Sex Hormones and Measures of Kidney Function in the Diabetes Prevention Program Outcomes Study

Catherine Kim,[1] Ana C. Ricardo,[2] Edward J. Boyko,[3] Costas A. Christophi,[4] Marinella Temprosa,[4,5] Karol E. Watson,[6] Xavier Pi-Sunyer,[7] and Rita R. Kalyani,[8] for the Diabetes Prevention Program Research Group

[1]Departments of Medicine, Obstetrics & Gynecology, and Epidemiology, University of Michigan, Ann Arbor, Michigan 48109; [2]Department of Medicine, University of Illinois, Chicago, Illinois 60612; [3]Department of Medicine, University of Washington, Veterans Affairs Puget Sound Health Care System, Seattle, Washington 98108; [4]Biostatistics Center, George Washington University, Rockville, Maryland 20852; [5]Department of Epidemiology & Biostatistics, George Washington University, Washington, DC 20852; [6]Department of Medicine, University of California, Los Angeles, California 90095; [7]Department of Medicine, Columbia University College of Physicians and Surgeons, New York City, New York 10032; and [8]Department of Medicine, Johns Hopkins University, Baltimore, Maryland 21287

ORCiD numbers: 0000-0001-9237-0532 (C. Kim).

Context: Despite sex differences in chronic kidney disease (CKD) onset and progression, it is unclear whether endogenous sex hormones are associated with kidney function in persons without CKD.

Design and Methods: We conducted a secondary analysis of the Diabetes Prevention Program (DPP) and its follow-up observational study, the DPP Outcomes Study, over 11 years. Participants included overweight and glucose-intolerant men (n = 889) and pre- and postmenopausal women (n = 1281) not using exogenous sex hormones and whose urine albumin-to-creatinine ratio (ACR) was <30 mg/g and normal estimated glomerular filtration ratio (eGFR) was ≥60 mL/min/1.73 m^2 at randomization. We examined the association between sex hormone levels and incidence of low eGFR and/or ACR ≥30 mg/g on at least one measurement.

Results: At randomization, the mean (SD) eGFR was 94 (15) mL/min/1.73 m^2; the median ACR (interquartile range) was 4.5 (3.3 to 7.6) mg/g. During follow-up, 187 men (24.6%) and 263 women (24.2%) had incident albuminuria and 136 men (17.9%) and 123 women (11.3%) had incident low eGFR. Among men, higher baseline sex hormone–binding globulin (SHBG) level was associated with reduced low eGFR risk (hazard ratio per SD, 0.80; 95% CI, 0.57 to 0.90) in adjusted analyses. No significant associations were observed among women. There were significant interactions between sex steroid levels and low eGFR by randomization arm.

Conclusion: Sex steroids were not associated with development of low eGFR or albuminuria. Among men, higher SHBG level was associated with reduced risk of low eGFR on at least one measurement. **(*J Clin Endocrinol Metab* 104: 1171–1180, 2019)**

Kidney function differs between women and men: Reports have noted that women and men have different measured glomerular filtration rates (1) and more women than men have chronic kidney disease (CKD), defined as estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 and/or the presence of albuminuria (2). Studies of animal models and *in vitro* reports suggest that the sex hormones that characterize sex differences may contribute to kidney disease (3–7). However, the relationship between endogenous sex hormones

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Abbreviations: ACE, angiotensin converting enzyme; ACR, albumin-to-creatinine ratio; BMI, body mass index; CKD, chronic kidney disease; CRP, C-reactive protein; CV, coefficient of variation; DHEAS, dehydroepiandrosterone; DPP, Diabetes Prevention Program; DPPOS, Diabetes Prevention Program Outcomes Study; eGFR, estimated glomerular filtration rate; HR, hazard ratio; SHBG, sex hormone–binding globulin.

and kidney function has not been examined in healthy women. Results of studies do not agree regarding the effects of postmenopausal estrogen therapy upon eGFR (8–10) and albuminuria (11–13).

Similarly, few reports of endogenous sex hormone concentrations and markers of kidney function exist in healthy men without end-stage renal disease (*i.e.*, those who are dialysis dependent). Results of two cross-sectional studies suggested that lower testosterone concentrations are associated with poorer eGFR among men (14, 15), with one small study noting that lower dehydroepiandrosterone sulfate (DHEAS) concentrations were associated with higher urinary albumin excretion among men with diabetes (16). Another report indicated higher estradiol levels may also be associated with more advanced CKD among men after adjustment for other CKD risk factors (15). To our knowledge, other sex steroids such as dihydrotestosterone have not been examined in relation to kidney markers, although dihydrotestosterone, rather than testosterone, is associated with incident diabetes (17), cardiovascular disease, and mortality (18), and administration of dihydrotestosterone and not testosterone affects sodium channel expression in rat models (19).

The Diabetes Prevention Program (DPP) was a randomized controlled trial in which men and women who were overweight and glucose intolerant were enrolled (20). The follow-up study, the Diabetes Prevention Program Outcomes Study (DPPOS), is in its 15th year and has continued to ascertain incident diabetes as well as microvascular complications including nephropathy (21). Estimated GFR and albumin-to-creatinine ratio (ACR) were assessed at baseline for the entire cohort, and serum measures of sex hormone–binding globulin (SHBG), dihydrotestosterone, testosterone, estradiol, estrone, and DHEAS were measured in stored samples. For the current report, we examined whether sex hormone concentrations were cross-sectionally associated with measures of kidney function, as estimated by eGFR, and kidney damage, as represented by ACR. We also examined whether endogenous sex hormone concentrations at baseline predicted at least one measure of subsequent low eGFR and/or incident albuminuria among the subset of participants without abnormalities in eGFR and albuminuria at baseline. On the basis of published reports in men, we hypothesized that lower total testosterone and higher estradiol concentrations would be associated with increased risk of low eGFR and higher risk of albuminuria in men. On the basis of reports of estrogen therapy in women, we hypothesized that higher endogenous estradiol levels would be associated with lower risk of reduced eGFR and higher risk of albuminuria in women.

Design and Methods

Participants

The design, methods, and baseline characteristics of the DPP have been described (22). Briefly, 3234 participants were recruited across 27 clinical centers located throughout the United States. Inclusion criteria were age ≥25 years, body mass index (BMI) ≥24 kg/m^2 (≥22 kg/m^2 for Asian Americans), a fasting plasma glucose level of 5.3 to 6.9 mmol/L (95 to 125 mg/dL), and a 2-hour plasma glucose level of 7.8 to 11.0 mmol/L (140 to 199 mg/dL) after an oral 75-g glucose load, with exclusion criteria previously described (21). Eligible participants were randomly assigned to one of three interventions: 850 mg of metformin twice daily, placebo twice daily, or lifestyle intervention. The goals of lifestyle intervention were to achieve and maintain a weight reduction of ≥7% through consumption of a low-calorie, low-fat diet, plus moderate physical activity for ≥150min/wk. Due to the possibility of random assignment to metformin use, men were excluded if their serum creatinine level was ≥1.4 mg/dL and women were excluded if their serum creatinine level was ≥1.3 mg/dL, and adults older than 80 years were excluded if their creatinine clearance was <75 mL/min based on a 24-hour urine collection (22). Use of thiazide diuretics or β adrenergic antagonists was an exclusion criterion, but other classes of antihypertensive agents including angiotensin-converting enzyme (ACE) inhibitors and angiotensin-receptor blockers were permitted. Each participating institution was overseen by its respective ethics review board.

At the conclusion of the DPP, participants had been followed for an average of 3.2 years. The placebo and metformin groups were unmasked as to their treatment assignment, and all participants were offered the lifestyle intervention in a group format during a 1-year bridge period (23). The surviving consenting members (n = 3149) of the three original treatment arms were invited to participate in DPPOS; 88% (n = 2776) joined. Maintenance group lifestyle sessions were offered quarterly to all DPPOS participants and metformin continued to be provided to participants originally randomly assigned to metformin treatment who remained eligible. The DPPOS is ongoing and participants in the current analysis had been followed for an average of 15 years since random assignment.

A substudy was conducted of participants who approved use of their blood samples for secondary analyses and who had sufficient prerandomization sera samples. Of the 1043 male participants, sex hormone levels were measured for 970 who were not using exogenous sex steroids at DPP baseline or DPP 1-year follow-up, and thus were included for this analysis. Of the 2191 female participants, sex hormone levels of 2008 were measured. Of these women, 1406 were not using exogenous sex steroids at baseline or 1-year follow-up, and thus were included for this analysis. In addition, we excluded two women who had testosterone levels >11 nmol/L in analyses of testosterone, and three women and one man with elevated estrone and estradiol concentrations consistent with exogenous supplementation.

Sex hormone measurements

Sex hormones were measured by Endoceutics (Quebec City, QC, Canada). SHBG levels were measured using an ELISA (Bioline, Taunton, MA) with interassay coefficients of variation of 7.8% and 5.0% at 18.2 nmol/L and 63.1 nmol/L, respectively. Sex steroids were measured using gas chromatography/mass spectrometry (24). Bioavailable testosterone concentrations that

incorporated testosterone and SHBG concentrations were calculated and also examined for men (25). The lower limit of detection for DHEAS was 20 ng/mL and for dihydrotestosterone, testosterone, estradiol, and estrone were 2, 10, 0.2, and 10 pg/mL, respectively. The lower limit of quantification for DHEAS was 100 ng/mL and for dihydrotestosterone, testosterone, estradiol, and estrone were 10, 50, 1, and 4 pg/mL, respectively. Interassay variation (coefficient of variation) was 8.9%, 10.7%, 7.0%, and 12.5% for dihydrotestosterone, testosterone, estradiol, and estrone, respectively, at the lower limits of quantification level. Values were extrapolated below the lower limit of quantification using MassHunter software (Agilent, Santa Clara, CA); briefly, because standard curves for estradiol and testosterone gave excellent coefficients of determination ($R^2 > 0.998$) with a *y*-intercept near zero, it can be acceptable to extrapolate concentration values with acceptable accuracy and precision using linear regression weighted 1/concentration.

Covariate measurements

At the time of random assignment during DPP, a screening questionnaire enquired about women's menses. Women were classified as postmenopausal if they reported absence of menses for at least 1 year, bilateral oophorectomy, and/or hysterectomy and age ≥55 years. BMI, blood pressure, history of smoking, and medication use information were obtained on annual examination using published methods (22). Glucose and insulin values, lipid profiles, and high-sensitivity C-reactive protein (CRP) were performed at the Central Biochemistry Laboratory (Northwest Lipid Research Laboratories, University of Washington, Seattle, WA) as previously reported (26). Insulin sensitivity was assessed by the homeostatic model assessment of insulin resistance (HOMA-IR) (27).

Kidney function measurements

At DPP baseline and annually during the DPPOS, serum and urinary creatinine concentrations were measured using Roche reagents on the Hitachi 917 autoanalyzer (Boehringer Mannheim, Mannheim, Germany). For serum creatinine, inter- and intra-assay CVs were 3.5% and 3.2%, respectively; urinary creatinine inter- and intra-assay CVs were 1.8% and 1.2%, respectively. We calculated eGFR using the 2009 Chronic Kidney Disease Epidemiology collaboration serum creatinine equation (28). Urinary albumin excretion was estimated from a morning fasting spot-urine sample by the ACR (*i.e.*, milligrams of albumin per gram of creatinine). Urinary albumin concentrations were measured using Behring reagents on the BN II nephelometer (Dade Behring, Deerfield, IL); inter- and intra-assay CVs were 4.4% and 4.3%, respectively.

Statistical analysis

Men and women were examined separately. For baseline characteristics, categorical variables are reported as number (%), and quantitative variables are reported as mean (SD) or median (interquartile range) with normal and skewed distributions, respectively (Table 1). Because of its skewed distribution, ACR was log transformed when examined as a continuous variable. For 184 participants with ACR values below detection limit, a value of 0.23 was assigned (29).

Linear regression models were created to examine cross-sectional associations between baseline measures of sex hormone levels with baseline measures of eGFR and ACR (Table 2). For these models, each regression unit was the standard deviation of the sex hormone value to enable comparisons of the strength of association across sex hormones. Models were adjusted for known risk factors for kidney disease at DPP baseline, including age, race or ethnicity, BMI, systolic and diastolic blood pressures, homeostatic model assessment of insulin resistance, high-density lipoprotein and CRP levels, and lack of protective medications including ACE inhibitors, angiotensin receptor blockers, and calcium channel blockers. Two interaction terms were evaluated: between sex hormones and menopausal status in women and between sex hormones and race or ethnicity. Interactions terms were not significant at $P < 0.10$, and thus results pool associations across racial or ethnic and menopausal groups.

Next, we examined the relationship between sex hormone levels and presence of low eGFR or albuminuria during the DPP and the DPPOS, using Cox proportional hazards models in men (Table 3) and women (Table 4). In these longitudinal models, only men (n = 889) and women (n = 1281) without kidney disease at baseline were included. In the longitudinal models, incident reduction in eGFR was defined as eGFR <60 mL/min/1.73 m^2 and incident albuminuria was defined as ACR ≥30 mg/g (2). Each regression unit was the standard deviation of the sex hormone to enable comparisons of the strength of association across sex hormones. Multivariable models were adjusted for baseline covariates noted in cross-sectional models, as well as baseline eGFR and baseline log ACR. The initial set of models stratified by DPP randomization arm and evaluated for heterogeneity of treatment group, and another set of models adjusted for randomization arm. Because not all associations were homogeneous across treatment group, stratified analyses are presented. The proportional hazards assumption of the models was confirmed using residuals.

We conducted several sensitivity analyses. First, we examined the relationship between sex hormone levels and change in eGFR and change in albuminuria, using linear regression models in men and women. We used repeated measures analysis (mixed models) the coefficients provide estimates of the increase or decrease in eGFR or the log of the albuminuria value over time from baseline for each SD increase in the hormone at baseline. In these models, we also examined associations in premenopausal women and postmenopausal women separately. Finally, we examined whether associations observed between SHBG level and renal function differed by strata of BMI. Analyses were performed using the SAS, version 9.2 (SAS Institute) and all tests were two sided with statistical significance set at $P < 0.05$.

Results

Table 1 lists participant characteristics at baseline by sex. The median (SD) age of men and women was 54 (11) years and 47 (10) years, respectively; approximately one-fifth of men and 45% of women were younger than 45 years. Among the women, 908 were premenopausal and 498 were postmenopausal. Approximately half of the participants were non-Hispanic white. In accord with DPP enrollment criteria, participants were overweight or obese and had elevated glucose levels. The majority of participants had blood pressure and lipid levels within normal range and did not smoke or use ACE inhibitors, angiotensin receptor blockers, or calcium channel blockers.

Table 1. Participant Characteristics at DPP Baseline Among Participants in the Sex Hormone Substudy

	Men (n = 969)	Women (n = 1406)	*P* Value
Treatment group			0.11
Placebo	311 (32.1)	502 (35.7)	
Metformin	338 (34.9)	440 (31.3)	
Intensive lifestyle intervention	320 (33.0)	464 (33.0)	
Age, y	53.9 ± 11.1	47.3 ± 10.2	<0.001
Age group, y			<0.001
25-44	204 (21.1)	632 (45.0)	
45-59	468 (48.3)	591 (42.0)	
≥60	297 (30.7)	183 (13.0)	
Race or ethnicity			<0.001
Non-Hispanic white	569 (58.7)	699 (49.7)	
African American	151 (15.6)	362 (25.7)	
Hispanic	162 (16.7)	246 (17.5)	
Asian	77 (7.9)	41 (2.9)	
American Indian	10 (1.0)	58 (4.1)	
Weight, kg	98.6 ± 19.4	94.3 ± 20.8	<0.001
Waist, cm	108.1 ± 13.3	104.7 ± 15.2	<0.001
BMI, kg/m^2	32.1 ± 5.6	35.6 ± 7.0	<0.001
Fasting glucose, mg/dL	108.7 ± 8.4	106.3 ± 8.1	<0.001
2-Hour glucose measurement, mg/dL	164.3 ± 16.9	164.2 ± 17.1	0.90
Fasting insulin, L/mIU	23.0 [16.0, 32.0]	25.0 [17.0, 35.0]	0.001
Systolic blood pressure, mm Hg	125.9 ± 13.9	122.3 ± 14.9	<0.001
Diastolic blood pressure, mm Hg	80.0 ± 9.4	77.7 ± 9.3	<0.001
High-density lipoprotein cholesterol, mg/dL	40.2 ± 8.8	46.2 ± 10.7	<0.001
Current cigarette use	70 (7.2)	105 (7.5)	0.82
ACE inhibitor use	75 (7.7)	75 (5.3)	0.018
Angiotensin-receptor blocker use	8 (0.8)	12 (0.9)	0.94
Calcium channel blocker use	79 (8.2)	67 (4.8)	<0.001
CRP, mg/L	0.31 ± 0.52	0.69 ± 0.76	<0.001
DHEAS, ng/mL	999.4 [600.4, 1551.6]	771.4 [478.4, 1154.5]	<0.001
Testosterone, nmol/L	10.95 [8.58, 13.66]	0.58 [0.42, 0.83]	<0.001
Estradiol, pg/mL	22.32 [18.16, 27.34]	32.31 [10.62, 83.12]	<0.001
Estrone, pg/mL	38.20 [31.48, 47.28]	83.43 [52.88, 126.47]	<0.001
SHBG, nmol/L	38.87 [26.39, 55.85]	39.74 [29.02, 59.24]	0.003
eGFR, mL/min/1.73 m^2	93.99 ± 15.70	102.49 ± 16.41	<0.001
eGFR at baseline, mL/min/1.73 m^2			<0.001
≥90	588 (60.7)	1065 (75.7)	
60-89	369 (38.1)	337 (24.0)	
45-59	11 (1.1)	4 (0.3)	
30-45	1 (0.1)	0 (0.0)	
ACR median (interquartile range), mg/g	4.72 [3.41, 8.51]	5.96 [3.98, 10.73]	<0.001
ACR category, mg/g			0.78
<30	898 (93.8)	1285 (93.2)	
30-300	56 (5.9)	88 (6.4)	
>300	3 (0.3)	6 (0.4)	

Data given as mean ± SD, median (interquartile range), or no. (%), unless otherwise indicated. Data in brackets indicate median (interquartile range).

Sixty percent of men and 76% of women had eGFR >90 mL/min/1.73 m^2, and approximately 93% of both men and women had ACR <30 mg/g.

Table 2 lists the cross-sectional associations between baseline sex hormone measures with baseline measures of continuous eGFR and ACR before and after adjustment for covariates in linear regression models. Among men, before adjustment for covariates, higher DHEAS concentration was associated with higher eGFR, and higher dihydrotestosterone, testosterone, and SHBG concentrations were associated with lower eGFR. However, after adjustment for covariates, higher concentrations of estradiol were significantly associated with lower eGFR, and higher concentrations of DHEAS were associated with higher levels of the log of the ACR. Among women, before adjustment for covariates, lower DHEAS, testosterone, estradiol, and SHBG levels were associated with lower eGFR. Higher DHEAS, testosterone, and SHBG levels were associated with higher levels of albuminuria. However, in adjusted models, only estrone was associated with eGFR.

During the 11 years of follow-up in DPPOS, albuminuria developed in 187 men (24.6%) and 263 women (24.2%) on at least one measurement and eGFR <60 mL/min/1.73 m^2

Table 2. Cross-Sectional Associations Between Continuous Measures of Sex Hormones and eGFR and ACR

	eGFR			log(ACR)		
Model	**No.**	**β^a**	***P* Value**	**No.**	**β^a**	***P* Value**
Men: unadjusted models						
DHEAS	963	4.134	<0.001	951	−0.010	0.717
Dihydrotestosterone	957	−1.858	<0.001	945	−0.027	0.339
Testosterone	956	−1.729	<0.001	944	−0.028	0.330
Estradiol	958	−0.742	0.142	946	0.036	0.207
Estrone	957	0.517	0.308	945	0.052	0.068
SHBG	968	−3.429	<0.001	956	0.017	0.558
Men: adjusted models[b]						
DHEAS	960	−0.884	0.068	948	0.075	0.023
Dihydrotestosterone	954	0.194	0.662	942	−0.015	0.609
Testosterone	953	−0.452	0.299	941	−0.010	0.733
Estradiol	955	−1.430	<0.001	943	0.031	0.277
Estrone	954	−0.021	0.960	942	0.043	0.130
SHBG	965	−0.174	0.696	953	−0.022	0.476
Women: unadjusted models						
DHEAS	1368	2.375	<0.001	1342	−0.074	0.003
Testosterone	1342	2.380	<0.001	1319	−0.043	0.087
Estradiol	1344	2.528	<0.001	1321	−0.013	0.587
Estrone	801	1.240	0.022	787	0.028	0.390
SHBG	1403	1.944	<0.001	1376	−0.050	0.040
Women: adjusted models[b]						
DHEAS	1364	−0.350	0.386	1338	−0.041	0.116
Testosterone	1338	0.607	0.115	1315	−0.026	0.281
Estradiol	1340	0.581	0.138	1317	0.019	0.458
Estrone	798	1.097	0.026	784	0.037	0.240
SHBG	1399	0.112	0.778	1372	−0.034	0.187

[a]The β coefficients express changes of kidney measure per SD unit increase of sex hormone in unadjusted and adjusted analyses.

[b]Adjusted for race or ethnicity; age at random assignment; BMI; systolic and, diastolic blood pressures; levels of fasting glucose, 2-hour glucose, fasting insulin, LDL, high-density lipoprotein, HbA1c, and CRP; use of ACE inhibitors, angiotensin-receptor blockers, and calcium channel blockers; and menopause status. None of the interactions between race and hormone or menopause and hormone (women) were statistically significant, so adjusted models are run in the overall group adjusted for race and/or menopause.

occurred in 136 men (17.9%) and 123 women (11.3%) on at least one measurement (Tables 3 and 4). Because of the heterogeneity of the associations by randomization arm in men and women for several hormone measures, we present pooled and stratified results by randomization arm; the pooled models are adjusted for randomization arm. Among men originally randomly assigned to placebo, 65 (25.4%) had incident albuminuria and 39 (15.2%) had incident low eGFR. Among men originally randomly assigned to lifestyle modification, 64 (25.6%) had incident albuminuria and 47 (18.8%) had incident low eGFR. Among men randomly assigned to receive metformin, 58 (22.9%) had incident albuminuria and 50 (19.8%) had incident low eGFR.

Table 3 lists the associations between baseline sex hormone measures and incident low eGFR and/or albuminuria among men after adjustment for covariates. Higher concentrations of SHBG at baseline were associated with lower risk of low eGFR in pooled analyses. For testosterone, estradiol, estrone, and dihydrotestosterone, association between sex hormone levels and incident low eGFR varied across randomization arm (*P* for test of homogeneity < 0.05), although the number of incident events was small within each randomization arm. Among men randomly assigned to placebo who thus underwent minimal intervention for diabetes risk reduction, higher levels of testosterone, bioavailable testosterone, estradiol, and estrone were protective from low eGFR, in contrast with the lifestyle group, which had higher risk for low eGFR with higher baseline testosterone levels. No sex hormone measures at baseline were associated with incident albuminuria.

Among women originally randomly assigned to the placebo group, incident albuminuria developed in 101 (25%) and incident low eGFR developed in 47 (11.7%). Among women originally randomly assigned to lifestyle modification, incident albuminuria developed in 82 (23.6%) and incident low eGFR developed in 35 (10.1%). Among women randomly assigned to receive metformin, incident albuminuria developed in 80 (23.6%) and incident low eGFR developed in 41 (12.1%). Table 4 lists the associations between baseline sex hormone measures and incident abnormalities among women after adjustment for covariates.

Among women, no sex hormones were consistently associated with low eGFR or albuminuria across randomization

Table 3. Among Men, Associations Between Sex Hormone Levels and Risk of Low eGFR or Albuminuria by Randomization Arm and Pooled With Adjustment for Randomization Arm

	Low eGFR or Albuminuria			Low eGFR[a]			Albuminuria[b]		
	HR[c]	95% CI		HR[c]	95% CI		HR[c]	95% CI	
Placebo (n = 256 overall)	n = 89 events			n = 39 events			n = 65 events		
DHEAS	1.03	0.77	1.37	1.31	0.82	2.09	0.91	0.65	1.26
Dihydrotestosterone	0.91	0.74	1.12	0.67[d]	0.45	1.02	0.97	0.77	1.23
Testosterone	0.87	0.66	1.14	0.58[d]	0.38	0.90	1.01	0.75	1.35
Bioavailable testosterone	0.58	0.12	2.84	0.05	0	0.82	0.81	0.13	4.90
Estradiol	0.87	0.69	1.10	0.52[d]	0.33	0.80	1.05	0.82	1.36
Estrone	0.79[d]	0.62	1.01	0.51[d]	0.32	0.81	0.88	0.67	1.16
SHBG	0.89	0.69	1.14	0.77	0.51	1.15	1.01	0.76	1.35
Lifestyle (n = 250 overall)	n = 91 events			n = 47 events			n = 64 events		
DHEAS	1.00	0.78	1.28	0.89	0.59	1.35	0.95	0.68	1.31
Dihydrotestosterone	1.11	0.87	1.41	1.38[d]	0.98	1.96	0.96	0.70	1.30
Testosterone	1.03	0.79	1.34	1.50	1.07	2.12	0.87	0.63	1.21
Bioavailable testosterone	2.37	0.53	10.6	8.97	1.13	71.4	1.34	0.21	8.41
Estradiol	1.14	0.92	1.41	1.19[d]	0.89	1.59	1.02	0.78	1.35
Estrone	1.23[d]	0.99	1.53	1.22[d]	0.93	1.62	1.16	0.88	1.54
SHBG	0.80	0.63	1.01	0.82	0.63	1.07	0.77	0.56	1.07
Metformin (n = 253)	n = 89 events			n = 50 events			n = 58 events		
DHEAS	0.91	0.67	1.25	1.12	0.69	1.79	0.85	0.57	1.25
Dihydrotestosterone	0.99	0.79	1.26	0.94[d]	0.68	1.30	0.97	0.71	1.34
Testosterone	1.08	0.88	1.32	1.13[d]	0.86	1.48	1.00	0.76	1.33
Bioavailable testosterone	2.94	0.72	12.1	7.68	1.09	54.2	1.08	0.18	6.64
Estradiol	0.93	0.76	1.14	0.82[d]	0.61	1.10	0.89	0.68	1.16
Estrone	0.98[d]	0.81	1.19	0.96[d]	0.72	1.28	0.94	0.72	1.21
SHBG	0.83	0.64	1.07	0.78	0.55	1.10	0.86	0.62	1.19
Pooled	n = 269 events			n = 136 events			n = 187 events		
DHEAS	0.96	0.82	1.12	1.03	0.82	1.31	0.89	0.74	1.08
Dihydrotestosterone	0.99	0.88	1.12	0.96	0.80	1.15	0.99	0.85	1.15
Testosterone	0.99	0.86	1.13	1.01	0.84	1.21	0.98	0.83	1.16
Bioavailable testosterone	1.60	0.69	3.67	2.24	0.70	7.18	1.21	0.44	3.31
Estradiol	0.97	0.86	1.09	0.89	0.75	1.06	0.98	0.85	1.13
Estrone	0.98	0.87	1.11	0.99	0.83	1.18	0.97	0.84	1.12
SHBG	0.85	0.74	0.97	0.80	0.68	0.95	0.89	0.75	1.06

[a] eGFR <60 mL/min/1.73 m^2.

[b] ACR ≥30 mg/g.

[c] HRs represent the incident kidney marker abnormality per change in standard deviation of sex hormone adjusted for baseline eGFR and log of ACR and randomization arm, age at random assignment, race or ethnicity, BMI, systolic and diastolic blood pressures, homeostatic model assessment of insulin resistance, levels of high-density lipoprotein and CRP, and use of ACE inhibitors, angiotensin-receptor blockers, and calcium channel blockers.

[d] $P < 0.05$ for homogeneity across treatment group in hormone and incident abnormalities.

arms. The association between DHEAS levels and incident abnormalities varied across randomization arm (P for test of homogeneity < 0.05). As with men, the number of incident events was small within each randomization arm. Sex hormones were not associated with impaired eGFR among participants randomly assigned to lifestyle or metformin treatment groups, with the exception of higher DHEAS concentrations among women randomly assigned to receive metformin. Bioavailable testosterone concentrations in women were low, with minimal variability, and estimates of association were unstable and thus not included.

In sensitivity analyses, higher concentrations of SHBG were associated with lesser declines in eGFR among men randomly assigned to lifestyle intervention [hazard ratio (HR), 1.09; 95% CI, 0.025 to 2.15] and metformin (HR, 1.17; 95% CI, 0.039 to 2.31), as well as pooled across study arms (HR, 0.96; 95% CI, 0.31 to 1.61). Other sex steroids were not associated with changes in eGFR, and no sex hormones were associated with changes in the log of the albuminuria value. Similarly, among women, sex hormones were not associated with changes in eGFR or changes in albuminuria. We did not observe that associations between sex steroid levels and declines in renal function were more pronounced in postmenopausal women than in premenopausal women. Finally, the association between SHBG level and risk of low eGFR was similar in men with BMI <30 kg/m^2 (HR, 0.78; 95% CI, 0.61 to 0.99) compared with men with BMI ≥30 kg/m^2 (HR, 0.75; 95% CI, 0.58 to 0.98).

Table 4. Among Women, Associations Between Sex Hormones and Risk of at Least One Measure of eGFR <60 mL/min/1.73 m^2 or ACR ≥30 mg/g by Randomization Arm and Pooled With Adjustment for Randomization Arm

	Low eGFR or Albuminuria			Low eGFR[a]			Albuminuria[b]		
	HR[c]	95% CI		HR[c]	95% CI		HR[c]	95% CI	
Placebo (n = 402 overall)	n = 131 events			n = 47 events			n = 101 events		
DHEAS	1.04	0.86	1.27	0.99[d]	0.68	1.45	0.99	0.79	1.23
Testosterone	1.11	0.92	1.34	1.05	0.78	1.42	1.04	0.82	1.31
Estradiol	0.94	0.76	1.18	0.80	0.50	1.29	1.02	0.81	1.29
Estrone	1.14	0.94	1.38	0.99	0.66	1.50	1.13	0.93	1.37
SHBG	0.86	0.67	1.11	0.54	0.26	1.10	0.94	0.73	1.21
Lifestyle (n = 348 overall)	n = 107 events			n = 35 events			n = 82 events		
DHEAS	0.95	0.75	1.21	0.67[d]	0.42	1.07	1.06	0.81	1.38
Testosterone	1.08	0.88	1.33	0.99	0.68	1.45	1.03	0.81	1.30
Estradiol	1.12	0.88	1.41	0.64	0.28	1.47	1.21	0.95	1.54
Estrone	1.16	0.89	1.52	1.64	0.56	4.78	1.16	0.88	1.53
SHBG	0.90	0.69	1.18	1.10	0.62	1.97	0.83	0.61	1.12
Metformin (n = 339 overall)	n = 105 events			n = 41 events			n = 80 events		
DHEAS	0.99	0.78	1.26	1.69[d]	1.11	2.57	0.87	0.66	1.14
Testosterone	1.06	0.88	1.29	0.96	0.66	1.39	1.06	0.85	1.31
Estradiol	1.09	0.90	1.32	1.05	0.76	1.46	1.02	0.82	1.26
Estrone	0.88	0.66	1.18	1.58	0.66	3.79	0.86	0.64	1.16
SHBG	1.07	0.88	1.31	1.08	0.71	1.65	1.08	0.87	1.33
Pooled (n = 1089 overall)	n = 343 events			n = 123 events			n = 263 events		
DHEAS	0.99	0.87	1.12	0.94	0.75	1.18	0.97	0.84	1.11
Testosterone	1.11	0.99	1.24	1.01	0.83	1.22	1.06	0.93	1.20
Estradiol	1.05	0.93	1.18	0.87	0.65	1.15	1.08	0.95	1.22
Estrone	1.06	0.93	1.22	1.03	0.74	1.43	1.05	0.91	1.21
SHBG	0.94	0.82	1.08	0.82	0.58	1.15	0.95	0.82	1.10

[a]eGFR <60 mL/min/1.73 m^2.

[b]ACR ≥30 mg/g.

[c]HRs represent the incident kidney marker abnormality per change in standard deviation of sex hormone adjusted for baseline eGFR and log of ACR and randomization arm, age at random assignment, race or ethnicity, BMI, systolic and diastolic blood pressures, homeostatic model assessment of insulin resistance, levels of high-density lipoprotein and CRP, and use of ACE inhibitors, angiotensin-receptor blockers, and calcium channel blockers.

[d]$P < 0.05$ for homogeneity across treatment group in hormone and incident abnormalities.

Discussion

Although epidemiologic reports have noted sex differences in the prevalence of kidney disease, few studies, to our knowledge, have examined whether endogenous sex steroid measures are associated with eGFR and albuminuria. No reports we found included women, and only one report studied men (15). We examined a cohort of healthy, midlife adults without CKD but with key risk factors for abnormalities such as obesity and glucose intolerance. Among men, SHBG level was associated with a lower risk of at least one measure of reduced eGFR across all treatment groups, even after adjustment for numerous other risk factors for CKD. Among women, few key associations across randomization arm were observed after adjustment for other known risk factors for kidney disease. Although the small number of events per randomization arm limited definitive conclusions, higher endogenous testosterone, estradiol, and estrone concentrations were associated with lower risk of reduced eGFR among men randomly assigned to the placebo group, whereas higher DHEAS levels were associated with impaired eGFR among women treated with metformin.

Our findings suggest that the relationship between sex hormone levels and markers of kidney function may differ by sex, in that low SHBG level may be a risk factor for abnormal kidney indices in overweight, glucose intolerant men but not women. Several population-based reports have noted that the prevalence of CKD differs between men and women, although the direction of this association differs by geographic region (30). Reports from Sweden (31) and the United States (32) noted a higher prevalence of stage 3 or higher advanced CKD in women compared with men, whereas reports from China noted a lower prevalence in women (33). Population-based reports have also noted that the prevalence of end-stage renal disease may be higher in men (34, 35). Although these observations of sex differences have led to speculation regarding the potential role of sex hormones, no studies, to our knowledge, have examined whether endogenous sex steroids are associated with eGFR or albuminuria in women.

Our findings regarding minimal associations between sex hormone levels and kidney markers across randomization arms, along with the favorable associations with estradiol, estrone, and testosterone concentrations among men randomly assigned to placebo differ from results of a previous study in men that suggested higher estradiol level was associated with lower eGFR. Using data from the National Health and Nutrition Examination Study, Yi *et al.* (15) reported that estradiol rather than testosterone concentrations were associated with kidney dysfunction. Our findings may have differed from prior studies because of the overweight and glucose-intolerant status of the DPP participants or due to our longitudinal study design. When we performed cross-sectional analyses, we observed that higher estradiol level was associated with lower continuous measures of eGFR. The change in the direction of the association suggests that higher estradiol at baseline may be associated with lower rates of progression over time. Results of studies of rat models have supported favorable effects of estradiol, specifically that estradiol inhibits podocyte apoptosis and tumor growth factor-β 1 expression even as testosterone induces such processes in female knock-out mice (36), and estradiol administration also decreases extracellular matrix production and glomerulosclerosis (37). Other reports have noted an association of estradiol with other favorable processes in the kidney, such as inhibition of the renin-angiotensin system, endothelin synthesis, and oxidative stress (3, 7, 38).

In studies of exogenous estrogen therapy, researchers have noted this treatment may result in poorer kidney markers in otherwise healthy postmenopausal women (8, 9, 11–13), suggesting that higher levels of endogenous estradiol or estrone might be associated with lower eGFR or higher levels of albuminuria in women. However, we did not find an association between estradiol or estrone concentration in women and kidney dysfunction, perhaps because we adjusted for other known risk factors for kidney disease such glucose, blood pressure, and lipid levels known to be associated with endogenous and exogenous estrogen. Among women randomly assigned to metformin treatment, we did find that women with higher endogenous DHEAS levels had increased risk of reduced eGFR. These findings suggest that increased androgenicity in women may be associated with increased risk of abnormal eGFR. However, the lack of associations between testosterone level with kidney markers and the lack of association across randomization arms suggests that such associations, if any, are modest. Our findings may have been biased to the null because estradiol fluctuates over the menstrual cycle for premenopausal women, and estradiol concentrations are also low in postmenopausal women generally.

Although we adjusted for BMI as well as insulin resistance, the association between SHBG level and kidney measures may be due to residual confounding by these measures. SHBG is manufactured primarily by the liver, and SHBG production is lower among persons with hepatic steatosis and associated visceral adiposity and glucose intolerance (39). However, it is also possible that the association between SHBG level and renal disease may be due to direct effects of SHBG upon the nephron. In mouse models, intracellular SHBG accentuates androgen-dependent mechanisms of proximal convoluted tubule cells, resulting in increased androgen uptake (40). Thus, SHBG may have ameliorated androgen-mediated tissue-specific effects.

The strengths of this report include a cohort well characterized for risk factors of CKD. We also used sensitive mass spectrometry to measure sex steroid concentrations. However, there are several limitations. We examined multiple sex steroids and SHBG, and due to the heterogeneous nature of associations across treatment arms, we performed multiple comparisons. Thus, some of the observed associations may have been due to chance and thus need to be replicated. Free fractions of sex steroids were not directly measured, and the optimal method of estimating bioavailable testosterone in lieu of direct measurements is controversial because of possible changes in binding affinity with age and population characteristics. Similar to previous reports of other data sets, we examined whether sex hormones were associated with one measure of reduced eGFR or albuminuria rather than sustained abnormalities due to the small number of participants who have developed chronic abnormalities. Finally, our results may not extend to persons who are not overweight or glucose intolerant. Previous reports have noted that body composition and glycemia may have bidirectional associations with sex hormones (41, 42) and thus may have altered the pattern of associations with kidney markers.

We conclude that the associations between sex hormone levels and abnormal kidney markers may differ between overweight and glucose-intolerant men and women. In particular, among men, low SHBG and low testosterone, estradiol, and estrone concentrations may be risk factors for abnormal kidney markers even after adjustment for multiple other risk factors for kidney disease. Studies should examine whether these associations exist in other cohorts with sustained CKD and whether the pattern of associations is similar to those observed in persons using exogenous sex steroid therapy.

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Correspondence and Reprint Requests: Catherine Kim, MD, MPH, c/o the Diabetes Prevention Program Outcomes Study Coordinating Center, George Washington University Biostatistics Center, 6110 Executive Boulevard, Suite 750, Rockville, Maryland 20852. E-mail: dppmail@bsc.gwu.edu.

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