Title: SHBG, but not testosterone, is associated with the metabolic syndrome in overweight and obese women with polycystic ovary syndrome

Short title: SHBG, metabolic syndrome and diabetes in PCOS

Moran, LJ[1,2], Teede, HJ[2, 6], Noakes, M[3], Clifton, PM[4,5], Norman, RJ[1] and Wittert, GA[5]

[1]The Robinson Institute, University of Adelaide, 55 King William Road, North Adelaide, 5006, South Australia, Australia

[2]School of Public Health and Preventive Medicine, 43-51 Kanooka Grove, Monash University; Diabetes Unit Southern Health, Clayton, 3168, Victoria, Australia

[3]CSIRO Food and Nutritional Sciences, PO Box 10041 Adelaide BC 5000, South Australia, Australia, 5000.

[4]Baker IDI Heart and Diabetes Institute, Playford Building, University of South Australia, Adelaide, 5001, South Australia, Australia

[5]The Discipline of Medicine, University of Adelaide, Eleanor Harrald Building, Frome Road, Royal Adelaide Hospital, Adelaide, 5005, South Australia, Australia

[6]Diabetes Unit, Southern Health, Monash Medical Centre, Clayton, 3168, Victoria, Australia

Corresponding author and reprint requests:

Dr Lisa Moran BSc (Hons), BND, PhD; The Robinson Institute, Research Centre for Reproductive Health, School of Paediatrics and Reproductive Health, University of Adelaide, 55 King William Road, North Adelaide, 5006, Australia

Email: lisa.moran@adelaide.edu.au; Telephone: +61 08 8313 1352; Fax: +61 08 8313 1355

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Abbreviations:

Body mass index: BMI

Cardiovascular disease: CVD

Diastolic blood pressure: DBP

European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine: ESHRE/ASRM

Free androgen index: FAI

High density lipoprotein cholesterol: HDL-C

Highly sensitive C-reactive protein: hsCRP

Homeostasis assessment of insulin resistance: HOMA

Impaired fasting glucose: IFG

Impaired glucose tolerance: IGT

Low density lipoprotein cholesterol: LDL-C

National Institute of Health: NIH

Oral glucose tolerance test: OGTT

Polycystic ovary syndrome: PCOS

Sex hormone binding globulin: SHBG

Systolic blood pressure: SBP

Thyroid stimulating hormone: TSH

Type 2 diabetes: T2DM

Abstract

Background: Polycystic ovary syndrome (PCOS) is associated with hyperandrogenism and an increased risk of type 2 diabetes and cardiovascular disease. Decreased sex hormone-binding globulin (SHBG) and elevated testosterone are associated with metabolic syndrome and glucose intolerance in women.

Aim: The aim of this study was to assess the relationship between SHBG and testosterone and metabolic syndrome and glucose intolerance in PCOS.

Material/Subjects and Methods: Cross-sectional study in overweight and obese premenopausal non-diabetic women with PCOS (n=178: n=55 metabolic syndrome, n=16 glucose intolerance). Data were analysed by multiple regression with metabolic syndrome, oral glucose tolerance test (OGTT) glucose or SHBG as dependent variables and reproductive hormones, insulin resistance, glucose tolerance, lipids or C-reactive protein as independent variables.

Results: Metabolic syndrome was independently associated with BMI (OR 1.084 95% CI 1.034-1.170, p=0.015) and SHBG (OR 0.961 95% CI 0.932-0.995 p=0.018). Glucose tolerance was independently associated with OGTT insulin (β=0.418 $p<0.001$), age (β=0.154 p=0.033) and prolactin (β=-0.210 p=0.002). SHBG was independently associated with OGTT insulin (β=-0.216 p=0.014) and PCOS diagnostic criteria (β=0.197 p=0.010).

Conclusions: SHBG, but not testosterone, is independently associated with the metabolic syndrome in overweight women with PCOS and is associated with insulin resistance and PCOS diagnostic criteria. .

Introduction

Polycystic ovary syndrome (PCOS) affects up to 18% of women of reproductive age (1) and is associated with menstrual irregularity, anovulation, hyperandrogenism and infertility (2). It is also associated with adverse metabolic health including impaired glucose tolerance (IGT), type 2 diabetes (T2DM), increased risk factors for cardiovascular disease (CVD) and apparent elevated CVD risk (2-5). Insulin resistance is a key aetiological factor in PCOS associated with both the reproductive and metabolic features (6, 7). It is also present in both lean and overweight women (8) with PCOS indicating an inherent, obesity-independent effect of PCOS status on insulin resistance and metabolic disease.

There is increasing interest in the independent contribution of both hyperandrogenism and insulin resistance, either as markers or mechanistic contributors, to metabolic disease in PCOS. These include elevated androgens, primarily testosterone, and reduced sex hormone binding globulin (SHBG). SHBG, synthesised in the liver, binds circulating sex steroids regulating their bioavailability and is proposed to be a putative marker of hepatic insulin resistance (9). In women in the general population, reduced SHBG levels and to a lesser extent, elevated testosterone, have been associated with increased T2DM and metabolic syndrome independent of obesity (10, 11). In keeping with this, the relationship between testosterone and incident T2DM is predominantly explained by adiposity and insulin resistance while SHBG is independently related to incident T2DM in post-menopausal women (12). Furthermore, SHBG, but not testosterone, has been associated with subclinical atherosclerosis (13, 14) in pre or post-menopausal women independent of factors including age, body mass index (BMI), insulin or lipids. This indicates the stronger relationship of SHBG as opposed to testosterone with metabolic abnormalities in women without PCOS.

In PCOS, the relationship between SHBG and testosterone and metabolic disease are less consistent. Some studies report SHBG as inversely related to metabolic syndrome (15) or impaired glucose tolerance (16) while others do not (17). The free androgen index (FAI) as an estimate of free testosterone, but not total testosterone, was additionally associated with metabolic syndrome in PCOS (10). The relationships between SHBG and testosterone with metabolic disease are therefore unclear and it is unknown whether testosterone or SHBG are primarily related to metabolic abnormalities in PCOS. To our knowledge there are also no studies in PCOS examining the association between SHBG and both abnormal glucose tolerance and the metabolic syndrome or the relationship of SHBG or testosterone to metabolic diseases independent of potential confounders such as adiposity, insulin resistance or the diagnostic criteria of PCOS. Tthe aim of this study was to therefore assess the independent relationship between SHBG and testosterone and the metabolic syndrome and glucose intolerance in PCOS.

Materials and methods

Subjects

This secondary analysis is a cross-sectional study of baseline measurements from three clinical trials (18-20) of women with PCOS where complete oral glucose tolerance test (OGTT) data were available (n=178). Sample sizes in the original studies were based on detectable differences in a change in weight between two dietary interventions in PCOS, a change in insulin resistance between three pharmacological interventions in PCOS or a difference in Diabetes Risk Score between women with and without PCOS (18-20). For the original trials, following baseline measurements women were either randomised to a lower dose oral contraceptive pill (20 µg ethinyl estradiol/100 µg levonorgestrel and aldactone 50 mg b.d.]), a higher dose oral contraceptive pill (35 µg ethinyl estradiol [EE]/2 mg

cyproterone acetate) or metformin (1 g b.d) (19) or a carbohydrate restricted or a fat restricted weight management diet (18). For one of the studies, this was soley a cross-sectional study and no intervention was involved following baseline measurements (20). The current analysis included data from all suitable and available subjects to maximise power. Study recruitment and inclusion and exclusion criteria have been previously described (18-20). The populations for all studies were premenopausal women aged 18-45 years who were overweight (n=42, BMI $\geq$ 25 kg/m^2), obese (n=95, BMI $\geq$ 30 kg/m^2) or morbidly obese (n=41, BMI $\geq$ 40 kg/m^2) according to World Health Organisation criteria (21). All women had PCOS as classified by the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) diagnosis (22). This comprises the presence of two of the three features of hyperandrogenism [either clinical (hirsutism by elevated Ferriman-Gallwey score) or biochemical (elevated testosterone or free androgen index (FAI)), oligo- or amenorrhoea and presence of polycystic ovaries on ultrasound]. This diagnosis incorporates both women diagnosed with PCOS based on either the older National Institute of Health (NIH) PCOS criteria (n=154), both clinical or biochemical hyperandrogenism and oligo- or amenorrhoea (23), or milder non-NIH PCOS criteria, defined as those whose diagnosis of PCOS would not meet the NIH criteria (ie presenting with the ESHRE/ASRM categories of PCO and menstrual dysfunction or PCOS and hyperandrogenism) non-NIH PCOS (n=24). The majority of women were Caucasian. For those with available data (n=49), n=2/47 were of South Asian ethnicity and n=47/49 were of Caucasian ethnicity. Exclusion criteria were T2DM, pregnancy, breastfeeding, and endocrine disorders (congenital adrenal hyperplasia, androgen-secreting tumours, Cushing's syndrome, hyperprolactinaemia, thyroid dysfunction and adrenal disorders). All participants had ceased insulin-sensitising or reproductive hormonal medication for at least 3 months prior to baseline measurements. Stable use of other medications (antihypertensives n=5 or lipid lowering medication or fish oil n=7) or

smoking (n=9 women) were not exclusion criteria and were adjusted for in all analyses. All women with highly sensitive C-reactive protein (hsCRP) > 10 mg/L (n=25) were excluded from analysis for hsCRP as this represents acute inflammation, however these women were not excluded from remaining analyses. The studies received ethics approval from Monash University, Southern Health, Commonwealth Scientific and Industrial Research Organisation Division of Health Sciences and Nutrition, The Royal Adelaide Hospital, and the Women's and Children's Hospital of South Australia and all participants gave written informed consent after full explanation of the purpose and nature of all procedures used.

Clinical and biochemical measurements

Following an overnight fast, height and weight (lightly clothed without shoes) were measured and BMI was calculated. Waist circumference was measured to the nearest 0.5 cm directly on the skin at the level of midway between the lateral lower rib margin and the iliac crest. Resting systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured as previously described (18-20). Fasting venous blood samples were taken for analysis of glucose, insulin, hsCRP, lipids (total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides), prolactin and thyroid stimulating hormone (TSH) as previously described(18-20). Total testosterone (bound and unbound) was measured by chemiluminescent immunoassay (ADVIA Centaur Assay, Bayer Corporation, Inc) (18) or (Beckman Coulter, Fullerton, CA) (19, 20) with intra- and inter-assay coefficients of variation of 2.3-6.2% and 1.4-4.7% and 1.67% and 4.78% respectively. SHBG was measured by a non-competitive liquid phase immunoradiometric assay (Orion Diagnostica, Espoo, Finland) (18) or chemiluminescent immunoassay (Immunolite 1000, EURO/Diagnostics Products Corp. Ltd. Los Angeles, CA) (19, 20) with intra- and inter-assay coefficients of variation of 3.2% and 1.11-9.98% and 4.1% and 5.8% respectively(19, 20). A

120-minute 75-gram OGTT was then performed and insulin and glucose measured at 30, 60, and 120 minutes. Impaired fasting glucose was defined as fasting glucose 6.1-6.9 mmol/L and 120-minute glucose < 7.8 mmol/L; and impaired glucose tolerance was defined as fasting glucose < 7.0 mmol/L and 120-minute glucose 7.8–11.0 mmol/L in accordance with the World Health Organisation report on the Diagnosis and Classification of Diabetes Mellitus (24). The metabolic syndrome was diagnosed by the 2009 Joint Scientific Statement Criteria (25) consisting of 3 of the following factors: elevated waist circumference by population and country-specific definitions (≥ 80 cm), triglycerides ≥ 1.7 mmol/L or specific treatment for this lipid abnormality, HDL-C < 1.3 mmol/L or specific treatment for this lipid abnormality, raised blood pressure (SBP ≥ 130 or DBP ≥ 85 mmHg) or treatment of previously diagnosed hypertension or raised fasting plasma glucose (≥ 5.6 mmol/L) or previously diagnosed T2DM. Homeostasis assessment of insulin resistance (HOMA) was calculated by [(fasting glucose x insulin)/22.5] and FAI was calculated by [(testosterone x SHBG)/100]. Where possible, women were assessed at day 3-7 of their menstrual cycle.

Statistics

Two-tailed statistical analysis was performed using SPSS for Windows 14.0 software (SPSS Inc, Chicago, USA) with statistical significance set at α level of $P \leq 0.05$. Data were assessed for normality using Kolmorgov-Smirnov tests and log transformed where non-normally distributed. Data are presented as mean ± SD except for non-normally distributed data (median ± interquartile range) and categorical data (proportions). Analyses were performed comparing women either with or without abnormal glucose tolerance or with or without the metabolic syndrome. Data were analysed using one-way ANOVA (parametric data) with BMI as a covariate or chi-square tests (categorical data) with metabolic syndrome or abnormal glucose tolerance status as the between subject factor. Logistic regression analysis

using simultaneous entry of preselected predictors was used to examine demographic, anthropometric and biochemical contributors to the categorical variable metabolic syndrome as the dependent variable and BMI, SHBG, TSH, testosterone and insulin 120 minutes OGTT as the independent variables. Separate multiple linear regression analyses using simultaneous entry of preselected predictors was used to examine the contribution of demographic, anthropometric and biochemical contributors to SHBG or to glucose tolerance (as assessed by 120-minutes OGTT glucose) as the dependent variables. For the SHBG model, insulin 120-minutes OGTT, PCOS diagnostic criteria, medication use, smoking, glucose 120-minutes OGTT, BMI, TSH and total cholesterol were used as the independent variables. For the glucose tolerance model, insulin 120-minutes OGTT, age, prolactin, medication use, smoking, PCOS diagnostic criteria, SHBG, testosterone, TSH, cholesterol, triglycerides, HDL-C and waist circumference were used as the independent variables. The independent variables were selected for each model based on hypothesis testing (for inclusion of SHBG or testosterone) or associations on correlations (with a P value <0.2 considered for inclusion). Regression models were constructed to avoid collinearity and assessed for the normality of residuals and all models were adjusted for smoking, PCOS diagnostic criteria and medication use as potential confounders. Post-hoc calculations were sufficiently powered to detect the observed difference in SHBG between women with and without metabolic dysfunction of 9.3±15.5 nmol/L to 88% power $p<0.05$.

Results

Anthropometric, reproductive and metabolic variables

This study comprised n=178 women with PCOS with a mean age of 33.2±6.3 years, mean weight of 94.9±18.7 kg and mean BMI of 35.2±6.4 kg/m^2. The baseline characteristics of all women are summarised in Table 1. N=55 had the metabolic syndrome, n=16 women had

abnormal glucose tolerance (n=1 impaired fasting glucose (IFG), n=15 IGT), and n=114 women had neither of these with incomplete data for metabolic syndrome determination for n=5 women.

Women with the metabolic syndrome had lower SHBG and HDL-C and elevated weight, BMI, waist circumference, FAI, TSH, triglycerides, hsCRP, fasting and OGTT glucose and insulin, HOMA, SBP and DBP. Women with abnormal glucose tolerance had lower SHBG and elevated FAI, triglycerides, fasting and 120-minute glucose and insulin, HOMA, SBP and DBP compared to women with normal glucose tolerance. There were no differences in testosterone between women with or without the metabolic syndrome or with or without abnormal glucose tolerance (Table 1). These relationships were maintained on adjustment for BMI with the exception of hsCRP for women with and without the metabolic syndrome and SHBG, fasting insulin and HOMA for women with and without abnormal glucose tolerance (Table 1).

Multiple regressions

The independent contribution of SHBG, testosterone, FAI and additional anthropometric and metabolic variables to the metabolic syndrome and abnormal glucose tolerance (through 120-minute OGTT glucose) were assessed through logistic and linear regression (Table 2). For the metabolic syndrome, an elevated BMI and a decreased SHBG were independently associated with the presence of the metabolic syndrome. The entire model for metabolic syndrome was statistically significant (non-significant Hosmer and Lemeshow goodness of fit test, p=0.755). For glucose tolerance, elevated 120-minute OGTT insulin and age and decreased prolactin were independently associated with elevated 120-minute OGTT glucose. These relationships were maintained on adjustment for PCOS diagnostic criteria, medication

use and smoking status. Testosterone and FAI were not independently associated with either the metabolic syndrome or glucose tolerance.

Following the independent association between SHBG and the metabolic syndrome, further linear regression analyses were conducted to identify the independent determinants of SHBG. Reduced 120-minute OGTT insulin and non-NIH PCOS diagnosis were associated with elevated SHBG. This relationship was maintained on adjustment for medication use and smoking status.

Discussion

We report here for the first time an independent association of SHBG with the metabolic syndrome but not abnormal glucose tolerance in PCOS. While this effect may be associated with insulin resistance, it appears to be independent of BMI or glucose tolerance. Testosterone was not associated with either the metabolic syndrome or abnormal glucose tolerance. We also report a less marked reduction in SHBG in non-NIH PCOS consistent with the less adverse reproductive and metabolic presentation of this diagnostic criteria (26) and indicating the need for further research in the assessment of metabolic disease across the diagnostic categories of PCOS.

We confirm here (27) previous reports of reduced SHBG for women with PCOS and the metabolic syndrome consistent with the general population (10). Of the limited literature assessing SHBG and metabolic syndrome in PCOS, few studies have assessed the independence of relationship. Chen et al reported decreased SHBG was associated with the metabolic syndrome independent of other risk factors including insulin resistance, adiposity or testosterone (15). Conversely, SHBG was not independently associated with the metabolic

syndrome in PCOS after adjustment for variables including BMI, insulin resistance, age, acanthosis nigricans, T2DM, luteinising hormone, free testosterone or FAI (17, 27, 28). We note the range of ethnicities examined (predominantly Caucasian in this current study compared to South or East Asian or a mixture of Caucasian, African-American, Hispanic or Asian) (17, 27, 28) and a lack of detail regarding medication status in prior studies (17, 28) which may alter risk factors for cardiovascular disease and T2DM and reproductive hormones (29) an d contribute to these discrepant results.

As a cross-sectional analysis, the causative role of SHBG in metabolic dysfunction in PCOS cannot be inferred. However, childhood (30) or pre-conception (31) SHBG predicts the later development of the metabolic syndrome (30) and gestational diabetes (31) in PCOS.Prior research also reports associations between SHBG gene single nucleotide polymorphisms and SHBG levels and T2DM risk in the general population and in PCOS(32-34). Surrogate markers of insulin resistance were also independently associated with SHBG in agreement with insulin resistance and hyperinsulinaemia reducing hepatic SHBG production (9). We note in this current study that SHBG was not related to other markers of the metabolic syndrome and instead was most tightly related to insulin resistance. This suggests that SHBG is associated with metabolic syndrome via its links to insulin resistance. SHBG may additionally reflect the status of hepatic de novo lipogenesis with in vitro monosaccharide-induced hepatic de novo lipogenesis inhibiting hepatic SHBG gene expression and protein secretion through altering hepatocyte nuclear factor (HNF)-4α levels(35). This is supported by human data showing removal of significant associations between SHBG and insulin on adjustment for liver fat, strong correlations of SHBG and liver fat independent of other modulators including adiponectin and increases in SHBG following lifestyle interventions related to reductions in liver fat independent of changes in total and visceral adiposity(36).

Furthermore, the presence of fatty liver was independently associated with reduced SHBG in association with insulin resistance, FAI and HDL-C in PCOS (37). This supports SHBG as a marker of metabolic dysfunction in PCOS and a potential indicator of hepatic insulin resistance.

Recent meta-analyses report both elevated testosterone and decreased SHBG are independently associated with T2DM and the metabolic syndrome in women (10, 11). It has also been previously proposed that the association between SHBG and metabolic disease may reflect the regulation of bioavailable androgens or oestrogens by SHBG. However, in this current study total testosterone was not independently associated with the metabolic syndrome or abnormal glucose tolerance or significantly different between women with or without these conditions. This is consistent with the bulk of the literature for PCOS identified in a recent systematic review (10) with no relationship between testosterone and metabolic syndrome on meta-analysis. In this current study, the difference in FAI between women with and without abnormal glucose tolerance or the metabolic syndrome likely reflects the contribution of SHBG. This is supported as SHBG, but not testosterone, was associated with subclinical atherosclerosis (13), risk factors for cardiovascular disease (38) or the metabolic syndrome (39) independent of age, BMI or insulin resistance in the general population. This suggests that SHBG may be acting as a marker of metabolic risk, independent of androgen status.

With regards to glucose levels, we report here no independent association between SHBG and abnormal glucose tolerance in PCOS in contrast to meta-analysis data from the general population (11). While reduced SHBG was previously associated with an elevated prevalence of IGT in PCOS (16, 40), this has not been reported independent of confounders such as

adiposity and insulin resistance. After correcting for confounders, our study showed SHBG was related to insulin but not glucose status. This may be related to the lack of women with T2DM and few with abnormal glucose tolerance in this study.. We confirm previously noted associations between age, insulin resistance and abnormal glucose tolerance (41, 42). Furthermore, a high prolactin level was independently associated with lower 2 hour OGTT glucose. This is consistent with prior associations of elevated prolactin with lower prevalence of diabetes and impaired glucose tolerance (43) and the association of elevated prolactin with the regulation of β-cell mass and glucose-stimulated insulin secretion in pregnancy (45) or animals (46). This may reflect a relationship between prolactin and the regulation of glucose and insulin homeostasis.

While some reproductive and metabolic parameters vary across the menstrual cycle, we were not able to standardise data collection at a specific menstrual cycle stage for all women due to irregular menstrual cycling or amenorrhoea for the majority of women. Use of more precise measures of insulin resistance and body composition may also have allowed for further elucidation of the relationship between these parameters, metabolic dysfunction and SHBG. However, we removed or adjusted for the effect of potential confounders of SHBG and testosterone regulation and metabolic risk such as hormonal and non-hormonal medication use, PCOS diagnostic criteria, smoking use, age and BMI. We note this is a clinic recruited population of overweight and obese women and does not represent a random sample. As such, the implications of this research are applicable to this population studied. Expansion of this population to include lean women in addition to overweight or obese women is also warranted given the common presence of insulin resistance in lean women with PCOS. However, we performed the multiple regression analysis utilising post-OGTT glucose as a continuous variable which allows assessment of the relationship between glucose intolerance and SHBG. Direct measurement of bioavailable or free testosterone in future studies would

also be a useful addition to this field. While meta-analyses have been performed in this area previously, these contain no studies or a lesser proportion of studies specifically assessing PCOS. This highlights the strength of this current study which combines several separate clinical trials and allows assessment of the relationship between SHBG and metabolic parameters on individual patient data.

We report an independent association between SHBG and the metabolic syndrome and no association between testosterone and the metabolic syndrome in overweight and obese women with PCOS. SHBG may therefore be acting as a marker of metabolic risk independent of androgen status. It is currently unclear if the association between SHBG and metabolic health is related to its status as an indirect marker of altered metabolic health or hepatic or overall insulin resistance or as a direct mechanistic contributor. Further research should examine the contribution of SHBG to metabolic disease independent of adiposity and determine thresholds for identification of higher risk categories of PCOS to aid screening and treatment of metabolic disease.

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Author contribution: LJM, HJT, MN, PC, RN and GAW conceived of and designed the study, LJM and GAW contributed to data analysis. LJM wrote the manuscript and HJT, MN, PC, RN and GAW contributed to manuscript revision and approval of the final manuscript.

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Tables

Table 1: Anthropometric, reproductive and metabolic variables in overweight or obese women with PCOS with or without abnormal glucose tolerance or the metabolic syndrome

Variable	n	All women N=178	NGT N=162	AGT N=16	P AGT	No Met Syn N=118	Met Syn N=55	P Met Syn
Age (years)	175	33.2 ±6.3	33.0 ±6.2	35.4 ±7.6	0.165	32.8 ±6.3	34.4 ±6.3	0.135
Weight (kg)	178	94.9 ±18.7	94.4 ±19.0	99.4 ±15.1	0.306	90.3 ±17.1	103.3 ±17.5	<0.001
BMI (kg/m2)	177	35.2 ±6.4	35.0 ±6.5	36.7 ±5.2	0.318	33.6 ±5.8	38.3 ±6.2	<0.001
WC (cm)	176	104.3 ±14.4	103.8 ±14.7	108.5 ±11.2	0.217	100.9 ±13.9	110.6 ±12.3	<0.001
Testosterone (nmol/L)	177	2.6 ±1.0	2.5 ±1.0	2.7 ±1.0	0.696	2.5 ±1.0	2.6 ±1.0	0.605
SHBG	178	31.4	32.1	24.3	0.047	34.6	25.3	<0.001**

(nmol/L)		±15.0	±14.9	±14.8		±15.5	±11.5	
FAI	177	10.4 ±7.7	9.8 ±6.1	16.0 ±16.1	0.002**	9.0 ±5.7	12.3 ±6.9	0.001**
TSH (mU/L) *	172	1.5 ±1.1	1.5 ±1.1	1.4 ±1.3	0.326	1.3 ±0.9	1.7 ±1.1	<0.001**
Prolactin (mIU/L)*	172	184.0 ±144.8	181.0 ±147.0	196.0 ±121.0	0.549	182.5 ±151.5	182.0 ±127.0	0.398
Cholesterol (mmol/L)	178	5.1 ±1.0	5.1 ±1.0	5.2 ±0.9	0.648	5.1 ±1.0	5.1 ±1.1	0.766
Triglycerides (mmol/L)	178	1.3 ±0.7	1.3 ±0.7	1.8 ±0.6	0.004**	1.1 ±0.4	1.9 ±0.8	<0.001**
HDL-C (mmol/L)	178	1.3 ±0.3	1.3 ±0.3	1.2 ±0.5	0.527	1.4 ±0.3	1.1 ±0.2	<0.001**
LDL-C (mmol/L)	178	3.2 ±0.9	3.2 ±0.9	3.2 ±0.8	0.834	3.2 ±0.9	3.2 ±1.0	0.879
hsCRP	151	3.9	3.8	4.9	0.119	3.4	4.6	0.013

(mg/L)		±2.6	±2.6	±2.7		±2.4	±2.9	
Glucose 0 min (mmol/L)	178	4.8 ±0.5	4.7 ±0.5	5.1 ±0.8	0.012**	4.7 ±0.4	5.0 ±0.6	<0.001**
Glucose 120 min (mmol/L)	178	5.7 ±1.5	5.4 ±1.1	8.9 ±1.1	<0.001**	5.4 ±1.2	6.3 ±1.8	<0.001**
Insulin 0 min (mU/L) *	177	15.2 ±13.2	14.3 ±13.0	21.1 ±24.3	0.045	13.3 ±11.0	20.4 ±17.9	<0.001**
Insulin 120 min (mU/L) *	177	78.4 ±99.4	68.6 ±77.7	205.8 ±213.5	<0.001**	66.7 ±80.0	100.2 ±152.6	<0.001**
HOMA *	177	3.1 ±2.9	3.1 ±2.8	5.1 ±4.7	0.027	2.8 ±2.3	4.7 ±3.7	<0.001**
SBP (mmHg)	174	118.9 ±11.0	118.4 ±11.0	125.1 ±9.9	0.022**	115.9 ±9.4	125.7 ±11.5	<0.001**
DBP (mmHg)	174	69.6 ±8.6	69.2 ±8.6	74.5 ±7.2	0.019**	67.8 ±7.1	73.7 ±10.0	<0.001**
Family history	136	55.1%	41.4%	50%	0.541	44.9%	36.4%	0.292

T2DM (%)								
PCOS diagnostic criteria	178	NIH: 87% Non-NIH:13%	NIH: 85.8% Non-NIH:14.2%	NIH: 93.8% Non-NIH:6.3%	0.700	NIH: 85.6% Non-NIH:14.4%	NIH: 87.3% Non-NIH:12.7%	1.00

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547 Data are presented as mean±SD or median±interquartile range where not normally distributed. Data were assessed by one-way ANOVA for
548 parametric data or chi-square for categorical data with abnormal glucose tolerance or metabolic syndrome status as between subject factor with
549 adjustment for BMI for all variables except for anthropometric variables.
550 P values reflect differences between women with NGT and AGT or between women with no Met Syn or Met Syn
551 * indicates data were not normally distributed
552 ** indicates significant relationship between NGT and AGT or no Met Syn and Met Syn subgroups was maintained on adjustment for BMI
553 AGT = abnormal glucose tolerance, BMI = body mass index, DBP = diastolic blood pressure, T2DM = type 2 diabetes mellitus, FAI = free
554 androgen index, HDL-C = high density lipoprotein cholesterol, hsCRP = highly sensitive C-reactive protein, LDL-C = low density lipoprotein
555 cholesterol, NGT = normal glucose tolerance, Met Syn = metabolic syndrome, SBP = systolic blood pressure, SHBG = sex hormone binding
556 globulin, TSH = thyroid stimulating hormone, WC = waist circumference

Table 2: Logistic and linear multiple regression for metabolic dysfunction, SHBG and 0 and 120 minute oral glucose tolerance test glucose and insulin

	Significant independent predictors	Model r^2
120 minute OGTT glucose [a]	Insulin 120-minutes OGTT: β=0.418 p<0.001	r^2=0.330 p<0.001
	Age: β=0.154 p=0.033	
	Prolactin: β=-0.210 p=0.002	
Metabolic syndrome [b]	BMI: OR 1.084, 95% CI 1.034-1.170, p=0.015	r^2=0.280
	SHBG: OR 0.961, 95% CI 0.932-0.995, p=0.018	
SHBG [c]	Insulin 120-minutes OGTT: β=-0.216 p=0.014	r^2=0.153 p<0.001
	PCOS diagnostic criteria*: β=0.197 p=0.010	

Data are presented as standardised β (linear regression) or odds ratio (OR) and 95% confidence intervals (logistic regression) and were analysed by logistic (metabolic syndrome) or linear (120 minute OGTT glucose, SHBG) multiple regression.

* PCOS diagnostic criteria tests non-NIH PCOS versus the reference category of NIH criteria

[a] Model also controlled for medication use, smoking, PCOS diagnostic criteria, SHBG, testosterone, TSH, cholesterol, triglycerides, HDL-C, waist circumference

[b] Model also controlled for TSH, testosterone, insulin 120-minutes OGTT. The entire model for metabolic syndrome was statistically significant (non-significant Hosmer and Lemeshow goodness of fit test, p=0.755).

[c] Model also controlled for medication use, smoking, glucose 120-minutes OGTT, BMI, TSH, cholesterol

BMI = body mass index, HDL-C = high density lipoprotein cholesterol, OGTT = oral glucose tolerance test, PCOS = polycystic ovary syndrome, SHBG = sex hormone binding globulin, TSH = thyroid stimulating hormone, WC = waist circumference

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