

Obesity, free testosterone, and cardiovascular risk factors in adolescents with polycystic ovary syndrome and regularly cycling adolescents

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Abstract

Adolescent girls with polycystic ovary syndrome (PCOS) have increased levels of factors constituting the metabolic syndrome: centripetal obesity, hypertension, hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-C), and hyperinsulinemia. Given the strong association reported between early, persistent obesity and development of metabolic syndrome 10 years later in girls, we speculated that if adolescent girls without PCOS had obesity measures similar to girls with PCOS, they would exhibit similar metabolic syndrome–cardiovascular disease risk factors. Within this context, we compared 37 adolescent girls with PCOS and 2 samples of normal, regularly cycling adolescent girls (controls) of similar ages, selected from the Cincinnati Clinic of the National Heart, Lung, and Blood Institute Growth and Health Study. The first sample included 157 controls selected using a stratified random sample based on age. As expected, girls with PCOS had higher body mass index (BMI), waist circumference, insulin, systolic blood pressure (SBP) and diastolic blood pressure, triglycerides (TGs), lower HDL-C, and higher low-density lipoprotein cholesterol (LDL-C) and free testosterone (FT) than controls. A second sample consisted of girls matched one to one with girls with PCOS for BMI and age. Comparisons of group differences were not significant for insulin, lipids, or blood pressure; girls with PCOS had a trend toward higher values for waist circumference (median, 92.7 vs 87.5 cm; $P = .07$) and much higher median FT (4.25 vs 1.42 ng/mL, $P = .0001$). After matching for BMI and age, by conditional regression analysis, we showed that the groups were not differentiated ($P > .15$) by insulin, HDL-C, LDL-C, TG, SBP, or diastolic blood pressure, but were differentiated by higher FT ($P = .0024$) and waist circumference ($P = .0024$) in PCOS than in controls. Prospective longitudinal analyses of NHGS controls showed that changes in BMI from ages 9 to 10 years to ages 15 to 16 years were positively associated with changes in waist circumference ($P < .0001$), LDL-C ($P = .01$), TG ($P = .008$), and SBP ($P = .002$). These findings suggest that if adolescent girls achieve adiposity equal to girls with PCOS, they then acquire major components of the metabolic syndrome, and excluding high FT and waist circumference, comparable increased cardiovascular disease risk.

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1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women, affecting approximately 6% of whites and more than 6% of Hispanics and African Americans [1]. Polycystic ovary syndrome manifests at menarche [2–6]. The PCOS phenotype commonly, but not

always, includes rapid, major weight gain from menarche to young adulthood [7,8], clinical hyperandrogenism (severe acne, hirsutism), oligo-amenorrhea, polycystic ovaries, and insulin resistance [9–20]. During adolescence, the phenotypic-clinical characteristics of PCOS worsen and subsequently are joined by infertility, frequent first-trimester miscarriage, and increased likelihood of gestational diabetes [13,15,16,19–25] and type 2 diabetes mellitus (DM) [26]. Plasminogen activator inhibitor 1 activity is positively and independently associated with first-trimester miscarriage in women with PCOS ($P = .004$), and there are several

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modifiable positive independent determinants of plasminogen activator inhibitor 1 activity in women with PCOS including body mass index (BMI) ($P < .0001$), serum insulin ($P < .0001$), and triglyceride (TG) ($P = .0009$) [27].

Adult women with PCOS may be at increased risk for atherosclerotic cardiovascular disease (CVD), attributed in part to the increased prevalence of obesity and central adiposity, hypertension, type 2 DM, dyslipidemia, hypofibrinolysis, and hyperinsulinemia [28]. After adjustment for age and BMI, PCOS remains a predictor of coronary artery calcification ($P = .05$) [28]. Total testosterone, which is elevated in women with PCOS, has been shown to be an independent risk factor for aortic calcification in women, even after controlling for PCOS, age, and BMI ($P = .03$) [28]. Adolescents with PCOS [26,29] present with clinical, metabolic, and endocrine profiles that, in aggregate, may be associated with increased CVD risk [28,30].

Rapid, major weight gain, which commonly occurs in girls with PCOS during adolescence [14], has been observed to a somewhat lesser degree in the general adolescent, female population during the past 2 decades [31]. In girls without PCOS, this weight gain is associated with marked increases in waist circumference, insulin, triglyceride, systolic blood pressure (SBP) and diastolic blood pressure (DBP), and decreases in high-density lipoprotein cholesterol (HDL-C) [31]. Although these CVD risk factors in girls from general populations are markedly lower than those in adolescent girls with PCOS, they have similar positive associations with both BMI and waist circumference [31]. Within this framework of strong correlations between obesity and central adiposity on the one hand and insulin resistance and CVD risk factors on the other, we hypothesized that (1) the increase in CVD risk factors that accompanies PCOS is due largely to the accompanying obesity and central adiposity; and (2) if normal ovulating girls were to develop BMI and central adiposity distributions similar to those in girls with PCOS, then their CVD risk factor distributions would be similar to those in girls with PCOS. Comparisons of adolescent girls with PCOS and girls from the Cincinnati Clinic of the 10-year National Heart, Lung, and Blood Institute Growth and Health Study [31] make possible preliminary examinations of these issues. Furthermore, the longitudinal nature of the Cincinnati NGHS study allows evaluations of changes in insulin, blood pressure, and lipids associated with increasing obesity and central adiposity in regularly cycling adolescents.

2. Materials and methods

2.1. Definition and sources of girls with PCOS

Girls with PCOS came from Ohio, Kentucky, West Virginia, Indiana, and Michigan and included all adolescent girls evaluated for PCOS at the Jewish Hospital Cholesterol Center from December 1997 to October 2003. They were referred by their pediatricians, gynecologists, or by first-

degree relatives with PCOS. There was no selection bias for obesity, with girls entering treatment in the consecutive order of their referral. The girls with PCOS were evaluated using a protocol approved by the Jewish Hospital Institutional Review Board, with signed informed consent. Procedures followed were in accordance with the ethical standards for human experimentation established by the Declaration of Helsinki. The diagnosis of PCOS was made on the basis of the revised 2003 Rotterdam ESHRE/ASRM consensus criteria [20], with girls with PCOS meeting 2 of the following 3 criteria after exclusion of other pathologies (pituitary insufficiency, persistent hyperprolactinemia, congenital adrenal hyperplasia): (1) oligomenorrhea or anovulation; (2) clinical and/or biochemical signs of hyperandrogenism; (3) polycystic ovaries. Additional exclusion criteria in the current study included serum creatinine level of more than 1.5 mg/dL, type 1 DM, type 2 DM on pharmacologic therapy, concurrent estrogen-progestin oral contraceptives, pregnancy at study entry, and older than 18 years. We used the definitions of Laven et al [32] for oligomenorrhea (bleeding intervals between 35 days and 6 months) or amenorrhea (bleeding interval >6 months).

At the initial pretreatment outpatient visit, weight, height, waist circumference, and SBP and DBP were measured, and blood was obtained after an overnight fast for measurement of serum insulin, free testosterone (FT), and lipid profile [13–16]. A single observer measured blood pressure with girls in a seated position, after a 5-minute resting period [33]. Weight at 14 years of age was documented (from pediatricians' records) for girls with PCOS whose first visit to the Cholesterol Center was at 14 years or older, allowing a comparison of weight at a mean age of 16 years when, on average, they were first evaluated by us.

2.2. Definition and source of comparison subjects

Comparison subjects were from the Cincinnati Clinic of the NGHS, a 10-year, multicenter cohort study of the development of obesity and its effects on CVD risk factors in black and white girls [34]. The Cincinnati NGHS cohort was drawn from urban, inner-city residential, and suburban public and parochial elementary schools, selected to provide a broad cross-section of socioeconomic levels in each ethnic group. Participants were 9 and 10 years old at enrollment and were seen under a collaborative protocol approved by the College of Medicine, University of Cincinnati, and the Cincinnati Children's Hospital Medical Center institutional review boards. Body mass index and blood pressure were determined annually; fasting blood was drawn for serum lipid profiles in years 1, 3, 5, 7, and 10. Ancillary studies measured FT in years 3, 5, and 7, and plasma insulin in years 1, 7, and 10. Starting in year 2, waist circumference was measured. A registered nurse conducted an interview annually to determine menstrual history, including the number of days since their last menstrual period for girls who reported having reached menarche. Because there were

no African Americans in the PCOS patient population, the comparison groups were restricted to white girls.

Because NGHS girls had data from multiple visits within the desired age range, only 1 NGHS visit per girl contributed data to the comparison groups. For these studies, we constructed 2 comparison samples of NGHS girls. First, stratified random sampling was used to select a comparison group from the total NGHS cohort, with the same percentage of girls in each single year of age as the PCOS cases. Second, also from the total NGHS cohort, we created a smaller comparison group of NGHS girls matched one to one with girls with PCOS for BMI and age. We also created a one-to-one matched comparison group matching on waist circumference and age, which was only used in 1 analysis.

2.3. Statistical methods

Differences in the distributions of age, BMI, waist circumference, insulin, SBP and DBP, lipids, and FT values between the cases and each comparison sample, separately, were tested for significance using the Wilcoxon nonparametric test because most of the data were not normally distributed.

The distributions of BMI in the 2 groups relative to published obesity standards were assessed by χ^2 tests, using the 85th and 95th percentiles of the age-specific percentile distributions for BMI in the Centers for Disease Control and Prevention Growth Charts [35], to identify normal BMI (<85th percentile), at risk for overweight (85th–95th percentile), and overweight (>95th percentile), respectively.

After matching for BMI and age, we compared median CVD risk factor levels and FT between girls with PCOS and controls. Because the BMI in the girls with PCOS was shifted sharply toward higher values than in the controls, only 30 of the 37 girls with PCOS could be matched with controls.

After covariance adjusting for BMI and age, we compared CVD risk factors and FT between girls with PCOS and controls ($n = 157$) by least square means [36].

Table 1

Median BMI (kg/m^2), waist circumference, insulin, FT, plasma high HDL-C and LDL-C, TGs, and SBP and DBP in adolescent girls with PCOS and controls

	Comparison group		PCOS group		Wilcoxon P
	n	Median	n	Median	
Age (y)	157	16.1	37	16.1	.24
BMI (kg/m^2)	157	21.2	37	31.0	<.0001
Waist (cm)	154	68.2	24	97.2	<.0001
Insulin ($\mu\text{U}/\text{mL}$)	68	11.4	37	21.0	<.0001
FT (ng/mL)	108	1.29	37	4.5	<.0001
HDL-C (mg/dL)	119	52.0	37	41.0	<.0001
LDL-C (mg/dL)	119	89.0	37	98.0	.048
TG (mg/dL)	119	77.0	37	107.0	.0057
SBP (mm Hg)	157	110.0	35	120.0	.0015
DBP (mm Hg)	157	70.0	35	76.0	.0005

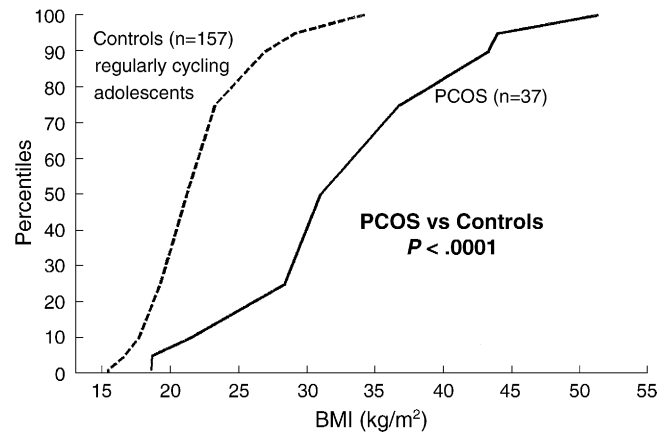


Fig. 1. Distribution of BMI in 37 adolescent girls with PCOS and in 157 adolescent female controls, with a median age of 16.1 years in both groups.

Conditional regression analysis [36] was used to compare endocrine and CVD risk factors in girls with PCOS and controls, after matching for BMI and age or after matching for waist circumference. We fit a mixed model with an indicator variable for the pairs as random effects to account for the matching. The fixed predictor was an indicator variable for girls with PCOS vs controls. Models of this form were run for different outcomes including FT, waist circumference, HDL-C and low-density lipoprotein cholesterol (LDL-C), TG, insulin, and SBP and DBP. For each outcome, we then conducted an F test to determine if there was a significant difference between the groups, controlling for the matched-pair design.

In prospective, longitudinal data from NGHS [31], linear regression was used to assess associations between changes in BMI and changes in metabolic syndrome–CVD risk factor levels from ages 9 and 10 years to ages 15 and 16 years, the age of most of the girls with PCOS.

All statistical tests were performed using SAS version 9.1 [36].

3. Results

3.1. Characteristics of PCOS

All 37 girls with PCOS had well-documented PCOS, using the 2003 consensus diagnostic criteria of the ESHRE/ASRM [20]. For the 37 girls with PCOS, mean \pm SD age at menarche was 11.8 ± 1.4 years. The mean \pm SD and median duration between menarche and first evaluation at the Cholesterol Center was 3.8 ± 1.8 and 3.8 years, respectively, with a range of 0.9 to 7.5 years. Severely obese at 14 years of age with median weight of 85.2 kg (range, 44.5–124.1 kg), the girls with PCOS became progressively more overweight, with median weight of 91.4 kg (range, 44.5–134.5 kg) ($P = .0001$) when first evaluated by us at 16 ± 1.0 years of age.

All controls were postmenarchal and had regular periods (ie, no reported time intervals between previous menstrual

periods and clinic visit of ≥ 35 days during NGHS); the mean \pm SD age at menarche for controls was 12.8 ± 1.2 years.

3.2. Case-control differences in endocrine and cardiovascular risk factors

Because the age range and age structure of the first NGHS sample ($n = 157$) matched that of the PCOS cases, the groups had similar age distributions (Table 1). There was a broad range of BMI in both girls with PCOS and controls, from 18.6 to 51.3 kg/m² in girls with PCOS and from 15.4 to 34.2 kg/m² in controls. The distribution of BMI in the girls with PCOS, however, was shifted sharply toward adult obesity (BMI ≥ 30 kg/m²) and extreme obesity (BMI ≥ 40 kg/m²) [33], and girls with PCOS had significantly higher median BMI (Fig. 1, Table 1). Moreover, the proportions of girls in the 2 groups with BMI below the 85th percentile for age (normal BMI), above the 95th percentile for age (obese), and between the 85th and 95th percentiles (at risk for obesity) were totally opposite. In girls with PCOS, 6 (16%) of 37 had normal BMI, 4 (11%) had at-risk BMI, and 27 (73%) were obese, whereas of the 157 controls, 131 (83%) had normal BMI, 18 (11%) had at-risk BMI, and 8 (5%) were obese ($\chi^2_2 = 95.6$, $P < .0001$).

Consistent with the differences in the BMI distributions, girls with PCOS had higher waist circumference, insulin, SBP and DBP, TG, and lower HDL-C than controls (Table 1). In addition, 9 (24%) of 37 girls with PCOS had HDL-C below the NIH Lipid Research Clinics age- and sex-specific 5th percentile [36] compared with 6 (5%) of 119 of controls ($P = .0005$). Of 37 girls with PCOS, 13 (35%) had TG above the Lipid Research Clinics 95th percentile [37] compared with 14 (12%) of 119 controls ($P = .001$).

The distributions of FT were markedly shifted to higher levels in girls with PCOS compared with controls; median FT levels in girls with PCOS and controls were 4.5 and 1.3 ng/dL, respectively ($P < .0001$, Table 1). No girls with PCOS had FT of less than 1 ng/dL and 70% had FT of 3 ng/dL or more. In sharp contrast, no controls had FT of 3 ng/dL or more and 30% of controls had FT of less than 1 ng/mL ($\chi^2_2 = 94.4$, $P < .0001$).

Table 2

Median age, BMI (kg/m²), waist circumference, insulin, FT, plasma HDL-C and LDL-C, TGs, and SBP and DBP in adolescent girls with PCOS and controls, one-on-one matched for age and BMI

	Comparison group		PCOS group		Wilcoxon P
	n	Median	n	Median	
Age (y)	30	15.6	30	16.0	.77
BMI (kg/m ²)	30	30.4	30	30.3	1.0
Waist (cm)	28	87.5	20	92.7	.07
Insulin (μ U/mL)	17	16.1	30	17.8	.66
FT (ng/mL)	15	1.4	30	4.25	.0001
HDL-C (mg/dL)	23	46.0	30	42.0	.74
LDL-C (mg/dL)	22	115.0	30	100.0	.22
TG (mg/dL)	23	101.0	30	99.0	.67
SBP (mm Hg)	30	114.0	28	119.0	.82
DBP (mm Hg)	30	75.0	28	76.0	.94

Table 3

Relationships between changes in BMI and associated changes in cardiovascular risk factors in NGHS girls, years 1 to 7

Variable	n	β	SE	P
Waist (cm)	124	2.0	0.1	<.0001
Insulin (μ U/mL)	58	0.5	0.46	NS
HDL-C (mg/dL)	81	-0.7	0.38	.056
LDL-C (mg/dL)	81	2.6	1.04	.01
TG (mg/dL)	81	3.3	1.95	.008
SBP (mm Hg)	140	1.07	0.3	.002
DBP (mm Hg)	135	0.6	0.33	.07

NS indicates not significant.

3.3. Analyses to explain case-control differences

We attempted to account for the marked differences in BMI by matching subjects with PCOS and subjects without PCOS one to one by age and BMI and (1) comparing median CVD risk factor levels and (2) evaluating CVD factors and FT for the 2 groups, using conditional regression analysis. Girls with PCOS had much higher FT (median, 4.25 vs 1.42 ng/dL, $P = .0001$) and a trend to higher waist circumference (median, 92.7 vs 87.5 cm, $P = .07$), after matching for age and BMI, but differences in insulin, lipids, and blood pressure were no longer significant (Table 2).

After covariance adjusting for BMI and age by analysis of variance [36], least square means between PCOS and controls differed only for waist circumference ($P < .0001$) and for FT ($P < .0001$).

After matching for BMI and age, conditional regression analysis [36] showed that the groups were not differentiated ($P > .15$) by insulin, HDL-C or LDL-C, TG, SBP, or DBP, but were differentiated by higher FT ($P = .0024$) and waist circumference ($P = .0024$) in girls with PCOS than in controls.

Only 17 of the 24 girls with PCOS who had measures of waist circumference could be matched for waist circumference with the controls because of much lower waist circumference in the controls. Polycystic ovary syndrome and control groups were differentiated by higher FT ($P = .017$) in PCOS, after matching for waist circumference, by conditional regression analysis [36], but the groups were not differentiated ($P > .15$) by insulin, lipids, or blood pressure.

3.4. Prospective associations between changes in BMI and CHD risk factors in control girls over 6 years of follow-up

Changes in BMI in NGHS girls from years 1 to 7 (ages 9 and 10 years to ages 15 and 16 years) were significantly associated with the changes in total cholesterol, HDL-C and LDL-C, TG, SBP, DBP, and waist circumference (years 2-7), but not with changes in insulin (Table 3).

4. Discussion

The importance of the diagnosis of PCOS in adolescence lies in primary prevention of downstream endocrinopathy,

obesity, infertility, hyperinsulinemia, type 2 DM, and, speculatively, increased CVD morbidity and mortality [3,8,14,26,28–30,38,39]. By a mean age of 16 years, in the current study, 27 (73%) of 37 girls with PCOS were above the 95th percentile (overweight) for American children [35] vs 8 (5%) of 157 controls; the difference in the distribution of weight categories was highly significant ($P < .0001$). The striking PCOS – control difference in BMI was associated with higher insulin, TG, SBP and DBP, FT, and LDL-C, and with lower HDL-C in girls with PCOS. After covariance adjusting for BMI and age, girls with PCOS had larger waist circumference than controls, and also after matching for BMI and age, by conditional regression, girls with PCOS were differentiated from controls by larger waist circumference ($P = .0024$), as testimony to their centripetal obesity. Waist circumference, a marker for intra-abdominal fat, is, along with TG, the best marker for the insulin resistance and visceral obesity components of the metabolic syndrome in adults [40]. Similarly, Morrison et al [31] recently showed in a longitudinal analysis of data from 2 NGHS clinical centers, from which the controls for this analysis were drawn, that waist circumference and TG at ages 9 to 11 years were the best predictors for developing the metabolic syndrome at ages 18 and 19 years. In the current analysis, after matching for BMI, both analysis of variance and conditional regression revealed that when control girls were as obese as girls with PCOS, they acquired hypertension, hyperinsulinemia, hypertriglyceridemia, and hypoalphalipoproteinemia equivalent to that in girls with PCOS. At the same time, despite matching for BMI, by both analysis of variance and conditional regression, girls with PCOS persisted with higher FT, evidence for the endocrinopathy of PCOS, independent of obesity. In postmenopausal women, higher FT was associated with the hyperinsulinemia and hyperglycemia components of the metabolic syndrome [41].

Adolescent obesity, which tracks into adulthood, is associated with carotid intimal medial thickening [42,43]. Higher BMI and SBP in PCOS vs controls in the current study thus portends increased risk for carotid artery disease [42,43] as adults with PCOS.

The insidious circular relationship of insulin resistance to centripetal obesity, characteristic of many girls with PCOS [3,8,14,44], also predisposes to later development of hyperinsulinemic type 2 DM. In PCOS, Glueck et al [15,16,22,23] have speculated that metformin, like troglitazone [45], may protect pancreatic beta cells from failure, thus lowering the later risk of developing type 2 DM. In the Diabetes Prevention Program [46], 3234 nondiabetic subjects with elevated fasting and postload glucose levels were randomized to placebo, metformin (1700 mg/d), or an intensive weight-loss exercise program, with average follow-up of 2.8 years [46]. The lifestyle and metformin interventions reduced the DM incidence by 58% and 31%, respectively, as compared with placebo [46].

In obese adolescents with fasting hyperinsulinemia and a family history of type 2 DM [39], metformin facilitated reduction in fasting blood glucose and insulin concentrations and moderated weight gain. In adolescents with PCOS, using metformin and diet, Glueck et al [14] reduced fasting insulin, glucose, and weight, all of which should have promise in prevention of later type 2 DM, an outcome similar to that of Freemark and Bursey [39].

Primary prevention of type 2 DM in children and adolescents is important, given the near epidemic status of type 2 DM in children and youth [47]. Legro [21] noted “because PCOS is associated with a 40% prevalence of abnormal glucose tolerance, every adolescent patient should be evaluated regularly for glucose intolerance with a 2-hour oral glucose tolerance test.”

Strengths of the current study include the presence of longitudinal data on accretion of weight and its associations with CVD–metabolic syndrome risk factors from the large, prospective NGHS study of girls followed from pre-adolescence through adolescence [31] and CVD–metabolic syndrome data from a large cohort of adolescent girls with well-characterized PCOS. Weaknesses of the analyses include the fact that the control girls did not include sufficient numbers of participants with extremely high BMI to match the highest number of patients with PCOS, thus limiting the results of the conditional regression to the range covered. Nevertheless, the findings suggest that if the pandemic of adolescent obesity continues and accelerates, and if normal ovulating girls achieve adiposity equal to girls with PCOS, they will then acquire major components of the metabolic syndrome—centripetal obesity, hypertension, hypertriglyceridemia, low HDL-C, and hyperinsulinemia—and become at much higher risk for later development of type 2 DM [41]. In the current study, differences in waist circumference and FT between girls with PCOS and control girls persisted after controlling for differences in BMI, characteristics of PCOS itself.

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