liver is unimpaired. Dunaif et al have reported on a subpopulation of PCOS subjects whose fibroblasts displayed increased insulin receptor serine phosphorylation in the basal state, as well as a reduction in insulin stimulation of tyrosine phosphorylation of the receptor.[39] The significance of this finding to cellular insulin resistance is uncertain, for these investigators also report that there were no correlations between receptor phosphorylation and insulin action. Our previous work in adipocytes found a modest reduction in maximal insulin-stimulated autophosphorylation in PCOS, with no difference in receptor kinase activity toward an exogenous substrate,[8] also suggesting either normal receptor kinase activity or that control of insulin sensitivity resides at some event other than the receptor kinase.

Another possible explanation for normal insulin action in PCOS fibroblasts is removal from the in vivo hormonal milieu during prolonged culture. While androgens have been shown, in some cases, to impair insulin action,[13,15] amelioration of in vivo hyperandrogenemia by surgical and pharmacologic means has little or no effect on in vivo insulin resistance in PCOS subjects.[40,41] In the current subjects, NC or PCOS, there was no correlation between serum testosterone levels and insulin sensitivity measured either in vitro (EC_{50} for glucose transport) or in vivo (S_I) (data not shown). Most likely, androgens contribute to but are not the primary cause of insulin resistance in some conditions of PCOS.

The lack of impaired insulin sensitivity in these PCOS fibroblasts could also mean that the defect lacks a genetic component and is solely the result of environmental factors. While there is still controversy over the genetic basis of PCOS,[42] due in part to the considerable heterogeneity of the condition, family studies support the contention that PCOS is a multigenetic disease with a dominant mode of inheritance.[42,43] It has been postulated that PCOS would involve two genetic defects: one leading to reproductive abnormalities and the other responsible for impaired insulin signaling and insulin resistance.[4,44] The combination of these genetic defects, together with changes in insulin and androgen levels, would give rise to the syndrome.

What might be the significance of cellular specificity of insulin resistance in PCOS? If impaired insulin sensitivity were limited to the major metabolic targets of insulin, skeletal muscle, and adipose tissue, then the resultant compensatory hyperinsulinemia could serve to overstimulate other tissues. Since in the ovary insulin can stimulate androgen production,[45] as well as upregulate IGF-1 receptors[46] further sensitizing the ovary to the androgenic effects of IGF-1, hyperinsulinemia could be a major cause of hyperandrogenism.[46] In the liver, hyperinsulinemia would decrease production of both IGF-binding protein-1[47,48] and sex hormone–binding globulin,[48,49] increasing the bioavailability of IGF-1 and androgens. Resistance for the metabolic effects of insulin could lead to a hormonal environment that would exacerbate the consequences of the genetic defect of PCOS regarding reproduction. Thus, a productive potential therapeutic approach for PCOS would be

to improve peripheral insulin action.[50,51] Since all of the subjects in this study were obese, and the relationship between the presence of PCOS and insulin resistance is not as strong in lean subjects,[3,5] the implications of the current findings might be limited to obese PCOS subjects.

In summary, from the current results and a consideration of the literature, we would draw several conclusions. The first is that, in some circumstances, insulin resistance in PCOS might not be present in all tissues. Second, this resistance appears to be limited to certain insulin actions, most clearly established for glucose transport and antilipolysis. As there is considerable heterogenity in many aspects of PCOS,[52-54] especially with regard to the roles of obesity[3,5,6] and ethnicity,[55] and even the presence of insulin resistance,[6,52,53] it is difficult to make generalizations about the condition. However, in the current group of subjects, who demonstrate insulin resistance both in vivo and in isolated adipocytes compared with weight-matched controls, it is clear that their fibroblasts do not reflect the insulin resistance seen at other levels. The relative contributions of genetic and environmental factors to insulin resistance in PCOS remain to be established.

ACKNOWLEDGMENT

We thank the staff of the Clinical Research Center for excellent patient care.

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