

ApoB48-Lipoproteins Are Associated with Cardiometabolic Risk in Adolescents with and without Polycystic Ovary Syndrome

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Context: Adolescents with polycystic ovary syndrome (PCOS) have increased incidence of cardiometabolic risk factors including dyslipidemia. Atherogenic apolipoprotein (apo) B-lipoprotein remnants are associated with increased cardiovascular disease (CVD) risk.

Objective: The aim of this study was to determine the concentrations of fasting plasma apoB-lipoprotein remnants, apoB48 and apoB100, and their association with cardiometabolic risk factors and androgen indices in adolescent girls with and without PCOS.

Design, setting and participants: Participants (n = 184) aged 17 years were recruited in the Menstruation in Teenagers Study from the Western Australian Pregnancy Cohort (Raine) Study.

The main outcome measures: Fasting plasma apo-B48 and -B100 lipoprotein remnant concentrations in adolescent girls with and without PCOS.

Results: Fasting plasma apoB48-lipoprotein remnants but not apoB100-lipoprotein remnants were elevated in adolescent girls with increased cardiometabolic risk compared with those with lower cardiometabolic risk (13.91 ± 5.06 vs 12.09 ± 4.47 $\mu\text{g/mL}$, $P < .01$). ApoB48-lipoprotein remnants were positively correlated with fasting plasma triglycerides ($b = .43$, $P < .0001$). The prevalence of increased cardiometabolic risk factors was 2-fold higher in those diagnosed with PCOS (35.3%) than in those without PCOS (16.3%).

Conclusion: Adolescents with PCOS have a 2-fold higher incidence of cardiometabolic risk factors than those without PCOS. Fasting apoB48-lipoprotein remnants are elevated in adolescent girls with a high prevalence of cardiometabolic risk factors.

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Key Words: ApoB-lipoprotein remnants, adiposity, metabolic syndrome, polycystic ovary syndrome, adolescence, cardiometabolic risk factors, atherosclerosis.

Abbreviations: ACVD, atherosclerotic cardiovascular disease; AEPCOSS, Androgen Excess and Polycystic Ovary Syndrome Society; apo, apolipoprotein; BMI, body mass index; cIMT, carotid intimal medial thickening; CVD, cardiovascular disease; DHEAS, dihydroepiandrosterone; FAI, free androgen index; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment index of insulin resistance; LDL, low-density lipoprotein; MetS, metabolic syndrome; NIH, National Institute of Health; PCOS, polycystic ovary syndrome; SHBG, sex hormone-binding globulin; T, testosterone; TC, total cholesterol; TG, triglyceride; WC, waist circumference.

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Polycystic ovary syndrome (PCOS) is a metabolic endocrine disorder that is highly associated with the metabolic syndrome (MetS) and cardiometabolic risk factors, including obesity, dyslipidemia, and insulin resistance [1]. Women with PCOS are reported to have increased incidence of type-2 diabetes and premature cardiovascular disease (CVD) [1-4]. In adolescents and young women (17-35 years) with PCOS and the MetS, increased incidence of early subclinical atherosclerotic CVD (ACVD), such as carotid intimal medial thickening (cIMT), has been reported [5-12]. Early development of ACVD may predispose women with PCOS to increased morbidity and mortality associated with ischemic CVD events [5-12]. A primary risk factor in the development of atherosclerosis and CVD is atherogenic dyslipidemia, and adolescents with features of the MetS and PCOS have been reported to have a higher incidence of dyslipidemia [10, 13-19].

Atherogenic dyslipidemia includes increased fasting plasma triglycerides (TGs), total apoB, and decreased high-density lipoprotein cholesterol (HDL-C) concentrations [20]. Evidence from the Copenhagen City Heart Study and ACCORD lipid study has also shown that elevated nonfasting plasma apoB-remnant lipoproteins are causally associated with cardiovascular ischemic events [21, 22]. These studies reveal the importance of plasma apoB-lipoprotein remnants in predicting CVD risk. ApoB-lipoprotein remnants are derived from both the intestine (chylomicrons and chylomicron remnants containing apoB48) and the liver (very low-density lipoprotein, and low-density lipoprotein [LDL] remnants containing apoB100) [22, 23]. Few studies report quantitation of both of these atherogenic apoB-lipoprotein remnants. Impaired apoB48-CM metabolism in the fasted and nonfasted state has been observed in normolipidemic conditions and in those with increased incidence of CVD, including obesity, MetS, and diabetes [22, 24].

Plasma testosterone (T) concentrations have been positively associated with plasma TG and total apoB, and an adverse lipid profile is highly correlated with androgens in adolescents and young women with PCOS [14, 25, 26]. We have shown adolescent girls with the MetS have elevated fasting and nonfasting plasma TG and apoB48 compared with healthy weight controls, and this is further exacerbated in those diagnosed with PCOS [27]. We have also shown fasting and nonfasting plasma TG and apoB48 are positively correlated with androgen indices, including free androgen index (FAI) and free T [27]. Whether these results are observed in a large population cohort of adolescent girls with PCOS remains unknown. The aim of this study was to determine the fasting plasma apoB48 and apoB100-lipoprotein remnant concentrations in adolescent girls aged 17 years with and without PCOS from the Raine Cohort Study, and the association with cardiometabolic risk factors and androgen indices. We hypothesized that apoB-lipoprotein remnants would be positively correlated with the prevalence of cardiometabolic risk factors and androgen indices in adolescents with and without PCOS.

1. Material and Methods

A. Study Population

The Western Australian Pregnancy Cohort (Raine) Study (<http://www.rainestudy.org.au>) was designed to measure the relationship between early life events and subsequent health and behavior [28]. The Raine study is a longitudinal pregnancy cohort study of 2868 children born between 1989 and 1992 in Perth, Western Australia. Recruitment and follow-up of the cohort has been previously described in detail [28]. The cohort has undergone serial cross-sectional assessments since birth and in the current study assessments were performed when the cohort reached the age of 17 years, and is representative of the Western Australia adolescent population [29, 30]. The study was conducted with institutional approval from the Human Research Ethics Committee of King Edward Memorial Hospital and Princess Margaret Hospital for Children. The adolescent and the primary caregiver provided written assent and consent, respectively. Adolescent girls (n = 244) from the Raine Study volunteered in the assessment of reproductive function in the Menstruation in Teenagers Study, and

the participants were at least 2 years post menarche (2.6 ± 0.3 -7.0 years), as previously described [17]. The timing or onset of menarche was prospectively determined from response to serial questionnaires [17]. The 2-year follow-up assessment at age, approximately 16 to 17 years, in this cohort has been previously published, including methodology for anthropometric and blood pressure measurements [31-33]. Participants were excluded from the current analyses if they did not have complete biochemical and anthropometric data sets, and at the time of the 2-year follow-up no participants were diagnosed with diabetes. In the current study, of the 244 participants in the Menstruation in Teenagers Study, at the 2-year follow-up, 184 participants were identified that had both a fasting plasma sample and a PCOS diagnostic screening available, and were aged 17.04 ± 0.23 years (Table 1).

B. PCOS Diagnosis and Androgen Status Assessment

The PCOS-National Institute of Health (NIH) and PCOS-Androgen Excess and Polycystic Ovary Syndrome Society (AEPCOSS) criteria were used to define PCOS when the study was conducted, and the incidence of PCOS was compared between the 2 criteria [17]. The latter AEPCOSS was considered more applicable for a rigorous diagnosis of PCOS in adolescents [34]. The PCOS-NIH criteria were met if menstrual cycles were oligo-ovulatory or anovulatory together with either clinical or biochemical hyperandrogenemia [34]. The AEPCOSS adolescent criteria for PCOS diagnosis was based on evidence of ovarian dysfunction, either using clinical evidence of oligo/anovulation, and either biochemical or clinical evidence of hyperandrogenism, and persistent oligomenorrhea for 2 to 3 years beyond menarche [34]. Polycystic ovary morphology was assessed by transabdominal ultrasound evaluation of ovarian size and morphology performed by 1 of 2 experienced gynecologic ultrasonographers. Polycystic ovary morphology was defined according to standard international criteria at the time of data collection (NIH/AEPCOSS), that is, 1 or more ovaries $>10 \text{ cm}^3$ or R12 follicles between 2 and 9 mm in diameter [34]. The presence of ovulation was assessed by initially screening with a prospective menstrual diary, collected over 3 months, to establish menstrual regularity. Irregular cycles were defined as those <25 or >35 days in duration or where the cycle length varied from month to month by >4 days [17, 34]. Other causes of oligo-ovulation or anovulation were excluded by measuring thyroid-stimulating hormone and prolactin concentrations. Clinical hyperandrogenism was assessed by the presence of hirsutism, with use of the Ferriman–Gallwey scoring system [34]. Biochemical hyperandrogenism was defined as concentrations in the upper 25th centile of free T (calculated free T), which was $>24.45 \text{ pmol/L}$ (conversion factor to conventional units divide by 0.347 for pm/dL) for this data set. Sex hormone-binding globulin (SHBG) was measured in duplicate by immunoassay with use of a noncompetitive liquid phase immunoradiometric assay (SHBG-IRMA kit; Orion Diagnostica, Espoo, Finland): intra-assay and interassay coefficients of variation were $<5\%$ to 8.6% and 15.4%, respectively. Total T was measured in duplicate by radioimmunoassay (Repromed Laboratory, Adelaide, Australia) (lower limit of sensitivity 0.347 nmol/L; normal female range 0.5-2.5 nmol/L; conversion factor to conventional units divide by 0.347 for nanograms per deciliter). The intra-assay and interassay coefficients of variation are 6% and 15% at the 1 nmol/L concentration, respectively. Calculated free T was calculated from the measured total T and SHBG concentrations, concentrations, and FAI was calculated as total T/SHBG $\times 100$ using with use of standardized methods [34] (<http://www.issam.ch/freetesto.htm>).

C. Biochemical Analysis

Venous blood samples were obtained following an overnight fast (12 hours) and plasma prepared following centrifugation of whole blood at 3000 rpm, and aliquots of samples stored -80°C [17, 32]. Standard plasma biochemistry analyses for the cohort were performed including, total cholesterol (TC), LDL-C, HDL-C, TGs, insulin, and glucose) in duplicate with a coefficient of variation of $<5\%$ intra-assay, and in multiple runs with a coefficient

Table 1. Fasting metabolic and biochemical parameters of adolescent girls with and without PCOS

Covariate	NIH diagnostic criteria			AEPPOS adolescent diagnostic criteria		
	All	non-PCOS		non-PCOS		PCOS-AEPCOS
		PCOS	PCOS	PCOS	PCOS	
N (%)	184 (100)	151 (82.0)	33 (18.0)	167 (90.7)	17 (9.2)	17 (9.2)
Age (years)	17.04 ± 0.23	17.03 ± 0.10	17.10 ± 0.30	17.05 ± 0.20	17.03 ± 0.21	17.03 ± 0.21
BMI (kg/m ²)	23.16 ± 4.33	22.57 ± 3.65	25.86 ± 6.02*	22.74 ± 3.69	26.68 ± 6.20*	26.68 ± 6.20*
Waist circumference (cm)	77.90 ± 10.88	76.70 ± 9.31	83.54 ± 15.38*	77.16 ± 9.57	86.58 ± 17.77**	86.58 ± 17.77**
SBP (mmHg)	109.33 ± 8.64	109.78 ± 8.69	107.31 ± 8.38	109.70 ± 8.78	106.15 ± 7.09	106.15 ± 7.09
DBP (mmHg)	59.47 ± 6.34	58.12 ± 6.29	59.99 ± 6.50	59.78 ± 6.39	56.26 ± 5.51	56.26 ± 5.51
Total T (nmol/L)	1.30 ± 0.52	1.19 ± 0.42	1.82 ± 0.66***	1.22 ± 0.43	2.00 ± 0.71***	2.00 ± 0.71***
SHBG (nmol/L)	52.65 ± 26.63	57.30 ± 26.50	31.44 ± 14.06***	55.28 ± 26.92	34.87 ± 15.97***	34.87 ± 15.97***
DHEAS (µmol/L)	3.43 ± 1.61	3.27 ± 1.50	4.20 ± 1.87	3.36 ± 1.54	4.06 ± 1.91	4.06 ± 1.91
Free T (pmol/L)	19.92 ± 12.14	16.52 ± 8.37	35.52 ± 14.43***	17.62 ± 8.97	38.10 ± 17.85***	38.10 ± 17.85***
FAI	3.33 ± 2.72	2.55 ± 1.64	6.89 ± 3.73*	2.82 ± 1.91	7.22 ± 4.49*	7.22 ± 4.49*
Glucose (mmol/L)	4.67 ± 0.34	4.65 ± 0.32	4.80 ± 0.37	4.66 ± 0.32	4.82 ± 0.34	4.82 ± 0.34
Insulin (mU/L)	9.21 ± 6.40	8.52 ± 4.78	12.82 ± 10.82**	9.05 ± 5.78	13.26 ± 10.62*	13.26 ± 10.62*
HOMA-IR	2.25 ± 4.46	1.79 ± 1.10	4.49 ± 10.28*	1.90 ± 1.31	2.85 ± 2.22*	2.85 ± 2.22*
High-risk metabolic cluster (%)	17.88	13.70	37.50**	16.34	35.29**	35.29**
Triglycerides (mmol/L)	0.99 ± 0.43	0.98 ± 0.43	1.01 ± 0.46	1.00 ± 0.43	1.04 ± 0.38	1.04 ± 0.38
Total cholesterol (mmol/L)	4.26 ± 0.74	4.26 ± 0.72	4.22 ± 0.82	4.48 ± 0.71	4.25 ± 0.96	4.25 ± 0.96
LDL-C (mmol/L)	2.39 ± 0.63	2.39 ± 0.63	2.35 ± 0.62	2.40 ± 0.62	2.43 ± 0.60	2.43 ± 0.60
HDL-C (mmol/L)	1.41 ± 0.32	1.42 ± 0.28	1.36 ± 0.46	1.42 ± 0.31	1.34 ± 0.44	1.34 ± 0.44
ApoB-100 (mg/mL)	4.14 ± 1.08	4.14 ± 1.15	4.13 ± 0.75	4.13 ± 1.12	3.96 ± 0.70	3.96 ± 0.70
ApoB-100 in high-risk metabolic cluster	4.18 ± 0.93	—	—	—	—	—
ApoB-100 in low-risk metabolic cluster	4.14 ± 1.13	—	—	—	—	—
ApoB-48 (µg/mL)	12.19 ± 4.64	12.51 ± 4.80	10.96 ± 3.43	12.44 ± 4.83	10.92 ± 3.20	10.92 ± 3.20
ApoB-48 high-risk metabolic cluster	13.91 ± 5.06***	—	—	—	—	—
ApoB-48 in low-risk metabolic cluster	12.09 ± 4.47	—	—	—	—	—

Data are presented as mean ± standard deviation. Parameters were compared within each diagnostic criteria for statistical difference.

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; DHEAS, dihydroepiandrosterone; FAI, free androgen index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; SBP, systolic blood pressure; SHBG, sex hormone-binding globulin; T, testosterone.

* $P < .05$, ** $P < .01$, *** $P < .0001$, **** $P = .022$, ApoB48-lipoprotein remnant concentration in high-risk compared to low-risk metabolic cluster.

of variation of <5% interassay for all assays, as previously described using accredited PathWest Laboratory at the Royal Perth Hospital [17]. LDL-C was calculated using the Friedewald formula [35]. Non-HDL-C cholesterol was calculated as TC (mmol/L) – HDL-C (mmol/L). The homeostasis model assessment index of insulin resistance (HOMA-IR) was calculated as insulin ($\mu\text{U}/\text{mL}$) \times glucose (mmol/L)/22.5 [36].

D. Quantification of ApoB-lipoproteins

ApoB48 and ApoB100 lipoprotein remnants were quantified using a Western blot method, as previously established in our laboratory [27, 37]. ApoB analyses was performed on an aliquot of plasma previously stored at -80°C and thawed for this analyses once. Briefly, total plasma (2 μL [1:100] loaded in sample buffer) proteins were separated on a 3% to 8% NUPAGE Tris-acetate polyacrylamide gel and then transferred onto a polyvinylidene difluoride membrane. Membranes were incubated with a primary polyclonal antibody specific for ApoB (RRID:AB_92217) [38] and a secondary antibody tagged with horseradish peroxidase (RRID:AB_628490) [39]. ApoB48 and B100 bands were visualized by enhanced chemiluminescence and quantified using linear densitometric comparison (Image J) with a known mass of purified human ApoB48 and ApoB100 standard, as shown in Fig. 1 [27, 37].

E. Cardiometabolic Cluster Analyses

The MetS definition in adults is based on cutoffs for metabolic biomarkers in adults and cannot be applied for use in adolescents. Therefore a 2-step cluster analysis algorithm technique for use in children and adolescents was originally developed to define those individuals with high and low cardiometabolic risk [40]. The cluster analyses uses the parameters waist circumference, fasting plasma TGs, LDL-C, HDL-C, glucose and insulin, and blood pressure. The cluster analyses is an effective tool to use in defining groups based on variables that have strong evidence of clustering, such as obesity, hypertension, dyslipidemia, and insulin resistance. The cardiometabolic cluster has been used previously to define high and low cardiometabolic risk in adolescents in the Raine cohort, including those with and without PCOS, and in nonalcoholic fatty liver disease [17, 31, 32, 41].

F. Statistical Analysis

Assumptions of normality, linearity, limited multi- and collinearity, and homoscedasticity were applied for continuous variables, and variables that did not assume a normal distribution of residuals (assessed by Q-Q plot) were log transformed. Categorical data were summarized with the use of frequency distributions. Univariate group comparisons were conducted with t-tests for continuous outcomes and chi-square tests for categorical outcomes. Select multiple linear regression models were used to further examine the relationship between primary lipid and apoB-lipoproteins and predictor variables of interest including, body mass index (BMI), and insulin and androgen indices. The level of statistical significance was set at $P < .05$. All statistical and graphical analyses were performed using Stata/IC 13.1 software (StataCorp LP).

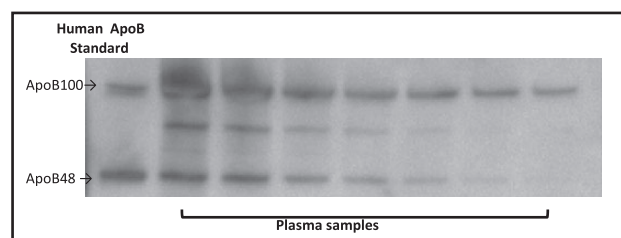


Figure 1. Plasma ApoB quantification.

2. Results

A. Metabolic and Biochemical Parameters

Metabolic and biochemical parameters in all adolescent girls and those diagnosed with PCOS according to NIH and AEPCOS Society criteria are shown in [Table 1](#). PCOS diagnosis was confirmed in 18% and 9.2% of individuals based on NIH and AEPCOS criteria, respectively. BMI was significantly elevated by 3-4 kg/m² in PCOS compared with the non-PCOS group. Waist circumference was approximately 8-9 cm (9-12%) higher in individuals with PCOS, but not different between the PCOS-NIH or PCOS- AEPCOS groups. Serum total T, free T, and FAI were approximately 2-fold higher ($P < .0001$) in the PCOS groups than in non-PCOS, and there was no difference between PCOS-NIH or PCOS-AEPCOS groups. Serum SHBG was significantly lower by approximately 40% in PCOS than in non-PCOS groups. Fasting plasma insulin concentration was >45% higher in those with PCOS using both NIH and AEPCOS diagnostic criteria. Consistently, HOMA-IR was 2.5- and 2-fold higher in those with PCOS-NIH and PCOS-AEPCOS, respectively, compared with non-PCOS. The high-risk metabolic cluster was associated with a 2-fold higher incidence of PCOS, consistent with our previous reports in this cohort [17], and consistent with other reports in adolescents with PCOS [10, 17, 42, 43].

B. Plasma Lipids and ApoB-lipoproteins

No significant difference was observed in fasting plasma lipids (TGs, LDL-C, HDL-C) or apoB-lipoprotein remnants (apoB48 and apoB100) in individuals with or without PCOS. Linear regression analyses investigated the relationship between fasting plasma apoB-lipoprotein remnants with cardiometabolic risk factors independent of PCOS status ([Table 2](#)). Fasting apoB48 remnants were positively associated with plasma TGs ($b = .43$, $P < .0001$), consistent with our previous findings in obese adolescents with and without PCOS [27]. ApoB48-lipoprotein remnants were inversely associated with fasting plasma HDL-C ($b = -.10$, $P = .015$). There was a positive association between apoB48 lipoprotein remnants and the high-risk metabolic cluster ($b = .152$, $P = .032$), predominately due to the strong correlation between plasma apoB48 remnants and plasma TGs. Individuals in the high-risk metabolic risk cluster had 1.82 µg/mL or approximately 15% higher apoB48-lipoprotein remnant concentration than in those in the low-risk cluster. ApoB-100 lipoprotein remnants were significantly and positively correlated with TGs, TC, and LDL-C, but not with the high-risk metabolic cluster ([Table 2](#)).

Regression analyses of apoB100 and apoB48 with free T, total T, FAI, SHBG and DHEAS showed that fasting plasma apoB100 and apoB48-lipoprotein remnants were not associated with biomarkers of androgen status in adolescent girls ([Table 2](#)).

3. Discussion

PCOS is associated with a higher incidence of cardiometabolic risk factors and a 2-fold higher incidence of ischemic CVD events later in life [1, 3, 44]. Early subclinical ACVD, including increased cIMT and endothelial dysfunction, and a higher incidence of the MetS and dyslipidemia have been observed in adolescents with PCOS [8, 10, 15, 17, 18]. However, limited data are available for atherogenic cholesterol dense apoB-lipoprotein remnants in adolescents with and without PCOS. Elevated concentrations of apoB-lipoprotein remnants are an early subclinical biomarker of CVD risk, and are causally associated with the development of atherosclerosis and increased incidence of end-stage ischemic CVD events [22, 24, 37, 45]. This is the first study to report apoB-lipoprotein remnant concentrations, apoB48 and apoB100, in a population-based cohort of adolescent girls with and without PCOS. We have shown that apoB48-lipoprotein remnants are elevated in adolescent girls with a high prevalence of cardiometabolic risk factors, including obesity, elevated plasma triglycerides, and

Table 2. Linear regression analysis of fasting plasma apoB-lipoprotein remnants with outcome variables including androgen status and cardiometabolic biomarkers in all adolescent girls

Variable	Total T	Free T	SHBG	FAI	DHEAS	TG	TC	LDL-C	HDL-C	Insulin	HOMA-IR	BMI	WC	High risk-metabolic cluster
ApoB48														
b	-.12	-.014	-.075	.003	-.0004	.43	.097	-.03	-.10	.021	.014	-.152	-.053	.152
P	.12	.80	.20	.09	.94	<.0001	.49	.95	.015	.64	.74	.26	.38	.032
ApoB100														
b	-.08	-.042	.002	-.02	.005	.10	.094	.11	.027	.022	.021	-.037	.074	.021
P	.11	.24	.96	.38	.97	.032	<.0001	<.0001	.75	.44	.45	.7	.097	.69

b is standardized beta-coefficient of linear regression, P is significance level of regression.

Abbreviation: WC, waist circumference.

HOMA-IR. Adolescents with PCOS had a 2-fold higher prevalence of cardiometabolic risk factors than those without PCOS. Therefore in adolescents with increased cardiometabolic risk factors with or without PCOS, an increase in apoB48 remnants may predispose these individuals to early development of CVD [21, 24, 46].

Consistent with previous findings, we have shown both ApoB48 and apoB100 lipoprotein remnants are highly correlated with fasting plasma TG concentrations[27]. We and others have shown ApoB-lipoprotein remnants are positively correlated with plasma TGs in the fasted and nonfasted states [27]. Plasma TG reflects TG-rich lipoproteins, including intestinal derived chylomicrons and hepatic very low-density lipoproteins containing apoB48 and apoB100, respectively. Evidence suggests that persistent elevations in apoB-lipoprotein remnants by 1 mmol/L in the nonfasted state are associated with a 2.8-fold risk for ischemic heart disease [21, 46]. Although nonfasting lipids and apoB-lipoproteins were not measured in this study, based on previous reports we can predict that individuals with higher fasting apoB48-lipoprotein remnants would also have elevated non-fasting TG and apoB-lipoprotein remnants in the nonfasted state or in response to a high-fat meal [27, 47].

The pathophysiology of dyslipidemia in PCOS has been attributed to elevated insulin and androgens that can independently upregulate lipogenesis [27, 48, 49]. We have previously shown in a PCOS-prone rodent model that androgens via the androgen receptor increase plasma TGs and apoB-lipoproteins, and intestinal secretion of TGs and apoB48 [48]. We have also reported that fasting and nonfasting plasma TGs and apoB48 are positively correlated with serum free T concentrations in adolescents with and without PCOS [27]. In contrast, in a case–control study in premenopausal women with PCOS no correlation of plasma apo-B48, total apo-B, or TGs with T was reported [47]. In the present study, fasting plasma apoB-lipoproteins were also found not to be correlated with androgen indices in adolescent girls from the Raine cohort.

We have previously shown in obese adolescents, with and without PCOS, that insulin resistance is associated with increased fasting and nonfasting plasma TGs and apoB48 [27]. In this study, we found no association between apoB-lipoprotein remnants with fasting plasma insulin or HOMA-IR. Furthermore, apoB-lipoprotein remnants were not associated with markers of adiposity including BMI or waist circumference. This is in contrast to our previous findings in adolescents and preadolescent children that have shown fasting and nonfasting plasma TGs and apoB48-lipoprotein remnants are associated with markers of adiposity, including BMI and waist circumference [27, 37, 45]. The discrepancy between studies may be due to greater heterogeneity in body weight, HOMA-IR, and androgen indices in the larger Raine population cohort than the smaller sample sizes in used case–control studies[27].

In the Raine population, participants with both nonalcoholic fatty liver disease and the high-risk metabolic cluster have been shown to have increased arterial stiffness, and >37% of those with nonalcoholic fatty liver disease were also diagnosed with PCOS [31, 32]. In this study we did not measure subclinical ACVD, such as arterial stiffness or cIMT. However our findings do highlight that increased apoB-lipoprotein remnants, in particular apoB48, may be an early biomarker for increased risk of developing CVD in adolescents who present with a high prevalence of cardiometabolic risk factors. [21]. Recent evidence shows that the presence of cardiometabolic risk factors in adolescence persist into adulthood, and this may contribute to the increased incidence of CVD observed in women with PCOS [1, 3, 50, 51]. Early apoB remnant dyslipidemia may predict increased premature development of CVD and increased incidence of ischemic CVD events in women with and without PCOS, consistent with findings in other populations [21]. However, given the cross-sectional design of our study we cannot determine the causal role of apoB-lipoprotein dyslipidemia on the long-term incidence of ischemic CVD events. Therefore, the relationship of atherogenic apoB-lipoprotein cholesterol-dense remnants with early diagnosis of subclinical ACVD, such as increased cIMT, and with long-term outcomes of ischemic CVD events in PCOS remains to be determined [1, 3]. We were also unable to account for family history of CVD, hypertension, dyslipidemia, apoE genotype, and type 2 diabetes, or other lifestyle factors such

as diet and exercise, which may contribute to cardiometabolic risk factors observed in this population.

The translation of our study findings to other studies using different PCOS diagnostic criteria will depend on the criteria used. However, we found no difference in primary outcomes of cardiometabolic risk factors, and apoB48 and apoB100 plasma concentrations between NIH-Rotterdam and AEPCOS PCOS diagnostic criteria. Therefore comparing other studies with different criteria to our study may not be a major issue for comparability of these outcomes. Furthermore, in the AEPCOS criteria we also used a PCOS diagnosis at >2 years post menarche, irregular cycle length of >35 days, and a pelvic ultrasound to assess ovarian morphology, consistent with current PCOS criteria in adolescents [3, 52]. Current guidelines do recommend that ovarian ultrasound assessment in adolescents is not required at <8 years post menarche due to the increased frequency of multifollicular ovaries in the normal developing ovary [3, 52]. Therefore, the impact of PCOS diagnostic criteria and the relationship to the primary outcome of apoB-lipoprotein remnants lipoproteins may be considered minimal when defining PCOS in this population.

In conclusion, elevated plasma apoB48-lipoprotein remnants are highly associated with cardiometabolic risk factors in adolescent girls with and without PCOS in the Raine cohort. In those with PCOS there is a 2-fold higher prevalence of cardiometabolic risk factors which may predispose this population of young women to premature development of atherosclerosis and CVD.

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Disclosure Summary: The authors have nothing to disclose.

Data Availability: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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