Determinants of Impaired Fasting Glucose Versus Glucose Intolerance in Polycystic Ovary Syndrome

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OBJECTIVE — To determine insulin resistance and response in patients with polycystic ovary syndrome (PCOS) and normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance, and combined glucose intolerance (CGI).

RESEARCH DESIGN AND METHODS — In this cross-sectional study, 143 patients with PCOS (diagnosed on the basis of National Institutes of Health criteria) underwent oral glucose tolerance testing (OGTT), and 68 patients also had frequently sampled intravenous glucose tolerance tests. Changes in plasma glucose, insulin, cardiovascular risk factors, and androgens were measured.

RESULTS — Compared with patients with NGT, those with both IFG and CGI were significantly insulin resistant (homeostasis model assessment 3.3 ± 0.2 vs. 6.1 ± 0.9 and 6.4 ± 0.5, $P < 0.0001$) and hyperinsulinemic (insulin area under the curve for 120 min 973 ± 69 vs. $1,470 \pm 197$ and $1,461 \pm 172$ pmol/l, $P < 0.0001$). Insulin response was delayed in patients with CGI but not in those with IFG (2-h OGTT, insulin $1,001 \pm 40$ vs. 583 ± 45 pmol/l, $P < 0.0001$). Compared with the NGT group, the CGI group had a lower disposition index ($1,615 \pm 236$ vs. 987 ± 296, $P < 0.0234$) and adiponectin level (11.1 ± 1.1 vs. 6.2 ± 0.8 ng/ml, $P < 0.0096$). Compared with the insulin-resistant tertile of the NGT group, those with IFG had a reduced insulinogenic index (421 ± 130 vs. 268 ± 68, $P < 0.05$). Compared with the insulin-sensitive tertile of the NGT group, the resistant tertile had higher triglyceride and high-sensitivity C-reactive protein (hs-CRP) and lower HDL cholesterol and sex hormone–binding globulin (SHBG). In the entire population, insulin resistance correlated directly with triglyceride, hs-CRP, and the free androgen index and inversely with SHBG.

CONCLUSIONS — Patients with PCOS develop IFG and CGI despite having significant hyperinsulinemia. Patients with IFG and CGI exhibit similar insulin resistance but very different insulin response patterns. Increases in cardiac risk factors and free androgen level precede overt glucose intolerance.

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Women with polycystic ovary syndrome (PCOS) are at risk for impaired glucose tolerance (IGT), type 2 diabetes, and gestational diabetes mellitus (1) owing to abnormalities in insulin secretion and action (2–4). The specific defect is increased serine and decreased tyrosine phosphorylation of the insulin receptor (5). Patients who develop glucose intolerance have a relative decrease in insulin secretion as well (4). Women with PCOS are hyperinsulinemic compared with weight-matched control subjects (1). Hyperinsulinemia worsens and insulin sensitizers improve ovarian dysfunction and hyperandrogenemia in PCOS (6). Therefore, assessing glucose homeostasis by an oral glucose tolerance test (OGTT) has become a common practice.

Definition and interpretation of glucose intolerance have changed in recent years. The fasting glucose cutoff for diabetes was reduced from 140 to 126 mg/dl and the new term, impaired fasting glucose (IFG), was introduced for the values between 110 and 125 mg/dl (7). In 2003, the cutoff value for normal fasting glucose was reduced to 100 mg/dl (8). In addition, recent research demonstrated that the pathophysiology of isolated IFG differs from that of isolated IGT (defined by glucose levels >140 mg/dl at 2 h of an OGTT). The former results from hepatic insulin resistance, whereas the latter results from peripheral insulin resistance (9,10). Those individuals who exhibit combined glucose intolerance (CGI) have resistance at both sites. In addition, cardiovascular risk factors are more commonly encountered with glucose intolerance (IGT and CGI) than with isolated IFG (11).

Although glucose intolerance is found in one-third of patients with PCOS, so far only one study investigated the insulin resistance and secretion in patients with PCOS with IFG or IGT/CGI (12). Our study was undertaken to determine the differences in insulin secretion and action in patients with PCOS exhibiting different types of glucose intolerance.

RESEARCH DESIGN AND METHODS — A total of 143 women (108 [76%] white, 17 [12%] African American, 8 [6%] Hispanic, 5 [3%] American Indian, and 5 [3%] Asian) with PCOS (diagnosed by National Institutes of Health criteria), aged 18–45 years (means $\pm$ SEM 26.1 ± 0.9 years) with BMI 20–50 kg/m^2 were recruited at the University of California (UC), Davis (Sacramento, CA) ($n = 68$) and Yale University School of Medicine (New Haven, CT) ($n = 75$). The study was approved by the institutional review boards of both institutions. The subjects at UC Davis provided written informed consent, and they

all were examined by either S.E.K. or A.J.D. Patients using insulin sensitizers or medicines affecting lipids, weight, or insulin sensitivity within 2 months; having diabetes, untreated hypothyroidism, or systemic illnesses (i.e., renal, hepatic, and gastrointestinal); smoking; and drinking >2 servings of alcohol per week were excluded. Pregnant, postpartum, or lactating women were excluded. The studies were carried out at the Clinical and Translational Science Center at UC Davis and at Yale University School of Medicine Reproductive Endocrinology and Fertility Center. The subjects consumed their habitual diets and were weight-stable.

Fasting blood tests and OGTT were done in all subjects. Subjects recruited at UC Davis also underwent a frequently sampled intravenous glucose tolerance test (FSIVGTT).

Anthropometric measurements
Weight was measured using a Tanita BWB800-P digital medical scale, and height was measured using an Ayrton model S100 stadiometer.

OGTT
A standard OGTT was performed using 75 g of glucose (Glucola). Blood samples were obtained every 30 min. The subjects remained supine in bed. Samples were collected in tubes containing sodium fluoride, EDTA, or heparin.

FSIVGTT
An intravenous catheter was placed in each forearm. The catheters were kept open with normal saline. Heating pads were used to maximize the blood flow. After blood samples were obtained at −20, −10, and 0 min, glucose (0.3 units/kg as 25% dextrose) was injected intravenously at time 0. Intravenous insulin (0.03 units/kg) (Humulin regular; Eli Lilly) was administered at time 20 min. Additional samples were obtained at 0, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 min. The samples were analyzed for glucose and insulin. Acute insulin response (AIR_g), β-cell function, insulin sensitivity index (S_i), and disposition index were calculated using the MINMOD Millennium software (13).

Laboratory assays
Glucose was measured with a YSI 2300 STAT Plus Glucose & Lactate Analyzer (YSI Life Sciences, Yellow Springs, OH) or

a Bayer Dax-48 system analyzer (Bayer Diagnostics, West Haven, CT). Triglyceride, cholesterol, and HDL cholesterol were measured using a Poly-Chem System clinical chemistry analyzer (Polymedco, Cortlandt Manor, NY) or an Olympus AU 600 autoanalyzer (Olympus, Melville, NY). The coefficients of variation (CVs) were 1% for glucose, 3.5% for cholesterol, 4% for triglyceride, and 3.6% for direct HDL. Insulin, leptin, and adiponectin were measured using radioimmunoassay kits (Millipore, St. Charles, MO) with CVs of 8.2, 4.3, and 6.5%, respectively. Insulin was also measured by chemiluminescence (Bayer Diagnostics). High-sensitivity C-reactive protein (hs-CRP) was measured using a highly sensitive latex–enhanced immunonephelometric assay with both interassay and intraassay CVs <5% or immunoturbidimetry with an image analyzer (Hitachi 917; Quest Diagnostics) with an intraassay CV of 8.7%.

Calculations
Peripheral insulin resistance was assessed by calculating Matsuda's sensitivity index ($ISI_{Matsuda}$), from the formula (10,000/square root of [(fasting glucose × fasting insulin) × (mean glucose × mean insulin during OGTT)]), and S_i was calculated by applying the MINMOD program to FSIVGTT data. Hepatic insulin resistance was calculated by homeostasis model assessment (HOMA), [(fasting insulin [microunits per milliliter] × fasting glucose [milligrams per deciliter]/405)], and the quantitative insulin sensitivity check index (QUICKI), 1/[log (fasting insulin) + log (fasting glucose)]). Early insulin secretion, the insulinogenic index, was calculated by dividing the increases in insulin and glucose in the first 30 min of the OGTT ($\Delta_{Insulin\ 0–30}/\Delta_{Glucose\ 0–30}$) and by calculating AIR_g from FSIVGTT. Pancreatic function was assessed by calculating the area under the curve (AUC) for insulin and calculating β-cell function during FSIVGTT. β-Cell compensation for insulin resistance was assessed by calculating the disposition index from AIR_g and S_i.

Statistical analysis
Statistical analyses were performed using SAS statistical software (version 9.1; SAS Institute, Cary, NC). Data were first examined to identify any significant variations between the populations from two sites, UC Davis versus Yale School of Medicine. The age distribution of the subjects from Yale was shifted to younger

ages. In addition, age appeared to be confounded with BMI. Therefore, the pooled data were analyzed by including age and BMI as covariates in all of the subsequent statistical analyses. After correction for age and BMI, the association between study site and any outcome was attenuated toward the null.

Descriptive statistics were calculated. A Spearman correlation coefficient and its P value for significance of correlation were calculated to assess the magnitude and direction of an association between two given outcomes based on their ordered ranks. The data were log-transformed to improve the normality of residuals and homoscedasticity of errors where appropriate before statistical analysis. Group comparisons for mean in the cross-sectional outcome were performed by ANCOVA, adjusted for the baseline values and covariates (age and BMI). When the overall difference among the group means was significant in ANCOVA, post hoc pairwise group comparisons were conducted using Bonferroni multiple comparisons to identify the groups with different means. The longitudinal trajectories of 120-min changes in glucose and insulin level were estimated by a repeated-measures ANOVA. Individual trajectories of change in glucose and insulin level over five time points, observed every 30 min over 2 h, were estimated from linear random-effects models. Each observed level was entered as the dependent variable. Group (i.e., type of glucose intolerance), time (in 30 min), and a group × time interaction term were entered as independent variables. The coefficients for the interaction term were used to estimate the additional changes in glucose and insulin level over time associated with type of glucose intolerance. To account for between-subject heterogeneity in the change of glucose or insulin level, intercept and time were modeled as random effects. Multiple comparisons were controlled by the Bonferroni method where appropriate. Two-sided $P < 0.05$ was considered significant.

RESULTS — Forty-six of 143 women with PCOS (32%) had glucose tolerance abnormalities. Sixteen (11%) had IFG, 10 (7%) had IGT, and 20 (14%) had CGI. The remaining 97 women (68%) had normal glucose tolerance (NGT). Different ethnic groups were similarly distributed among NGT, IFG, IGT, and CGI. None of the minorities were overrepresented in any of the groups.

Table 1—Clinical and biochemical variables of the women with PCOS with NGT, IFG, IGF, and CGI

	NTG	IFG	IGT	CGI	P (ANOVA)
n	97	16	10	20	
Anthropometric					
Age (years)	26.1 ± 0.8	30.9 ± 2.2	35.8 ± 2.4†	30.6 ± 1.7	0.0003
Weight (kg)	89.0 ± 2.6	108.1 ± 4.4*	84.7 ± 7.5	101.4 ± 5.2	0.0001
BMI (kg/m^2)	32.9 ± 0.8	38.7 ± 1.5*	33.1 ± 2.9	38.1 ± 1.8	0.0072
Fasting glucose (mmol/l)	4.9 ± 0.0	6.1 ± 0.2	5.1 ± 0.1	6.0 ± 0.1	<0.0001
Insulin (pmol/l)	88 ± 5	131 ± 16	112 ± 22	145 ± 12	0.0002
Adiponectin (ng/ml)†	11.1 ± 1.1	8.4 ± 1.1	8.2 ± 1.0	6.2 ± 0.8*	0.0132
HOMA	3.3 ± 0.2	6.1 ± 0.9*	4.4 ± 0.3	6.4 ± 0.5*	<0.0001
QUICKI	0.33 ± 0.003	0.30 ± 0.006*	0.32 ± 0.011	0.30 ± 0.004*	<0.0001
OGTT					
ΔGlucose$_{0-30}$	2.4 ± 0.1	3.4 ± 1.0	4.0 ± 0.5	2.9 ± 0.5	0.0012
ΔInsulin$_{0-30}$	446 ± 34	689 ± 124	395 ± 82	462 ± 70	0.3,141
Insulinogenic index	257 ± 46	268 ± 68	104 ± 23	154 ± 11	0.5,114
AUC$_{Glucose\ 0-30}$	3.1 ± 0.0	3.9 ± 0.1*	3.6 ± 0.1*	3.7 ± 0.1*	<0.0001
AUC$_{Insulin\ 0-30}$	156 ± 11	238 ± 38*	155 ± 30	188 ± 22	0.049
AUC$_{Glucose\ 0-120}$	12.8 ± 0.2	16.1 ± 0.6*	18.1 ± 0.6*	18.3 ± 0.8*‡	<0.0001
AUC$_{Insulin\ 0-120}$	973 ± 69	1,470 ± 197*	1,170 ± 197	1,461 ± 172*	<0.0001
ISI$_{Matsuda}$	4.61 ± 0.34	2.12 ± 0.29*	2.76 ± 0.62*	1.90 ± 0.28*	<0.0001
FSIVGTT†					
S_i†	3.47 ± 0.68	1.58 ± 0.34	2.46 ± 0.31	2.27 ± 0.44	0.1468
AIR$_g$†	655 ± 106	725 ± 204	583 ± 152	471 ± 83	0.482
β-Cell function†	236 ± 25	266 ± 55	350 ± 76	317 ± 31	0.0901
Disposition index†	1,615 ± 236	879,187	1,225 ± 226	988 ± 296*	0.0238
CVD risk factors					
Triglyceride (mmol/l)	1.22 ± 0.07	1.29 ± 0.16	1.78 ± 0.29	1.29 ± 0.14	0.2421
Cholesterol (mmol/l)	4.89 ± 0.10	4.65 ± 0.20	4.99 ± 0.21	4.91 ± 0.22	0.7987
LDL cholesterol (mmol/l)	3.03 ± 0.10	2.99 ± 0.22	3.10 ± 0.11	3.22 ± 0.19	0.8518
HDL cholesterol (mmol/l)	1.23 ± 0.30	1.08 ± 0.04	1.09 ± 0.11	1.10 ± 0.10	0.1678
hs-CRP (mg/l)	5.8 ± 0.8	6.4 ± 1.5	4.3 ± 1.5	6.4 ± 1.3	0.4786
Androgens					
Testosterone (nmol/l)	2.46 ± 0.10	2.43 ± 0.21	2.53 ± 0.35	3.09 ± 0.24	0.0722
SHBG (nmol/l)	42.1 ± 3.7	27.5 ± 2.5	37.4 ± 2.5	35.5 ± 4.8	0.3932
FAI	9.1 ± 0.7	8.8 ± 4.4	5.4 ± 2.0	11.1 ± 1.3	0.1633
DHEAS (μmol/l)	0.59 ± 0.03	0.57 ± 0.09	0.46 ± 0.10	0.54 ± 0.08	0.6062

Data are means ± SEM. *$P < 0.05$ compared with the NGT group. †Studies done in the subpopulation of $n = 68$ (NGT 36, IFG 8, IGT 8, and CGI 16). ‡$P < 0.05$ compared with the IFG group. CVD, cardiovascular disease; DHEAS, dehydroepiandrosterone sulfate; FAI, free androgen index.

Baseline differences among NGT, IFG, IGT, and CGI groups

The IGT group was older and the IFG group was more obese than the NGT group (Table 1). By definition, the IFG, IGT, and CGI groups had higher glucose levels than the NGT group. All glucose-intolerant groups had higher fasting insulin than the NGT group (IFG 131 ± 16 pmol/l, IGT 112 ± 22 pmol/l, and CGI 145 ± 12 pmol/l vs. NGT 88 ± 5 pmol/l, $P = 0.0002$). The difference between the fasting insulin values of the CGI and IGT groups was also significant ($P = 0.0047$).

Insulin resistance

Both IFG and CGI groups had lower ISI$_{Matsuda}$ values than the NGT groups (2.12 ± 0.29 and 1.90 ± 0.28 vs. 4.61 ± 0.34, $P = 0.0004$ and $P < 0.0001$, respectively) (Table 1, Fig. 2). In 68 women who underwent FSIVGTT, differences in S_i did not reach significance (NGT 3.47 ± 0.60, IFG 1.58 ± 0.34, IGT 2.46 ± 0.29, and CGI 2.27 ± 0.44, $P = 0.1468$). On the other hand, the disposition index was significantly reduced in the CGI group compared with the NGT group (987 ± 296 vs. 1,615 ± 236, $P = 0.0234$). The CGI group also had lower serum adiponectin than the NGT group (6.24 ± 0.8 vs. 11.1 ± 1.1 ng/ml, $P = 0.0096$). Compared with the NGT group, both IFG and CGI groups had higher HOMA (6.1 ± 0.9 and 6.4 ± 0.5 vs. 3.3 ± 0.2, $P = 0.0006$ and $P < 0.0001$) and lower QUICKI (0.30 ± 0.006 and 0.30 ± 0.004 vs. 0.33 ± 0.003, $P = 0.0009$ and $P < 0.0001$).

Insulin response

Compared with the NGT group, the IFG group had an overall increase in insulin response during an OGTT, and the differences were significant at every time point (Table 1, Fig. 1), whereas the IGT and CGI groups exhibited a delayed response. During the first half of the OGTT, the IFG group had higher insulin levels than the CGI group (30 min 820 ± 42 vs. 508 ± 54 pmol/l and 60 min 1,032 ± 54 vs. 738 ± 72 pmol/l, $P < 0.001$ for both). After 60 min, the insulin response pattern changed; at 120 min the CGI group had higher insulin than the IFG group (996 ± 34 vs. 583 ± 45 pmol/l, $P < 0.001$). In 68
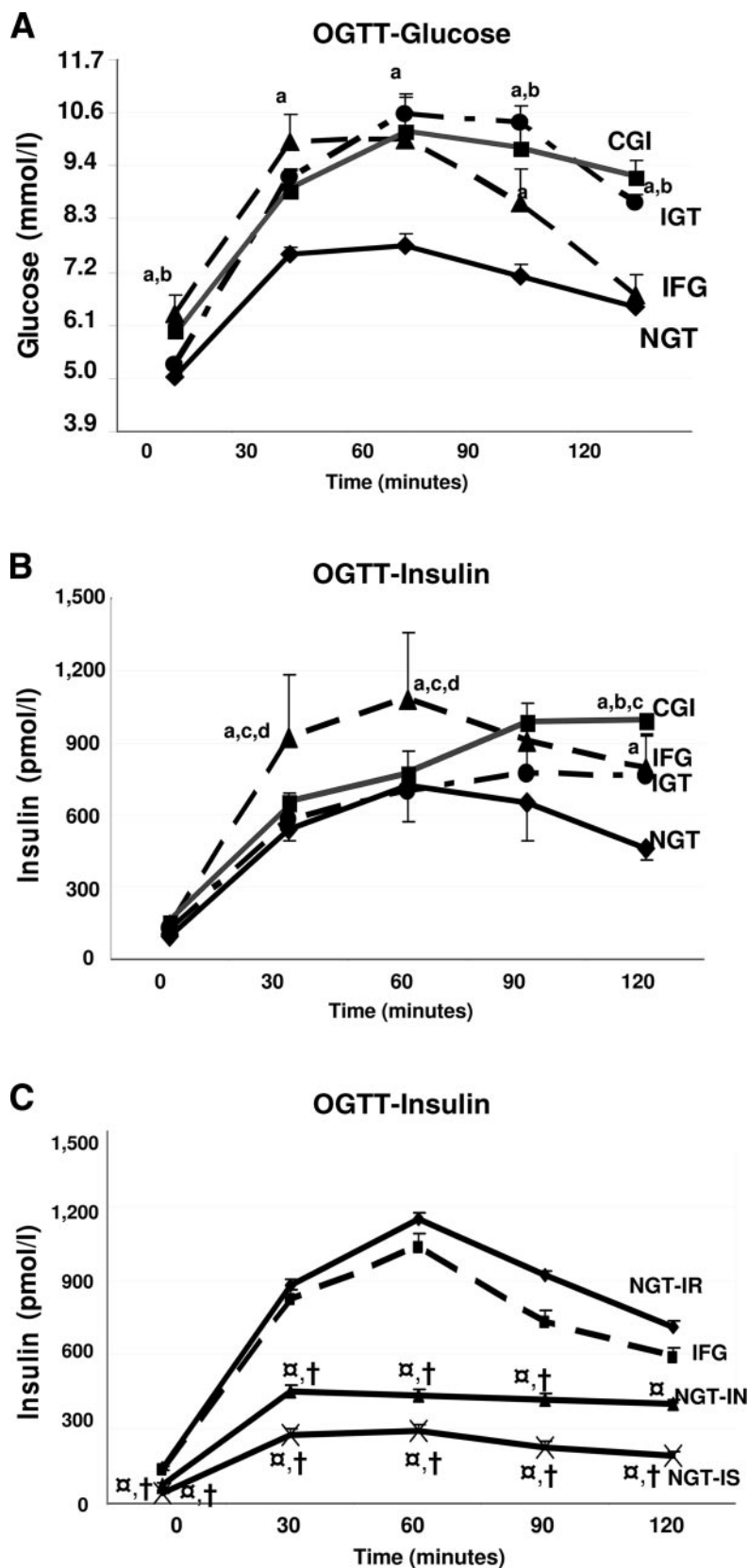
Figure 1—*Changes in glucose and insulin during OGTTs. A and* B: ♦*, NGT,* n = 97; ▲ *with broken line, IFG,* n = 16; ●*, IGT,* n = 10; ■*, CGI,* n = 20. C: ✕*, NGT-IS,* n = 33; ■*, NGT-IN,* n = 32; ▼*, NGT-IR,* n = 32; ▲ *with broken line, IFG,* n = 16. ¤*,* P < 0.05 *compared with NGT-IR.* †P < 0.05 *compared with IFG. Data are means* ± *SEM. a,* P < 0.05 *compared with NGT; b,* P < *0.05 compared with IFG; c,* P < *0.05 compared with IGT.*

individuals who underwent FSIVGTTs, there were no significant differences in AIR_g ($P = 0.4842$) or β-cell function ($P = 0.0901$).

Insulin response in NGT tertiles, divided based on insulin resistance

The IFG and CGI groups had similar insulin resistance based on HOMA (6.1 ± 0.9 vs. 6.4 ± 0.5), QUCKI (0.30 ± 0.006 vs. 0.30 ± 0.004), and $ISI_{Matsuda}$ (2.12 ± 0.29 vs. 1.90 ± 0.28) but different insulin response patterns (Table 2) (Fig. 1). Insulin response in insulin-resistant subjects with NTG was also investigated. When divided into tertiles based on $ISI_{Matsuda}$, the insulin-resistant (NGT-IR) tertile was similar to subjects with IFG and CGI, based on their HOMA (5.6 ± 0.3), QUICKI (0.30 ± 0.002), and $ISI_{Matsuda}$ (1.90 ± 0.09). Their BMI (39.9 ± 1.2 kg/m^2) was similar to those of the subjects with IFG (38.7 ± 1.5 kg/m^2) and CGI (38.1 ± 1.8 kg/m^2). The NGT-IR group had higher a insulinogenic index than the IFG group (421 ± 130 vs. 268 ± 68, $P < 0.05$).

Differences among the NGT tertiles

The tertiles were referred to as insulin-sensitive (NGT-IS), intermediate (NGT-IN), and NGT-IR (Table 2) (Fig. 2). In these tertiles, fasting glucose and insulin increased stepwise (from 4.8 ± 0.02 to 5.0 ± 0.02 and to 5.1 ± 0.02 mmol/l, $P < 0.0001$ and from 41 ± 2 to 79 ± 2 and to 146 ± 2 pmol/l, respectively, $P < 0.0001$). The NGT-IN and NGT-IR tertiles had higher BMI, fasting glucose, HOMA, $\Delta Insulin_{0-30}$, hs-CRP, and free androgen index and lower QUICKI and HDL cholesterol than the NGT-IS tertile. In addition, the NGT-IR tertile had higher insulinogenic index, $AUC_{Glucose\ 0-120}$, $AUC_{Insulin\ 0-120}$, and triglyceride and lower sex hormone–binding globulin than the NGT-IS tertile. Even after correction for BMI, differences in fasting glucose, fasting insulin, $\Delta Insulin_{0-30}$, $AUC_{Glucose\ 0-120}$, $AUC_{Insulin\ 0-120}$, and HDL cholesterol remained significant.

Next, partial correlations among insulin resistance parameters, cardiovascular risk factors, and androgens were calculated after adjustment for the differences in BMI. Plasma triglyceride and hs-CRP correlated directly with HOMA ($r = 0.316$ and 0.253, $P = 0.009$ and $P = 0.037$, respectively) and inversely with $ISI_{Matsuda}$ ($r = -0.282$ and -0.306, $P = 0.020$ and 0.011, respectively).

Table 2—*Clinical and biochemical variables of the NGT group divided into tertiles based on degree of insulin resistance*

	Sensitive	Intermediate	Resistant	ANOVA* P_1	ANOVA* P_2
n	33	32	32		
Anthropometric					
Age (years)	26.4 ± 1.23	25.9 ± 1.5	25.9 ± 1.5	0.9641	0.449
BMI (kg/m^2)	27.4 ± 1.0	31.6 ± 1.2†	39.9 ± 1.2†‡	<0.0001	—
Fasting					
Glucose	4.8 ± 0.02	5.0 ± 0.002†	5.1 ± 0.02†‡	<0.0001	<0.0001
Insulin	41 ± 2	79 ± 2†	146 ± 2†‡	<0.0001	<0.0001
HOMA	1.44 ± 0.10	2.9 ± 0.1†	5.6 ± 0.3†‡	<0.0001	<0.0001
QUICKI	0.37 ± 0.004	0.33 ± 0.002†	0.30 ± 0.002†‡	<0.0001	<0.0001
OGTT					
ΔGlucose$_{0-30}$	2.2 ± 0.3	2.3 ± 0.2	2.6 ± 0.2‡	0.3,801	0.4358
ΔInsulin$_{0-30}$	235 ± 23	391 ± 32†	736 ± 73†‡	<0.0001	<0.001
Insulinogenic index	134 ± 26	221 ± 45I	421 ± 130†§	0.0355	0.3425
AUC$_{Glucose\ 0-120}$	11.6 ± 0.4	13.0 ± 0.3†	14.2 ± 0.3†§	<0.0001	0.0006
AUC$_{Insulin\ 0-120}$	463 ± 32	781 ± 29†	1,689 ± 129†‡	<0.0001	<0.0001
ISI$_{Matsuda}$	8.12 ± 0.6	3.71 ± 0.12†	1.90 ± 0.09†‡	<0.0001	<0.0001
CVD risk factors					
Triglyceride (mmol/l)	0.91 ± 0.07	1.18 ± 0.09	1.51 ± 0.17†	0.0006	0.1873
Cholesterol (mmol/l)	4.76 ± 0.14	5.04 ± 0.17	4.86 ± 0.18	0.4407	0.1721
LDL cholesterol (mmol/l)	2.73 ± 0.19	3.25 ± 0.17	3.14 ± 0.14	0.0671	0.1361
HDL cholesterol (mmol/l)	1.43 ± 0.07	1.25 ± 0.05†	1.03 ± 0.05†‡	<0.0001	0.0359
hs-CRP (mg/l)	1.9 ± 0.3	5.9 ± 2.1†	10.6 ± 1.9†‡	<0.0001	0.1055
Androgens					
Testosterone	2.25 ± 0.14	2.53 ± 0.14	2.60 ± 0.21	0.2176	0.3996
SHBG (nmol/l)	57.9 ± 7.7	37.9 ± 7.0	25.0 ± 3.3†	0.0025	0.0661
FAI	6.6 ± 1.0	10.1 ± 1.4†	12.8 ± 1.7†‡	0.0010	0.0741
DHEAS (μmol/l)	0.61 ± 0.0.6	0.61 ± 0.06	0.54 ± 0.07	0.4783	0.8567

Data are means ± SEM. *P_1, significance by ANCOVA; P_2, significance by ANCOVA after adjustment for BMI. †$P < 0.05$ compared with the NGT group. ‡$P < 0.05$ compared with the IFG group. §$P < 0.05$ compared with the IFG group shown in Table 1, analyzed using the Bonferroni multiple-comparisons procedure in ANCOVA. DHEAS, dehydroepiandrosterone sulfate; FAI, free androgen index.

CONCLUSIONS — This study demonstrated that, first, patients with PCOS with IFG exhibited severe peripheral insulin resistance and developed IFG despite having an increased early insulin response. Second, having normal glucose levels during an OGTT did not indicate normal insulin sensitivity or a low risk for cardiovascular disease. Third, in the NGT group, the BMI, sex hormone–binding globulin, HDL cholesterol, and hs-CRP levels appeared to have value in assessing insulin resistance. Because we did not have a control group of age- and weight-matched women with normal reproductive function, we cannot conclusively state that these findings are specific to patients with PCOS. However, our observations differ significantly from those obtained from middle-aged men and women (14).

Diabetes risk increases with increasing age and obesity (15). We found age and weight to be important in development of glucose intolerance and insulin resistance, respectively (Tables 1 and 2).

Per definition, glucose levels were higher in patients with IFG, IGT, and CGI compared with patients with NGT. Insulin responses of patients with IFG, IGT, or CGI were not decreased. In fact, AUC$_{Insulin-120}$ was higher in both patients with IFG and CGI, indicating that patients with PCOS can develop IFG and CGI in the presence of hyperinsulinemia. This was consistent with the findings of Kulshreshtha et al. (12), who reported increased insulin responses in patients with PCOS with IGT/CGI or type 2 diabetes. The time course of the insulin response was the most significant difference between patients with IFG versus those with IGT and CGI. Patients with IFG had a brisk early insulin response that declined during the second half of OGTT. In contrast, patients with CGI and IGT exhibited a decreased early insulin response followed by delayed hyperinsulinemia. Previous reports showed similar response patterns in those with IFG versus those with IGT and CGI among subjects without PCOS (12,14,16,17).

The brisk, early insulin response of patients with IFG appeared to be specific to PCOS because several studies in different populations, ethnic groups, sex, and age distributions have reported decreased cumulative and early insulin response in patients with IFG (14,17–20), although a recent report in healthy nondiabetic men and women showed an increased insulin response (16). The only available study in PCOS demonstrated an increased insulin response similar to ours (12).

In patients with PCOS who have IGT, the early and cumulative insulin responses (AUC$_{Insulin-30}$ and AUC$_{Insulin-120}$) did not differ significantly from the responses of those with NGT, whereas Kulshreshtha et al. (12) reported increased insulin response in glucose-intolerant patients with PCOS. However, their study did not distinguish between IGT and CGI, and the subjects were less obese and of different ethnicities.

Studies using intravenous glucose tolerance tests in subjects without PCOS reported that AIR$_g$ was decreased by ~30%
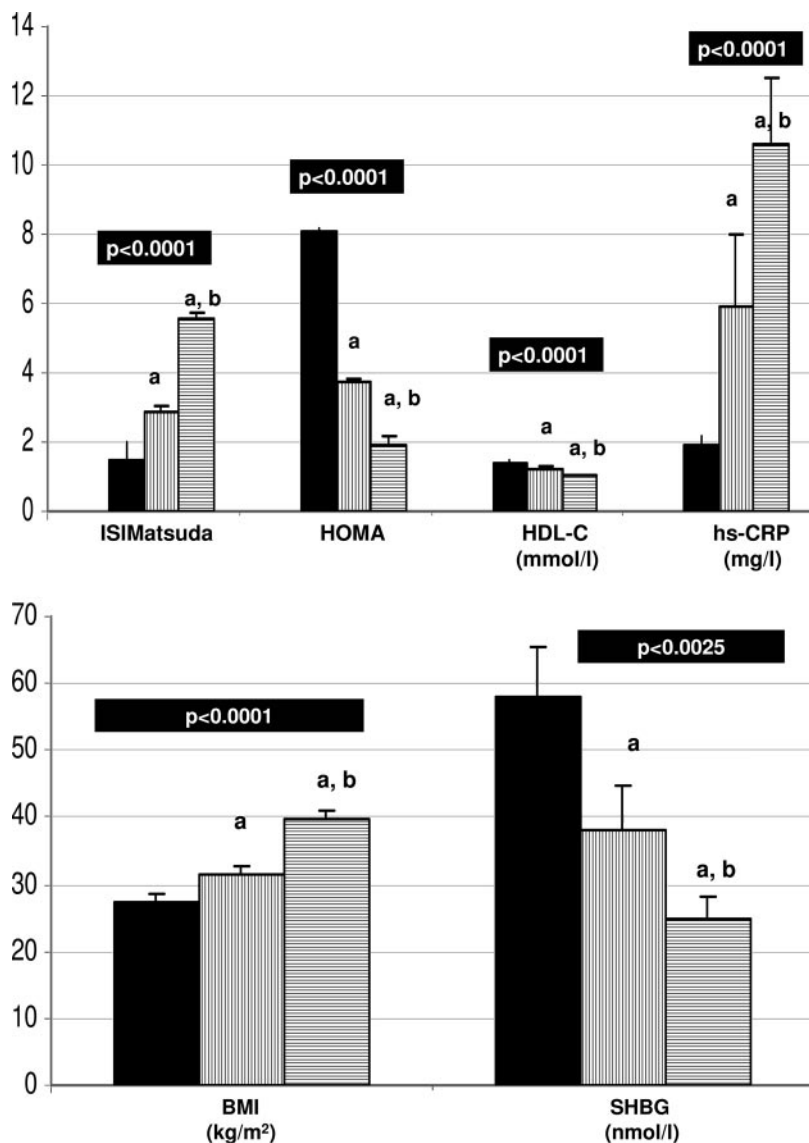
Figure 2—*HOMA, HDL cholesterol, hs-CRP, BMI, and SHBG values in subjects with NGT divided into tertiles based on their ISI$_{Matsuda}$ values (■, NGT-IS, n = 33; ▥, NGT-IN, n = 32; ▤, NGT-IR, n = 32). Data are means ± SEM. a, P < 0.05 compared with NGT-IS; b, P < 0.05 compared with NGT-IN.*

in those with IFG and by 8–18% in those with IGT (20–22). As seen in Table 1, AIR$_g$ did not decrease in our patients with PCOS with IFG. Taken altogether, these findings indicate that patients with PCOS can develop fasting hyperglycemia and glucose intolerance even with increased early and cumulative insulin responses.

The literature indicates that primary sites of insulin resistance differ in IFG, IGT, and CGI (9,10,14): hepatic in IFG; peripheral in IGT; and both hepatic and peripheral in CGI. These distinctions cannot be made without using a hyperinsulinemic clamp. The surrogate measures of hepatic insulin resistance include HOMA and QUICKI. Consistent with the litera-

ture, we found increased HOMA and decreased QUICKI only when fasting glucose was impaired (IFG/CGI) but not in IGT (Table 1). In contrast with the literature, the surrogates for peripheral insulin resistance, ISI$_{Matsuda}$ and S_i, of our IFG group were similar to those of the IGT and CGI groups, indicating that patients with PCOS with isolated IFG also have peripheral insulin resistance.

The cause of IFG was an enigma because this group had significant early and late hyperinsulinemia. Thus, we compared the IFG group to subjects with equal insulin resistance with NTG (NGT-IR). Although the NGT-IR group and those with IFG had similar HOMA,

QUICKI, and ISI$_{Matsuda}$ values, the NGT-IR group had a higher insulinogenic index. As shown in Fig. 1, patients with IFG also had decreased overall insulin secretion relative to the degree of insulin resistance.

The studies of tertiles demonstrated that patients with PCOS who have NGT can still be severely insulin-resistant. In addition, cardiovascular risk factors and hyperandogenemia worsen before overt hyperglycemia. Consistent with these findings, a recent report indicated that low HDL cholesterol levels correlate with hyperinsulinemia in PCOS (23). Previous studies had found increased cardiovascular risk factors only in subjects with IGT and CGI without PCOS (11,21).

We propose the natural course of glucose intolerance in PCOS as follows. Insulin resistance increases with weight gain, as suggested by the stepwise increase in BMI in the NGT tertiles (Fig. 2). As long as insulin response can compensate, plasma glucose remains within the "normal" range. A relatively small decrease in overall insulin response results in isolated IFG. A decrease in the early insulin response results in IGT/CGI, even with late hyperinsulinemia. Factors leading to impairment of the early versus overall response are not known, although genetic factors may be important (4,24).

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