

# Early metabolic abnormalities in adolescent girls with polycystic ovarian syndrome

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**Objective:** To investigate insulin sensitivity and secretion in young adolescent girls with childhood onset polycystic ovarian syndrome (PCOS) and to identify the early metabolic derangement(s).

**Study design:** Twelve obese girls with PCOS (age  $12.0 \pm 0.7$  years) were compared with 10 obese nonhyperandrogenic girls (control group). The groups were matched for age, percent body fat, and abdominal fat. All subjects underwent a 3-hour hyperinsulinemic (80  $\mu\text{u}/\text{m}^2/\text{min}$ )-euglycemic clamp to determine in vivo insulin sensitivity and a 2-hour hyperglycemic clamp (225  $\text{mg}/\text{dL}$ ) to determine insulin secretion. Fasting hepatic glucose production was determined with the use of  $[6,6\text{-}^2\text{H}_2]\text{glucose}$ .

**Results:** Fasting glucose and hepatic glucose production were comparable between the 2 groups, but fasting insulin was 2-fold higher in the PCOS group. The fasting glucose to insulin ratio was lower in the PCOS group versus the control group ( $1.9 \pm 0.3$  vs  $3.1 \pm 0.3$ ,  $P = .02$ ). During the hyperinsulinemic-euglycemic clamp, insulin sensitivity was lower in the PCOS group ( $1.4 \pm 0.2$  vs  $2.7 \pm 0.3$   $\text{mg}/\text{kg}/\text{min}$  per  $\mu\text{u}/\text{mL}$ ,  $P = .002$ ). During the hyperglycemic clamp, insulin secretion was significantly higher in the PCOS group. Insulin sensitivity correlated negatively with fasting insulin ( $r = -0.71$ ,  $P = .0002$ ) and positively with the fasting glucose to insulin ratio ( $r = 0.79$ ,  $P < .0001$ ).

**Conclusion:** Adolescent girls with PCOS have profound metabolic derangements detected early in the course of the syndrome, including (1) ~50% reduction in peripheral tissue insulin sensitivity, (2) evidence of hepatic insulin resistance, and (3) compensatory hyperinsulinemia. These observations may predict an increased risk of type 2 diabetes mellitus in adolescents with PCOS. (*J Pediatr* 2001;138:38-44)

Polycystic ovarian syndrome is one of the most common endocrine disorders of premenopausal women, increasingly recognized in adolescent girls with hyperandrogenism.<sup>1-5</sup> Although PCOS has been defined in multiple ways, the currently accepted criteria are based on standards developed at a 1990 consensus conference of the National Institute of Child Health and Human Development, The National Institute of Health, USA.<sup>6</sup> These criteria are chronic anovulation and hyperandrogenism excluding other causes such as congenital adrenal hyperplasia, hyperprolactinemia, Cushing's syndrome, and androgen secreting tumors. The presence of "polycystic ovaries on ultrasonography" is not a requirement. Adult women with PCOS have profound insulin resistance with  $\beta$ -cell dysfunction and are at increased risk for impaired glucose tolerance and type 2 diabetes.<sup>7-10</sup> Traditionally, type 2 diabetes has been considered rare in the pediatric population. However, lately there has been a disturbing trend of increasing cases of obesity-related type 2 diabetes in adolescents, with an over-representation of females, especially those with hyperandrogenism, irregular periods, and acanthosis nigricans.<sup>11-12</sup>

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Supported by United States Public Health Service grant RO1 HD27503 (S.A.), MO1-RR00084 General Clinical Research Center, HD34808 (S.F.W.), Renziehausen Trust Fund (SA), and Eli-Lilly and Company.

Presented at the 1999 Pediatric Academic Societies' Annual Meeting, May 1-4, San Francisco, Calif.

Submitted for publication Jan 25, 2000; revision received Apr 19, 2000; accepted June 19, 2000.

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0022-3476/2001/\$35.00 + 0 9/21/109603

doi:10.1067/mpd.2001.109603

OGTT Oral glucose tolerance test  
PCOS Polycystic ovarian syndrome

The aim of this study was to investigate in vivo insulin sensitivity and insulin secretion in adolescents with recent onset PCOS to identify the early metabolic derangements and the risk factors for type 2 diabetes.

## EXPERIMENTAL DESIGN

### Subjects

Twenty-two obese (body mass index  $\geq 90\%$  for age) adolescent girls participated in the study. Twelve (8 black, 4 white) were referred to the endocrinology service for evaluation of oligomenorrhea ( $n = 4$ ), hirsutism ( $n = 5$ ), and acne ( $n = 3$ ). All had clinical and biochemical evidence of hyperandrogenism by careful physical examination and elevated serum total and free testosterone levels and/or androstenedione and/or dehydroepiandrosterone sulfate levels. The diagnosis of PCOS was made after other causes of hyperandrogenism including congenital adrenal hyperplasia were excluded. Ten (5 black, 5 white) nonhyperandrogenic girls (Table I) were the control group recruited through newspaper advertisement. These were otherwise normal adolescents with exogenous obesity. Five had normal menses, and the other 5 were not menarcheal yet. Research participants and parents gave written informed consent after they received a thorough explanation of the research. None of the subjects was receiving any medications. Hematologic, renal, and liver function was normal in all subjects. All studies were approved by the Human Rights Committee of Children's Hospital of Pittsburgh.

## METHODS

Each subject was studied twice, 1 to 2 weeks apart, once during a 3-hour hyperinsulinemic-euglycemic clamp to assess insulin sensitivity and once during a 2-hour hyperglycemic clamp to assess insulin secretion, in random order. Clamp experiments were performed after a 10- to 12-hour overnight fast. For each study, 2 intravenous catheters were inserted after the skin and subcutaneous tissues were anesthetized with Emla cream (Astra Pharmaceutical Products, Westborough, MA). One

**Table I.** Physical and hormonal characteristics of subjects in study

	PCOS (n = 12)	Control group (n = 10)	P value
Age (y)	12.0 $\pm$ 0.7	12.1 $\pm$ 0.6	NS
Tanner Stage			
II-III	4	5	
IV	4	2	
V	4	3	
BMI (kg/m <sup>2</sup> )	33.1 $\pm$ 1.8	31.4 $\pm$ 1.3	NS
Total testosterone (ng/dL)	31.8 $\pm$ 6.0	17.1 $\pm$ 3.4	.025
Free testosterone (pg/mL)	7.2 $\pm$ 1.4	3.4 $\pm$ 1.0	.02
%BF	43.2 $\pm$ 1.4	45.6 $\pm$ 1.1	NS
FM (kg)	34.8 $\pm$ 2.9	34.0 $\pm$ 2.2	NS
Leptin (ng/mL)	36.6 $\pm$ 3.5	38.8 $\pm$ 4.3	NS
SAF (cm <sup>2</sup> )	465.6 $\pm$ 41.8	424.4 $\pm$ 37.6	NS
VAF (cm <sup>2</sup> )	80.5 $\pm$ 15.6	59.2 $\pm$ 12.0	NS
TAF (cm <sup>2</sup> )	546.1 $\pm$ 48.9	483.6 $\pm$ 41.9	NS

NS, Not significant; BMI, body mass index; FM, fat mass; % BF, percent body fat; VAF, visceral abdominal fat; SAF, subcutaneous abdominal fat; TAF, total abdominal fat.

**Table II.** Fasting metabolic data

	PCOS	Control group	P value
Glucose (mg/dL)	97.2 $\pm$ 2.4	94.4 $\pm$ 2.0	NS
Insulin ( $\mu$ u/mL)	65.6 $\pm$ 10.6	33.7 $\pm$ 3.6	.013
HGP (mg/kg/min)	2.2 $\pm$ 0.3	1.9 $\pm$ 0.1	NS
G/I ratio	1.9 $\pm$ 0.3	3.1 $\pm$ 0.3	.02
IGF-I (ng/mL)	401 $\pm$ 40	331 $\pm$ 54	NS
IGF-BP <sub>1</sub> (ng/mL)	2.3 $\pm$ 0.6	5.0 $\pm$ 1.4	.06

HGP, Hepatic glucose production; G/I ratio, fasting glucose (mg/dL) to insulin ( $\mu$ u/mL) ratio; IGF-I, insulin-like growth factor-I; IGF-BP<sub>1</sub>, insulin-like growth factor binding protein<sub>1</sub>.

catheter was placed in a forearm vein for administration of stable isotopes, insulin, and glucose. The second catheter was placed in the dorsal contralateral hand vein, which was heated for sampling of arterialized blood.

### In Vivo Glucose Metabolism and Insulin Sensitivity

Hepatic glucose production was measured with a primed (2.2  $\mu$ mol/kg) constant infusion of [6,6-<sup>2</sup>H<sub>2</sub>]glucose (Isotech, Miamisburg, OH) at 0.22  $\mu$ mol/kg/min for a total of 2 hours as described by us previously.<sup>13</sup> Blood was sampled at the start of the stable isotope infusion (-120 minutes) and

every 10 minutes from -30 to 0 time (basal period) for determination of plasma glucose, insulin, and isotopic enrichment of glucose. Fasting turnover calculations were made over the last 30 minutes (-30 to 0) of the basal 2-hour infusion period.

After the 2-hour baseline isotopic infusion period, in vivo insulin sensitivity was evaluated during a 3-hour hyperinsulinemic-euglycemic clamp in conjunction with indirect calorimetry. The insulin infusion rate was 80  $\mu$ u/m<sup>2</sup>/min. Plasma glucose was clamped at approximately 100 mg/dL with a variable rate infusion of 20% dextrose in water. The rate of glucose infusion was

adjusted based on arterialized plasma glucose measurements every 5 minutes. During the hyperinsulinemic-euglycemic clamp, blood was sampled every 10 to 15 minutes for determination of insulin concentrations.

Continuous indirect calorimetry by a ventilated hood system (Deltatrac Metabolic Monitor, Sensormedics, Anaheim, CA) was performed to measure carbon dioxide production, oxygen consumption, and respiratory quotient.<sup>13</sup>

### *In Vivo Insulin Secretion*

First- and second-phase insulin secretions were assessed during a 2-hour hyperglycemic (225 mg/dL) clamp as described by us before.<sup>14</sup> Plasma glucose was increased rapidly to 225 mg/dL by a bolus infusion of 50% dextrose and maintained at that level by a variable rate infusion of 20% dextrose for 2 hours.

### *Body Composition and Abdominal Fat*

Body composition was assessed at the Obesity/Nutrition Research Center of the University of Pittsburgh with dual-energy x-ray absorptiometry.<sup>13</sup> Intra-abdominal fat was assessed by a 10-mm single axial computed tomography scan of the abdomen at the level of L<sub>4-5</sub> vertebrae as described by us previously.<sup>13</sup>

### *Biochemical Measurements*

Plasma glucose was measured by the glucose oxidase method with a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin and insulin-like growth factor-I were analyzed by radioimmunoassay.<sup>15</sup> Total and free testosterone and insulin like growth factor binding protein<sub>1</sub> were measured by radioimmunoassay at Endocrine Sciences Laboratories (Calabasas Hills, CA). Serum leptin was measured by double-antibody radioimmunoassay.<sup>16</sup> Urinary nitrogen was measured by the Kjeldahl method.<sup>15</sup> Deuterium enrichment of glucose in the plasma was determined on a Hewlett-

Packard 5971 mass spectrometer coupled to a 5890 series II gas chromatograph as reported by us previously.<sup>13</sup> Plasma samples were deproteinized with methanol. The aldolnitrile pentaacetate derivative of glucose was analyzed for <sup>2</sup>H enrichment in the electron impact mode. Selective ion monitoring software was used to monitor mass-to-charge ratio for (*m/z*) 200 and 202 reflecting unlabeled and labeled glucose. Standard curves of known enrichments were performed with each assay.

### *Calculations*

The basal rate of appearance of glucose or hepatic glucose production was calculated during the last 30 minutes of the basal fasting period according to steady-state tracer dilution equations as reported by us previously.<sup>17</sup> Insulin-stimulated glucose disposal was calculated during the last 30 minutes of the 80  $\mu\text{u}/\text{m}^2/\text{min}$  hyperinsulinemic clamp. Basal and insulin-stimulated carbohydrate oxidation rates and lipid oxidation rates were calculated from indirect calorimetric data by averaging the data over the 30-minute period of measurements during each period according to Frayn formulas.<sup>17</sup> Glucose storage or nonoxidative glucose disposal during hyperinsulinemia was estimated by subtracting glucose oxidation from total glucose disposal. Insulin sensitivity was calculated by dividing insulin-stimulated glucose disposal by steady-state plasma insulin concentration during the hyperinsulinemic clamp as described previously.<sup>18</sup>

During the hyperglycemic clamp, the first-phase insulin concentration was calculated as the mean of 5 determinations every 2.5 minutes during the first 15 minutes of the clamp, and the second phase was calculated as the mean of 8 determinations from 15 to 120 minutes.<sup>14</sup>

### *Statistical Analysis*

Comparisons between the PCOS group and the control group were made with the use of Student *t* test.

Least-squares regression analysis was used for univariate relationships, and multiple regression analysis was applied to assess multivariate relationships. Data are presented as mean  $\pm$  SEM. Statistical significance was considered as  $P \leq .05$ .

## RESULTS

### *Physical and Hormonal Characteristics*

The 2 groups were comparable with respect to age, body mass index, percent body fat, fat mass, abdominal adiposity, and serum leptin levels (Table I). Both total and free testosterone levels were significantly higher in the PCOS group than in the control group (Table I). Fasting glucose levels were normal in all of the subjects and were similar between the 2 groups (Table II). Hepatic glucose production was not different between the 2 groups; however, fasting insulin levels were higher in the PCOS group (Table II). The insulin-like growth factor binding protein<sub>1</sub> concentration was lower in the PCOS group, with no difference in insulin-like growth factor-I. The fasting glucose to insulin ratio was lower in the PCOS versus the control group ( $1.9 \pm 0.3$  vs  $3.1 \pm 0.3$ ,  $P = .02$ ) (Table II).

### *Insulin Sensitivity*

Steady-state plasma glucose and insulin concentrations during the hyperinsulinemic-euglycemic clamp were not different between the PCOS and control groups ( $100 \pm 0.3$  and  $99 \pm 0.6$  mg/dL and  $369.9 \pm 31.4$  and  $303.0 \pm 25.6$   $\mu\text{u}/\text{mL}$ , respectively). Insulin-stimulated glucose disposal, glucose oxidation, and glucose storage were lower in the PCOS group compared with the control group ( $4.7 \pm 0.6$  vs  $7.6 \pm 0.6$  mg/kg/min,  $P = .002$ ;  $2.4 \pm 0.3$  vs  $3.1 \pm 0.1$  mg/kg/min,  $P = .04$ ;  $2.2 \pm 0.4$  vs  $4.5 \pm 0.5$  mg/kg/min,  $P = .003$ , respectively) (Fig 1). In a similar fashion, insulin sensitivity was lower in the PCOS group ( $1.4 \pm 0.2$  vs  $2.7 \pm 0.3$  mg/kg/min per  $\mu\text{u}/\text{mL}$ ,  $P = .002$ ). Data

were similar when expressed per fat-free mass. No group differences were found in rates of fat oxidation during hyperinsulinemia ( $0.47 \pm 0.1$  vs  $0.34 \pm 0.1$  mg/kg/min).

### Insulin Secretion

First-phase and second-phase insulin levels were higher in the PCOS group compared with the control group (first phase,  $358.5 \pm 59.3$  vs  $211.1 \pm 31.7$   $\mu$ u/mL,  $P = .04$ ; second phase,  $367.4 \pm 51.4$  vs  $255.9 \pm 50.5$   $\mu$ u/mL,  $P = .07$ ) (Fig 2).

### Correlations

Insulin sensitivity correlated negatively with fasting insulin ( $r = -0.71$ ,  $P = .0002$ ) (Fig 3) and positively with the glucose to insulin ratio ( $r = 0.79$ ,  $P < .0001$ ) (Fig 4).

In a multiple regression analysis with insulin sensitivity as the dependent variable and fasting insulin and body mass index as independent variables, the best predictive equation was (insulin sensitivity =  $-0.02$  fasting insulin  $- 0.06$  body mass index +  $5.25$ , with a standard error of estimate of  $0.72$ ,  $R^2 = 0.60$ ,  $P = .0002$ ). With the glucose to insulin ratio as the independent variable, the best predictive equation was (insulin sensitivity =  $0.70$  glucose to insulin ratio +  $0.26$ , standard error of estimate of  $0.67$  and  $R^2 = 0.62$ ).

## DISCUSSION

There is clear evidence that hyperinsulinemia plays an important role in the pathogenesis of reproductive abnormalities in patients with PCOS by stimulating ovarian androgen production.<sup>7,19-21</sup> This evidence comes from observations that correction or suppression of hyperinsulinemia either through weight loss,<sup>22</sup> diazoxide, metformin, and troglitazone administration<sup>19,23-25</sup> leads to lowering of androgen concentrations and improvement of ovulatory function. We recently reported the case of an adolescent with

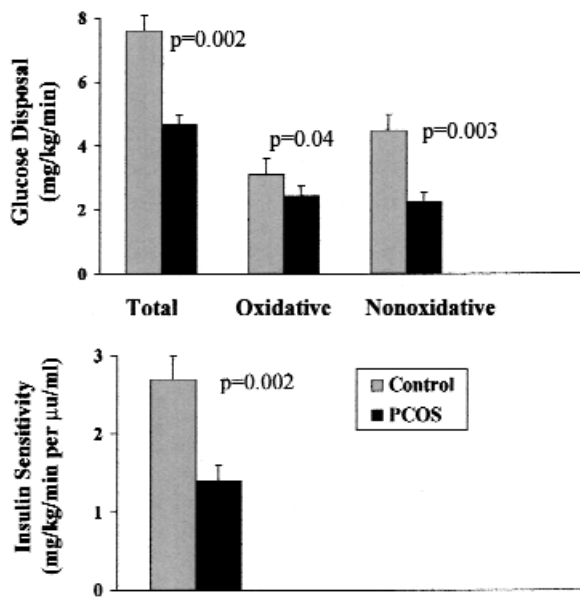


Fig 1. Insulin-stimulated total, oxidative, and nonoxidative glucose disposal during 80  $\mu$ u/m<sup>2</sup>/min hyperinsulinemic clamp (upper) and insulin sensitivity (lower) in PCOS versus control groups.

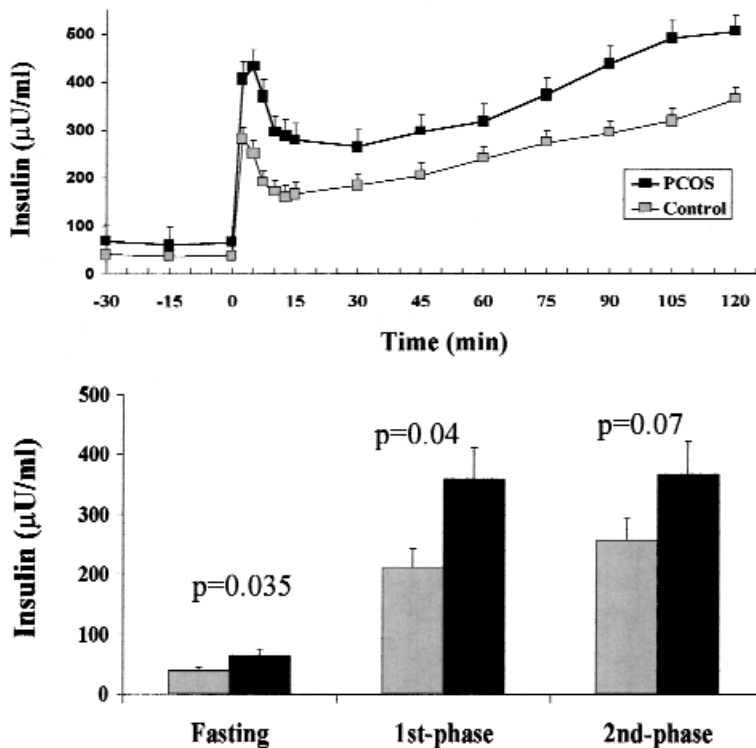


Fig 2. Insulin secretion during hyperglycemic clamp (upper) and mean of fasting, first-phase, and second-phase insulin levels in PCOS versus control groups.

PCOS who, when hyperinsulinemic/insulin resistant, had exaggerated androgen response to human chorionic gonadotropin stimulation, but during spontaneous insulin deficiency, the androgen response to hCG was flat.<sup>26</sup>

This result provided further support of the role of hyperinsulinemia in promoting hyperandrogenism.

In women with PCOS, menstrual cycle irregularities and signs of hyperandrogenism can be traced to adoles-

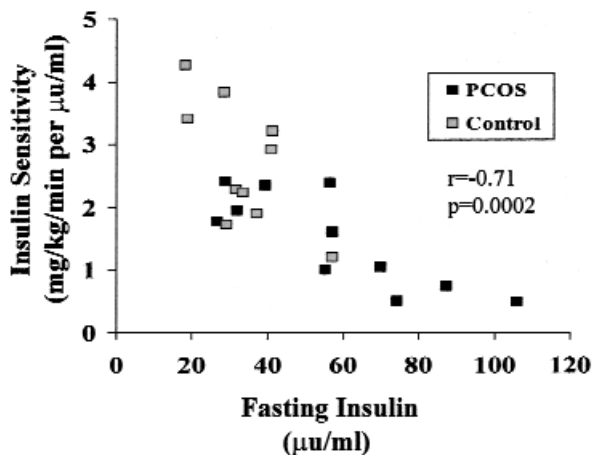


Fig 3. Correlation between insulin sensitivity and fasting insulin levels in PCOS and control groups.

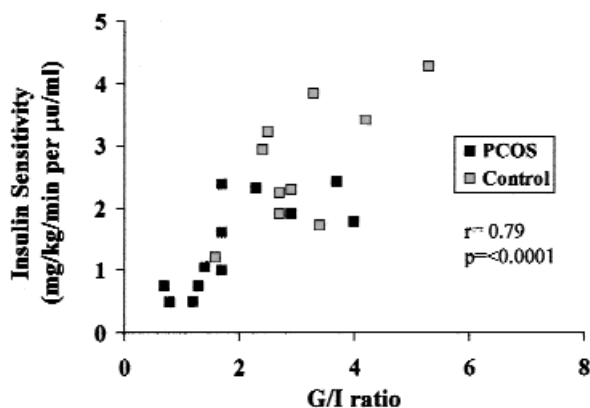


Fig 4. Correlation between insulin sensitivity and fasting glucose to insulin ratio.

cent development, pointing to the pubertal onset of the syndrome.<sup>1</sup> In accord with the neuroendocrine features found in adult women with PCOS, adolescents with hyperandrogenism have augmented luteinizing hormone pulsatility, an increased luteinizing hormone/follicle stimulating hormone ratio, and a selective elevation of ovarian androgens.<sup>27</sup>

To determine whether the metabolic features characteristic of premenopausal women with PCOS are detected early in the course of PCOS or evolve over time, we investigated insulin sensitivity and secretion in adolescents with PCOS. In agreement with the adult literature,<sup>7</sup> adolescents with PCOS have profound insulin re-

sistance independent of body composition and abdominal obesity. Compared with a normal group of obese adolescents with similar body composition and body fat topography, peripheral insulin sensitivity is 50% lower in hyperandrogenic adolescents. Also, adolescents with PCOS have evidence of hepatic insulin resistance, because with 2-fold higher fasting insulin levels, hepatic glucose production was not suppressed in the PCOS group but rather was comparable with that in the control group. This severe degree of insulin resistance in obese adolescents with PCOS is compensated for by increased insulin production from the pancreatic  $\beta$ -cells as detected by 70% higher first-phase and 44% higher sec-

ond-phase insulin secretion during the hyperglycemic clamp. This finding is in contrast to adult studies that have shown  $\beta$ -cell dysfunction with reduced insulin secretory response to meals and during frequently sampled intravenous glucose tolerance.<sup>28-30</sup> This contrasting finding regarding  $\beta$ -cell function between adolescents and adults with PCOS could be attributed to the duration of PCOS. In adolescents with short duration of PCOS and insulin resistance, the  $\beta$ -cell is able to compensate with increased insulin production. However, in adults with long-standing PCOS and insulin resistance,  $\beta$ -cell exhaustion may ensue, with ultimate failure to compensate for the insulin resistance. Such differences between adolescents and adults may have important implications in the choice of therapy for abnormalities in glucose metabolism. Very few studies have addressed the issue of insulin sensitivity and secretion in adolescents with PCOS. In one study adolescents with PCOS were found to have higher fasting and stimulated insulin responses to intravenous glucose tolerance test compared with a control group. However, the PCOS group was significantly heavier, with a body mass index of 28.0 versus 22.1 kg/m<sup>2</sup>, thus clouding interpretation.<sup>31</sup> Evidence of hepatic insulin resistance was found in obese hyperandrogenic adolescents, but peripheral insulin sensitivity was not assessed.<sup>32</sup> In another study serum insulin concentrations during oral glucose tolerance test were higher in adolescent girls with ovarian hyperandrogenism compared with those in eumenorrheic girls, but insulin sensitivity was not different.<sup>3</sup> Insulin sensitivity, however, was estimated during the OGTT, which is not a reliable and sensitive method of assessing in vivo insulin sensitivity.

Insulin resistance is the primary abnormality early in the course of type 2 diabetes mellitus. This is initially offset by a compensatory increase in insulin secretion. With time, insulin secretion

declines with failure of the pancreatic  $\beta$ -cell to compensate and overcome insulin resistance.<sup>33</sup> Our findings of severe insulin resistance in adolescents with PCOS would predict that this group could be at an increased risk for type 2 diabetes mellitus. Such a prediction is in accord with the observation that there is female over-representation of type 2 diabetes in adolescents, especially those with menstrual irregularities and hyperandrogenism.<sup>11,12,34</sup> The prevalence of glucose intolerance in women with PCOS is reported to be substantially higher than that in age- and weight-matched populations of women without PCOS. In 2 very recent studies from 2 independent groups, the prevalence of impaired glucose tolerance was 31.1% and 35%, and that of type 2 diabetes was 7.5% and 10%, based on a standard OGTT.<sup>9,10</sup> More important, in both studies the fasting glucose concentration did not reliably predict the 2-hour glucose concentration after OGTT, thus failing to detect a significant number of women with PCOS and diabetes. The fasting glucose to insulin ratio has been proposed as a useful screening test for identifying women with PCOS and insulin resistance.<sup>35</sup> In these women a value of  $<4.5$  for a fasting glucose to insulin ratio was considered abnormal. In our study the mean glucose to insulin ratio in adolescents with PCOS was much lower at 1.9. This result could be due to better pancreatic reserve and higher fasting insulin concentrations in adolescents, especially considering that 43% of the reported women were glucose intolerant as a result of  $\beta$ -cell failure. In this study the glucose to insulin ratio shows the highest correlation with insulin sensitivity (Fig 4) and predicts 62% of its variability with a low standard error of estimate. Thus the fasting glucose to insulin ratio could be a useful screening test for insulin resistance in obese adolescents with PCOS.

Heightened awareness of increased risk of type 2 diabetes and careful screening with OGTT in such patients

may unravel silent abnormalities in glucose tolerance/type 2 diabetes.

*The authors thank Lynnette Orlansky and Kathy Brown for indelible effort in recruiting and coordinating subject participation, the General Clinical Research Center for their expert nursing assistance, and Pat Antonio for secretarial assistance.*

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