Coffee and Caffeine Consumption in Relation to Sex Hormone–Binding Globulin and Risk of Type 2 Diabetes in Postmenopausal Women

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OBJECTIVE—Coffee consumption has been inversely associated with type 2 diabetes risk, but its mechanisms are largely unknown. We aimed to examine whether plasma levels of sex hormones and sex hormone–binding globulin (SHBG) may account for the inverse association between coffee consumption and type 2 diabetes risk.

RESEARCH DESIGN AND METHODS—We conducted a case-control study nested in the prospective Women's Health Study (WHS). During a median follow-up of 10 years, 359 postmenopausal women with newly diagnosed type 2 diabetes were matched with 359 control subjects by age, race, duration of follow-up, and time of blood draw.

RESULTS—Caffeinated coffee was positively associated with SHBG but not with sex hormones. Multivariable-adjusted geometric mean levels of SHBG were 26.6 nmol/l among women consuming ≥4 cups/day of caffeinated coffee and 23.0 nmol/l among nondrinkers (P for trend = 0.01). In contrast, neither decaffeinated coffee nor tea was associated with SHBG or sex hormones. The multivariable-adjusted odds ratio (OR) of type 2 diabetes for women consuming ≥4 cups/day of caffeinated coffee compared with nondrinkers was 0.47 (95% CI 0.23–0.94; P for trend = 0.047). The association was largely attenuated after further adjusting for SHBG (OR 0.71 [95% CI 0.31–1.61]; P for trend = 0.47). In addition, carriers of rs6259 minor allele and noncarriers of rs6257 minor allele of *SHBG* gene consuming ≥2 cups/day of caffeinated coffee had lower risk of type 2 diabetes in directions corresponding to their associated SHBG.

CONCLUSIONS—Our findings suggest that SHBG may account for the inverse association between coffee consumption and type 2 diabetes risk among postmenopausal women. ***Diabetes* 60: 269–275, 2011**

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Previous prospective studies have documented an inverse association between coffee consumption and type 2 diabetes risk (1,2), especially in women (2). Coffee intake may improve glucose tolerance via activation of energy metabolism and enhancement of insulin sensitivity and β-cell function (2,3)—although much of the molecular mechanism remains unknown. Previous cross-sectional studies have associated coffee intake with plasma levels of sex hormones or sex hormone–binding globulin (SHBG) (4,5). In addition, a large body of observational and experimental data has implicated the important roles of sex hormones in the development of type 2 diabetes (6–8). Notably, recent experiments indicate that SHBG not only regulates the biologically active fraction of sex hormones but may bind to its own receptors at the plasma membranes of a variety of cells, directly mediating intracellular signaling of sex hormones (9). More recently, prospective studies of men and women incorporating both genetic and phenotypic assessment of SHBG revealed a strong inverse association between SHBG levels and type 2 diabetes risk (10). However, no studies have comprehensively evaluated the interrelationships of coffee consumption in relation to sex hormones and SHBG with respect to type 2 diabetes risk. To examine whether and to what extent sex hormones or SHBG may account for the potential protective effect of coffee intake against type 2 diabetes, we analyzed data from a prospective case-control study of women. In particular, we evaluated the associations of coffee consumption with plasma levels of sex hormones and SHBG, as well as the direct association between coffee consumption and type 2 diabetes risk during a 10-year follow-up. Moreover, we investigated whether the association of coffee consumption with type 2 diabetes risk was attenuated by further adjusting for plasma sex hormones or SHBG. Finally, we examined whether coffee intake may interact with specific SHBG genotypes in affecting diabetes risk.

RESEARCH DESIGN AND METHODS

The Women's Health Study (WHS) is a randomized, double-blind, placebo-controlled trial originally designed to evaluate the balance of benefits and risks of low-dose aspirin and vitamin E in the primary prevention of cardiovascular disease and cancer (11). Of the 39,876 participants aged 45 years and older, 98% of participants completed a 131-item semiquantitative food frequency questionnaire (SFFQ). At baseline, participants were asked if they were willing to provide blood samples by mail. Women who responded affirmatively and were eligible to be enrolled into the run-in phase were mailed a blood collection kit. Of the 28,345 (71%) who provided baseline blood samples, we restricted our study to 6,574 postmenopausal women who were not using hormone replacement therapy (HRT) at the time of blood collection. By February 2005, 366 of these initially healthy women reported developing

TABLE 1
Baseline characteristics between participants with incident cases of type 2 diabetes and control participants among 718 women

	Case subjects	Control subjects	P^*
n	359	359	
Age (years)	60.3 ± 6.1	60.3 ± 6.1	
Caucasian (%)	93.5	93.5	
BMI (kg/m^2)	30.9 ± 6.1	26.0 ± 5.0	<0.001
Alcohol (g/day)	2.62 ± 7.4	4.19 ± 8.3	0.008
Current smoking (%)	14.5	13.7	0.74
Physical activity ≥once/week (%)	30.7	38.7	0.02
Family history of diabetes (%)	48.5	24.0	<0.001
Past postmenopausal hormone use (%)	34.0	29.3	0.17
Ever oral contraceptive use (%)	50.4	48.0	0.57
Age at menopause (years)	48.0 ± 6.2	48.0 ± 5.8	0.79
Years since menopause	12.2 ± 8.2	12.2 ± 8.0	0.77
Age at menarche <12 (%)	25.4	21.7	0.23
Age at first pregnancy of ≥6 months, <25 (%)	63.4	57.2	0.37
Pregnancies ≥5 (%)	18.7	19.9	0.69
Currently married (%)	65.7	68.2	0.28
Caffeine-related beverages			
Caffeinated coffee, ≥4 cups/day (%)	13.8	20.9	0.01
Decaffeinated coffee, ≥4 cups/day (%)	2.3	4.3	0.20
Tea, ≥4 cups/day (%)	5.2	2.9	0.13
Caffeine, ≥500 mg/day (%)	14.3	21.1	0.02
Sex hormones			
SHBG (nmol/l)	22.3 ± 13.8	36.9 ± 17.4	<0.001
Estradiol (pg/ml)	24.6 ± 15.9	20.5 ± 11.3	<0.001
Testosterone (ng/dl)	29.8 ± 19.1	28.9 ± 19.1	0.49
DHEAS (μg/dl)	91.0 ± 61.3	92.6 ± 53.7	0.67

Data are means ± SD. *Baseline characteristics were compared between case patients and control subjects using the paired t test for continuous variables and the McNemar test for categorical variables.

incident type 2 diabetes. Control subjects were matched in 1:1 ratio to case subjects by age (within 1 year), duration of follow-up (within 1 month), race, and fasting status at time of blood draw (82% provided fasting blood samples, defined as ≥10 h since the last meal). Based on these eligibility criteria, 359 case subjects and 359 control subjects were included in our analyses. Written informed consent was obtained from all participants. This study was approved by the Institutional Review Boards of Brigham and Women's Hospital, Harvard Medical School, and the University of California, Los Angeles (UCLA).

Assessment of dietary intake. In the SFFQ, participants were asked how often on average during the previous year they had consumed caffeinated and decaffeinated coffee ("one cup"), tea ("one cup or glass"), different types of caffeinated soft drinks ("one glass, bottle, or can"), and chocolate products (e.g., "bar or packet"). Participants could choose from nine responses (never or <1/month, 1–3/month, 1/week, 2–4/week, 5–6/week, 1/day, 2–3/day, 4–5/day, and ≥6/day). Using U.S. Department of Agriculture food composition data supplemented with other sources, we estimated that the caffeine content was 137 mg per cup of coffee, 47 mg per cup of tea, 46 mg per bottle or can of cola beverage, and 7 mg per serving of chocolate candy (12). A validation study from a similar cohort of women reported high correlations between intake of coffee and other caffeinated beverages assessed with SFFQ and with four 1-week diet records (coffee, $r = 0.78$; tea, $r = 0.93$; and caffeinated sodas, $r = 0.85$) (13).

Ascertainment of incident type 2 diabetes. Details regarding ascertainment of incident type 2 diabetes in our cohorts have been reported previously (14). After excluding those with diabetes at baseline, all participants were asked annually whether and when they had a diagnosis of diabetes since baseline. Using the diagnostic criteria of the American Diabetes Association (15), all self-reported cases of type 2 diabetes were confirmed by a supplemental questionnaire. Self-reported diabetes in the WHS was validated against physician-led telephone interviews, supplementary questionnaires, and medical record reviews, all yielding positive predictive values >91% (16).

Laboratory procedures. A mailed blood collection kit contained instructions, three 10-ml EDTA vacutainer tubes, three 4.5-ml sodium citrate tubes, supplies needed to draw a sample of blood, a completed overnight courier air bill, and a gel-filled freezer pack. The gel-filled freezer pack was frozen overnight to serve as a coolant for mailing. Women were asked to have a morning fasting blood sample drawn into two EDTA and two citrate tubes, and to return the completed blood kit via overnight courier. All samples

arrived in our laboratory within 24–30 h of venipuncture. Upon receipt, samples were kept chilled until processed. After centrifugation for 20 min (2,500 rpm, 4°C) each sample was pipetted into 2 ml Nunc vials. Samples were stored in liquid nitrogen tanks until the time of laboratory analyses. Laboratory personnel were blinded to case-control status, and matched case-control pairs were handled identically and assayed in random order in the same analytical run. Plasma concentrations of sex hormones and SHBG were measured using chemiluminescent immunoassays (Elecsys autoanalyzer 2010; Roche Diagnostics, Indianapolis, IN), which have been validated for measuring plasma sex hormones and SHBG (17–19). For the hormone levels in this study, the coefficients of variation from blinded quality control samples were 5.2% for estradiol, 7.4% for testosterone, 2.8% for dehydroepiandrosterone sulfate (DHEAS), and 2.8% for SHBG. Detailed methods for single nucleotide polymorphism (SNP) selection and genotyping of SHBG SNPs were described previously (10). Two informative SNPs associated with plasma SHBG levels were included in our study: rs6259 in exon 8, encoding an amino acid substitution of asparagine for aspartic acid, which may lead to reduced clearance rate of SHBG, and rs6257 in intron 1.

Statistical analysis. Following conventional practice in previous studies, we categorized caffeinated coffee, decaffeinated coffee, and tea consumption by aggregating nine possible responses for caffeinated coffee, decaffeinated coffee, and tea from SFFQ into four categories (0 cups/day, ≤1 cups/day, 2–3 cups/day, and ≥4 cups/day). We also categorized caffeine consumption into four categories (≤50, 51–250, 251–500, and >500 mg/day).

Baseline characteristics were compared between case patients and control subjects using the paired t test for continuous variables and McNemar test for categorical variables. To assess the association of caffeine-related beverage consumption (caffeinated coffee, decaffeinated coffee, tea, and caffeine) with sex hormones (estradiol, testosterone, and DHEAS) and SHBG, we calculated the geometric means of sex hormones and SHBG plasma levels according to the four categories of caffeine-related beverage consumption. We used multiple linear regression models to adjust for matching factors (age, race, duration of follow-up, and time of blood draw), smoking status (never, past, and current smokers), physical activity (rarely/never, <1, 1–3, and ≥4 times/week), alcohol use (rarely/never, 1–3 drinks/month, 1–6 drinks/week, and ≥1 drinks/day), total calories (≤1,500, 1,501–2,000, 2,001–2,500, and >2,500 kcal/day), and BMI (continuous). To test for a linear trend across increasing categories of caffeine-related beverage consumption, we computed the me-

TABLE 2
Geometric mean levels of plasma SHBG (nmol/l) according to caffeinated coffee, decaffeinated coffee, tea, and caffeine consumption

	Categories of intake				*P for trend
	0 cups/day	≤1 cups/day	2–3 cups/day	≥4 cups/day	
Caffeinated coffee, median (*n*)	0 (185)	1.0 (187)	2.5 (212)	4.5 (122)	
Match-adjusted model§	23.1 (16.1–33.1)	22.7 (15.8–32.7)	24.7 (17.2–35.5)	27.6 (19.1–39.9)	0.002
Categorical model†	23.0 (16.5–32.0)	22.8 (16.3–31.8)	23.6 (16.9–33.0)	26.6 (18.9–37.4)	0.01
Spline model‡	22.9 (16.5–31.9)	22.7 (16.2–31.8)	24.1 (17.2–33.6)	26.0 (18.5–36.5)	
Decaffeinated coffee, median (*n*)	0 (401)	0.4 (188)	2.5 (84)	4.5 (23)	
Match-adjusted model§	24.5 (17.1–35.2)	24.3 (16.8–35.4)	25.2 (17.1–37.3)	26.7 (17.4–40.9)	0.44
Categorical model†	23.7 (17.0–33.1)	22.3 (15.8–31.3)	23.2 (16.2–33.3)	22.9 (15.5–33.9)	0.75
Spline model‡	23.6 (16.9–32.9)	23.5 (16.8–33.0)	23.6 (16.5–33.6)	23.5 (16.2–34.3)	
Tea, median (*n*)	0 (242)	0.4 (351)	2.5 (76)	4.5 (28)	
Match-adjusted model§	25.6 (17.9–36.7)	23.6 (16.4–33.8)	24.6 (16.9–35.8)	21.6 (14.3–32.5)	0.27
Categorical model†	24.5 (17.6–34.1)	23.3 (16.7–32.6)	23.3 (16.5–32.8)	21.4 (14.7–31.3)	0.24
Spline model‡	24.4 (17.5–33.9)	23.7 (17.0–33.1)	22.5 (16.1–31.5)	22.7 (15.9–32.3)	
Caffeine category (mg/day)	≤50	51–250	251–500	>500	
Caffeine intake (mg/day), median (*n*)	13 (131)	140 (209)	366 (230)	656 (123)	
Match-adjusted model§	22.9 (15.8–33.0)	22.5 (15.6–32.5)	23.6 (16.4–34.0)	26.9 (18.5–39.0)	0.008
Categorical model†	22.9 (16.5–32.0)	23.2 (16.7–32.3)	23.0 (16.5–32.1)	26.6 (19.0–37.4)	0.02
Spline model‡	23.0 (16.5–32.1)	23.0 (16.6–32.0)	23.7 (17.0–33.0)	25.6 (18.3–35.8)	

Data are geometric means (95% CI) unless otherwise indicated. *P values for trend are based on median values in categories of the participants. §Match-adjusted model: adjusted for age, race, duration of follow-up, and time of blood draw. †Categorical model: adjusted for matching factors, smoking status, physical activity, alcohol use, total calories, and BMI. ‡Spline model: estimates at category medians from quadratic spline regression models with one knot at the middle category boundaries, adjusted for covariates used in categorical model.

dian value for each category and included this as a continuous variable in the multiple linear regression models.

To assess the relations of caffeine-related beverage consumption with type 2 diabetes risk, we used conditional logistic regression models to adjust for matched pairs (match-adjusted model). We further adjusted for smoking status (never, past, and current smokers), physical activity (rarely/never, <1, 1–3, and ≥4 times/week), family history of diabetes (yes or no), alcohol use (rarely/never, 1–3 drinks/month, 1–6 drinks/week, and ≥1 drinks/day), total calories (≤1,500, 1,501–2,000, 2,001–2,500, and >2,500 kcal/day), and BMI (continuous) (categorical model). To test for a linear trend across increasing categories of caffeine-related beverage consumption, we computed the median value for each category and included this as a continuous variable in the conditional logistic regression models. Because caffeine and caffeinated coffee consumption were associated with plasma SHBG levels but not with sex hormones, in subsequent analyses, we further included plasma SHBG levels

(≤20.0, 20.1–25.0, 25.1–30.0, and >30.0 nmol/l) in the models (categorical model + SHBG).

To further provide a visual representation of the dose-response curve, we fitted quadratic spline models by including transformed variables of caffeine-related beverage consumption to multiple regression models using a single knot at the middle category boundary used in each categorical model (20). Finally, we examined potential effect modification by two informative *SHBG* SNPs, rs6259 and rs6257, using the dominant genetic model. We calculated adjusted plasma SHBG levels and odds ratios (ORs) of type 2 diabetes for combinations of *SHBG* genotypes and caffeinated coffee intake levels (≥2 cups/day vs. <2 cups/day). Wald tests were used to test for statistical interaction by entering product terms to the regression models. We performed the χ^2 test to evaluate Hardy-Weinberg equilibrium for rs6259 and rs6257 among the control subjects. All statistical analyses were conducted using SAS (version 9.2; SAS institute, Cary, NC).

TABLE 3
Geometric mean levels of total estradiol, total testosterone, and DHEAS according to caffeinated coffee, decaffeinated coffee, tea, and caffeine consumption

	0 cups/day	≤1 cups/day	2–3 cups/day	≥4 cups/day	P for trend
Caffeinated coffee, median (*n*)	0 (185)	1.0 (187)	2.5 (212)	4.5 (122)	
Total estradiol (pg/ml)	23.6 (17.9–31.1)	21.2 (16.1–28.1)	23.9 (18.0–31.6)	22.0 (16.5–29.2)	0.76
Total testosterone (ng/dl)	20.7 (13.4–32.0)	21.5 (13.8–33.3)	22.8 (14.6–35.4)	22.3 (14.3–35.0)	0.23
DHEAS (µg/dl)	87.8 (57.6–133.7)	89.0 (58.2–136.1)	94.4 (61.6–144.6)	93.3 (60.4–144.0)	0.28
Decaffeinated coffee, median (*n*)	0 (401)	0.4 (188)	2.5 (84)	4.5 (23)	
Total estradiol (pg/ml)	23.0 (17.4–30.5)	21.6 (16.2–28.8)	22.7 (16.8–30.8)	21.8 (15.7–30.3)	0.67
Total testosterone (ng/dl)	21.0 (13.6–32.4)	18.7 (11.9–29.2)	19.7 (12.3–31.6)	19.9 (11.9–33.3)	0.55
DHEAS (µg/dl)	86.3 (56.5–131.8)	83.1 (53.8–128.5)	84.5 (53.5–133.6)	88.5 (53.7–145.6)	0.99
Tea, median (*n*)	0 (242)	0.4 (351)	2.5 (76)	4.5 (28)	
Total estradiol (pg/ml)	22.9 (17.3–30.2)	23.5 (17.8–31.1)	24.6 (18.5–32.7)	22.4 (16.3–30.8)	0.60
Total testosterone (ng/dl)	21.7 (14.1–33.6)	23.0 (14.8–35.6)`	21.9 (14.0–34.3)	19.7 (11.9–32.4)	0.49
DHEAS (µg/dl)	87.8 (57.7–133.5)	94.9 (62.2–144.8)	88.2 (57.2–136.1)	78.1 (48.3–126.2)	0.36
Caffeine category (mg/day)	<50	50–249	250–499	≥500	
Caffeine intake, median (mg/day) (*n*)	13 (131)	140 (209)	366 (230)	656 (123)	
Total estradiol (pg/ml)	23.0 (17.4–30.4)	22.4 (16.9–29.5)	24.2 (18.3–32.0)	22.4 (16.9–29.8)	0.79
Total testosterone (ng/dl)	19.4 (12.5–30.0)	21.9 (14.2–33.8)	22.1 (14.2–34.2)	22.1 (14.2–34.5)	0.21
DHEAS (µg/dl)	84.2 (55.1–128.7)	90.2 (59.1–137.6)	92.3 (60.3–141.3)	90.5 (58.7–139.5)	0.42

Data are geometric means (95% CI). *P for trend are based on median values in categories of the participants. All models were adjusted for matching factors (age, race, duration of follow-up, and time of blood draw), smoking status, physical activity, alcohol use, total calories, and BMI.

RESULTS

Compared with control participants, diabetic subjects had a greater proportion of traditional risk factors at baseline (Table 1). Diabetic subjects had higher levels of plasma total estradiol and lower levels of plasma SHBG, but plasma total testosterone and DHEAS appeared to be similar between case and control subjects.

Caffeinated coffee and caffeine intakes were positively associated with plasma SHBG levels but not with sex hormones (Table 2 and Table 3). For caffeinated coffee, the multivariate-adjusted geometric mean levels of plasma SHBG were 26.6 nmol/l (95% CI 18.9–37.4) in women consuming ≥4 cups/day and 23.0 nmol/l (16.5–32.0) in nondrinkers (P for trend = 0.01). For caffeine, the multi-variate-adjusted geometric mean levels of plasma SHBG were 26.6 nmol/l (19.0–37.4) in women consuming >500 mg/day and 22.9 nmol/l (16.5–32.0) in women consuming ≤50 mg/day (P for trend = 0.02) (Table 2). We found similar results using quadratic spline models that imposed smooth dose-response relations (Table 2 and Fig. 1A). The spline plots indicated that heavy drinkers of caffeinated coffee (>2 cups/day) were associated with higher levels of plasma SHBG (Fig. 1A). In contrast, decaffeinated coffee and tea intakes were not associated with plasma SHBG levels and sex hormone levels (Table 3).

Caffeinated coffee and caffeine intakes were also in-versely associated with risk of type 2 diabetes (Table 4). The multivariate-adjusted ORs of type 2 diabetes were 0.47 (95% CI 0.23–0.94; P for trend = 0.047) for ≥4 cups/day of caffeinated coffee compared with nondrinkers and 0.56 (0.27–1.15; P for trend = 0.18) for >500 mg/day compared with ≤50 mg/day of caffeine. Little or no association of decaffeinated coffee and tea consumption with type 2 diabetes was observed. After further adjusting for plasma SHBG levels, the inverse associations of caffeinated coffee and caffeine with type 2 diabetes risk were attenuated (categorical model + SHBG). Compared with nondrinkers, the ORs for ≥4 cups/day of caffeinated coffee changed from 0.47 to 0.71 (0.31–1.61; P for trend = 0.47). Similarly, compared with <50 mg/day of caffeine, the ORs for ≥500 mg/day were changed from 0.56 to 0.89 (0.38–2.10; P for trend = 0.91). In contrast, further adjustment for plasma sex hormones instead of SHBG did not change the asso-ciation. Similar results were shown in our fitted spline logistic regression models (Table 4, Spline model). In the spline plots, before adjusting for plasma SHBG, an inverse trend between caffeinated coffee and risk of type 2 diabe-tes was observed above 2 cups/day of caffeinated coffee (Fig. 1B). This trend disappeared after further adjusting for plasma SHBG (Fig. 1C).

Finally, we estimated the multivariable-adjusted geo-metric mean levels of plasma SHBG and multivariable-adjusted ORs of type 2 diabetes for combinations of SHBG genotypes and caffeinated coffee intake levels (≥2 cups/day vs. <2 cups/day) (Table 5). We detected no departure from Hardy-Weinberg equilibrium for rs6259 and rs6257 SNPs among the control subjects (P = 0.24 and P = 0.06, respectively). Carriers of the rs6259 minor allele and noncarriers of the rs6257 minor allele who reported high intake of caffeinated coffee had a lower risk of type 2 diabetes in directions corresponding to their associated plasma SHBG levels. Compared with low-drinkers (<2 cups/day) without the rs6259 minor allele, high-drinkers (≥2 cups/day) with the minor allele had 20% higher plasma SHBG levels (27.8 vs. 23.2 nmol/l) and were associated
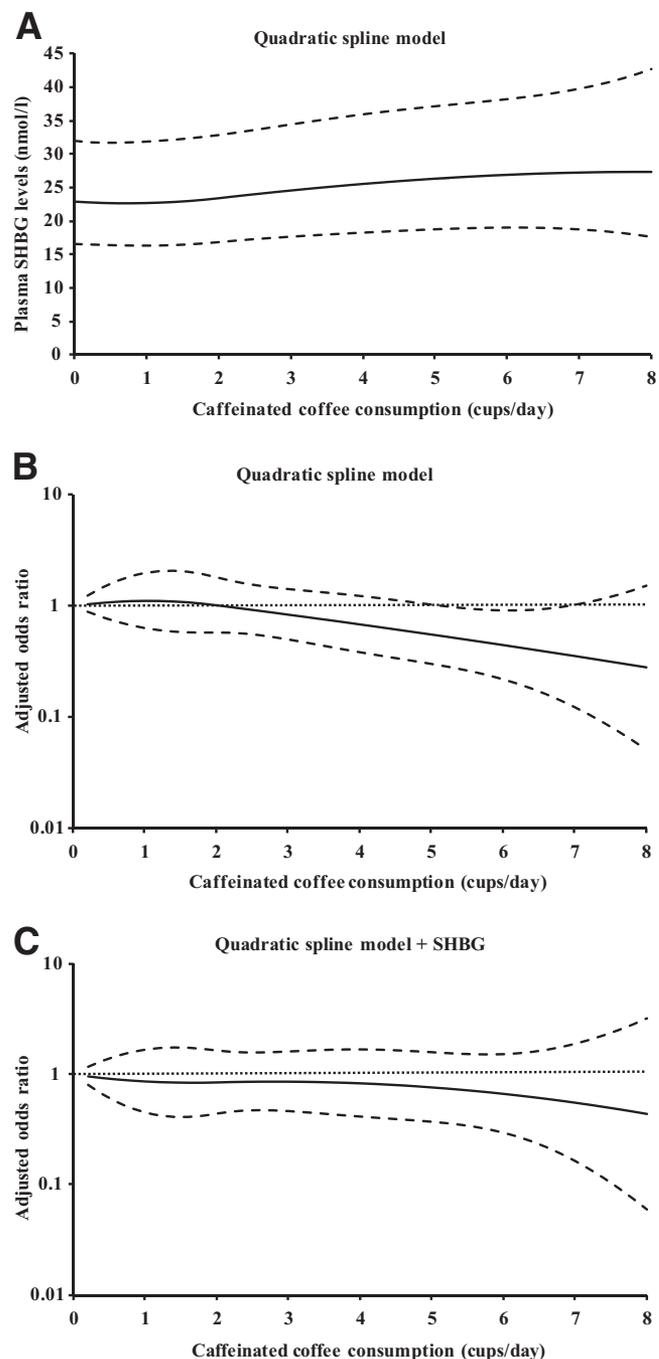
FIG. 1. Estimated plasma SHBG and risk of type 2 diabetes in women according to caffeinated coffee consumption. A: The geometric mean SHBG levels adjusted for matching factors, smoking status, physical activity, family history of diabetes, alcohol use, total calories, and BMI from quadratic spline model (solid curve) with pointwise 95% confi-dence limits (dashed curves). B: The OR of type 2 diabetes from quadratic conditional logistic spline model adjusted for matching factors, smoking status, physical activity, family history of diabetes, alcohol use, total calories, and BMI (solid curve) with pointwise 95% confidence limits (dashed curves). C: The multivariate-adjusted OR of type 2 diabetes from quadratic conditional logistic spline model with further adjustment for plasma SHBG.

with lower risk of type 2 diabetes (OR 0.54 [95% CI 0.26–1.11]). Similarly, compared with low-drinkers (<2 cups/day) with the rs6257 minor allele, high-drinkers (≥2 cups/day) without the minor allele had 24% higher plasma SHBG levels (25.2 vs. 20.3 nmol/l) and were associated with lower risk of type 2 diabetes (0.38 [0.18–0.83]).

TABLE 4
ORs for type 2 diabetes according to caffeinated coffee, decaffeinated coffee, tea, and caffeine consumption

	0 cups/day	≤1 cups/day	2–3 cups/day	≥4 cups/day	P for trend*
Caffeinated coffee (case subjects/control subjects)	99/86	103/84	105/107	49/73	
Median (cups/day)	0	1.0	2.5	4.5	
Match-adjusted model†	1.00	1.04 (0.68–1.60)	0.82 (0.55–1.23)	0.55 (0.34–0.90)	0.008
Categorical model‡	1.00	0.95 (0.52–1.74)	0.94 (0.53–1.67)	0.47 (0.23–0.94)	0.047
Categorical model + SHBG§	1.00	0.92 (0.46–1.84)	0.96 (0.48–1.94)	0.71 (0.31–1.61)	0.47
Spline model‖	1.00	1.11 (0.63–1.97)	0.92 (0.55–1.54)	0.61 (0.34–1.11)	
Spline model + SHBG¶	1.00	0.87 (0.45–1.68)	0.86 (0.47–1.58)	0.80 (0.39–1.65)	
Decaffeinated coffee (case subjects/control subjects)	211/190	94/94	37/47	8/15	
Median (cups/day)	0	0.4	2.5	4.5	
Match-adjusted model†	1.00	0.87 (0.60–1.26)	0.63 (0.38–1.03)	0.49 (0.20–1.20)	0.03
Categorical model‡	1.00	1.16 (0.70–1.95)	0.77 (0.38–1.55)	0.72 (0.19–2.69)	0.39
Categorical model + SHBG§	1.00	1.03 (0.56–1.90)	1.11 (0.46–2.71)	1.33 (0.27–6.49)	0.71
Spline model‖	1.00	1.30 (0.90–1.88)	0.61 (0.33–1.14)	0.47 (0.17–1.29)	
Spline model + SHBG¶	1.00	1.31 (0.84–2.03)	0.91 (0.42–2.00)	0.92 (0.26–3.27)	
Tea (case subjects/control subjects)	114/128	180/171	37/39	18/10	
Median (cups/day)	0	0.4	2.5	4.5	
Match-adjusted model†	1.00	1.21 (0.87–1.69)	1.06 (0.64–1.74)	2.03 (0.91–4.54)	0.23
Categorical model‡	1.00	1.12 (0.70–1.77)	1.22 (0.59–2.50)	1.53 (0.43–5.47)	0.46
Categorical model + SHBG§	1.00	0.97 (0.55–1.72)	1.14 (0.48–2.72)	1.74 (0.44–6.93)	0.42
Spline model‖	1.00	1.39 (0.99–1.93)	1.52 (0.83–2.77)	1.32 (0.51–3.38)	
Spline model + SHBG¶	1.00	1.37 (0.93–2.03)	1.37 (0.67–2.78)	1.57 (0.54–4.58)	
Caffeine category (mg/day)	≤50	51–250	251–500	>500	
Caffeine intake (mg/day), median	13	140	366	656	
(case subjects/control subjects)	67/64	109/100	118/112	49/74	
Match-adjusted model†	1.00	1.01 (0.65–1.56)	0.99 (0.63–1.54)	0.62 (0.37–1.04)	0.06
Categorical model‡	1.00	1.00 (0.56–1.82)	1.26 (0.68–2.32)	0.56 (0.27–1.15)	0.18
Categorical model + SHBG§	1.00	0.94 (0.47–1.88)	1.44 (0.68–3.04)	0.89 (0.38–2.10)	0.91
Spline model‖	1.00	1.56 (0.80–3.04)	1.56 (0.81–3.01)	0.92 (0.48–1.76)	
Spline model + SHBG¶	1.00	1.53 (0.71–3.30)	1.70 (0.79–3.62)	1.32 (0.61–2.82)	

Data are OR (95% CI) unless otherwise indicated. *P for trend values are based on median levels in categories. †Match-adjusted model: stratified on matched pairs using conditional logistic regression models. ‡Categorical model: further adjusted for smoking status, physical activity, family history of diabetes, alcohol use, total calories, and BMI. §Categorical model + SHBG: further adjusted for plasma SHBG. ‖Spline model: ORs comparing odds at category medians from quadratic logistic spline models with one knot at the middle category boundaries adjusted for covariates used in categorical model. ¶Spline model + plasma SHBG: further adjusted for plasma SHBG.

DISCUSSION

In this prospective study of postmenopausal women, caffeinated coffee and caffeine intakes were positively associated with plasma SHBG levels. Also, we observed an inverse association between intake of caffeinated coffee and caffeine and risk of type 2 diabetes. The associations were largely attenuated after adjustment for SHBG levels. Finally, carriers of the rs6259 minor allele and noncarriers of the rs6257 minor allele who reported high intake of caffeinated coffee had a lower risk of type 2 diabetes in directions corresponding to their associated plasma SHBG levels. These findings suggest that SHBG may account for

TABLE 5
Caffeinated coffee consumption in relation to plasma SHBG levels and type 2 diabetes stratified by SHBG SNPs

	SHBG genotype							
	rs6259				rs6257			
	GG (wild type)		AG or AA (variant)		CT or CC (variant)		TT (wild type)	
Plasma SHBG levels (nmol/l)*	n		n		n		n	
Caffeinated coffee intake								
Low (<2 cups per day)	277	23.2 (16.6–32.5)	70	23.3 (16.4–32.9)	69	20.3 (14.2–29.0)	285	23.2 (16.6–32.4)
High (≥2 cups per day)	246	24.4 (17.4–34.3)	76	27.8 (19.4–39.7)	72	21.6 (15.2–30.8)	244	25.2 (17.9–35.4)
P for interaction†		0.18				0.84		

	(Case subjects/control subjects)		(Case subjects/control subjects)		(Case subjects/control subjects)		(Case subjects/control subjects)	
ORs‡								
Caffeinated coffee intake								
Low (<2 cups/day)	152/125	1.00 (reference)	40/30	0.90 (0.40–2.00)	42/27	1.00 (reference)	149/136	0.41 (0.19–0.88)
High (≥2 cups/day)	122/124	0.70 (0.42–1.16)	26/50	0.54 (0.26–1.11)	34/38	0.40 (0.16–1.02)	109/135	0.38 (0.18–0.83)
P for interaction†		0.79				0.13		

Data are OR (95% CI) and n. *The multivariate-adjusted geometric mean SHBG levels with 95% CIs for combinations of SHBG genotypes and caffeinated coffee intake levels (≥2 vs. <2 cups/day) adjusted for matching factors, smoking status, physical activity, family history of diabetes, alcohol use, total calories, and BMI. †Wald tests were used to test for statistical interaction by entering product terms into the regression models. ‡The multivariate-adjusted ORs and 95% CIs of type 2 diabetes risk for combinations of SHBG genotypes and caffeinated coffee intake levels (≥2 vs. <2 cups/day) adjusted for matching factors, smoking status, physical activity, family history of diabetes, alcohol use, total calories, and BMI.

the inverse association between caffeinated coffee and type 2 diabetes risk.

The inverse associations of caffeinated coffee and caffeine intake with type 2 diabetes risk observed in our study are consistent with findings from previous studies (1,2). Several possible explanations have been put forth to explain the protective effect of coffee consumption on type 2 diabetes risk, including effects on insulin sensitivity and β-cell function by varying coffee components such as magnesium, potassium, chlorogenic acid, and caffeine (2). To date, however, little is known about the underlying mechanisms. Evidence from a systematic review suggests the sex differences in the inverse association between coffee and type 2 diabetes risk (2). Moreover, both observational and experimental data indicate the important roles of sex hormones in the development of type 2 diabetes (6–8). SHBG is synthesized primarily in the liver and binds androgens with high affinity and estrogens with low affinity, thereby regulating the biologically active fraction of sex hormones (21). Recently, it has been shown that the plasma membranes of a variety of cells are able to bind SHBG specifically and with high affinity, and SHBG mediates sex hormones signaling at the cell membrane through the SHBG receptors (9). This discovery of the function of SHBG as a mediator of a steroid-signaling system has drawn much interest to the biological effects of SHBG. We first reported that lower levels of SHBG may be causally associated with type 2 diabetes risk using Mendelian randomization analyses (10), findings of which have been replicated by a large consortium of case-control studies (22). Taken together, we hypothesized that caffeinated coffee consumption may lower the risk of type 2 diabetes possibly by altering SHBG metabolism.

We found that caffeine and caffeinated coffee intakes were positively associated with plasma SHBG levels, which is consistent with earlier studies (4,5,23–25). Little or no association between decaffeinated coffee and plasma SHBG levels suggest that caffeine may be a key component of coffee responsible for determining plasma SHBG levels. Moreover, our findings of little or no relations between caffeine-related beverage consumption and sex hormones suggest that caffeine may increase the level of plasma SHBG without directly altering sex hormones levels. Caffeine and other major components of coffee (cafestol and kahweol) alter expression and activity of liver enzymes (26–29). Because SHBG is synthesized and metabolized primarily in the liver (21), coffee intake may affect SHBG metabolism in the liver and influence the plasma levels of SHBG (5).

Coffee may increase plasma SHBG levels, resulting not only in affecting the biological actions of sex hormones by binding to circulating androgens and estrogens but also in exerting direct metabolic effects (9). Our findings thus provide a new explanation for the potential protective effect of coffee consumption on the type 2 diabetes risk. Notably, we found that carriers of the rs6259 minor allele and noncarriers of the rs6257 minor allele who reported high intake of caffeinated coffee had a lower risk of type 2 diabetes in directions corresponding to their associated plasma SHBG levels. These findings may further support the notion that SHBG may account for the potential protective effect of caffeinated coffee on type 2 diabetes. In contrast, the role of specific sex-steroids in relation to the coffee-diabetes relation remains to be determined.

The strengths of our study include its prospective study design with 10-year follow-up with comprehensive assessment of baseline variables, blood samples, and SHBG genotypes. Nevertheless, our study has several limitations. First, cross-sectional analyses of coffee consumption and plasma SHBG may be a concern, although it is not likely that endogenous sex hormones or SHBG would influence the consumption. Second, we cannot exclude the possibilities of residual confounding from unmeasured or incompletely measured covariates even though we have adjusted for many major risk factors for type 2 diabetes. Third, misclassifications of dietary intakes and biomarker measures are inevitable. For example, there may be measurement errors of plasma sex hormones and SHBG because of the limitations of stored samples. However, because case subjects were identified prospectively and case-control pairs were matched and handled in an identical fashion in the same analytical run, any potential misclassifications should affect case and control subjects equally. Therefore, such misclassifications were likely to be nondifferential, which would lead to an underestimation of the associations. Fourth, there is a concern about the possibility of residual confounding from unmeasured time-dependent confounders when a standard method is performed to adjust for both an exposure and a measured intermediate variable. However, we consider it is less likely that such residual confounding would substantially explain our findings, because our observed associations appear to be consistent with the observed genetically determined SHBG levels when stratifying by SHBG genotypes. Finally, our study only included postmenopausal women, which may limit the generalizability of our findings to premenopausal women or men.

In conclusion, our results suggest that SHBG levels may account for the potential protective effect of habitual coffee consumption against type 2 diabetes risk among postmenopausal women. A better understanding of the underlying mechanisms requires further investigation in both observational and experimental settings.

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