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Interrelation Between Sex Hormones and Plasma Sex Hormone-Binding Globulin and Hemoglobin A1c in Healthy Postmenopausal Women

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Abstract

Background: Androgenicity, as measured by low sex hormone-binding globulin (SHBG) and elevations in testosterone and free androgen index (FAI), is associated with adverse cardiovascular (CV) outcomes, possibly due to effects on insulin resistance and glycemia.
Methods: Glycosylated hemoglobin (HbA1c) concentration, SHBG, and sex hormones were available in 200 nondiabetic postmenopausal women who were not using hormone therapy (HT) in the Women's Health Study. Of these, 98 were cardiovascular disease (CVD) cases; the remainders were matched controls. To achieve normality, continuous values were log transformed and geometric means were calculated. Associations between sex hormones and HbA1c were examined using general linear models (GLM), partial correlations, and multiple linear regression analyses.
Results: Lower SHBG levels and higher FAI and HbA1c values were found among the CVD cases, and all analyses were adjusted for this factor. In GLM, higher values of HbA1c were observed in the highest quartiles of FAI and the lowest quartiles of SHBG. However, the correlation between SHBG and HbA1c across quartiles was eliminated after adjusting for body mass index (BMI). In partial correlations, HbA1c values were inversely associated with SHBG ($r = -0.19$, $P = 0.008$) and positively associated with FAI ($r = 0.19$, $P = 0.01$), even after adjusting for age, CVD case–control status, and BMI. In multivariate models, a significant inverse association between SHBG and HbA1c persisted, as well as a significant positive association between FAI and HbA1c.
Conclusions: Androgenicity, as measured by low SHBG and high FAI, is associated with glycemia, and thereby may contribute to CVD risk in postmenopausal women.

Introduction

Androgenicity, as measured by low sex hormone-binding globulin (SHBG) and elevations in testosterone concentrations and free androgen index (FAI), has been associated with adverse cardiovascular (CV) risk factors and CV outcomes in women.[1–6] These negative effects may be partially related to the influence of androgens on glucose and insulin metabolism. FAI and bioavailable testosterone are positively correlated with glucose, insulin, and insulin resistance.[6–9] Experiments in oophorectomized rats demonstrate that testosterone administration impairs insulin-mediated glucose uptake by inhibition of glycogen synthase expression.[10] Similarly, studies in women using hyperglycemic and euglycemic hyperinsulinemic clamp techniques suggest that, although testosterone administration does not affect endogenous glucose production, it decreases glucose utilization, inducing insulin resistance and mild elevations in glycemia.[11,12] Simultaneously, low levels of hyperglycemia may evoke androgen production via an insulin-mediated mechanism because insulin has been shown to stimulate testosterone release from ovarian stroma and theca.[13,14]

Low SHBG levels have also been associated with impaired glucose tolerance.[15] *In vitro* studies in hepatic cell lines have demonstrated that insulin is a potent inhibitor of SHBG production.[16] Data from healthy women reveal a negative correlation between plasma SHBG and insulin as evinced by a

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stepwise decrease in SHBG levels with increases in plasma insulin concentrations.[17,18] Case–control and prospective studies further reinforce the association between androgenicity and insulin and glucose metabolism. Postmenopausal diabetic women have repeatedly been found to have higher levels of free testosterone than nondiabetic controls.[19,20] Similarly, higher testosterone levels and lower SHBG levels appear to predict incident type 2 diabetes mellitus (T2DM), independent of fasting insulin concentrations.[21–23]

Whereas several studies have examined the relationship between androgenicity and both insulin resistance and diabetes, the influence of androgenicity on glycosylated hemoglobin (HbA1c) values has not been analyzed previously in postmenopausal women. Recent investigations have demonstrated that increased HbA1c levels, even within the normal range, are associated with increased cardiovascular mortality.[24–27] The relationship between androgenicity and glycemia may have an important influence on cardiovascular disease (CVD) risk in postmenopausal women. Therefore, we examined the relationship between HbA1c levels and testosterone, FAI, and SHBG in a nested case–control study of women from the Women's Health Study.

Materials and Methods

Participants

The Women's Health Study is a randomized, double-blind, placebo-controlled trial of low-dose aspirin and vitamin E for the primary prevention of CVD and cancer among female health professionals aged 45 years and older and free of known CVD and cancer (except for nonmelanoma skin cancer).[28] Of the 39,876 participants enrolled from November, 1992, to July, 1994, 28,345 (71%) provided fasting baseline blood samples.

We used SHBG, FAI, total testosterone, estradiol, free estrogen index (FEI) values, and HbA1c concentrations from a prior CVD nested case–control study of 229 postmenopausal women, who did not use hormone therapy.[2] Furthermore, current hormone users were excluded because exogenous hormones drastically alter endogenous hormone levels. We also excluded 28 women with a history of diabetes and 1 woman who had HbA1c equal or greater than 8.5%. Of the 200 women with available data, 98 were cases of CVD, whereas 102 matched controls were not.[2] The study protocol was approved by the Brigham and Women's Hospital Institutional Review Board for Human Subjects Research.

Assays

Before randomization, all blood samples were collected in citrate and ethylenediamine-tetra-acetic acid (EDTA) and stored in liquid nitrogen freezers at −70°C until the time of analysis. Estradiol levels were measured at Quest Diagnostics (Capistrano, CA) by radioimmunoassay preceded by extraction and purification by Celite column chromatography.[29] The lower limit of detection for the assay was 5 pg/mL; the coefficient of variation was 9.5% for estradiol. Estradiol levels from citrated plasma were slightly lower than from EDTA (r = 0.89). Total testosterone levels and SHBG assays were performed at the Massachusetts General Hospital Reproductive Endocrine Laboratory (Boston, MA). Total testosterone was measured using a solid-phase radioimmunoassay (Diagnostic Products Corporation), with a lower limit of sensitivity of 4.0 ng/dL. SHBG was measured using a fully automated system (Immunolite, Diagnostic Products Corporation), which used a solid-phase, two-site immunometric assay. Coefficients of variation for SHBG and total testosterone were 4.5% and 4.8%, respectively. To estimate free testosterone (nonprotein bound), the FAI was calculated as the molar ratio of total testosterone/SHBG multiplied by 100, which is highly correlated with free testosterone.[30,31] To estimate the free estradiol concentration, the free estrogen index (FEI) was calculated as the molar ratio of total estradiol/SHBG.[30] A single measurement of sex hormones has previously been demonstrated to be relatively stable in postmenopausal women over a 2- to 3-year period.[32]

HbA1c was analyzed using a Roche diagnostic assay; details are described elsewhere.[25] HbA1c levels have been shown to be stable for approximately 1 week at 4°C and demonstrate long-term stability when stored below −70°C.[33,34] The HbA1c assay has been approved for clinical use by the United States Food and Drug Administration (FDA) and has been certified by the National Glycohemoglobin Standardization Program.

Statistical analysis

Because of the skewed distribution of sex hormones and HbA1c values, these covariates were natural logarithm (ln) transformed to achieve normality and then geometric means were obtained.

First, the baseline characteristics were compared according to CVD case–control status. The chi-square was used to assess for differences in proportions for categorical covariates, whereas paired Student t-tests were used for continuous covariates. Second, quartiles for endogenous hormones were created based on the distribution of values in the study population, and HbA1c values were compared by the General Linear Models (GLM) procedure across the quartiles of sex hormones and SHBG. A linear test of trend was performed across the quartiles. In addition, correlations between HbA1c concentrations, SHBG, and hormone levels were assessed using partial (Spearman) correlation coefficients. For all analyses, additional adjustments for age at randomization, body mass index (BMI) and CVD case–control status were performed. Baseline BMI (self-reported weight in kilograms divided by the square of self-reported height in meters [kg/m^2]) was analyzed as a continuous covariate.[35] Self-reported weight was highly correlated (r = 0.96) with a similar population of female health professionals.[36]

Finally, stepwise forward multivariable linear regression models between log- transformed HbA1c and each sex hormone in separate were performed and unstandardized beta-coefficients were calculated. Adjustments for the same potential confounding covariates mentioned before were also performed. A two-tailed P value of 0.05 was considered to represent a statistically significant result. Data were analyzed using SAS software (version 8.0, SAS Institute, Inc., Cary, NC).

Results

Baseline characteristics of the 200 postmenopausal women in the study are presented in Table 1 according to their CVD case–control status. Women who were actively using hormone therapy at baseline, had a history of diabetes, or an HbA1c level ≥8.5% were excluded. As expected, women with CVD events had significantly higher BMI values ($P = 0.006$). Furthermore, SHBG levels were significantly lower, whereas FAI ($P = 0.03$) and HbA1c ($P = 0.05$) values were higher among women who developed CVD compared to controls. No significant differences in age, hormone therapy (HT) use, or levels of total estradiol, testosterone, or FEI were observed according to presence or absence of CVD during follow up. All subsequent analyses controlled for presence or absence of CVD during follow up.

As shown in Table 2, a consistent and significant increase in HbA1c values across increasing quartiles of FAI was found, which persisted even after adjustment for CVD status, age, and BMI (P for trend = 0.04). HbA1c values decreased across higher SHBG quartiles adjusted for CVD during follow up and age (P for trend = 0.003), but adjustment for BMI eliminated a significant association. No significant trends in HbA1c concentrations across quartiles of testosterone, estradiol, and FEI were found.

In partial correlations, HbA1c values were inversely associated with SHBG ($r = -0.19$, $P = 0.008$) and positively associated with FAI ($r = 0.19$, $P = 0.01$), even after adjustment for age, CVD during follow-up, and BMI (Table 3). In multivariable linear models adjusted for age, BMI, and CVD during follow up, a significant inverse association between SHBG and HbA1c persisted, while a significant positive association between FAI and HbA1c was found (Table 4). Each unit change in ln-transformed SHBG was associated with a decrease of 0.03 units in HbA1c levels. On the other hand, each unit change in ln-transformed FAI was associated with an increase of 0.02 units in HbA1c.

Discussion

Overall, we found that HbA1c was positively associated with FAI and inversely associated with SHBG levels among nondiabetic postmenopausal women not using hormone therapy. The relationship between FAI and HbA1c concentrations persisted, even after adjustment for BMI. Although the association between SHBG and HbA1c concentrations did not persist across the quartiles of SHBG (Table 2) after adjustment for BMI, in analyses of partial correlations and linear multiple regression models the association remained significant, even after adjusting for BMI (Tables 3 and 4).

Although the observed association between FAI and HbA1c levels has not been described previously, this finding is consistent with previous observations.[7,21] In a cohort of 1956 postmenopausal women from the Multi-Ethnic Study of Atherosclerosis, a significant association between bioavailable testosterone and impaired fasting glucose was observed.[7] Women in the highest quartile of bioavailable testosterone had more than a two-fold increased odds of having impaired fasting glucose.[7] Similarly, among postmenopausal women in the prospective Rancho Bernardo Study who were not taking hormone therapy, bioavailable testosterone was positively correlated with fasting glucose levels and with glucose levels during oral glucose tolerance testing.[21] In addition, baseline evaluation of the 845 postmenopausal women participating in the Postmenopausal Estrogen/Progestin Intervention (PEPI) trial revealed a linear association between bioavailable testosterone and insulin resistance.[8]

The observed association between HbA1c and SHBG in this study is also compatible with the findings of previous

Table 1. Baseline Characteristics of Postmenopausal Women According to Cardiovascular Disease Status in the Women's Health Study

	HT nonusers (n = 200)		
Characteristics	*Cases (n = 98)*	*Controls (n = 102)*	*P value*[a]
Hormone therapy (%)			
Never	55.4	58.3	0.68
Past	44.6	41.7	
Mean age, years (± SD)	64.8 ± 7.0	65.0 ± 6.8	0.84
Mean BMI (± SD), kg/m^2	28.1 ± 5.9	25.1 ± 4.8	0.006
SHBG, nmol/L[b]	41.95	48.4	0.05
Testosterone, ng/dL[b]	19.90	18.10	0.28
Estradiol, pg/mL[b]	10.25	10.91	0.50
FAI, nmol/L[b]	0.02	0.01	0.03
FEI, pmol/L[b]	0.001	0.001	0.51
HbA1c, %[b]	5.20	5.01	0.05

[a]P values were obtained from paired Student t-test for continuous variables and chi-square for categorical variables.

[b]Geometric means.

Abbreviations: HT, hormone therapy; SD, standard deviation; BMI, body mass index; SHBG, sex hormone-binding globulin; FAI, free androgen index; FEI, free estrogen index; HbA1c, glycosylated hemoglobin.

Table 2. HbA1c Concentrations by Hormonal Quartile Among 200 Non-HT Using Postmenopausal Women in the Women's Health Study

Serum markers	*Model 1*[a]	*Model 2*[b]
SHBG, nmol/L[c]		
Quartile 1	5.34	5.31
Quartile 2	5.14	5.11
Quartile 3	5.08	5.111
Quartile 4	5.09	5.14
P for trend	0.003	0.09
Testosterone, ng/dL[c]		
Quartile 1	5.10	5.11
Quartile 2	5.19	5.21
Quartile 3	5.13	5.12
Quartile 4	5.24	5.22
P for trend	0.12	0.37
Estradiol, pg/mL[c]		
Quartile 1	5.07	5.12
Quartile 2	5.17	5.19
Quartile 3	5.23	5.22
Quartile 4	5.15	5.08
P for trend	0.25	0.25
FAI, nmol/L[c]		
Quartile 1	5.04	5.09
Quartile 2	5.12	5.13
Quartile 3	5.22	5.20
Quartile 4	5.28	5.24
P for trend	0.003	0.04
FEI, nmol/L[c]		
Quartile 1	5.07	5.12
Quartile 2	5.07	5.09
Quartile 3	5.21	5.20
Quartile 4	5.29	5.22
P for trend	0.22	0.64

[a]Model 1, adjusted for development of CVD and age.
[b]Model 2, adjusted for development of CVD, age, and BMI.
[c]Geometric means.
Abbreviations: HbA1c, glycosylated hemoglobin; HT, hormone therapy; SHBG, sex hormone-binding globulin; FAI, free androgen index; FEI, free estrogen index.

Table 3. Partial Correlation Coefficients for HbA1c Concentrations and Sex Hormones Among 200 Non-HT Using Postmenopausal Women in the Women's Health Study

	Model 1[a]		*Model 2*[b]	
Serum marker	*r*	*P value*	*r*	*P value*
SHBG, nmol/L[c]	−0.26	0.0003	−0.19	0.008
Testosterone, ng/dL[c]	0.13	0.08	0.08	0.25
Estradiol, pg/mL[c]	−0.04	0.58	−0.03	0.68
FAI nmol/L[c]	0.25	0.0004	0.19	0.01
FEI nmol/L[c]	0.18	0.01	0.09	0.19

[a]Model 1, adjusted for development of CVD and age.
[b]Model 2, adjusted for development of CVD, age, and BMI.
[c]HbA1c and sex hormones were log-transformed values.
Abbreviations: HbA1c, glycosylated hemoglobin; HT, hormone therapy; SHBG, sex hormone-binding globulin; FAI, free androgen index; FEI, free estrogen index.

studies. Cross-sectional data reveal an independent inverse association between SHBG and impaired glucose tolerance in women, although this finding is less consistent among postmenopausal women.[15,18,22,23] Additionally, SHBG appears to be an independent predictor of T2DM.[22,23]

In the present analyses, we did not observe an association between total testosterone levels and HbA1c. The absence of an association is consistent with other studies that failed to identify a relationship between total testosterone and impaired fasting glucose, insulin resistance, and incident T2DM.[7,8,21] This is likely because total testosterone levels only partially reflect bioactive androgen exposure.

This study has several strengths and limitations. To our knowledge, this is the first study to investigate the relationship between sex hormones and SHBG and HbA1c in nondiabetic women. The association between HbA1c and androgenicity has not previously been described. Although only a single measurement of hormones was used for each participant, levels have been shown to be relatively stable in postmenopausal women.[32] Additionally, although we did not directly measure free estrogen or free androgen levels, calculated FEI and FAI correlate well with measured values.[30,31] Although we adjusted for CVD case–control status, age, and BMI, residual confounding by other anthropometrical indexes that are not measured is possible. Lastly, due to the cross-sectional nature of this study, it is not possible to determine whether androgenicity and elevations in HbA1c are causally related. Indeed, on the basis of our data, it is not possible to address directly the interrelationship between CVD and sex hormones. However, data suggesting both an independent effect of testosterone administration on glucose use[10–12] and a direct effect of insulin on androgen release[14,17] raise the possibility that the cause–effect relationship between androgenicity and HbA1c may be bidirectional.

Higher glycemia, even within the "normal" range, may be associated with increased CVD events and mortality.[24–27] In this study, there was a difference of approximately 0.15 units in HbgA1c between extreme quartiles of SHBG and FAI for nondiabetic women. Although these differences are relatively small, they may contribute to the overall impact of sex hormones on the risk of CVD in postmenopausal women. A HbA1c level of ≥5.2 was associated with increased CVD mortality in women.[25]

In this study of postmenopausal, nondiabetic women, a more androgenic profile, characterized by higher FAI and lower SHBG levels, was generally associated with higher HbA1c levels. Prospective studies in postmenopausal women are needed to enhance our understanding of the relationship between androgenicity and glycemia and consequently their contribution to CVD risk.

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Table 4. Stepwise Forward Multivariable Linear Regression Models With HbA1c as Dependent variable Among 200 Nonhormone-Using Women

	HbA1c[c]		
	Beta coefficient[b]	*Standard error*	*P value*
Model 1[a]			
SHBG[c]	−0.04	0.00961	0.0003
Total testosterone[c]	0.01	0.00834	0.09
Estradiol[c]	−0.003	0.00820	0.55
FAI[c]	0.02	0.00057	0.0004
FEI[c]	0.01	0.00594	0.008
Model 2[d]			
SHBG[c]	−0.03	0.01058	0.01
Total testosterone[c]	0.009	0.00835	0.24
Estradiol[c]	−0.003	0.00846	0.68
FAI[c]	0.02	0.00614	0.01
FEI[c]	0.01	0.00672	0.19

Each hormone in separate model.

[a]Model 1, adjusted for development of CVD and age.

[b]Beta coefficient represents the unit change in ln-transformed HbA1c for every 1-unit increase in ln-transformed sex hormone concentrations.

[c]HbA1c and sex hormones were log transformed values.

[d]Model 2, adjusted for development of CVD, age, BMI.

Abbreviations: HbA1c, glycosylated hemoglobin; SHBG, sex hormone-binding globulin; FAI, free androgen index; FEI, free estrogen index; CVD, cardiovascular disease; BMI, body mass index.

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