Associations of Total Testosterone with Cardiometabolic Biomarkers among Women with Polycystic Ovary Syndrome

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Abstract

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Background: Polycystic ovary syndrome (PCOS) affects 6–12% of women of reproductive age in the United States. Women with PCOS are at a higher risk of experiencing cardiovascular and metabolic diseases such as diabetes; however, mechanisms linking PCOS to the development of diabetes remain unclear. This study aimed to investigate the association of total testosterone with cardiometabolic risk factors among women with PCOS and the potential effect modifying role of obesity.

Methods: A retrospective cross-sectional study was conducted using data extracted from the medical records of women attendants (2014–2021) of the University of Washington Endocrinology Clinical and Diabetes Institute. Participants (n=170) were 18–50 years old and met at least two Rotterdam criteria for PCOS: Irregular menstrual periods, elevated androgen levels, and the presence of polycystic ovaries on an ultrasound. Blood samples were collected for biomarker measurements. Unadjusted and adjusted (for age, smoking, and race) regression models were used to examine associations of total testosterone with glucose metabolism

biomarkers (fasting glucose, fasting insulin, hemoglobin A1C [HbA1c], and Homeostatic Model Assessment of Insulin Resistance [HOMA-IR] and lipid biomarkers (HDL-c, LDL-c, total cholesterol, and triglycerides). Additionally, we examined whether associations differed in women with and without obesity, defined by a body mass index (BMI) ≥30 kg/m2.

Results: The average total testosterone of participants was 65.36 ng/dL (SD=27.14 ng/dL). Overall, we found no significant associations of total testosterone with glucose metabolism biomarkers or lipid biomarkers. There was some suggestive evidence that associations of total testosterone with lipid biomarkers differed by obesity status although association estimates from obesity-stratified models did not reach statistical significance: Among participants without obesity, total testosterone was inversely related to LDL-c (adj. β=-0.229; 95% CI: -0.551,0.093) and total cholesterol (adj. β=-0.296; 95% CI: -0.717, 0.125), while among participants with obesity, total testosterone was positively related to LDL-c (adj. β=0.096; 95% CI: -0.232,0.424) and total cholesterol (adj. β=0.101; 95% CI: -0.289,0.491) (p for interaction > 0.05).

Conclusion: We found no evidence for overall associations between total testosterone and cardiometabolic biomarkers, contrary to previous reports of associations between elevated androgen and cardiometabolic risk. We found suggestive evidence that obesity may modify associations of total testosterone with lipid levels. Future research using prospective study designs and careful phenotyping is needed to understand factors contributing to cardiometabolic disease among women with PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine condition that affects 6–12% of women of reproductive age in the United States(1). PCOS is diagnosed based on meeting at least two of the following criteria (irregular menstrual periods, elevated androgen levels, and presence of polycystic ovaries on an ultrasound), adopted from criteria by the National Institute of Health (NIH), the Androgen Excess and PCOS Society (AES-PCOS), and the Rotterdam criteria(2)(3)(4).

Women with PCOS may experience hormonal and other metabolic abnormalities that put them at greater risk for cardiovascular and metabolic diseases, including insulin resistance, hyperinsulinemia, glucose intolerance, dyslipidemia, and type 2 diabetes (T2D)(2)(5)(6)(7). Recently, Zhu et al. found no associations between genetically predicted PCOS and cardiovascular and metabolic diseases (type 2 diabetes, coronary heart disease, and stroke), a finding supporting the role of other features of PCOS (such as obesity, elevated testosterone, and low sex hormone-binding globulin) in the associations of PCOS with cardiometabolic diseases(8).

A study comparing androgen levels among women with different types of ovarian dysfunction, including PCOS, found that a higher free androgen index is associated with increased cardiovascular risk factors such as elevated insulin, HOMA-IR, and triglyceride(9). While literature reviews have indicated relationships between overexposure to androgens and insulin resistance, T2D, and hyperinsulinemia(10)(5), putative mechanisms accounting for relationships among women with PCOS have not been comprehensively described. Studies have also found that if an individual with PCOS has a high body mass index (BMI), they have an even higher metabolic risk which leads to worse cardiovascular outcomes(6)(5)(11). Because obesity is common among women with PCOS (30–70% among women with PCOS), cardiometabolic risk in this sub-population is of significant public health and clinical importance(11)(12). In sum, it is important to have a clear understanding of the relationships of

hormones and other metabolic biomarkers with cardiometabolic risk and the determinants of these relationships among women with PCOS.

We investigated associations of total testosterone with cardiometabolic risk factors among women with PCOS and the potential effect modifying role of obesity. Study findings can clarify mechanisms for cardiovascular and metabolic diseases among women with PCOS to inform preventative strategies and clinical practice.

Methods

Study Setting

The current study was conducted using data extracted from the medical records of women attendants of the University of Washington (UW) Endocrinology Clinic and the Diabetes Institute in Seattle, Washington.

Study Design and Study Population

We conducted a retrospective cross-sectional study using electronic health records data. Study participants were 18–50-year-old women attendants of the UW Endocrinology clinic and the Diabetes Institute in Seattle, WA, between 2014 and 2021 with a diagnosis of PCOS or hirsutism. To qualify for this study, women had to have met at least two of the following Rotterdam criteria for PCOS: Irregular menstrual periods, elevated androgen levels, and the presence of polycystic ovaries on an ultrasound. We excluded participants with existing diabetes. One hundred seventy participants met the inclusion and exclusion criteria and were enrolled in the study. The study was approved by the institutional review board at the University of Washington and the requirement for written informed consent was waived.

Data Collection, Independent, Dependent, and Adjustment Variables

Information on covariates (e.g., demographics and clinical characteristics) were

extracted from clinical notes and laboratory records. The independent variable was total testosterone. Total testosterone quantifies the level of free and protein-bound testosterone in the blood. A total testosterone of less than 70ng/dL is considered normal. Dependent variables included cardiometabolic biomarkers (glucose metabolism markers and lipid markers). We evaluated fasting glucose, fasting insulin, hemoglobin A1C (HbA1c), and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) to characterize glucose metabolism biomarkers. The normal range for fasting glucose after an overnight fast is 99 mg/dL or lower. After not eating for at least 8 hours, the normal range for fasting insulin is less than 25 microIU/mL. HbA1c measures the average blood glucose level over the past 2 or 3 months and having an HbA1c less than 5.7% is considered normal. We calculated HOMA-IR based on this formula: HOMA-IR= [(fasting insulin level (mU/mL) * fasting plasma glucose (mmol/L)]/22.5. A HOMA-IR greater than 2.5 indicates insulin resistance. We evaluated HDL-c, LDL-c, total cholesterol, and triglycerides to characterize lipid biomarkers. For lipid biomarkers, we dichotomized values based on “optimal” levels from the Centers for Disease Control recommendations for women (13): HDL-c ≥50 mg/dL, LDL-c <100 mg/dL, total cholesterol <150 mg/dL, and total triglycerides <150 mg/dL.

We adjusted for age (continuous in years), smoking (current, former, never), and race (White, Black or African American, Asian, American Indian/Alaska Native, Native Hawaiian or other Pacific Islander, more than one race). Additionally, we investigated whether the association of total testosterone with cardiometabolic biomarkers differ by obesity, measured using BMI. BMI measures a person’s body fat based on height and weight. We categorized obesity as a binary variable where a BMI greater than or equal to 30 kg/m^2 indicated obesity.

Data analysis

We calculated summary statistics using mean (and standard deviation) for continuous measures and frequency distributions (and percentages) for categorical measures to describe

the study population. Regression models were used to assess associations of total testosterone with cardiometabolic biomarkers. We fitted independent unadjusted and adjusted linear regression models with total testosterone (continuous) as the exposure and each of the cardiometabolic biomarkers (continuous) as outcomes. We included age, smoking, and race as covariates in adjusted models. We assessed effect modification by obesity by fitting unadjusted and adjusted linear regression models among participants with (BMI≥30 kg/m^2) and without (BMI<30 kg/m^2) obesity separately. We fit unadjusted and adjusted linear regression models that included exposure (total testosterone), each outcome, obesity status, covariates (for adjusted models: age, smoking, and race), and interaction terms between total testosterone and obesity. The p-value of the interaction term was used to assess the statistical significance of interactions. We conducted a sensitivity analysis where we dichotomized total testosterone and cardiometabolic biomarkers based on normal/optimal range cutoffs (as described above) and fit similar unadjusted and adjusted logistic regression models, as described above. Statistical testing was two-sided, and significance was determined at $\alpha = 0.05$. All statistical analyses were conducted using R 4.2.2.

Results

Selected characteristics of study participants are presented in **Table 1**. The majority of study participants were White (69%), non-Hispanic (93%), utilized private insurance (66%) and never smoked (84%). The average BMI of participants was 32.63 kg/m^2 (SD=10kg/m^2). In addition, the average total testosterone of participants was 65.36 ng/dL (SD=27.14 ng/dL).

Overall, we found no significant association (all p-values>0.05) of total testosterone with glucose metabolism biomarkers (**Table 2**). While not statistically significant, the estimates suggested inverse correlations between total testosterone and all glucose metabolism biomarkers. Adjustment for covariates did not change findings. Similar results were observed in

models stratified by obesity status. The interaction terms of total testosterone and obesity status on glucose metabolism biomarkers were not significant (interaction p-values>0.05).

Findings from models for associations of total testosterone with lipid biomarkers are shown in **Table 3**. Overall, we found no significant associations of total testosterone with lipid biomarkers (all p-values>0.05). In adjusted models, while not statistically significant, estimates suggested positive associations of total testosterone with LDL-c and triglyceride, as well as inverse associations of total testosterone with HDL-c and total cholesterol. Interestingly, there were some differences in associations comparing participants with and without obesity. Among participants without obesity, total testosterone was inversely related to LDL-c (adj. β=-0.229; 95% CI: -0.551,0.093) and total cholesterol (adj. β=-0.296; 95% CI:-0.717, 0.125), while among participants with obesity, total testosterone was positively related to LDL (adj. β=0.096; 95% CI:-0.232,0.424) and total cholesterol (adj. β=0.101; 95% CI:-0.289,0.491) although these associations were not significant at a pre-specified α<0.05. The interaction terms of total testosterone and obesity status on lipid biomarkers were not significant (interaction p-values>0.05).

In sensitivity analyses conducted using dichotomized measures of exposure and outcomes, high testosterone (total testosterone >70 ng/dL) was not significantly associated with higher odds of abnormal glucose metabolism biomarkers or abnormal lipid biomarkers (**Table 4**).

Discussion

The primary aim of this study was to investigate associations of total testosterone with cardiometabolic biomarkers among women with PCOS. Overall, we found no significant association of total testosterone with glucose metabolism biomarkers or lipid biomarkers. There was some suggestive evidence that associations of total testosterone with lipid biomarkers differed by obesity status although association estimates from obesity-stratified models did not

reach statistical significance. Among participants without obesity, total testosterone was inversely related to LDL-c and total cholesterol, while among participants with obesity, total testosterone was positively related to LDL-c and total cholesterol; however, these associations did not reach statistical significance (interaction p-values>0.05).

Significant research has been conducted to understand the association between high testosterone levels and cardiometabolic biomarkers among women with PCOS. Dann et al. conducted a cross-sectional study that evaluated ovarian dysfunction associated with cardiometabolic abnormalities and found statistically significant associations of high free androgen index (FAI) with elevated triglycerides (*β* log FAI for PCOS: 0.45, $P < 0.001$), insulin (*β* log FAI for PCOS: 0.77), HOMA-IR ((*β* log FAI for PCOS: 0.82), and increased glucose (*β* log FAI for PCOS: 0.05) in women with PCOS(9). Shirazi et al. conducted a cross-sectional study comparing androgen levels with insulin and insulin resistance among women with PCOS (N=80). After adjusting for BMI, they found a significant correlation between insulin level and free androgen index (correlation coefficient: 0.266 and P = 0.018)(15). Rasool et al. conducted a case-control study evaluating the association of serum fasting insulin concentration with cardiovascular and metabolic risk factors in women with PCOS. Their sample included 249 women with PCOS and 100 age-matched healthy controls. They found that fasting insulin in the PCOS group had a positive correlation with free androgen index ($r = 0.141$, $p < 0.05$), total testosterone ($r = 0.227$, $p < 0.001$), and HOMA-IR ($r = 0.092$, $p < 0.001$. In the control group, they found a positive correlation between fasting insulin and testosterone ($r=0.251$, $p=0.012$) and HOMA-IR ($r=0.846$, $p<0.001$). However, they didn't find a correlation between fasting insulin and FAI ($p>0.05$) in the control group(16). In the current analysis, we did not observe statistically significant associations of total testosterone with triglycerides, fasting glucose, fasting insulin, or HOMA-IR. Reasons for these differences might include differences in the assays used. FAI is recognized as a more accurate proxy for hyperandrogenism than total testosterone(17).

Alternatively, the cohorts might have differed by factors such as age or BMI. Lastly, we may have been underpowered to detect these differences.

Women with PCOS present with hyperandrogenism, and androgen hormones can potentially interact with hormones and metabolites from other organs in the body(5)(18)(19). In particular, hyperandrogenism may contribute to pancreatic and/or hepatic dysfunction. Metabolic perturbations such as insulin resistance, T2D, and glucose intolerance are accelerated by obesity, contributing to excess risk for cardiovascular events(20)(14). We saw this relationship when we evaluated effect modification based on obesity status. Among participants without obesity, total testosterone was inversely related to LDL-c and total cholesterol, while among participants with obesity, total testosterone was positively associated with LDL-c and total cholesterol, even though this association wasn't statistically significant. This is consistent with the findings of a meta-analysis that investigated the effects of overweight, obesity, and central obesity on metabolic, reproductive, and psychological features of PCOS. They found that in overweight or obese women with PCOS, obesity significantly worsened all metabolic and reproductive outcomes except for hirsutism compared to normal weight women with PCOS(21). It is essential to pay close attention to the biomarker levels of women with high BMI because they are more likely to experience worse cardiovascular and metabolic risks.

Total testosterone was utilized to evaluate hyperandrogenism. Another proxy measure used to assess hyperandrogenism that was used in other studies is the FAI. In the original study design, we planned to examine associations of FAI and total testosterone with cardiometabolic biomarkers; however, roughly half of the participants in our cohort were missing data on sex hormone-binding globulin (SHBG) level, a biomarker needed to calculate FAI. This missingness was not due to chance; hence we couldn't calculate the FAI. FAI is a more reliable proxy for hyperandrogenism than testosterone alone(17).

In addition to having a lot of missingness, using total testosterone exclusively, and possibly not having high study power, other limitations deserve consideration. We conducted a

cross-sectional study, making it hard to make causal inferences because we only looked at a single moment in time (18). We didn't have a group without PCOS for comparison. Few participants had a transvaginal ultrasound, so we could not examine subtypes of PCOS. Lastly, we only had fasting glucose/insulin metabolism measures rather than measures of glucose and insulin from a two-hour oral glucose tolerance test that would provide more comprehensive characterization of glucose metabolism.

In conclusion, this study found no associations of total testosterone with cardiometabolic biomarkers among a cohort of women with PCOS, contrary to other studies. We found suggestive evidence indicating potential roles of obesity in the relationships between total testosterone and lipid biomarkers among women with PCOS. Future research using prospective study designs and careful phenotyping is needed to understand factors contributing to cardiometabolic disease among women with PCOS.

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Table 1: Selected characteristics of female clinic patients with a diagnosis of polycystic ovary syndrome (N=170)

Age (years)*	31.39 (6.45)
Race	
White	117 (69%)
Black or African American	7 (4%)
Asian	18 (11%)
American Indian/Alaska Native	1 (1%)
Native Hawaiian or Other Pacific Islander	2 (1%)
More than One Race	3 (2%)
Body Mass Index (kg/m^2) *	32.63 (10.00)
Ethnicity	
Hispanic	11 (7%)
Non-Hispanic	141 (93%)
Insurance	
Public Medicare	2 (1%)
Public Medicaid	34 (25%)
Private	90 (66%)
Other	5 (4%)
Smoking	
Current	8 (5%)
Former	19 (11%)
Never	141 (84%)
Systolic blood pressure (mmHg)*	120.24 (11.58)
Diastolic blood pressure (mmHg)*	75.13 (8.67)
Glucose metabolism biomarkers*	
Fasting glucose (mg/dL)	87.47 (8.73)
Fasting insulin (microU/mL)	15.23 (14.66)
Hemoglobin A1C (%)	5.22 (0.39)
HOMA-IR	3.36 (3.30)
Lipid biomarkers*	
High density lipoprotein-cholesterol (mg/dL)	51.99 (12.03)
Low density lipoprotein-cholesterol (mg/dL)	103.27 (30.45)
Total cholesterol (mg/dL)	178.55 (36.10)
Triglycerides (mg/dL)	110.17 (57.29)
Total testosterone (ng/dL) *	65.36 (27.14)

*mean (standard deviation), otherwise number (%)

Table 2: Associations of total testosterone with glucose metabolism biomarkers among women with a diagnosis of polycystic ovary syndrome (N=170)

	All participants				Participants without obesity (BMI <30)				Participants with obesity (BMI ≥30)			
	Unadjusted models		Adjusted models*		Unadjusted models		Adjusted models*		Unadjusted models		Adjusted models*	
	ß	95% CI	ß	95% CI	ß	95% CI	ß	95% CI	ß	95% CI	ß	95% CI
Fasting glucose	-0.028	-0.094,0.038	-0.039	-0.107,0.029	-0.009	-0.082,0.065	-0.030	-0.107,0.048	-0.060	-0.180,0.059	-0.057	-0.193,0.079
Fasting insulin	-0.015	-0.159,0.129	-0.061	-0.195,0.074	-0.093	-0.229,0.043	-0.123	-0.278,0.032	0.029	-0.237,0.294	-0.013	-0.251,0.225
HbA1c	-0.000	-0.003,0.002	-0.000	-0.003,0.002	-0.001	-0.004,0.002	-0.002	-0.004,0.001	-0.002	-0.005,0.002	-0.001	-0.005,0.003
HOMA-IR	-0.005	-0.037,0.027	-0.016	-0.046,0.014	-0.021	-0.054,0.012	-0.029	-0.067,0.008	0.002	-0.054,0.058	-0.008	-0.054,0.039

*Adjustment covariates: age, smoking, and race.
Note: Interaction term p-values between total testosterone and obesity were all p>0.05.
Abbreviations: BMI: Body Mass Index, HbA1c: Hemoglobin A1C, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance

Table 3: Associations of total testosterone with lipid biomarkers among women with a diagnosis of polycystic ovary syndrome (N=170)

	All participants				Participants without obesity (BMI <30)				Participants with obesity (BMI ≥30)			
	Unadjusted models		Adjusted models*		Unadjusted models		Adjusted models*		Unadjusted models		Adjusted models*	
	ß	95% CI	ß	95% CI	ß	95% CI	ß	95% CI	ß	95% CI	ß	95% CI
HDL-c	-0.050	-0.130,0.029	-0.025	-0.110,0.060	-0.020	-0.143,0.102	0.042	-0.088,0.172	-0.009	-0.091,0.073	-0.022	-0.106, 0.062
LDL-c	-0.012	-0.209,0.185	0.018**	-0.197,0.232	-0.115	-0.415,0.186	-0.229	-0.551,0.093	0.013	-0.274,0.300	0.096	-0.232,0.424
Total cholesterol	-0.093**	-0.340,0.153	-0.034**	-0.299,0.232	-0.248	-0.634,0.138	-0.296	-0.717,0.125	-0.026	-0.370,0.318	0.101	-0.289, 0.491
Triglycerides	0.113	-0.293,0.520	0.093	-0.344,0.531	-0.121	-0.450,0.208	-0.235	-0.582,0.112	-0.163	-0.763,0.437	0.115	-0.532,0.763

* Adjustment covariates: age, smoking, and race.
Note: Interaction term p-values between total testosterone and obesity were all p>0.05.
Abbreviations: BMI: Body Mass Index; HDL-c: High density lipoprotein-cholesterol; LDL-c: Low density lipoprotein-cholesterol

Table 4: Associations of categorical total testosterone with categorical glucose metabolism biomarkers among women with a diagnosis of polycystic ovary syndrome (N=170)

	Unadjusted models		Adjusted models*	
	OR	95% CI	OR	95% CI
Fasting glucose ≥100 mg/dL	2.800	0.243,32.309	0.681	0.028,16.330
Fasting insulin >25 (microU/mL)	1.375	0.358,5.285	0.867	0.174,4.308
HbA1c >5.7%	0.750	0.066,8.549	0.562	0.032,10.006
HOMA-IR >2.5	1.004	0.380,2.653	0.824	0.277,2.452

* Adjustment covariates: age, smoking, and race.
Total testosterone was dichotomized based on the cut off: <70=normal.
Abbreviations: HbA1c: Hemoglobin A1C, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance

Table 5: Associations of categorical total testosterone with categorical lipid biomarkers among women with a diagnosis of polycystic ovary syndrome (N=170)

	Unadjusted models		Adjusted models*	
	OR	95% CI	OR	95% CI
HDL-c <50 mg/dL	1.509	0.677,3.364	1.326	0.562,3.128
LDL-c >100 mg/dL	1.164	0.525,2.580	1.078	0.451,2.577
Total cholesterol >150 mg/dL	0.917	0.347,2.421	0.995	0.342,2.890
Triglycerides>150 mg/dL	1.419	0.557,3.614	1.309	0.471,3.632

*Adjustment covariates: age, smoking, and race.
Total testosterone was dichotomized based on the cut off: <70=normal.
Abbreviations: HDL-c: High density lipoprotein-cholesterol; LDL-c: Low density lipoprotein-cholesterol