Racial and ethnic differences in metabolic disease in adolescents with obesity and polycystic ovary syndrome

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Abstract

Purpose: Polycystic ovary syndrome (PCOS) is common and associated with the metabolic syndrome. In the general population, metabolic disease varies by race and ethnicity. The interaction of race and ethnicity with PCOS-related metabolic disease in adolescent youth has not been extensively examined.

Methods: Secondary analysis of data from girls (age 12-21 years) with overweight or obesity (>90 BMI%ile) and PCOS. Measurements included fasting hormone and metabolic measures, a 2-hour oral glucose tolerance test (OGTT) and MRI for hepatic fat. Groups were categorized by race or ethnicity.

Results: Participants included 39 non-Hispanic white (NHW age 15.7±0.2 years; BMI 97.7±0.2 %ile), 50 Hispanic (HW 15.2±0.3 years; 97.9±0.3 %ile) and 12 non-Hispanic black (NHB 16.0±0.6 years; 98.6±0.4 %ile) adolescents. Hepatic markers of insulin resistance were worse in NHW, including lower sex hormone binding globulin and higher triglyceride over high density lipoprotein cholesterol (TG/HDL-C) ratio (p=0.002 overall, HW vs NHB [p=0.009] vs NHW [p=0.020]), although HOMA-IR was worst in NHB (p=0.010 overall, NHW vs NHB p=0.014). Fasting and 2-hour OGTT glucose were not different between groups, although HbA1c was lowest in NHW (overall p<0.001, NHW 5.2±0.3 vs HW 5.5±0.3 p<0.001 vs 5.7±0.4%, p<0.001). The frequency of hepatic steatosis (HW 62%, NHW 42%, NHB 25%, p=0.032); low HDL-C <40 mg/dL (HW 82%, NHW 61%, NHB 50%, p<0.001) and pre-diabetes HbA1c 5.7-6.4% (NHB 50%, HW 36%, NHW 5%, p<0.001) were different between the groups.

Conclusions: Adolescents with PCOS appear to show similar racial and ethnic variation to the general population in terms of metabolic disease components.

Keywords: polycystic ovary syndrome, metabolic syndrome, adolescent, race, ethnicity
Introduction

Polycystic ovary syndrome (PCOS) affects between 10-20% of women worldwide (1). Metabolic disease is common in women with PCOS—50-90% of these women have insulin resistance (IR) and up to 60% of them will develop dysglycemia or type 2 diabetes (T2D) (1). The annual health care cost of PCOS alone is $4.3 billion (2). Female hyperandrogenemia (HA) is associated with the metabolic and reproductive dysfunction seen in PCOS (3). The metabolic syndrome (MetS) and IR are known risk factors for progression to cardiovascular disease (CVD) and T2D (4); thus, understanding racial and ethnic differences in components of the metabolic syndrome in women and girls with PCOS is critical for developing targeted early prevention and treatment. This has been explored in adult women with PCOS but not in adolescent girls.

Within the adult PCOS population, racial and ethnic differences have been observed (5). Non-Hispanic-black (NHB) (6) and Hispanic White (HW) (5) women with PCOS have an increased risk for and a higher mortality due to CVD and T2D compared to non-Hispanic white (NHW) women with PCOS. Similar to women without PCOS, HW women with PCOS display more severe metabolic dysfunction and a higher prevalence of the MetS (5) than NHB and NHW women. Interestingly, NHB women with PCOS display fewer characteristics of MetS (lower triglyceride levels, higher high-density lipoprotein cholesterol (HDL-C) levels, and lower central adiposity) with less overall incidence of MetS compared to HW and NHW women, yet NHB women have high rates mortality due to CVD and T2D (5,7).

The triglyceride to HDL-C ratio (TG/HDL-C) has been developed as a clinical biomarker for IR, validated in NHW and HW women, and displays a higher degree of IR in HW women, but has not been found to be as robust in predicting IR in NHB women (8). Increasing TG/HDL-C ratios have been shown to be a rising burden globally in asymptomatic non-diabetic individuals, increasing subclinical coronary atherosclerosis (9). However, racial differences in this biomarker have not been examined in PCOS.

Within adolescent PCOS populations, studies on the effects of race and ethnicity on metabolic disease are limited (10,11). A retrospective meta-analysis study showed that NHB
adolescents and young adults with PCOS had an increased prevalence of metabolic syndrome compared with their NHW counterparts and that in contrast, there was no difference in risk of MetS between non-PCOS NHB and NHW adolescents and adult women in the NHANES dataset (6). After adjusting for possible confounding influences of socioeconomic status and lifestyle, there were no differences in MetS prevalence among girls, though NHB girls exhibited less hypertriglyceridemia (10). Here, we examine racial and ethnic differences in insulin resistance and metabolic syndrome markers in adolescent girls who are overweight or obese with PCOS.

**Materials and Methods**

**Study design, Setting and Participants**

The study was a secondary analysis including participants from three cross-sectional cohorts with nearly identical enrollment criteria involving metabolic characterization of adolescents with PCOS: 1) Androgens and Insulin Resistance Study (AIRS, prior to NCT, N=76) (12-14), 2) Liver and Fat Regulation in Overweight Girls (APPLE, NCT02157974, N=92) and 3) Post-Prandial Liver Glucose Metabolism in PCOS (PLUM, NCT03041129, N=17). Participants were enrolled between 2012 and 2018. The participants were recruited via general endocrine clinics and lifestyle intervention obesity clinics at Children’s Hospital Colorado. AIRS inclusion criteria comprised: females with obesity (BMI ≥ 95th percentile) or normal weight (BMI< 85th percentile), with or without PCOS, who were physically inactive (exercising less than 3.0 hours a week) and ages 12-21 years old. Identical inclusion criteria were used for APPLE and PLUM except that the BMI range was expanded to include females with overweight or obesity (BMI ≥ 90th percentile). The NIH classification of PCOS (hyperandrogenism with oligomenorrhea and no use of ovary ultrasound) was used with oligomenorrhea in adolescents defined as the presence of irregular menstrual cycles for at least 1.5 years (AIRS) or 2 years (APPLE and PLUM) after menarche, consistent with 2013 Endocrine Society guidelines (15,16). A pediatric endocrinologist (MCG, KJN or MMK) performed a physical exam at the screening visit to determine clinical hyperandrogenism. The Ferriman-Gallaway scale (FGS) was used to rate hirsutism (17). For all studies,
exclusion criteria included diabetes (defined as HbA1c ≥ 6.5%), AST or ALT > 125 IU/L, uncontrolled hypertension defined as persistent blood pressure >140/80 mm Hg or medication for hypertension and weight > 325 pounds, due to equipment limitations. All participants were selected from the above studies if they had 1) PCOS, 2) a BMI ≥90th %ile and 3) were not prescribed oral contraceptives or metformin. The OGTT was not performed in 17 AIRS participants and OGTT data from APPLE participants who received Exenatide were not included in analysis (N=10, 3 NHB, 3 HW, 4 NHW).

The University of Colorado Anschutz Medical Campus institutional review board and the Children’s Hospital Colorado Research Institute approved the studies. All participants aged 18 to 21 years provided written informed consent, and the parents and participants provided written consent and assent, respectively, for all participants aged <18 years.

**Data collection**

*Overall study design:* For APPLE and PLUM, physical exam, fasting laboratory measures, MRI and OGTT were performed within a 24-hour period. For AIRS, physical exam, fasting measurements and MRI were performed within 24 hours, and the OGTT within 6 weeks of the fasting measures and MRI.

Waist circumference, BMI and BMI percentile per Center for Disease Control and Prevention BMI growth charts (18) were obtained. All fasting laboratory measurements were obtained following a monitored inpatient 12 hour fast. The OGTT included 75 grams of Glucola, and for APPLE and PLUM, an additional 25 grams of fructose. Blood samples were collected at baseline, 30, 60, 90 and 120 minutes from the drink.

**Hepatic fat fraction**

Hepatic fat fraction was assessed with the DIXON technique as previously described (19). MRI imaging was obtained on a 3 Tesla Magnet (Siemens Magnetom Skryra, Tarrytown, NY or GE Healthcare, Milwaukee, WI). The weighted average of the mean fat
fraction was calculated for each participant. Hepatic steatosis was defined as hepatic fat fraction ≥5.5%.

**Laboratory measurements**

Analyses were performed by the University of Colorado Anschutz Research core laboratory or the Children’s Hospital Colorado clinical laboratory. Plasma total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) (Hitachi 917 autoanalyzer; Boehringer Mannheim Diagnostics, Indianapolis, IN) and TG (Beckman Coulter, Brea, CA) were analyzed enzymatically. Insulin was analyzed with radioimmunoassay (Millipore, Billerica, MA). Plasma glucose was measured at the bedside using the StatStrip® Hospital Glucose Monitoring System (Nova Biomedical, Waltham, MA, USA). HbA1c, was measured with the Siemens DCA Vantage (Siemens Medical Solutions, CA). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined via VITROS 5600 (Ortho Clinical Diagnostics, Rochester, NY). Sex hormone binding globulin (SHBG) was measured using an electrochemiluminescence immunoassay (Esoterix, Calbassas Hills, CA). Total testosterone was analyzed using a liquid chromatography–tandem mass spectrometry and free testosterone with equilibrium dialysis (Esoterix, Calbassas Hills, CA).

**Calculations:**

IR was estimated using HOMA-IR and calculated as (fasting plasma glucose [mg/dL] x fasting plasma insulin [mg/dL] / 405) (20). The Matsuda insulin sensitivity index (M) was calculated based on the results of OGTT: M=10 000/(G0 × I0 × Gmean × Imean)^1/2, where G and I represents plasma glucose [mmol dl−1] and insulin [mU l−1] concentrations, respectively, and ‘0’ and ‘mean’ indicates fasting value and mean value during the OGTT, respectively (21). The free androgen index (FAI) was calculated as the ratio of total testosterone divided by the SHBG (both expressed in the same units) and multiplied by 100 to yield numerical results comparable in free testosterone concentration (22,23). TG:HDL-C
ratio was measured by dividing fasting serum TG by the HDL-C (9). HOMA-%B, a measurement of insulin secretion, was calculated by \( \frac{20 \times \text{fasting insulin (uU/mL)}}{\text{fasting glucose (mg/dL)} - 3.5} \) (24). Fasting insulin clearance was calculated as the ratio of fasting c-peptide to fasting insulin (25). The Ferrimann Gallwey Score (FGS) (26) was determined by examining each of the nine body areas most sensitive to androgen and each was assigned a score from 0 (no hair) to 4 (frankly virile). These separate scores were summed to provide a total hormonal hirsutism score.

**Metabolic Syndrome Definitions**

Cardiovascular risk factors were defined by American Heart Association guidelines (27) as 1) systolic blood pressure of ≥ 130 mmHg (prehypertension), HDL-C ≤ 40 mg/dL, and TG ≥ 150 mg/dL. Pre-diabetes was defined per American Diabetes Association criteria (28): fasting glucose 100-125 mg/dL, 2 hour OGTT glucose 140-199 mg/dL or HbA1c 5.7-6.4%. Thus, impaired fasting glucose (IFG) is a fasting glucose greater than or equal to 100 mg/dL, impaired glucose tolerance (IGT) is a 2-hour OGTT greater than or equal to 140 mg/dL, and an abnormal HbA1c is equal to or greater than 5.7%. The Met-S was defined by the version of the National Cholesterol Education Program Adult Treatment Panel III (ATP III) definition updated by the American Heart Association and the National Heart Lung and Blood Institute in 2005 (29). By this definition, Met-S is present if three or more of the following five criteria are met: waist circumference > 40 inches (men) or 35 inches (women), systolic blood pressure > 130 mmHg, fasting TG > 150 mg/dL, fasting HDL-C < 40 mg/dL (men) or 50 mg/dL (women) and fasting blood sugar >100 mg/dL.

**Statistical analysis**

Descriptive statistics were expressed as mean ± standard deviation (SD, if normally distributed) and median (interquartile range, if non-normally distributed). ANOVA with Kruskal-Wallis post-hoc testing was used to determine differences between the groups and
Chi squared testing was utilized for categorical variables. Correlations were performed with simple linear regression. Adjustments for multiple comparisons were not performed. Statistical analyses were performed using GraphPad Prism v. 8.3 (GraphPad Software, San Diego, CA).

Results

Participant Characteristics

The participant characteristics are detailed in Table 1. Thirty-nine NHW, 50 HW and 12 NHB adolescent girls with obesity or overweight and PCOS were included. Groups were similar in age and BMI percentile, although absolute BMI was higher in NHB than NHW (overall ANOVA p=0.042, post-hoc p=0.037). Waist circumferences were larger in NHB compared to NHW (overall ANOVA p=0.008, post-hoc p=0.007) but waist-to-hip ratios were not significantly different between groups. Systolic blood pressure is lower in NHB compared to NHW (overall ANOVA p=0.011, post-hoc p=0.009) and HW (post-hoc p=0.020).

Measurements associated with PCOS

Total testosterone was higher in NHB compared to HW (overall ANOVA p=0.009, post-hoc p=0.026), and SHBG was lower in HW compared to NHW (overall ANOVA p=0.018, post-hoc p=0.016). (Table 1). Free androgen index (FAI), free testosterone, and hirsutism scores were similar between groups (Table 1).

Measurements of liver function, body fat, lipid and glycemic parameters

There were no differences between liver fat and AST between groups. ALT was different between the groups, but none of the post-hoc testing was significant. CRP was higher in NHB compared to NHW (overall ANOVA p=0.004, post-hoc p=0.003) and HW (post-hoc p=0.014) (Table 2). Body composition was similar between the groups. Total cholesterol and LDL-C concentrations were similar in all three groups. HDL was lower in HW compared to NHW (overall ANOVA p=0.004, post-hoc p=0.011) and NHB (post-hoc p=0.027), whereas TG was lower in NHB compared to NHW (overall ANOVA p=0.011, post-hoc p=0.026).
Regarding glycemic parameters, fasting and 2-hour OGTT glucose and insulin concentrations were similar in all three groups; however, fasting c-peptide was higher in HW compared to NHW girls, (overall ANOVA p=0.017, post-hoc p=0.013) (Table 2). NHB exhibited higher HbA1c than NHW (overall ANOVA p<0.001, post-hoc p<0.001) and HW (post-hoc p=0.027) girls with PCOS. HbA1c was also higher in HW compared to NHW (post-hoc p<0.001).

**Measurements Insulin Sensitivity**

HOMA-IR (Figure 1A) is worse (higher) in NHB compared to NHW, as was 1/fasting insulin (Figure 1D) However there was no difference in Matsuda scores (Figure 1B). HW girls had a higher TG/HDL (Figure 1C) compared to NHW and NHB girls. There were no differences between the groups in terms of fasting insulin clearance (Figure 1E) or insulin secretion as assessed by HOMA-%B (Figure 1F).

**Proportion of Metabolic Syndrome Components**

The proportion of participants who met criteria for the Met-S are shown in Figure 2. Dysglycemia is shown as IFG, IGT and HbA1c (Figure 2A). Whereas all groups had similar rates of IGT, there was no IFG in NHB, yet NHB had a higher proportion with abnormal HbA1c. Few participants had a systolic blood pressure ≥ 130 mm/Hg although the frequency of hypertension was different between groups (Figure 2B). Hepatic steatosis prevalence was different between the groups, and highest in HW (Figure 2C). A low HDL-C was common, with differences across the groups; however, fewer NHB girls with PCOS had a low HDL-C compared to the other two groups (Figure 2D). Whereas 41% of HW girls had elevated serum TG, none of the NHB participants did (Figure 2E). All participants had a waist circumference > 80 cm, and 90% had a waist greater than 90 cm, with no difference between groups (data not shown).

**Correlation Analysis**

The relationship between the FAI and liver fat% was significant only in HW girls (Figure 3A, $r^2=0.107, p=0.023$) whereas FAI and HOMA-IR were only related in NHW girls (Figure 3B, $r^2=0.272, p=0.001$). HbA1c did not significantly correlate to FAI in any group,
although the slope in NHB girls was the highest. Liver fat % related to HOMA-IR in both HW (Figure 3D, $r^2=0.225$, $p=0.001$) and NHW girls ($r^2=0.301$, $p=0.001$), with no relationship found in NHB ($r^2<0.001$, $p=0.950$). HbA1c related to hepatic fat in NHW (Figure 3E, $r^2=0.133$, $p=0.022$) and HW ($r^2=0.083$, $p=0.042$) but to HOMA-IR only in NHW girls (Figure 3F, $r^2=0.159$, $p=0.016$). The 2-hour insulin from the OGTT related to hepatic fat in HW (Figure 3G, $r^2=0.129$, $p=0.027$), and NHW ($r^2=0.214$, $p=0.013$) and to HbA1c only in NHW girls (Figure 3H, $r^2=0.169$, $p=0.030$).

Discussion

We found that racial and ethnic differences in IR, Met-S, and glycemia displayed similar patterns in girls with PCOS as those previously reported in adolescent females in the NHANES cohort (10,11) and adult women with PCOS (5). We found that girls with HW ethnicity are more likely to have markers of IR, and elevated markers of hepatic and lipid dysmetabolism; whereas NHB girls have more evidence of dysglycemia. We confirmed that HW girls with PCOS have greater prevalence of hepatic steatosis. Despite having an overall more favorable hepatic and lipid metabolic profile (lower ALT, less hepatic steatosis, higher subcutaneous fat, higher lean mass, lower TG/HDL-C), NHB girls with PCOS displayed a higher degree of insulin resistance per HOMA-IR and 1/fasting insulin along with higher HbA1c. These differences in patterns for metabolic disease risk by race and ethnicity need to be included in the consideration of treatment options for these adolescents.

Racial and ethnic differences have been examined in adult women with PCOS, however, there is a paucity of data regarding racial and ethnic differences in girls with PCOS. NHB and NHW women with PCOS who were morbidly obese (BMI > 35) had waist-hip ratio, total testosterone, and SHBG that were similar between groups (30). Another study that compared NHW, NHB, and Hispanic adults with obesity and PCOS found no difference in waist circumference and total testosterone by race/ethnicity (5). A meta-analysis examining metabolic disease in NHW and NHB adults with PCOS found similar lipid patterns as our data (7). In contrast to adults, NHB girls with obesity or overweight and PCOS in our
study had a larger waist than NHW and HW girls; and HW girls had the lowest total testosterone of the three groups. ALT followed a similar pattern in adults with obesity (30) and in our adolescents with PCOS (Table 2), where NHB adolescents had lower ALT than NHW adolescents. TG also followed similar racial and ethnic patterns in adults with obesity (5,30) and our adolescents with PCOS (Table 2): HW had the highest TG and NHB the lowest between the three groups. In women with chronic androgen excess, plasma testosterone is positively correlated with waist circumference, an index of visceral obesity, suggesting that testosterone and visceral obesity are related (31). Within the HW girls, androgens were highly related to the degree of central adiposity and insulin resistance (32). HDL-C was highest in HW adults (5), but in contrast was lowest in HW adolescents, compared to other racial and ethnic groups. Interestingly, fasting glucose and fasting insulin also displayed racial and ethnic differences in adults (5,30) but not in our sample of girls with PCOS. Differences in BMI (33,34), physical activity (35), and sleep (36) in girls compared to adult women may contribute to the differences we observed in adolescents compared to adults with PCOS. Our data are also similar to those from the largest study describing metabolic differences by race/ethnicity in youth (10,11,37). However, in both of these NHANES datasets, PCOS was not specifically excluded from the analysis, and this it is likely that 5-15% of the patients described have PCOS. Additionally, lower insulin clearance and lower insulin sensitivity in youth compared to adults as shown in the RISE study (38) may contribute to the differences we observed in girls in this study compared to adults in previous findings. These latter data suggest that as is the case for T2D, we cannot simply view adult and adolescent PCOS as identical phenomena, thus implying the need for therapeutic interventions that are tailored to an adolescent population.

Pancreatic β-cell insufficiency with dysglycemia has been demonstrated in blacks previously (39,40), and this finding persists in girls with PCOS (41). NHB girls are more likely to have dysglycemia and impaired β-cell function (42) than NHW and Hispanics, which is thought to underlie the increased rates of T2D in blacks (43). β-cell insufficiency may be preceded by early excess insulin secretion, and mechanisms for this may vary with race.
One study showed an ethnicity-specific relationship of β-cell function and pancreatic TG content (40). In HW and NHW, high pancreatic TG levels potentially represent a risk factor for β-cell failure (40). However, in NHB with obesity, hypersensitivity of β-cells to elevated pancreatic TGs and subsequent exaggerated glucose-stimulated insulin secretion may represent risk factors for progression to β-cell failure (40). Alternatively, greater postprandial hyperinsulinemia was observed in NHB compared to NHW pre-menopausal and postmenopausal women and was associated with lower hepatic insulin clearance and heightened β-cell capacity to rapid changes in glucose, but not to higher insulin secretion (44). Moreover, racial differences including lower insulin clearance in NHB vs. NHW youth have been reported (45). We did not find differences in in fasting insulin clearance or β-cell clearance when all of the girls had PCOS. Clearly, further study is needed to understand race and ethnicity related differences in β-cell function prior to and through the development of dysglycemia.

Studies using HbA1c as a glycemic marker have shown that Latino Americans, African Americans, and Asian Americans have poorer control of their diabetes (46). NHB adults have been shown to have higher HbA1c for a similar mean glucose concentration than NHW adults, in both individuals with and without diabetes (47). This is thought to be related to genetic variants in the beta chain of Hb (48). Kelsey et al and others have shown that black youth with normal weight have higher HbA1c (47,48) NHW adolescents. In youth aged 5-24 years who were part of NHANES-3, mean HbA1c was progressively higher in NHW to HW to NHB (37). These differences have been demonstrated to persist through the lifespan (49). Even when adjusting for adherence to prescribed therapies, in adults with T2D, HbA1c remained higher in NHB compared to NHW (50). These racial differences could be the results of higher glucose, or HbA1c may be higher in blacks due to increased glycation of hemoglobin (51). Other theories for the difference in HbA1c between race and ethnicity groups include differences in red blood cell turnover, hemoglobinopathies such as thalassemia or sickle cell disease (52), and genetic variation of the beta chain of Hb (48).
The racial difference in HbA1c has significant implications in terms of diagnosing T2D, thus is currently a topic of debate.

The TG/HDL ratio is potentially not an ideal marker for insulin resistance when including a cohort with multiple racial ethnic backgrounds. The TG/HDL-C ratio value to predict insulin resistance differs by race and ethnicity in adults (53,54) as shown by a study in NHW, NHB and Mexican-Americans. In NHB, TG and TG/HDL-C were not dependable indicators of insulin resistance, as measured by the insulin sensitivity index (55). In adolescents, we similarly found that whereas the TG/HDL-C ratio was low and would not indicate insulin resistance in NHB, HOMA-IR and Matsuda indexes for insulin sensitivity were worst in NHB.

An increased risk of central obesity and insulin resistance have been well described in people with Hispanic ethnicity. In a study of over 8,000 women of various Hispanic ancestries from 4 US cities, Met-S was present in 36% of women, with a prevalence that ranged from 27% in South Americans to 41% in Puerto Ricans (56). Abdominal obesity was present in 96% of these women and 62% had hyperglycemia and insulin resistance (56). In a large cohort of adults of both sexes, Hispanics had increased hepatic fat and serum triglycerides, whereas hepatic and visceral fat was low in blacks, as were serum triglycerides (57). Similar trends of differences in hepatic fat were reported in adolescents with obesity (58). However, we did not find that Hispanics had a large waist circumference, greater visceral or total percent body fat when all participants had PCOS. Rather, we found that NHB girls had a larger waist circumference, although their absolute BMI was also great, and this may just be a reflection of this.

Our study has several strengths and limitations. Notable strengths include the fact that our data were collected in a rigorous research environment with monitored fasting, used the most strict NIH PCOS criteria and included gold-standard MRI measures of hepatic steatosis, and hormone assays. Limitations include the fact that the exclusion criteria of the parent protocols included hypertension or treatment for hypertension, which may have reduced the prevalence of hypertension in this population. Although some participants were
recruited from the community, others were recruited from specialty clinics at a tertiary care regional referral center, which may have increased overall rates of metabolic disease. Our race and ethnicity distribution in our cohort is similar to that in our surrounding demographic area, leading to a smaller NHB cohort size than would be ideal. This potential underrepresentation of black girls and overall moderate sample size is another limitation, particularly given the number of comparisons, and could be an alternative explanation for differences from reported data in adults. Finally, we do not have a local non-PCOS control population, but rather are comparing to published rates of disease. With the exception of smaller studies where PCOS was excluded in a control group, information from large data sets such as NHANES did not specifically exclude a diagnosis of PCOS and thus 5-15% of these girls likely have PCOS. Conversely, 85-95% will not have PCOS, and thus these groups are still acceptable as a comparison reference.

In conclusion, we have demonstrated that there are racial differences in metabolic outcomes in girls with obesity or overweight and PCOS. While some of these are similar to those described in women with and without PCOS, other factors in our youth differed from data reported in adults. Moreover, whereas TG/HDL-C may be a good clinical biomarker for IR and metabolic syndrome in NHW and HW girls, this ratio has a lower utility in NHB girls. However, NHB girls with obesity or overweight and PCOS are just as likely to have IR as HW, and are known to be at higher risk for T2D than NHW girls, thus should be followed closely for the development of T2D. Our initial findings need to be replicated in a larger racially and ethnically balanced cohort and studied across the lifespan in order to develop more accurate patient-specific screening and treatment algorithms. These studies will help build cultural competency for improved PCOS care and prevention of T2D and CVD and their complications.
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Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.
Figure titles and legends:

**Figure 1 Measurements of insulin sensitivity and secretion.** Data shown compare the three groups of women with PCOS (NHW, HW, and NHB) as individual values in a scatter plots as mean ± standard error bars. TG/HDL-C = triglyceride to high density lipoprotein cholesterol ratio.

**Figure 2 Proportions of the metabolic syndrome.** Data are presented comparing the three groups of women with PCOS (white, Hispanic, and black) as percentages graphed as a column bar graph. Since these are percentages, there is no standard error bars. Statistical analyses were performed using ordinary ANOVA tests where significance was p<0.05. According to the American Diabetes Association, a diagnosis of metabolic syndrome requires displaying three of the five following criteria: (A) dysglycemia measured by fasting glucose ≥100 mg/dL (impaired fasting glucose), two-hour glucose levels of 140 to 199 mg per dL (7.8 to 11.0 mmol) on the 75-g oral glucose tolerance test (impaired glucose tolerance), and/or elevated HbA1c 5.7-6.4% (pre-diabetes HbA1c); (B) blood pressure ≥130/85 mm Hg or being treated for high blood pressure (hypertension); (C) liver fat > 5% (hepatic steatosis has been included in place of waist circumference as a standard waist circumference by race has yet to be fully determined); (D) high density lipoprotein cholesterol <40 mg/dL (HDL-C); and (E) triglycerides ≥150 mg/dL (TG).

**Figure 3 Correlations between metabolic and androgen parameters.** Statistical relationships between parameters of fat content, insulin sensitivity, and androgen concentrations were performed with Spearman correlations.
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Table 1: Participant Description

|                      | NHW          | HW           | NHB          | Overall ANOVA p-value |
|----------------------|--------------|--------------|--------------|-----------------------|
| **Biometric**        |              |              |              |                       |
| Participants         | 39           | 50           | 12           |                       |
| Age (years)          | 16 (15, 17)  | 15 (13, 16.3)| 16.5 (15, 17.8)| 0.253                |
| BMI (kg/m²)          | 35.2 (31.3, 36.8) | 35.1 (31.9, 38.8) | 38.5 (36.5, 42.0) | 0.042                |
| BMI%ile              | 98.3 (96.8, 98.7) | 98.5 (97.2, 99.1) | 99.2 (97.9, 99.4) | 0.051                |
| BMI-z score          | 1.99 ± 0.06  | 2.08 ± 0.06  | 2.24 ± 0.12  | 0.179                |
| Waist Circumference  | 101 ± 2      | 106 ± 2      | 113 ± 3**    | 0.008                |
| Hip Circumference    | 116 ± 1      | 116 ± 2      | 123 ± 3      | 0.134                |
| Waist/Hip Ratio      | 0.87 (0.83, 0.92) | 0.91 (0.87, 0.96) | 0.92 (0.90, 0.95) | 0.059                |
| Systolic blood pressure (mmHg) | 122 ± 9 | 120 ± 9 | 112 ± 8** ‡ | 0.011                |
| Diastolic blood pressure (mmHg) | 73 ± 9 | 72 ± 8 | 67 ± 9       | 0.111                |
| **Measurements of Hyperandrogenism** | | | | |
| Total Testosterone   | 43.5 (35.8, 57.0) | 35.0 (28.8, 48.0) | 57 (36.5, 96.3) ‡ | 0.009                |
| SHBG (nmol/L)        | 21.5 (14.9, 31.9) | 15.6 (12.1, 20.3)* | 18.9 (13.3, 27.7) | 0.018                |
| FAI                  | 7.2 (4.9, 12.1) | 8.4 (6.1, 11.0) | 10.4 (5.8, 14.2) | 0.393                |
| Free Testosterone    | 7.6 (5.8, 11.3) | 7.3 (5.2, 9.9) | 9.5 (6.8, 14.5) | 0.386                |
| Hirsutism (FGS scale)| 6 (2,13)      | 6 (3,10)     | 8 (3,12)     | 0.840                |

BMI=body mass Index, FAI= free androgen index, FGS= Ferriman Gallwey score, SHBG= sex hormone binding globulin. Data are mean ± standard deviation or median (25th, 75th). * post-hoc p<0.05-0.01 compared to NHW participants, ** post-hoc p<0.01-0.001 compared to NHW participants, *** post-hoc p<0.001 compared to NHW participants, ‡ post-hoc p<0.05-0.01 compared to HW participants, ‡‡ post-hoc p<0.01-0.001 compared to HW participants.
| Table 2: Metabolic Parameters | NHW | HW | NHB | Overall ANOVA p-value |
|-------------------------------|-----|----|-----|-----------------------|
| Liver Measures               |     |    |     |                       |
| Liver Fat %                  | 4.4 (2.6, 8.5) | 6.3 (4.0, 12.0) | 4.9 (3.5, 5.7) | 0.057                 |
| AST (IU/L)                   | 32 (27, 41)  | 38 (32, 52)     | 37 (28, 49)     | 0.081                 |
| ALT (IU/L)                   | 35 (27, 41)  | 39 (30, 50)     | 27 (21, 40)     | 0.041                 |
| hs-CRP (mg/L)                | 2.1 (1.0, 4.1) | 2.5 (1.0, 5.5)  | 6.4 (4.4, 9.4)** ‡ | 0.004                 |
| Body Composition             |     |    |     |                       |
| Visceral Fat (g)             | 85 ± 5 | 85 ± 4 | 78 ± 7 | 0.428                 |
| Subcutaneous Fat (g)         | 456 ± 18 | 468 ± 18.94 | 541 ± 43 | 0.672                 |
| Fat Mass (%)                 | 44 (41, 46) | 43 (40, 47) | 45 (42, 48) | 0.719                 |
| Lean Mass (%)                | 50 ± 1 | 49 ± 1 | 57 ± 2.4 | 0.966                 |
| Lipid Parameters             |     |    |     |                       |
| Total Cholesterol (mg/dL)    | 156 ± 5 | 151 ± 5 | 162 ± 9 | 0.999                 |
| HDL-C (mg/dL)                | 39 ± 1 | 34 ± 1* | 39 ± 2 ‡ | 0.004                 |
| LDL-C (mg/dL)                | 93 (73, 127) | 83 (66, 111) | 96 (79, 133) | 0.260                 |
| TG (mg/dL)                   | 109 (76, 148) | 128 (105, 165) | 95 (79, 115) ‡ | 0.011                 |
| Glycemic Parameters          |     |    |     |                       |
| Fasting Glucose (mg/dL)      | 84 (81, 92) | 89 (84, 95) | 88 (86, 94) | 0.202                 |
| Fasting Insulin (mU/mL)      | 22 (16, 30) | 29 (18, 40) | 25 (19, 54) | 0.158                 |
| Fasting C-peptide (mU/mL)    | 2.6 ± 0.1 | 3.4 ± 0.2* | 3.0 ± 0.4 | 0.017                 |
|                        | HbA1c (%)          | OGTT 2 hour Glucose (mg/dL) | OGTT 2 hour Insulin (mU/mL) |
|------------------------|--------------------|-----------------------------|----------------------------|
|                        | 5.2 (5.1-5.4)      | 129 (114, 151)              | 168 (92,355)               |
|                        | 5.5 (4.9-5.7)**    | 134 (121, 158)              | 256 (153, 513)             |
|                        | 5.7 (5.2-6.1)***   | 132 (119, 180)              | 123 (86, 275)              |
|                        |                    | 0.479                       | 0.076                      |
|                        | <0.001             |                              |                            |

AST = aspartate aminotransferase, ALT = alanine aminotransferase, CRP = C-reactive protein, HDL-C = high density lipoprotein cholesterol, IGF=impaired fasting glucose, IGT=impaired glucose tolerance, LDL-C = low density lipoprotein cholesterol, SAT = subcutaneous adipose tissue, SBP =systolic blood pressure, TG= triglycerides, VAT = visceral adipose tissue. Data are mean ± standard deviation or median (25th, 75th). * post-hoc p<0.05-0.01 compared to NHW participants, ** post-hoc p<0.01-0.001 compared to NHW participants, *** post-hoc p<0.001 compared to NHW participants, ‡ post-hoc p<0.05-0.01 compared to HW participants, ‡‡ post-hoc p<0.01-0.001 compared to HW participants.
Figure 2

A. % Impaired Fasting Glucose
   - White: 10%
   - Hispanic: 15%
   - Black: 20%
   - $p = 0.001$

B. % Hypertension
   - White: 20%
   - Hispanic: 25%
   - Black: 30%
   - $p = 0.004$

C. % Hepatic Steatosis
   - White: 40%
   - Hispanic: 60%
   - Black: 60%
   - $p < 0.001$

D. % HDL < 40 mg/dL
   - White: 40%
   - Hispanic: 60%
   - Black: 70%
   - $p < 0.001$

E. % TG > 150 mg/dL
   - White: 20%
   - Hispanic: 40%
   - Black: 50%
   - $p < 0.001$
Figure 3

A

Liver Fat %

FAI

B

HOMA-IR

FAI

C

HbA1c (%)

FAI

D

Liver Fat %

HOMA-IR

E

HbA1c (%)

Liver fat %

F

HbA1c (%)

HOMA-IR

G

Liver Fat %

2 hour OGTT insulin (mU/L)

H

HbA1c (%)

2 hour OGTT insulin (mU/L)