The Journal of Clinical Endocrinology & Metabolism, 2025, **110**, 3380–3390
https://doi.org/10.1210/clinem/dgaf256
Advance access publication 28 April 2025
Clinical Research Article

SHBG, Testosterone, and Type 2 Diabetes Risk in Middle-Aged African Women: Exploring the Effect of HIV and Menopause

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Abstract

Context: Sex hormone–binding globulin (SHBG) and testosterone are differentially associated with type 2 diabetes (T2D) risk.

Objective: This work aimed to investigate whether the associations between SHBG, testosterone, and T2D risk differ by HIV and menopausal status in Black African women living with HIV (WH) and without HIV (WOH).

Methods: This cross-sectional observational study took place at the Health Research Unit in Soweto, Johannesburg, South Africa. A total of 81 premenopausal (57 WOH, 24 WH) and 280 postmenopausal (236 WOH, 44 WH) women from the Middle-Aged Soweto Cohort (MASC) participated. Main outcome measures included circulating SHBG and sex hormones, body composition (dual-energy x-ray absorptiometry), insulin sensitivity (Matsuda index), secretion (insulinogenic index) and clearance, and β-cell function (disposition index, DI). Dysglycemia was defined as either impaired fasting or postprandial glucose or T2D.

Results: SHBG was higher and total and free testosterone were lower in postmenopausal WH than WOH (all $P \leq .023$). Irrespective of HIV serostatus, SHBG was positively associated with Matsuda index, insulin clearance, and DI and inversely with HOMA-IR (all $P < .011$). The association between SHBG and Matsuda index was stronger in premenopausal than postmenopausal women ($P = .043$ for interaction). Free testosterone (and not total testosterone) was only negatively associated with basal insulin clearance ($P = .021$) and positively associated with HOMA-IR (homeostatic model assessment of insulin resistance) in premenopausal and not postmenopausal women ($P = .015$ for interaction).

Conclusion: We show for the first time that midlife African WH have higher SHBG and lower total and free testosterone than WOH, which corresponded to their higher β-cell function, suggesting a putative protective effect of SHBG on T2D risk in WH.

Received: 28 January 2025. **Editorial Decision:** 24 April 2025. **Corrected and Typeset:** 13 May 2025

Key Words: sex hormone–binding globulin, androgens, total testosterone, free testosterone, estrogen, HIV, postmenopausal, premenopausal, Africa

Abbreviations: ART, antiretroviral; BMI, body mass index; DI, disposition index; E2, estrogen; FM, fat mass; FMI, fat mass index; FSH, follicle-stimulating hormone; HbA_{1c}, glycated hemoglobin A_{1c}; HNF-4α, hepatocyte nuclear factor 4 α; HOMA-IR, homeostatic model assessment of insulin resistance; IFG, impaired fasting glucose; IGI, insulinogenic index; IGM, impaired glucose metabolism (IFG and/or IGT); IGT, impaired glucose tolerance; LH, luteinizing hormone; MAD, median absolute deviation; MASC, Middle-Aged Soweto Cohort; MASLD, metabolic dysfunction–associated steatotic liver disease; NGT, normal glucose tolerance; NNRTIs, nonnucleoside reverse transcriptase inhibitors; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome; SA, South Africa; SAT, subcutaneous adipose tissue; SHBG, sex hormone–binding globulin; SSA, Sub-Saharan Africa; T2D, type 2 diabetes (fasting plasma glucose ≥7.0 mmol/L and/or 120 minute post glucose load ≥11.1 mmol/L) and/or on diabetes medication; VAT, visceral adipose tissue; WH, women living with HIV; WOH, women living without HIV.

Type 2 diabetes (T2D) has reached epidemic proportions globally and is associated with obesity and aging (1). Sub-Saharan Africa (SSA) is the region with the highest projected relative increase in T2D (129% by 2045) (1), with South Africa (SA) (2) having the highest number of people living with T2D in SSA (3). Midlife SA women are disproportionately affected by T2D (4), with putative drivers being HIV, the menopausal transition, and the high prevalence of obesity (5, 6). Hormonal changes emerge as a key factor connecting these health challenges, with studies suggesting that sex hormones play a critical role in the development and progression of T2D (7-11).

The menopausal transition is characterized by an increase in the androgen to estrogen (E2) ratio, which reflects the substantial decline in E2 production and a slower rate of decline in androgen biosynthesis (9). Higher testosterone in women has been associated with increased risk for T2D (7, 11-13). However, there is evidence to suggest that the association between testosterone and T2D risk may be driven (directly or indirectly) by the effects of sex hormone–binding globulin (SHBG) (7, 14). Indeed, recent mendelian randomization studies showed an independent association between SHBG and T2D (7, 8, 15).

Given the effect of SHBG and androgens on the risk of T2D, it is important to investigate their potential independent roles in the prevalence of dysglycemia among two high-risk groups: women living with HIV (WH) and postmenopausal women. On average, SHBG decreases over the menopausal period (16), with SHBG being significantly lower in postmenopausal compared to premenopausal women (17). Results from the Women's Interagency HIV study have shown that WH have lower E2 and total testosterone, but higher SHBG, compared to women living without HIV (WOH) (18-21). However, this study did not explore the role of SHBG and androgens on insulin dynamics (ie, insulin sensitivity, insulin response, and β-cell function), which is integral to the development of T2D (22). We recently showed that SHBG was associated with incident T2D, insulin sensitivity, and β-cell function in a cohort of middle-aged Black SA men without HIV (23). This may be because the already elevated SHBG levels in men living with HIV may have reached a saturation point, limiting further effects (23). Since women have higher SHBG levels than men (24), it is important to understand how elevated SHBG affects insulin dynamics and T2D risk in WH. This is especially relevant for African women who present with a phenotype of low insulin sensitivity and hyperinsulinemia (25).

We hypothesized that higher SHBG and lower total and free testosterone in SA WH before and after menopause would be associated with favorable insulin dynamics that may protect against the development of T2D. Accordingly, the aim of the study was to investigate associations between androgen hormones, SHBG, and T2D risk in premenopausal and postmenopausal Black SA women living with and without HIV.

Materials and Methods

Design, Study Population, and Setting

Recruitment and data collection were conducted between January 2017 and August 2018 at the South African Medical Research Council/Wits Developmental Pathways for Health Research Unit at the Chris Hani Baragwanath Hospital in Soweto, Johannesburg, South Africa. The starting sample for this cross-sectional study included women (n = 501) selected from the Middle-Aged Soweto Cohort (MASC) (26). Exclusion criteria included using menopausal hormone therapy (n = 7), hormonal contraceptives (n = 30), had a hysterectomy (n = 47), had no HIV data (n = 1) or blood samples (n = 1), or were perimenopausal (n = 54), resulting in a final sample of 361 participants. The final sample included 81 premenopausal women (57 WOH, 24 WH), and 280 postmenopausal women (236 WOH, 44 WH).

The study, conducted in accordance with the Declaration of Helsinki, was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (clearance certificate No. M160604). Informed written consent was obtained from all participants.

Testing Procedures

Administered questionnaires

Interviewer-administered questionnaires were captured onto REDCap (version 14.6.7, Vanderbilt University, 2024). Data collected included age, housing density (number of people per room), and asset index (percentage from a possible 12 assets), current medication use, smoking status (current smoker/nonsmoker), and alcohol consumption (currently consumes/does not consume). Menopausal status was classified using the date of final menstrual period. Premenopause was defined as currently having a regular menstrual cycle and postmenopause was defined as cessation of the menstrual cycle for 12 months or longer (27).

HIV testing and CD4 count

Women without a previous HIV positive diagnosis completed a HIV antibody test (One Step HIV-1/2, Guangzhou Wondfo Biotech, catalog No. W050). In WH, venous blood was analyzed for CD4 count using flow cytometry (Beckman Coulter). The number of years since HIV diagnosis, number of years on HIV treatment, and the medication used were recorded.

Body composition

Weight and height were measured using standard techniques. Waist circumference was measured in the mid-axillary line at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest at the end of normal expiration, and hip circumference was measured as the greatest protrusion of the buttocks (28). Subtotal fat mass (FM) and regional

fat distribution were measured using dual-energy x-ray absorptiometry (DXA; Hologic Discovery-A [S/N 86254], APEX software version 13.4.2:3). Regional fat distribution included trunk and leg FM reported relative (%) to FM (29). Abdominal visceral (VAT) and subcutaneous adipose tissue (SAT) were estimated (30). Fat mass index (FMI) was calculated as FM (kg) divided by height squared (m^2).

Fasting venous blood and oral glucose tolerance test

At approximately 0800 hours and following an overnight fast (10-12 hours), baseline venous blood was drawn for the determination of glycated hemoglobin A_{1c} (HbA_{1c}), glucose, insulin, C-peptide, SHBG, follicle-stimulating hormone (FSH), luteinizing hormone (LH), E2, and testosterone concentrations. Participants then completed a standard 120-minute oral glucose tolerance test (OGTT). After ingestion of glucose (75 g), venous blood samples (~5 mL) were drawn at 30, 60, 90, and 120 minutes for the determination of glucose, insulin, and C-peptide concentrations. Participants with known diabetes (on medication) did not complete the OGTT. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated (31). Basal insulin clearance was estimated using the ratio of fasting C-peptide to insulin (32) and peripheral insulin sensitivity was estimated using the Matsuda Index (33). Insulin response and secretion were estimated using the insulinogenic index (IGI, [$\Delta Insulin_{0\text{-}30}$/$\Delta Glucose_{0\text{-}30}$]) (34) and C-peptide index ($\Delta C\text{-}peptide_{0\text{-}30}$/$\Delta Glucose_{0\text{-}30}$) (35), respectively. The disposition index (DI), which is an estimate of β-cell function, was calculated (IGI*Matsuda index) (34). World Health Organization criteria were used for the classification of glucose tolerance status (36). The participants were classified as normal glucose tolerance (NGT), impaired glucose metabolism (IGM) including those with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), or T2D (36). Dysglycemia was defined as the combination of IGM and T2D.

Biochemical analysis

Plasma glucose was analyzed on the Randox RX Daytona Chemistry Analyzer using enzymatic methods (Randox Laboratories Ltd). Serum insulin and C-peptide were analyzed on the Immulite 1000 Immunoassay System (Siemens Chemiluminescent Healthcare GmbH). HbA_{1c} levels were measured on whole-blood samples using the D-10 Hemoglobin Analyzer (Bio-Rad Laboratories Inc). Serum FSH, LH, and SHBG were analyzed using chemiluminescent microparticle immunoassays (Architect assays, Abbott Laboratories), and albumin was analyzed using a colorimetric (Bromcresol Green) assay (Alinity c, Abbott Laboratories). Endogenous steroid hormones (E2 and total testosterone) were quantified by ultra-high-performance liquid chromatography tandem mass spectrometry (37, 38). Free testosterone was calculated (39).

Statistical Analysis

Statistical analysis was performed using STATA version 18 (StatCorp). The Shapiro-Wilk test was used to assess the distribution of continuous variables. Unadjusted mean ± SD, median and (25th-75th percentile), or count (%) are presented for normally distributed, skewed, or categorical data, respectively. Within menopausal groups, differences between WOH and WH were assessed using unpaired *t* tests, 2-sample Wilcoxon rank sum test, or Fisher exact test for normally distributed, skewed, and categorical data, respectively. Logistic (or multinomial logistic), linear, or quantile regressions were used to explore the overall effects of HIV and menopause in the combined sample, including an HIV × menopausal interaction term. Overall group differences in sex hormones as well as glycemia and insulin parameters were adjusted for age. Associations between androgens and SHBG, and prevalent dysglycemia were explored using logistic regression, adjusting for age and FMI. Data are reported as odds ratio (95% CI). In women living without T2D, associations between SHBG, and total and free testosterone with continuous glycemic and insulin parameters, were explored using quantile regression at the 50th percentile, adjusting for age and FMI. Data are reported as β coefficient (β) (95% CI). In all logistic and quantile regressions, we explored whether HIV or menopausal status altered these relationships by including interaction terms with sex hormones in the models separately. Age was included in all models to explore the effect of menopausal status independent of chronological age. For both the logistic and quantile regression models, robust *Z* scores, calculated from the median and scaled median absolute deviation (MAD), were derived for SHBG, total and free testosterone, and the glycemic and insulin measures to facilitate comparisons of the magnitude of the associations using a standardized measure. The robust *Z* scores represent the number of MADs that a data point lies from the median. The MADs were scaled by a constant factor of 1.4826 so that the robust *Z* scores are more directly comparable to a traditional *Z* score, with 1 robust *Z* score indicating a value approximately 1 SD from the median. As missing data were minimal (<10%) and missing at random (no differences between groups), pairwise deletion was used when handling missing data.

Results

Participant Characteristics

Characteristics of participants stratified by HIV and menopausal status are described in Table 1. Postmenopausal women were older than premenopausal women, and within the postmenopausal group, WH were younger than WOH. Socioeconomic status, characterized by housing density and asset index, did not differ by HIV or menopausal group. Postmenopausal women were less likely to drink alcohol and smoke cigarettes than premenopausal women. Dietary intake and physical activity did not differ between HIV and menopausal groups (data not shown). A greater proportion of postmenopausal women reported taking antihypertensive medication compared to premenopausal women, while diabetes and lipid-lowering medications did not differ by menopause or HIV. In WH, 81% of women were taking antiretroviral (ART) therapy, with the majority (73%) using nonnucleoside reverse transcriptase inhibitors (NNRTIs) and this, together with CD4 count, did not differ by menopausal group.

Differences in Body Composition

Total body fatness (body mass index [BMI] or body fat %) and body fat distribution (waist circumference, trunk and leg fat mass, VAT and SAT areas) between the menopausal and HIV groups are reported in Table 1. The only difference between the groups was body fat percentage, which was higher in postmenopausal compared to premenopausal women. When excluding participants with T2D, weight and FMI

Table 1. Participant characteristics

	Premenopausal			Postmenopausal			*P*		
	WOH (n = 57)	**WH (n = 24)**	***P***	**WOH (n = 236)**	**WH (n = 44)**	***P***	**HIV**	**Meno**	**HIV × Meno**
Sociodemographic and lifestyle factors									
Age, y	48 (46-51)	46 (45-49)	.065	58 (54-61)	54 (50-59)	**<.001**	.208	**<.001**	.484
Housing density, people per room	1.2 (0.9-1.5)	1.0 (0.8-1.3)	.271	1.2 (0.8-1.6)	1.1 (0.6-1.6)	.447	.277	.767	.368
Asset index, % of 12	75.0 (66.7-83.3)	75.0 (58.3-91.6)	.664	75.0 (58.3-83.3)	75.0 (58.3-83.3)	.871	>.999	>.999	>.999
Current smoker, n (%)	7 (12.3)	0	.079	10 (4.2)	2 (4.5)	.931	.931	**.026**	
Current alcohol consumer, n (%)	24 (42.1)	13 (54.2)	.320	53 (22.5)	11 (25.0)	.712	.321	**.003**	.578
Medication use, n (%)									
Diabetes medications	4 (7.0)	1 (4.2)		25 (10.6)	6 (13.6)		.630	.421	.501
Hypertension medications	10 (17.5)	5 (21.7)		99 (41.9)	22 (50.0)		.664	**.001**	.942
Lipid medications	3 (5.3)	1 (4.4)		21 (8.9)	2 (4.5)		.865	.371	.710
HIV information									
Established on ART, n (%)		18 (75.0)			37 (84.1)			.221	
PIs, n (%)		0			1 (2.3)			.466	
NNRTIs, n (%)		13 (72.2)			27 (73.0)			.721	
CD4 count, cell/mm^3		474 (361-619)			611 (416-858)			.128	
CD4 WCC, cell/mm^3		5 (4-6)			5 (4-7)			.862	
CD4 lymphocytes, cell/mm^3		33 (21-40)			34 (23-40)			.463	
Body composition									
Height, cm	158.1 (154.1-162.5)	157.1 (153.0-160.2)	.267	158.2 (154.4-162.0)	156.3 (153.3-160.4)	.108	.506	.915	.844
Weight, kg	81.8 (77.0-97.4)	75.6 (63.7-88.3)	.065	81.3 (72.4-95.1)	79.9 (64.3-92.8)	.106	.755	.873	.846
BMI, kg/m^2	33.3 (29.4-37.7)	31.6 (26.8-35.7)	.121	33.1 (29.1-37.8)	33.1 (26.6-36.5)	.184	.173	.868	.271
Waist circumference, cm	95.5 (89.6-103.0)	95.5 (77.5-106.0)	.432	96.5 (89.6-105.6)	96.6 (85.1-103.8)	.384	.806	.686	.973
Subtotal body fat mass, %	43.8 (41.8-46.7)	43.8 (39.0-45.6)	.364	46.2 (42.2-49.0)	44.3 (38.6-49.3)	.170	.990	**.014**	.335
FMI, kg/m^2	14.2 ± 3.5	12.5 ± 3.7	.059	14.5 ± 4.3	13.5 ± 4.8	.151	.102	.683	.570
Leg fat mass, %	44.7 ± 7.1	43.9 ± 7.2	.681	44.2 ± 6.4	44.1 ± 7.2	.972	.803	.775	.959
Trunk fat mass, %	42.7 ± 6.3	42.8 ± 6.1	.924	43.2 ± 6.0	43.2 ± 6.3	.973	.869	.997	.703
VAT, cm^2	102 (60-128)	77 (59-108)	.223	108 (78-140)	109 (64-132)	.385	.128	.518	.196
SAT, cm^2	464 (372-515)	413 (309-535)	.274	483 (392-582)	443 (296-580)	.158	.222	.529	.723
VAT/SAT	0.21 (0.16-0.26)	0.20 (0.15-0.25)	.628	0.22 (0.18-0.27)	0.22 (0.18-0.30)	.814	.912	.277	.849

Values are median (interquartile range) or mean ± SD. Bolded values highlights statistical significance.
Abbreviations: ART, antiretroviral therapy; BMI, body mass index; FFSTM, fat-free soft tissue mass; FMI, fat mass index; NNRTIs, nonnucleoside reverse transcriptase inhibitors, PI, protease inhibitors; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WCC, white cell count; WH, women living with HIV; WHR, waist-hip-ratio; WOH, women living without HIV.

were statistically significantly lower in WH than WOH ($P = .037$ and .028, respectively; data not shown).

Differences in sex hormone–binding globulin and sex hormones

In age-adjusted analyses, LH and FSH were higher and E2 was lower in postmenopausal compared to premenopausal women (Table 2). While LH and FSH did not differ by HIV status, E2 was lower in postmenopausal WH than WOH (HIV × menopause interaction; $P < .001$), while SHBG was higher in postmenopausal WH than WOH. In contrast, total and free testosterone were lower in premenopausal and postmenopausal WH compared to WOH. Total testosterone was lower in postmenopausal compared to premenopausal women, but free testosterone and SHBG did not differ by menopausal status. The findings did not differ when excluding participants with T2D.

Table 2. Differences in sex hormone–binding globulin, sex hormones, and fasting and oral glucose tolerance test–derived parameters between menopausal and HIV groups

	Premenopausal			Postmenopausal			*P* adjusted for age		
	WOH (n = 52)	**WH (n = 21)**	***P***	**WOH (n = 225)**	**WH (n = 41)**	***P***	**HIV**	**Meno**	**HIV × Meno**
Sex hormones									
LH, IU/L	6.7 (4.0-14.5)	4.5 (3.1-12.0)	.275	21.8 (16.0-29.3)	22.7 (17.3-31.1)	.218	.495	**<.001**	.448
FSH, IU/L	11.9 (6.0-23.6)	8.3 (4.7-18.9)	.212	55.3 (40.8-69.3)	63.8 (43.8-75.3)	.092	.380	**<.001**	.128
E2, pmol/L	198.0 (77.5-560.5)	333.0 (96.0-483.0)	.991	**24.0 (15.0-39.0)**	**8.0 (4.3-19.5)**	**<.001**	**<.001**	**<.001**	**<.001**
SHBG, nmol/L	55.4 (43.4-81.7)	64.2 (51.3-111.3)	.176	**50.6 (37.8-65.2)**	**72.1 (54.2-103.0)**	**<.001**	.326	.370	.180
Total testosterone, nmol/L	**0.45 (0.25-0.56)**	**0.24 (0.18-0.34)**	**.008**	**0.35 (0.21-0.57)**	**0.25 (0.09-0.48)**	**.023**	**.026**	**.038**	.294
Free testosterone, pmol/L	**5.4 (3.5-6.9)**	**2.5 (1.3-4.3)**	**.012**	**4.8 (2.5-8.0)**	**2.0 (1.0-3.6)**	**<.001**	**.011**	.907	.921
Glycemic status, n (%)									
NGT	46 (80.7)	19 (86.4)	.703	154 (65.8)	34 (77.3)	.065	Ref	Ref	Ref
IGM	5 (8.8)	2 (9.1)		44 (18.8)	2 (4.5)		.870	.639	.188
T2D	6 (10.5)	1 (4.5)		36 (15.4)	8 (18.2)		.486	.764	.449
Dysglycemia	11 (19.3)	3 (13.6)	.555	80 (34.2)	10 (22.7)	.136	.711	.603	.861
Fasting parameters including participants with T2D									
n	57	22		232	44				
HbA_{1c}, $\%A_{1c}$	5.7 (5.3-6.3)	5.7 (5.3-5.9)	.569	6.0 (5.6-6.6)	6.1 (5.7-6.6)	.543	.698	.529	.326
Fasting glucose, mmol/L	5.0 (4.5-5.5)	5.0 (4.7-5.5)	.577	5.0 (4.5-5.7)	5.2 (4.5-5.8)	.592	.753	.262	.604
Fasting insulin, mmol/L	8.5 (5.2-14.0)	10.3 (6.3-14.0)	.697	9.0 (4.9-14.4)	9.9 (5.6-16.2)	.497	.392	.627	.756
Fasting C-peptide, nmol/L	1.6 (1.3-2.3)	1.8 (1.4-2.2)	.710	1.8 (1.3-2.5)	1.9 (1.3-2.7)	.457	.512	.522	.828
HOMA-IR	1.7 (1.2-3.6)	2.3 (1.3-3.4)	.706	2.0 (1.1-3.6)	2.4 (1.3-4.2)	.430	.257	.843	.706
Basal insulin clearance	0.20 (0.15-0.26)	0.18 (0.16-0.27)	.969	0.20 (0.16-0.27)	0.20 (0.15-0.27)	.602	.297	.599	.446
Fasting and OGTT-derived parameters excluding participants with T2D									
n	51	22		199	37				
HbA_{1c}, $\%A_{1c}$	5.6 (5.2-6.1)	5.6 (5.3-5.8)	.775	6.0 (5.6-6.3)	6.1 (5.6-6.4)	.635	.990	.174	.539
Fasting glucose, mmol/L	4.8 (4.4-5.3)	5.0 (4.7-5.4)	.577	4.9 (4.4-5.4)	5.1 (4.5-5.6)	.492	.481	.485	.638
Fasting insulin, mmol/L	8.0 (5.1-13.8)	10.2 (6.3-13.4)	.623	8.1 (4.7-13.2)	9.6 (5.4-15.7)	.357	.301	.518	.814
Fasting C-peptide, nmol/L	1.6 (1.3-2.3)	1.8 (1.4-2.1)	.696	1.7 (1.3-2.3)	1.7 (1.3-2.7)	.667	.318	.523	.485
HOMA-IR	1.7 (1.1-3.0)	2.3 (1.3-2.8)	.537	1.7 (1.0-3.2)	2.2 (1.2-3.4)	.354	.209	.660	.894
Basal insulin clearance	0.21 (0.15-0.27)	0.18 (0.16-0.27)	.840	0.21 (0.16-0.28)	0.21 (0.16-0.26)	.390	.477	.533	.696
Matsuda index	5.7 (3.1-8.1)	4.4 (3.0-8.4)	.763	5.3 (3.2-8.1)	4.9 (2.8-8.0)	.869	.390	.462	.435
Insulinogenic index	32.7 (17.5-56.2)	39.8 (19.6-56.5)	.824	**23.4 (13.0-42.3)**	**38.4 (19.5-65.5)**	**.002**	.582	.490	.357
C-peptide index	3.2 (1.8-5.6)	3.6 (2.5-4.3)	1.000	**2.8 (1.6-4.4)**	**4.0 (2.4-6.6)**	**.019**	.444	.777	.360
Disposition index	169.0 (73.5-253.2)	139.7 (63.3-232.5)	.760	**126.2 (57.1-215.4)**	**214.2 (90.6-461.9)**	**.007**	.418	.622	**.019**

Values are median (interquartile range). Bolded values highlights statistical significance.
Abbreviations: AIR_g, acute insulin response to glucose; DI, disposition index; Dysglycemia, IGM and/or T2D; E2, estrogen; FSH, follicle-stimulating hormone; HbA_{1c}, glycated hemoglobin A_{1c}; HOMA-IR, homeostatic model assessment of insulin resistance; IFG, impaired fasting glucose (fasting plasma glucose: 6.1-6.9 mmol/L); IGM, impaired glucose metabolism (IFG and/or IGT); IGT, impaired glucose tolerance (120 minute post glucose load: 7.8-11.0 mmol/L); LH, luteinizing hormone; NGT, normal glucose tolerance (fasting plasma glucose <6.1 mmol/L and 120 minute post glucose load <7.8 mmol/L); OGTT, oral glucose tolerance test; Ref, reference; T2D, type 2 diabetes (fasting plasma glucose ≥7.0 mmol/L and/or 120 minute post glucose load ≥11.1 mmol/L) and/or on diabetes medication; SHBG, sex hormone–binding globulin; S_I, insulin sensitivity; Sg, glucose effectiveness; WH, women living with HIV; WOH, women living without HIV.

Differences in glycemic and insulin parameters

Overall, 70.7% of participants presented with NGT, 14.8% with IGM, and 14.5% with T2D, and these did not differ by HIV or menopausal status (see Table 2). The overall prevalence of dysglycemia was 29.2% (95% CI, 24.6%-34.3%), with 32% of WOH presenting with dysglycemia compared to 20% of WH.

Differences in fasting parameters were evident only when excluding those with T2D (n = 309). In postmenopausal women without T2D, the IGI, C-peptide index, and DI were higher in WH compared to WOH.

Association between sex hormone–binding globulin, and total and free testosterone with glycemic and insulin parameters

In the total sample, each approximately 1-SD increase in SHBG was associated with 40% lower odds of dysglycemia (Table 3), with the association being statistically significant in WOH (OR [95% CI], 0.50 [0.35-0.71]; $P < .001$) but not in WH (OR [95% CI], 0.90 [0.62-1.31]; $P = .592$) (SHBG × HIV interaction $P = .055$; Fig. 1). The association between SHBG and dysglycemia did not differ by menopausal status.

SHBG was positively associated with Matsuda Index, DI, and basal insulin clearance, and negatively associated with fasting glucose, insulin, and HOMA-IR, irrespective of HIV status, age, and FMI (see Table 3). Notably, the association between SHBG and Matsuda Index differed by menopausal status ($P = .043$ for SHBG × menopause; Fig. 2A), with the association stronger in premenopausal (standardized β [95% CI], 0.46 [0.22-0.70]; $P < .001$) compared to postmenopausal women (standardized β [95% CI], 0.29 (0.16-0.43); $P < .001$).

Total testosterone was not associated with dysglycemia or any glycemic or insulin parameters (see Table 3). The association between free testosterone and HOMA-IR was statistically significant only in the premenopausal women (standardized β [95% CI], 0.40 [0.13-0.67]; $P = .005$) and not the postmenopausal women (standardized β [95% CI], 0.05 [−0.02 to 0.12]; $P = .200$). Free testosterone was negatively associated with basal insulin clearance, independent of age, HIV status, and FMI.

Discussion

This is the first study to report sex hormones in Black African women at different stages of the menopausal transition, explore differences by HIV serostatus, and investigate how SHBG and testosterone levels relate to T2D risk. We showed that SHBG was higher in postmenopausal WH compared to WOH. Notably, higher SHBG was associated with a T2D-protective phenotype, including higher insulin sensitivity, β-cell function, and basal insulin clearance, as well as lower fasting glucose, insulin, and HOMA-IR, regardless of HIV status. In contrast, total and free testosterone were lower both in premenopausal and postmenopausal WH than WOH. Lower free testosterone was linked to higher basal insulin clearance, and lower HOMA-IR in premenopausal, but not postmenopausal, women. These findings support our hypothesis that higher SHBG and lower free testosterone in WH compared to WOH may be associated with reduced risk of T2D. This study also provides a putative mechanism underlying the lower prevalence of T2D in WH and obesity compared to women living with obesity but without HIV (40). However, longitudinal studies are required to understand the clinical implications of the findings, particularly as premenopausal women transition into postmenopause.

Higher SHBG levels have been consistently and causally associated with lower risk for T2D both in men and women (7, 8, 12, 14-16, 23, 41, 42). Accordingly, one may hypothesize that the higher SHBG in postmenopausal WH may confer

Table 3. Associations between sex hormone–binding globulin, androgen hormones, and glycemic and insulin parameters

Parameter	SHBG (scaled robust *Z* score)		Total testosterone (scaled robust *Z* score)		Free testosterone (scaled robust *Z* score)	
	Odds ratio (95% CI)	***P***	**Odds ratio (95% CI)**	***P***	**Odds ratio (95% CI)**	***P***
Dysglycemia	**0.60 (0.45-0.79)**[a]	**<.001**	0.95 (0.80-1.12)	.540	1.20 (0.12-11.97)	.876
Standardized values (scaled robust *Z* scores)	**β (95% CI)**	***P***	**β (95% CI)**	***P***	**β (95% CI)**	***P***
HbA_{1c}	−0.08 (−0.18 to 0.02)	.106	−0.06 (−0.12 to 0.01)	.106	−0.05 (−0.12 to 0.02)	.195
Fasting glucose	**−0.13 (−0.22 to −0.04)**	**.003**	−0.02 (−0.09 to 0.05)	.541	−0.01 (−0.09 to 0.06)	.673
Fasting insulin	**−0.12 (−0.21 to −0.03)**	**.011**	0.07 (−0.003 to 0.14)	.060	0.06 (−0.01 to 0.12)	.079
HOMA-IR	**−0.14 (−0.23 to −0.05)**	**.003**	0.06 (−0.02 to 0.13)	.132	**0.05 (−0.02 to 0.11)**[a]	.176
Basal insulin clearance	**0.14 (0.04 to 0.24)**	**.005**	−0.06 (−0.14 to 0.02)	.147	**−0.09 (−0.16 to −0.01)**	**.021**
Insulinogenic index	−0.03 (−0.15 to 0.09)	.592	0.0002 (−0.08 to 0.08)	.995	0.03 (−0.05 to 0.11)	.535
C-peptide index	0.10 (−0.03 to 0.22)	.139	−0.05 (−0.14 to 0.03)	.204	0.001 (−0.08 to 0.08)	.977
Matsuda index	**0.31 (0.21 to 0.41)**[b]	**<.001**	−0.04 (−0.12 to 0.04)	.354	−0.04 (−0.13 to 0.04)	.289
Disposition index	**0.29 (0.16 to 0.42)**	**<.001**	−0.02 (−0.11 to 0.08)	.690	−0.01 (−0.11 to 0.78)	.770

Results from logistic and quantile regression models adjusted for age, fat mass index, and HIV status. Robust *Z* scores, calculated from the median and MAD, were derived for SHBG, total and free testosterone, and the glycemic and insulin measures to facilitate comparisons of the magnitude of the associations. The robust *Z* scores represent the number of MADs that a data point lies from the median of the variable in the data set. The MADs were scaled by a constant factor of 1.4826 so that the robust *Z* score is more directly comparable to a traditional *Z* score. Bolded values highlights statistical significance.

Abbreviations: HbA_{1c}, glycated hemoglobin A_{1c}; HOMA-IR, HOMA-IR, homeostatic model assessment of insulin resistance; MAD, median absolute deviation; SHBG, sex hormone–binding globulin.

[a]Sex hormone × HIV interaction;

[b]Sex hormone × menopausal status interaction.

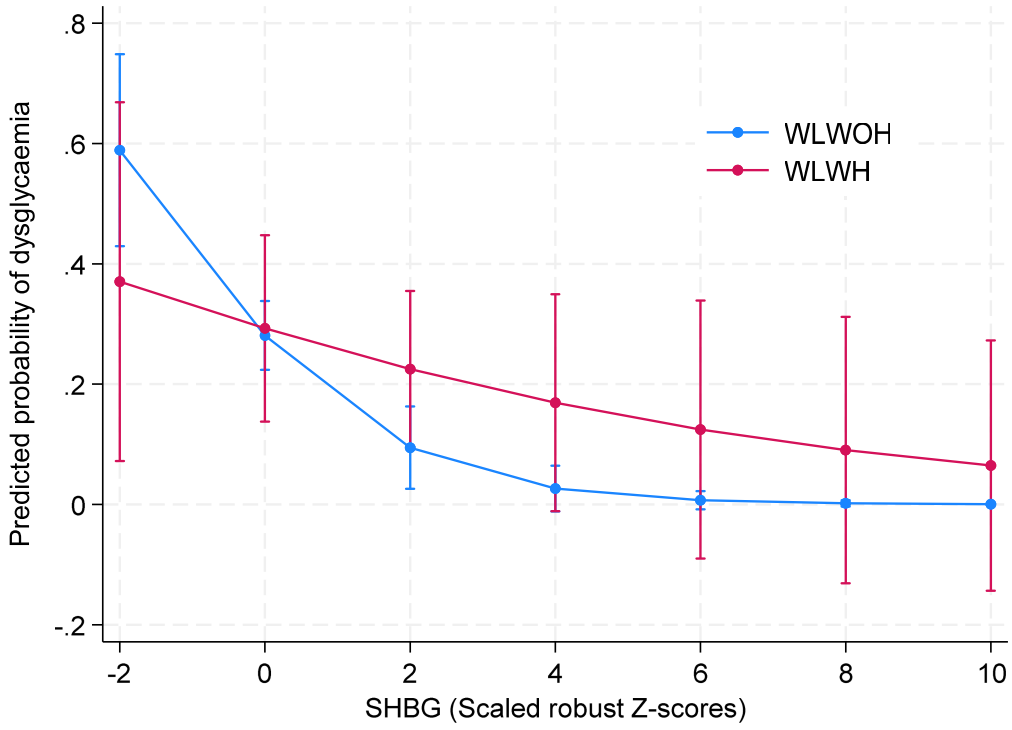

Figure 1. The association between sex hormone–binding globulin (SHBG) and prevalent dysglycemia by HIV status ($P = .055$ for SHBG × HIV interaction). Higher SHBG was associated with lower odds of dysglycemia in women living without HIV (WOH) (OR; 95% CI, 0.50; 0.35-0.71; $P < .001$) but not in women living with HIV (WH) (OR (95% CI): 0.90 (0.62-1.31; $P = .592$). Model adjusted for age and fat mass index. SHBG presented as scaled robust *Z* scores, calculated from the median and median absolute deviation (MAD), with MAD scaled by a constant factor of 1.4826 so that the robust *Z* score is comparable to a traditional *Z* score.

reduced risk for T2D compared to WOH. Indeed, higher SHBG levels in postmenopausal WH were linked to greater β-cell function compared to WOH. Further, SHBG was also associated with lower risk of T2D-related traits, such as lower fasting glucose and insulin, and higher insulin sensitivity, with these associations being independent of FM and unaffected by HIV serostatus. Similar alterations in SHBG and androgens have been observed in middle-aged African men, along with significant associations between SHBG, insulin dynamics, and incident T2D. However, these associations were significant only in men without HIV and not those living with HIV (23). The reasons for these differences are unclear, but the association between SHBG and T2D risk is stronger in women, suggesting sexually dimorphic effects (13). Further, recent results from the KORA study reported higher SHGB levels in women compared to men contributed to sex differences in fasting glucose and incident T2D (24).

Higher SHBG levels in postmenopausal WH than WOH is consistent with previous studies (18-20). Higher SHBG has previously been linked to chronic viral infections including HIV, especially in women and those with HIV RNA greater than 400 copies/mL (20). The regulation of SHBG is complex, but proinflammatory cytokines like interleukin-1β and tumor necrosis factor α reduce SHBG messenger RNA expression by downregulating its main transcription factor, hepatocyte nuclear factor 4 α (HNF-4α) (43-45). While cytokines were not measured in this study, we can only speculate that elevated SHBG levels in WH may be a compensatory mechanism to protect against systemic inflammation associated with HIV (20). SHBG is also downregulated by hepatic de novo lipogenesis (46), and SHBG has been shown to be the mediator of the association between intrahepatic lipid content and T2D (47). Notably, adiponectin, which activates adenosine 5′-monophosphotase and suppresses hepatic lipid accumulation, also increases SHBG via upregulation of HNF-4α (48). Although we did not measure intrahepatic lipid content in this study, we have previously shown that WH have higher adipose messenger RNA expression and circulating adiponectin levels than WOH (49). Further, there is evidence to suggest that people living with HIV with metabolic dysfunction–associated steatotic liver disease (MASLD) have lower intrahepatic lipid content than their HIV-seronegative controls (50). Taken together, these findings suggest that hepatic lipid content and adiponectin may mediate SHBG elevation in WH, although further studies are required to verify this hypothesis.

SHBG levels are also regulated by sex hormones (51-53). Mendelian randomization studies have shown that higher SHBG is inversely associated with free testosterone, but not with total testosterone (7). Indeed, our study shows that higher SHBG in the postmenopausal WH was accompanied with lower free testosterone concentrations, which corroborates the findings of the Women's Interagency HIV study (18). While E2 is known to positively regulate SHBG (53), we observed lower E2 levels in postmenopausal WH, suggesting a direct effect of HIV or ARTs on SHBG production. Since most of the women were being treated with NNRTIs, it is difficult to differentiate the effect of HIV from those of ARTs on endocrine function. Further studies are needed to clarify the mechanism underlying these hormonal alterations.

Nonetheless, our study showed that the association between SHBG and prevalent dysglycemia was statistically significant only in WOH. We postulate that the lower total and free testosterone concentrations in WH may confer reduced risk for T2D, with higher levels of SHBG having no additional benefit. In contrast, in WOH who present with higher total

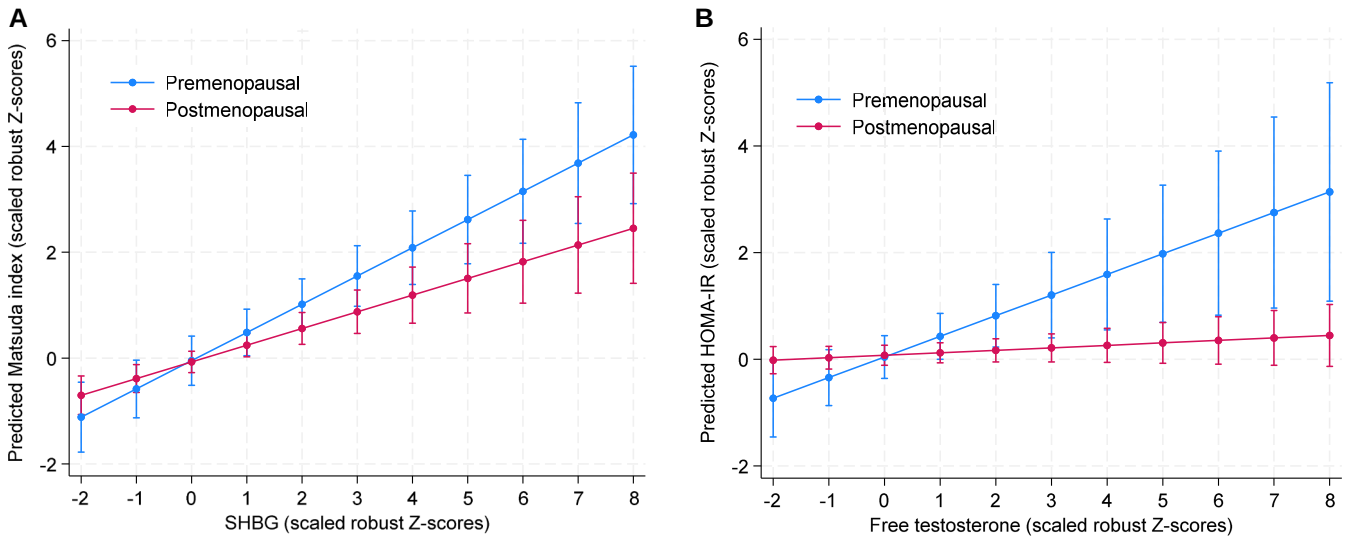

Figure 2. The association between sex hormone–binding globulin (SHBG) and free testosterone and insulin parameters by menopausal status, adjusting for age, HIV status, and fat mass index. A, The association between SHBG and insulin sensitivity (Matsuda index) differs by menopausal status ($P = .043$ for SHBG × menopause), with the association being stronger in premenopausal (standardized β [95% CI], 0.46 (0.22-0.70); $P < .001$) compared to postmenopausal women (standardized β [95% CI], 0.29 (0.16-0.43); $P < .001$). B, The association between free testosterone and HOMA-IR differs by menopausal status ($P = .015$ for free testosterone × menopause), with the association statistically significant only in the premenopausal women (standardized β [95% CI], 0.40 (0.13-0.67); $P = .005$) and not the postmenopausal women (standardized β [95% CI], 0.05 (−0.02 to 0.12); $P = .200$).

and free testosterone levels, higher SHBG may reduce T2D risk by lowering the bioavailability of testosterone. This is supported by our findings, which showed that the association between SHBG and insulin sensitivity was stronger in premenopausal women, who have higher total testosterone levels compared to postmenopausal women (Fig. 2A). A recent mendelian randomization study confirmed a causal link between the bioavailability of testosterone and increased risk of T2D in women (7). Similar to our study and others (14, 16, 21, 42, 54), the mendelian randomization study showed no association between total testosterone and T2D risk, suggesting that the association between SHBG and bioavailable testosterone is likely driven by SHBG, either directly or in combination with free testosterone (7).

The exact mechanisms by which SHBG may influence T2D risk are not fully understood. However, SHBG has been shown to act as a hepatokine, mediating the link between intrahepatic lipids, insulin sensitivity, and T2D risk, with stronger effects in women than men (47, 55). SHBG has also been shown to protect against endoplasmic reticulum stress (56, 57), and it has anti-inflammatory and lipolytic properties (58). Our research has also shown that overexpressing SHBG in mice protects against development of MASLD and obesity by different molecular mechanisms (59-61). Studies to determine whether intrahepatic lipids mediate the association between SHBG and T2D risk in our context are required. Further, SHBG binds to GPRC6A, stimulating insulin release in a dose-dependent manner (62), providing a potential mechanism for the observed relationships between SHBG and β-cell function.

Our study showed that free testosterone was associated with lower basal insulin clearance and higher HOMA-IR in premenopausal women. This could be due to the lower total androgen load after menopause. These results are supported by other studies showing that higher free testosterone in premenopausal women is associated with reduced hepatic insulin clearance and lower insulin sensitivity, but not with obesity-related insulin secretion (63). Other studies also suggest that the relationship between free testosterone and incident T2D is mediated by adiposity and insulin resistance (42). Taken together, our results indicate that higher SHBG, coupled with lower free testosterone levels, may confer reduced risk for T2D in WH.

Despite significantly lower E2 in the postmenopausal women, no differences in body composition or T2D risk were observed between the menopausal groups, findings contrary to others (64). However, others have shown that Black African women may not gain as much abdominal adiposity across the menopause transition due to both higher abdominal adiposity and smaller fluctuations in sex steroid hormones in the years leading up to menopause (65). In our study, the majority (67.1%) of the women were living with obesity, limiting the potential for further increases in body fat and related changes in glycemic and insulin parameters. A comparative study conducted in SSA revealed no significant differences in anthropometric and cardiometabolic risk factors between premenopausal and postmenopausal women from SA and East Africa who presented with a high prevalence of obesity (32%-66%) (5, 66). In contrast, women from West Africa, with a lower prevalence of obesity (1%-5%), showed distinct variations in cardiometabolic risk between menopausal stages (5).

Strengths and Limitations

This study comprehensively phenotypes Black African women, including OGTT-derived measures of glycemia and insulin dynamics, as well as high-precision measurement of sex hormones using ultra-high-performance liquid chromatography tandem mass spectrometry. The study is however limited by the cross-sectional design that precludes inferences about causality, and limits our understanding of the long-term effect of changes in SHBG and testosterone on the risk of T2D. No adjustments were made for multiple comparisons, which may increase the chance of type 1 error. Free testosterone was not

measured, and we relied on calculated free testosterone (39). In addition, the relatively small sample of premenopausal women and WH limits our analysis and interpretation of the results, and the exclusion of perimenopausal women, those on contraceptives, and with a history of hysterectomy may have led to selection bias in the sample. Further, sex hormone measurements in premenopausal women were not taken at a set time during the menstrual cycle; however, we do not believe that the phase of menstrual cycle influenced the androgen results as differences in these hormones by HIV serostatus were consistent at each menopausal stage. While we excluded all participants on menopausal hormone therapy or hormonal contraceptives from the analysis, we have no information on the minimum time without these treatments. A further limitation to the study is the lack of information on liver disease and polycystic ovary syndrome (PCOS). It is well known that hepatic steatosis and fibrosis alters SHBG production, hepatic glucose metabolism, and the risk for T2D (46, 47). Similarly, PCOS is closely associated with SHBG, hyperandrogenism, obesity, insulin resistance, and T2D (67). Inadvertently including participants with PCOS in the analysis may confound the relationship between SHBG and T2D risk.

Conclusion

For the first time, we show that midlife Black African women with HIV have higher SHBG, and lower total and free testosterone concentrations compared to their HIV-negative counterparts, which may contribute to their lower T2D risk. These findings highlight a complex relationship between androgenic hormones, HIV, menopause, and T2D risk. However, longitudinal studies are required to determine the clinical implications of these associations across the menopausal transition.

Acknowledgments

We are grateful to the participants of the MASC and the research staff who were involved in this study.

Funding

This work was jointly supported by the South African Medical Research Council via the South African National Department of Health, the Medical Research Council (United Kingdom) (via the Newton Fund), the GlaxoSmithKline Africa Non-Communicable Disease Open Lab (grant project No. ES/N013891/1), and the South Africa National Research Foundation (grant No. UID:99108). The AWI-Gen Collaborative Centre is funded by the National Human Genome Research Institute, the National Institute of Environmental Health Sciences, the Office of AIDS Research, and the National Institute of Diabetes and Digestive and Kidney Diseases, of the National Institutes of Health under award number U54HG006938, as part of the H3Africa Consortium, and by the Department of Science and Innovation, South Africa, award number DST/CON 0056/2014. I.D.S. is also supported by the Fogarty International Center grant number D43TW010937 (University of Pittsburgh HIV Comorbidities Research Training Program in South Africa—Pitt-HRTP-SA, principal investigators Prof Jean B. Nachega and Prof Soraya Seedat) and the Department of Science and Innovation and the South Africa National Research Foundation Professional Development Programme. J.H.G., A.P.L., and I.D.S. are supported by the South African Medical Research Council. The contents of this publication are solely the responsibility of the authors and do not represent the official views of the sponsors.

Disclosures

The authors have nothing to disclose.

Data Availability

Data are available from the authors on reasonable request.

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