Androgens, Bilateral Oophorectomy, and Cardiovascular Disease Mortality in Postmenopausal Women With and Without Diabetes: The Study of Osteoporotic Fractures

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OBJECTIVE

Diabetes elevates cardiovascular disease (CVD) risk more markedly in women than in men. Because the high risk of CVD among women with type 2 diabetes (DM2) may be partly due to increased ovarian androgen production, we investigated whether a history of bilateral salpingo oophorectomy (BSO) is inversely associated with CVD mortality among women with DM2.

RESEARCH DESIGN AND METHODS

Data were obtained from 7,977 women (a random subset of 564 had measurements of sex-steroid hormones) enrolled in the Study of Osteoporotic Fractures (SOF), a community-based, multicenter study that monitored women aged ≥65 years for a mean of 15.1 years. Adjusted hazard ratios (HRs) and 95% CIs were calculated using Cox proportional hazards regression.

RESULTS

The average age at baseline was 71.5 years, with 6.3% and 18% of participants reporting a history of diabetes or BSO, respectively. In the subset of the SOF cohort with sex-steroid hormone measurements, those with DM2 had 43.6% significantly higher levels of free testosterone that were partly explained by age and adiposity, whereas total and free testosterone levels were lower in women with BSO than in those with intact ovaries. CVD mortality was elevated in women with DM2 without BSO (HR 1.95, 95% CI 1.62–2.35) as well as in women with DM2 and BSO (HR 2.56, 95% CI 1.79–3.65; *P* = 0.190 for interaction). Overall, BSO was not associated with CVD mortality (HR 1.05, 95% CI 0.89–1.23).

CONCLUSIONS

The association of diabetes with CVD was not reduced by BSO, suggesting that ovarian hyperandrogenemia may not be a primary mechanism to explain the high risk for CVD among women with DM2.

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Diabetes is thought to be a stronger cardiovascular disease (CVD) risk factor in women than in men because women with type 2 diabetes (DM2) are more likely to develop heart failure (1) and die of a CVD event than those without diabetes or men of the same age with or without diabetes (2). However, the reasons for the sex difference in diabetes-associated CVD are not well understood.

Diabetes usually occurs in the presence of other cardiometabolic factors, such as hypertension, adiposity, dyslipidemia, and insulin resistance, which all appear to be accentuated in DM2 and may predispose to a greater CVD risk. Meta-analyses suggest, however, that the vulnerability to CVD among individuals with DM2 may exist after accounting for these factors (3–5) and may reflect interacting effects of estrogens and insulin on cardiomyocytes (5) or a disparity in the recognition and care for heart disease in DM2 (6).

Androgens are thought to contribute to the earlier development of heart disease in men (7). Testosterone levels are higher in postmenopausal women with diabetes compared with those without diabetes independent of age or adiposity (7–10), and several reports have suggested that androgen production may play a role in their elevated CVD risk (8,9). Lower levels of sex-hormone binding globulin (SHBG) in DM2 further increase the non-SHBG (bioavailable) testosterone and may amplify its actions. Androgens have been associated with subclinical CVD (11,12), but evidence for an association between androgens and incident cardiovascular end points among women with diabetes in longitudinal studies is lacking.

The source of excess androgens in DM2 has not been studied in detail; however, results in women with polycystic ovary syndrome (PCOS), who are also obese and insulin resistant, suggest a predominant ovarian source (13), although adrenal steroid production might also be increased (14). Bilateral salpingo oophorectomy (BSO) causes a pronounced reduction in androgen levels (15). Some studies found BSO is associated with CVD risk (16–18), but others did not (19–21), and no studies have focused on DM2. If elevated androgen levels increase the risk for CVD in DM2, we tested the hypothesis that BSO would be associated with a reduced risk of CVD mortality among women with DM2 using data from the Study of Osteoporotic Fractures (SOF).

RESEARCH DESIGN AND METHODS

Study Population

SOF is a multicenter prospective observational study of women aged 65 or older, with clinical sites at Baltimore, MD; Minneapolis MN; Portland, OR; and Monongahela Valley near Pittsburgh, PA. Originally, 9,704 women, who were predominantly Caucasian, were recruited from September 1986 to October 1988 through population-based listings (22). African Americans were initially excluded due to their low incidence of hip fracture. Women who had a history of bilateral hip replacement or were unable to walk without assistance were excluded (23). Participants returned approximately every 2 years for a clinical evaluation. Follow-up contacts for ascertaining vital status were performed every 4 months. To date, rates of follow-up have exceeded 95%. All participants provided written informed consent, and human subject research approval was obtained by each clinical center's Institutional Review Board.

For the present analyses, we excluded women who had missing or unknown age at hysterectomy, oophorectomy, or last menstrual period (n = 87); unilateral or unknown oophorectomy status (n = 795); or intact uterus but underwent BSO (n = 43) and those with prevalent CVD (n = 802), as defined by a physician-diagnosed heart attack, coronary, or myocardial infarction, or stroke, yielding an analytic sample of 7,977 women. Sex hormone measurements were assayed in a random subsample of the cohort and were restricted to women who reported they were not taking postmenopausal hormone therapy (n = 954). For this present analysis of sex hormones, only women who had no missing baseline values for androgens were included (n = 564). Participants in this analytic subsample were not different from those excluded in age, education, smoking status, BMI, waist girth, BSO status, parity, physical activity, age at menarche, and age at menopause.

Determinants and Covariates

Information on age, race, education, and smoking status was self-reported. Height, weight, BMI, waist circumference, hip girth, and waist-to-hip ratio were measured according to standard protocols (22). Recreational and sports activities from the previous 12 months were recorded using a modified version of the Harvard Alumni Questionnaire and were converted into weekly caloric expenditure by intensity level (23). Physical activity was defined as a continuous variable of total kilocalories expended per week. Participants were asked at baseline and follow-up about their medical and reproductive health histories, including gynecological surgeries. Age at menarche and natural menopause, hysterectomy status, parity, and hormone therapy use were all self-reported. Women in whom both ovaries were surgically removed were defined to have BSO, with the age at which this occurred recorded. Participants were asked "had a doctor had ever told you that you have diabetes or 'sugar' diabetes?" Women who answered "yes" were also asked about current insulin use. A systolic blood pressure above 140 mmHg and/or diastolic blood pressure above 90 mmHg defined a diagnosis of hypertension.

Biochemical Analyses

At baseline, nonfasting blood samples were drawn from all participants between the hours of 8:00 A.M. and 2:00 P.M. Participants were instructed to avoid fatty foods on the morning of the examination to minimize lipemia, which may interfere with assays. Blood was drawn after the participant had been seated for at least 10 min and was immediately frozen to −20°C for up to 2 weeks and stored in liquid nitrogen at −190°C until assays were done (24–26). Total testosterone was measured by radioimmunoassay after extraction and aluminum oxide column chromatography with interassay coefficient of variation of 6.1–13.4% (24–26). Free testosterone was measured using an ammonium sulfate precipitation procedure with an interassay coefficient of variation of 10.7–15.5% (24–26), with adjustment for the albumin concentration (coefficient of variation for intra-assay and total assay 5% and 5.4%, respectively; sensitivity 34.7 pmol/L) (27). Dehydroepiandrosterone sulfate (DHEAS) was measured using radioimmunoassay with sample dilution (coefficient of variation for intra-assay and interassay 6–11% and 9–12%, respectively (27). SHBG was measured using a displacement technique (24–26). All biochemical analyses were performed at Nichols Institute (San Juan Capistrano, CA) or Endocrine Sciences Esoterix (Calabasas Hills, CA). In both

laboratories, the intra-assay and interassay coefficients of variation, respectively, for total testosterone ranged from 4 to 12% and from 9 to 11% for the assays done by Nichols and from 3 to 13% and from 9 to 14% for assays done by Endocrine Sciences Esoterix (24–26). Blood samples from 51 women were selected to determine the stability of these hormones in stored serum. Accordingly, samples obtained at baseline and after 3.5 years of follow-up stored at –190°C were analyzed. The correlations between testosterone measured at these two time points was $r = 0.99$ ($P < 0.001$) (26).

Outcome

We obtained copies of original death certificates of participants who died during follow-up. All deaths were centrally adjudicated. CVD-related mortality was defined using ICD-9 codes 402, 404, 410–414, and 426–445, which represented deaths from atherosclerosis, stroke, or coronary heart disease, respectively.

Statistical Analyses

Descriptive statistics were calculated for baseline characteristics among participants. Comparisons for categorical variables were assessed using χ^2, and continuous variables were tested using the *t* test and ANOVA. Analyses of covariance with adjustment for age and BMI were used for comparison of sex hormones. Quantitative measures that were skewed were normalized by natural log transformation. The Tukey adjustment method was used for multiple comparisons of sex hormones among the groups with diabetes-BSO. In time-to-event analysis, the Kaplan-Meier method was used to estimate the risk of CVD mortality. Survival curves were produced to graphically depict incident events with differences among groups assessed by means of the log-rank test. Estimation of adjusted hazard ratios (HR) and 95% CI for CVD mortality were calculated using Cox proportional hazards regression models. The proportionality assumption was tested using cumulative sums of martingale residuals with a Kolmogorov-type supremum test and also by visually inspecting plots of Schoenfeld residuals versus time. Because testosterone levels decrease during the menopausal transition (28) and our previous study of women in the National Health and Nutrition Examination Survey (29) suggested that BSO before age 45 years increased the CVD risk for DM2, we examined all-cause and CVD mortality in women stratified by age at menopause (≤45 vs. >45 years), a cut point that others have also used (30). A two-tailed *P* value of <0.05 was considered statistically significant. In sensitivity analyses, we calculated HRs using Fine and Gray methodology (31) for competing risks, taking into account the competing risk of death from other non-CVD causes.

RESULTS

Baseline characteristics of the participants are presented in Table 1 according to diabetes and BSO status. The mean ± SD age at baseline was 71.5 ± 5.3 years, with a 6.3% prevalence of diabetes. Approximately 37% of participants had undergone hysterectomy. Of these, 49% had concomitant BSO at a mean age of 45.6 years (95% CI 45.3–46.0). A greater proportion of women with BSO, compared with women with intact ovaries, were current users of hormone therapy (25.0 vs. 9.7%), nulliparous (22.1 vs. 18.3%), or hypertensive (41.0 vs. 37.4%). Women with diabetes, compared with those without diabetes, were more likely to never use hormone therapy (67.1 vs. 52.8%) or report a hysterectomy (42.0 vs. 36.9%) and were hypertensive (54.3 vs. 37.0%). With regard to differences by ovarian status among DM2, women with BSO reached menopause at an earlier age (44.7 vs. 48.8 years), were more likely to be current hormone therapy users (15.8 vs. 4.0%), and were more often nulliparous (21.8 vs. 17.2%) compared with those with intact ovaries, with all differences meeting statistical significance.

Sex-Steroid Hormone Levels

Sex-steroid hormones levels according to DM2 and oophorectomy status are reported in Table 2. Diabetes and ovarian status both significantly influenced sex hormones levels. On the one hand, women with diabetes had lower SHBG ($P < 0.001$) and higher free testosterone levels ($P = 0.026$) than women without diabetes that was independent of age and BMI. On the other hand, women with DM2 with intact ovaries had higher estrone levels than women without DM2 ($P < 0.001$). As expected, total testosterone ($P < 0.001$) and free testosterone ($P < 0.001$) levels were lower in women with BSO than in women with intact ovaries. BSO was associated with slightly lower SHBG levels in women with DM2 but with higher levels ($P = 0.001$) in women without DM2. No statistically significant differences were found for estrone or DHEAS levels by diabetes diagnosis or ovarian status. These observations remained even after adjusting for age and BMI. In analysis restricted to DM2, those with BSO had significantly lower levels of total testosterone and a higher percentage of free testosterone than women with DM2 and intact ovaries. However, after adjustment for age and BMI, only the variation in percentage of free testosterone by ovarian status persisted among women with DM2.

Incident CVD

After a mean follow-up of 15.1 years, 4,797 deaths occurred, with 1,638 (34.1%) attributed to CVD. CVD-related mortality rates per 1,000, according to diabetes and BSO status, were no diabetes or BSO, 12.9; BSO without diabetes, 12.7; diabetes without BSO, 25.9; and diabetes with BSO, 31.4. The incidence of CVD mortality was elevated in women with diabetes, with or without BSO ($P < 0.001$) (Fig. 1). Among women with diabetes, the risk of CVD mortality was elevated for those with intact ovaries (HR 1.95, 95% CI 1.62–2.35) and for those with BSO (HR 2.56, 95% CI 1.79–3.65) (Table 3). Although CVD mortality in women with diabetes and BSO was slightly higher compared with women with diabetes and intact ovaries, this difference did not meet statistical significance (HR 1.49, 95% CI 0.90–2.46, $P = 0.120$). Overall, BSO was not associated with CVD mortality (HR 1.05, 95% CI 0.89–1.23). In addition, no significant interaction was found between diabetes-BSO status and hormone therapy ($P = 0.340$).

We next performed analyses stratified by age at menopause (≤45 or >45 years) (Table 3) and found that CVD mortality risk was elevated for women with diabetes regardless of age at menopause. However, in analyses restricted to DM2, with those who had intact ovaries as referent, the adjusted risk of CVD mortality was elevated for women with DM2 who had BSO before or at 45 years of age (HR 2.75, 95% CI 1.24–6.11, $P = 0.012$), whereas the risk among women with DM2 who had surgery at or after age 45 years was not statistically different

Table 1—Baseline characteristics of 7,977 participants according to diabetes and BSO status from the SOF

	No diabetes		Diabetes			
	No BSO (n = 6,135)	BSO (n = 1,340)	No BSO (n = 401)	BSO (n = 101)	P value*	P value†
Age (years)	71.7 (5.4)	71.0 (4.8)	71.7 (5.1)	71.3 (4.8)	0.001	0.462
High school education or less (%)	60.2	64.2	73.6	73.3	0.001	0.972
BMI (kg/m^2) (%)					0.001	0.187
<25.0	44.7	45.8	26.7	35.2		
25.0–29.9	37.2	35.6	36.9	30.5		
≥30.0	18.1	18.6	36.4	34.3		
Smoking status (%)					0.543	0.525
Never	61.6	59.2	63.0	61.9		
Former	28.6	30.1	29.0	26.7		
Current	9.8	10.7	8.0	11.4		
Waist girth (cm)	82.7 (10.8)	82.9 (11.0)	90.4 (11.8)	90.6 (12.9)	0.001	0.944
Waist-to-hip ratio	0.81 (0.1)	0.81 (0.1)	0.85 (0.1)	0.84 (0.1)	0.001	0.606
Physical activity (total kcal/week)‡	6.80 (1.2)	6.83 (1.2)	6.37 (1.3)	6.32 (1.5)	0.001	0.739
Age at menarche (%)					0.459	0.846
<12 years	21.3	20.0	25.4	22.9		
12–14 years	65.9	67.0	62.1	64.7		
>14 years	12.8	13.0	12.5	12.4		
Age at menopause (%)					0.001	0.001
<45 years	21.4	32.2	23.2	41.9		
45–49 years	28.0	21.9	24.9	15.2		
>49 years	50.6	45.9	51.9	42,9		
Hysterectomy (%)	23.1	87.2	27.4	96.2	0.001	0.001
Parity (%)					0.001	0.030
None	18.4	22.5	17.1	21.9		
1–2 live births	41.0	43.9	39.2	48.6		
3–4 live births	31.0	27.8	32.2	25.7		
≥5 live births	9.6	5.8	11.5	3.8		
Hormone therapy use (%)					0.001†	0.001
Never	57.5	31.5	73.8	41.9		
Former	32.5	43.3	22.2	42.9		
Current	10.0	25.2	4.0	15.2		
Hypertension (%)	36.3	40.1	55.5	50.5	0.001	0.344

Values are displayed as percentages or mean (SD). P values are based on the χ^2 test for categorical variables and ANOVA for normally distributed continuous variables. *P value comparing all four groups. †P value comparing women with diabetes and no BSO to women with diabetes and BSO. ‡Log-transformed values.

from that for women with DM2 with intact ovaries (HR 0.94, 95% CI 0.48–1.85, P = 0.864 and P = 0.097 for interaction). Similar results were obtained when the analyses were stratified by the average age (50 years) at natural menopause (data not shown). In a sensitivity analyses, we observed that these associations were similar when deaths from other non-CVD causes were censored or treated as competing events (Supplementary Table 1).

Table 2—Hormone levels according to diabetes and BSO status among a subsample of participants (n = 564) in the SOF

	BSO and diabetes status								
	No diabetes		Diabetes						
	No BSO (n = 431)	BSO (n = 91)	No BSO (n = 37)	BSO (n = 5)	P value*	P value†	P value‡	P value§	P value‖
Testosterone									
Total (ng/dL)	18.0 (11.0–28.0)	12.0 (7.5–17.0)	21.0 (13.0–33.0)	12.0 (6.0–16.0)	<0.001	<0.001	0.045	0.096	0.798
Free (pg/mL)	2.0 (1.1–3.2)	1.2 (0.7–1.7)	2.7 (1.9–4.0)	1.8 (1.3–2.9)	<0.001	<0.001	0.289	0.432	0.026
% Free	1.1 (0.9–1.4)	1.0 (0.8–1.3)	1.3 (1.1–1.7)	1.8 (1.8–1.9)	<0.001	0.247	0.027	0.040	<0.001
DHEAS (μg/dL)	45.0 (25.0–73.0)	39.0 (24.0–70.0)	45.0 (26.0–78.0)	55.0 (37.0–61.0)	0.573	0.648	0.399	0.446	0.970
SHBG (μg/dL)	1.4 (1.0–2.1)	1.7 (1.2–2.5)	1.0 (0.6–1.2)	0.5 (0.4–0.6)	<0.001	<0.001	0.710	0.818	<0.001
Estrone (pg/mL)	22.0 (15.0–30.0)	24.0 (14.0–47.0)	28.0 (23.0–32.0)	16.0 (14.0–46.0)	<0.001	<0.001	0.963	0.896	0.489

Data are shown as the median (interquartile range). Reported P values were adjusted for multiple comparisons using the Tukey method. *P value comparing all four categories based on ANOVA for normally distributed continuous variables after log transformation. †P value for subgroup analyses restricted to only women without diabetes by BSO status. ‡P value for subgroup analyses restricted to only women with diabetes by BSO status. §P value for subgroup analyses restricted to only women with diabetes by BSO status adjusted for age and BMI, based on ANCOVA. ‖P value for comparing women with and without diabetes regardless of BSO status adjusted for age and BMI.

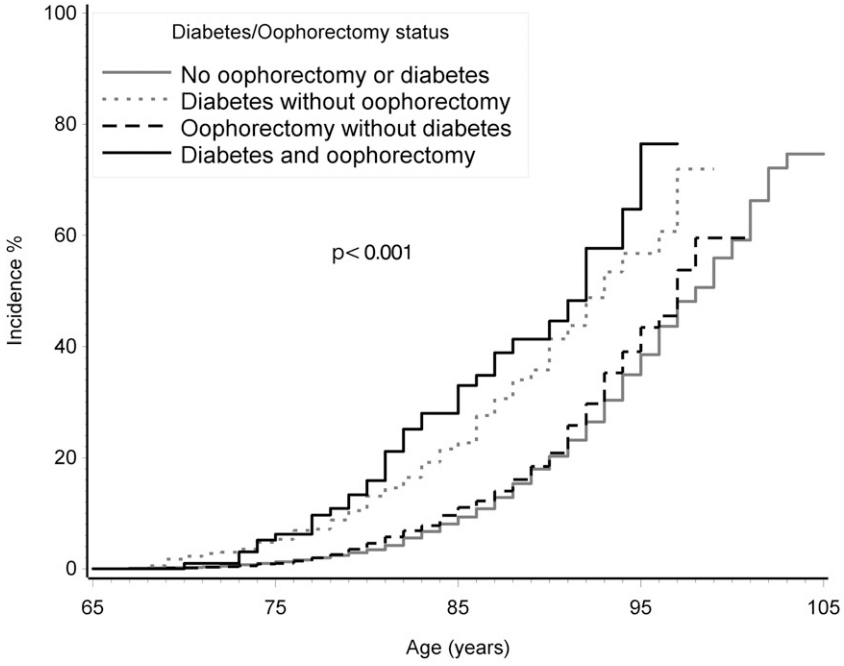

Figure 1—Kaplan-Meier cumulative incidence estimates of CVD mortality according to diabetes and oophorectomy status. The log-rank *P* value of <0.001 represents differences among all groups.

CONCLUSIONS

To our knowledge, this is the first study to assess the association between BSO, serum androgens, and CVD mortality in a large population of women with or without diabetes. We found that diabetes in postmenopausal Caucasian women was associated with a higher CVD mortality risk that appeared to be influenced by the age at surgical menopause. As in previous studies, androgen levels were higher in women with diabetes (7–10). Because androgens have been linked to CVD risk, possibly due to insulin resistance and glycemia, and to adverse effects on the coronary vasculature (32), cardiac myocytes (33), and carotid artery intima-media thickness (11,12), we hypothesized that BSO would actually reduce the CVD risk in women with DM2. However, our analysis of this prospective cohort of 7,977 women aged 65 years and older does not support this hypothesis.

The CVD risk among women with DM2 appeared to be greater in women who had BSO before or at age 45 years. Although this risk may be explained by estrogen deficiency (30,34), BSO before age 45 years did not increase CVD mortality risk in women without diabetes. Thus it is intriguing to propose that a unique underlying disease process links BSO and CVD in DM2. On the one hand, no difference was found in BMI, waist circumference, age at menarche, or prevalence of hypertension between women with DM2 with BSO and those without BSO. On the other hand, parity was less among women with DM2 with BSO, and SHBG levels tended to be lower rather than increased as in women without diabetes. Few studies (30,34) have included the reason for pelvic surgery in their analysis, but in women from Olmstead County, MN (34), elevated mortality was limited to those who were younger than age 45 years at BSO and those who underwent BSO for benign tumors or inflammation but not the larger group of women with endometriosis. We propose that further investigation that includes indications for BSO in younger women with DM2 may provide insight into why they are at higher risk for CVD. Further, we hypothesize that hysterectomies

Table 3—All-cause and CVD mortality according to diabetes and SBO status and age at menopause from the SOF

		All-cause mortality			CVD mortality		
	N	Events (*n*)	Age-adjusted HR (95% CI)	Multivariable-adjusted* HR (95% CI)	Events (*n*)	Age-adjusted HR (95% CI)	Multivariable-adjusted* HR (95% CI)
No diabetes or BSO	5,009	3,632	1 (Referent)	1 (Referent)	1,209	1 (Referent)	1 (Referent)
Diabetes; no BSO	401	311	1.83 (1.63–2.06)	1.66 (1.47–1.88)	132	2.33 (1.95–2.79)	1.95 (1.62–2.35)
BSO; no diabetes	1,340	776	1.10 (1.02–1.19)	1.02 (0.92–1.12)	261	1.11 (0.97–1.27)	1.02 (0.86–1.21)
Diabetes and BSO	101	78	2.36 (1.88–2.95)	2.15 (1.70–2.72)	35	3.20 (2.28–4.48)	2.56 (1.79–3.65)
$P_{interaction}$				0.039			0.190
Age at menopause							
Diabetes							
No BSO ≤45 years	133	100	1 (Referent)	1 (Referent)	44	1 (Referent)	1 (Referent)
BSO ≤45 years	47	35	1.45 (0.98–2.14)	1.64 (0.99–2.71)	17	1.60 (0.91–2.81)	2.75 (1.24–6.11)
No BSO >45 years	268	211	1 (Referent)	1 (Referent)	88	1 (Referent)	1 (Referent)
BSO >45 years	54	43	1.20 (0.86–1.66)	1.14 (0.73–1.79)	18	1.19 (0.72–1.98)	0.94 (0.48–1.85)
No diabetes							
No BSO ≤45 years	1,838	1,131	1 (Referent)	1 (Referent)	389	1 (Referent)	1 (Referent)
BSO ≤45 years	488	284	1.07 (0.94–1.22)	1.02 (0.88–1.20)	102	1.12 (0.90–1.38)	1.05 (0.81–1.36)
No BSO >45 years	4,297	2,501	1 (Referent)	1 (Referent)	820	1 (Referent)	1 (Referent)
BSO >45 years	852	492	1.10 (1.00–1.22)	0.99 (0.87–1.13)	159	1.10 (0.93–1.30)	0.96 (0.77–1.20)

*Adjusted for age, education, age at menopause, hysterectomy, parity, postmenopausal hormone use, BMI, waist-to-hip ratio, hypertension, smoking status, and physical activity.

concomitant with BSO may be performed in young women because of ovarian cysts in conjunction with irregular menstrual bleeding in whom the diagnosis is PCOS, a high-risk group for insulin resistance, diabetes, and CVD.

BSO, particularly at a young age, has been associated with increased overall and CVD mortality in the general population in some studies but not in others (30,34). The Nurses' Health Study (16) found a 17% higher risk for CVD in women with BSO and a 44% higher risk among those women with BSO before age 45 years. The Women's Health Initiative (19) found no statistically significant differences in the rates of fatal and nonfatal CHD, stroke, or total CVD in women with hysterectomy concomitant with BSO compared with women with hysterectomy alone. The California Teachers Study found no elevated CVD risk or mortality with BSO irrespective of hormone therapy use or age at BSO (35). The prevalence of diabetes in each of these studies was low compared with estimates from the current study.

In this cohort of postmenopausal women, SHBG levels were lower and free testosterone levels were higher among women with diabetes independent of age and adiposity. Several previous studies have found similar results (8,9); however, the source and pathogenesis of the androgen excess are not well understood. By extrapolating the findings in insulin-resistant premenopausal women with PCOS, Korytkowski et al. (8) proposed that elevated androgens in postmenopausal women with diabetes are of ovarian origin. However, results after dexamethasone suppression and ACTH stimulation in postmenopausal women with a history of PCOS suggest that the ovary and adrenal cortex both contribute to the elevated androgen levels in these subjects (14). In the current study, free testosterone levels among women with BSO were slightly but not statistically higher in women with diabetes than in those without diabetes after accounting for the effect of age and adiposity. The level of DHEAS (an androgenic steroid produced by the adrenal cortex) was similar in the two groups as in other studies (8). These results favor the ovary as the predominant but not the sole source of androgens in postmenopausal women with diabetes.

This study has several notable strengths. The use of a large sample of postmenopausal women with sufficiently long follow-up, coupled with minimal loss to follow-up of participants, enhances the assessment of mortality outcomes, which were adjudicated. In addition, total testosterone was measured by radioimmunoassay after extraction and aluminum oxide column chromatography, a method with good precision and sensitivity and a high accuracy comparable to liquid chromatography-tandem mass spectrometry for the low values in women (36).

Several limitations should be acknowledged, however. The analytic sample consisted of Caucasian women, limiting any generalization to women of other races. Further, because sex hormones were measured in a small subset of only five women with diabetes and BSO, we could not test whether sex steroids modify the relationship between BSO and diabetes on mortality. A single measure of sex hormones at baseline was used to characterize each woman's hormonal status. However, a single measurement for plasma sex hormones in postmenopausal women can reliably categorize average levels over a period of at least 3 years (37). Adjustment for lipids in multivariable models was not feasible because only a subsample of women had measures of cholesterol and triglycerides. A failure to recall the actual age at menopause and oophorectomy occurring many years before study enrollment could potentially lead to misclassification. A woman's accuracy in recalling age at oophorectomy seems to surpass recall of age at natural menopause (38). The use of death certificates to identify cardiovascular deaths may have resulted in some misclassification of cause of death (39) especially among elderly women, who often have multiple medical problems. Our CVD findings were consistent, however, with all-cause mortality, suggesting that the influence of such misclassification on our results, if present, may be minimal. Lastly, the diagnosis of diabetes was based on self-report of physician diagnosis and no doubt is an underestimate because many women could have had undiagnosed diabetes. Regardless, this random misclassification would have been expected to bias the results toward the null given the observational design of our study.

In summary, women with DM2 have a higher CVD mortality risk that is not attenuated by a history of BSO. These findings imply that ovarian hyperandrogenemia may not be the primary mechanism to explain CVD mortality among postmenopausal women with DM2. The apparent influence of age at menopause on the relation between BSO and diabetes with CVD mortality raises the possibility that joint factors may influence the risk for diabetes, CVD, and the decision for BSO in younger women. These results warrant further investigations among younger women with diabetes and BSO to determine if this risk is due to BSO or is a marker for risk factors that could be associated with PCOS. The prevalence of diabetes is increasing worldwide. As such, it is essential and prudent that we understand and identify the underlying factors that predispose women with diabetes to cardiovascular events to aid in the early detection and management of high-risk women in a timely fashion.

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