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. During a median followup of 10 years, 359 postmenopausal women with newly diagnosed type 2 diabetes were matched with 359 controls 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 non-drinkers (*P* for trend = 0.01). In contrast, neither decaffeinated-coffee nor tea was associated with SHBG or sex hormones. Multivariable-adjusted odds ratio (OR) of type 2 diabetes for women consuming ≥4 cups/day of caffeinated-coffee compared with non-drinkers 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 non-carriers 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.

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 signalling 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. Further, 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. 28,345 (71%) provided baseline blood samples, of which 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 incident type 2 diabetes. Controls were matched in 1:1 ratio to cases 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 $\geq$ 10 h since the last meal). Based on these eligibility criteria, 359 cases and 359 controls 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 at 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 less than one per month, one to three per month, one per week, two to four per week, five to six per week, one per day, two to three per day, four to five per day, and six or more per 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 hours of venipuncture. Upon receipt, samples were kept chilled until processed. After centrifugation for 20 minutes (2500 rpm, 4 ºC) 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, USA), 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 SNP selection and genotyping of SHBG single nucleotide polymorphisms (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 (no cups per day, less than one cup to one cup per day, 2 to 3 cups per day, and 4 or more cups per 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 controls using the paired t-test for continuous variables and McNemar's 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/months, 1-6 drinks/week, ≥1 drinks/day), total calories (≤1500, 1501-2000, 2001-2500, and >2500 kcal/day), and body mass index (BMI) (continuous). 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 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/months, 1-6 drinks/week, ≥1 drinks/day), total calories (≤1500, 1501-2000, 2001-2500, and >2500 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 of type 2 diabetes for combinations of *SHBG* genotypes and caffeinated-coffee intake levels (2 cups/day or more vs. less than 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 controls. All statistical analyses were conducted using SAS (version 9.2; SAS institute, Cary, NC).

RESULTS

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

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% confidence interval [CI], 18.9-37.4) in women consuming ≥4 cups/day and 23.0 nmol/L (95% CI, 16.5-32.0) in non-drinkers (*P* for trend = 0.01). For caffeine, the multivariate-adjusted geometric mean levels of plasma SHBG were 26.6 nmol/L (95% CI, 19.0-37.4) in women consuming >500 mg/day and 22.9 nmol/L (95% CI, 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 Figure 1A). The spline plots indicated that heavy drinkers of caffeinated-coffee (>2 cups/day) were associated with higher levels of plasma SHBG (Figure 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 inversely associated with risk of type 2 diabetes (Table 4). The multivariate-adjusted odds ratios (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 non-drinkers, and 0.56 (95% CI: 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 non-drinkers, the ORs for ≥4 cups/day of caffeinated-coffee changed from 0.47 to 0.71 (95% CI, 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 (95% CI, 0.38 –

2.10; *P* for trend = 0.91). In contrast, further adjustment for plasma sex hormones instead of SHBG did not change the association. 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 diabetes was observed above 2 cups/day of caffeinated-coffee (Figure 1B). This trend disappeared after further adjusting for plasma SHBG (Figure 1C).

Finally, we estimated the multivariable-adjusted geometric 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 controls (*P*=0.24 and *P*=0.06, respectively). Carriers of the rs6259 minor allele and non-carriers of the rs6257 minor allele who consumed high caffeinated-coffee had a lower risk of type 2 diabetes in directions corresponding to their associated plasma SHBG levels: as 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 with lower risk of type 2 diabetes (OR =0.54; 95% CI, 0.26-1.11). Similarly, as 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 (OR =0.38; 95% CI, 0.18-0.83).

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 non-carriers of the rs6257 minor allele who consumed high 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 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 biologic 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 via 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 biologic 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 non-carriers of the rs6257 minor allele who consumed high 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 due to the limitations of stored samples. However, as cases were identified prospectively and case-control pair were matched and handled in an identical fashion in the same analytical run, any potential misclassifications should affect case and control equally. Therefore, such misclassifications were likely to be non-differential, which would lead to an underestimation of the associations. Forth, 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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REFERENCES

1. Huxley R, Lee CM, Barzi F, Timmermeister L, Czernichow S, Perkovic V, Grobbee DE, Batty D, Woodward M: Coffee, decaffeinated coffee, and tea consumption in relation to incident type 2 diabetes mellitus: A systematic review with meta-analysis. *Arch Intern Med* 169:2053-2063, 2009

2. van Dam RM, Hu FB: Coffee consumption and risk of type 2 diabetes: a systematic review. *JAMA* 294:97-104, 2005

3. Isogawa A, Noda M, Takahashi Y, Kadowaki T, Tsugane S: Coffee consumption and risk of type 2 diabetes mellitus. *Lancet* 361:703-704, 2003

4. London S, Willett W, Longcope C, McKinlay S: Alcohol and other dietary factors in relation to serum hormone concentrations in women at climacteric. *Am J Clin Nutr* 53:166-171, 1991

5. Kotsopoulos J, Eliassen AH, Missmer SA, Hankinson SE, Tworoger SS: Relationship between caffeine intake and plasma sex hormone concentrations in premenopausal and postmenopausal women. *Cancer* 115:2765-2774, 2009

6. Ding EL, Song Y, Malik VS, Liu S: Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA* 295:1288-1299, 2006
7. Ding EL, Song Y, Manson JE, Rifai N, Buring JE, Liu S: Plasma sex steroid hormones and risk of developing type 2 diabetes in women: a prospective study. *Diabetologia* 50:2076-2084, 2007
8. Rincon J, Holmang A, Wahlstrom EO, Lonnroth P, Bjorntorp P, Zierath JR, Wallberg-Henriksson H: Mechanisms behind insulin resistance in rat skeletal muscle after oophorectomy and additional testosterone treatment. *Diabetes* 45:615-621, 1996
9. Rosner W, Hryb DJ, Khan MS, Nakhla AM, Romas NA: Sex hormone-binding globulin mediates steroid hormone signal transduction at the plasma membrane. *J Steroid Biochem Mol Biol* 69:481-485, 1999
10. Ding EL, Song Y, Manson JE, Hunter DJ, Lee CC, Rifai N, Buring JE, Gaziano JM, Liu S: Sex hormone-binding globulin and risk of type 2 diabetes in women and men. *N Engl J Med* 361:1152-1163, 2009
11. Buring JE, Hennekens CH: The Women's Health Study: summary of the study design. *J Myocard. Ischemia* 4:27-29, 1992
12. Watt BK, Merrill AL: *Composition of Foods: Raw, Processed, Prepared, 1963-1992: Agriculture Handbook no. 8.*, Washington, DC, U.S. Department of Agriculture, 1993
13. Salvini S, Hunter DJ, Sampson L, Stampfer MJ, Colditz GA, Rosner B, Willett WC: Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. *Int J Epidemiol* 18:858-867, 1989
14. Song Y, Manson JE, Buring JE, Liu S: A prospective study of red meat consumption and type 2 diabetes in middle-aged and elderly women: the women's health study. *Diabetes Care* 27:2108-2115, 2004
15. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183-1197, 1997
16. Liu S, Lee IM, Song Y, Van Denburgh M, Cook NR, Manson JE, Buring JE: Vitamin E and risk of type 2 diabetes in the women's health study randomized controlled trial. *Diabetes* 55:2856-2862, 2006
17. Reynders M, Anckaert E, Schiettecatte J, Smitz J: Evaluation of a new automated electrochemiluminescent sex hormone-binding globulin (SHBG) immunoassay. *Clin Chem Lab Med* 43:86-89, 2005
18. Sanchez-Carbayo M, Mauri M, Alfayate R, Miralles C, Soria F: Elecsys testosterone assay evaluated. *Clin Chem* 44:1744-1746, 1998
19. Yang DT, Owen WE, Ramsay CS, Xie H, Roberts WL: Performance characteristics of eight estradiol immunoassays. *Am J Clin Pathol* 122:332-337, 2004
20. Greenland S: Dose-response and trend analysis in epidemiology: alternatives to categorical analysis. *Epidemiology* 6:356-365, 1995
21. Anderson DC: Sex-hormone-binding globulin. *Clin Endocrinol (Oxf)* 3:69-96, 1974
22. Perry JR, Weedon MN, Langenberg C, Jackson AU, Lyssenko V, Sparso T, Thorleifsson G, Grallert H, Ferrucci L, Maggio M, Paolisso G, Walker M, Palmer CN, Payne F, Young E, Herder C, Narisu N, Morken MA, Bonnycastle LL, Owen KR, Shields B, Knight B, Bennett A, Groves CJ, Ruokonen A, Jarvelin MR, Pearson E, Pascoe L, Ferrannini E, Bornstein SR, Stringham HM, Scott LJ, Kuusisto J, Nilsson P, Neptin M, Gjesing AP, Pisinger C, Lauritzen T, Sandbaek A, Sampson M, Zeggini ME, Lindgren CM, Steinthorsdottir V, Thorsteinsdottir U, Hansen T, Schwarz P, Illig T, Laakso M, Stefansson K, Morris AD, Groop L, Pedersen O, Boehnke M,

Barroso I, Wareham NJ, Hattersley AT, McCarthy MI, Frayling TM: Genetic evidence that raised sex hormone binding globulin (SHBG) levels reduce the risk of type 2 diabetes. *Hum Mol Genet* 19:535-544
23. Lucero J, Harlow BL, Barbieri RL, Sluss P, Cramer DW: Early follicular phase hormone levels in relation to patterns of alcohol, tobacco, and coffee use. *Fertil Steril* 76:723-729, 2001
24. Nagata C, Kabuto M, Shimizu H: Association of coffee, green tea, and caffeine intakes with serum concentrations of estradiol and sex hormone-binding globulin in premenopausal Japanese women. *Nutr Cancer* 30:21-24, 1998
25. Ferrini RL, Barrett-Connor E: Caffeine intake and endogenous sex steroid levels in postmenopausal women. The Rancho Bernardo Study. *Am J Epidemiol* 144:642-644, 1996
26. Park BK, Kitteringham NR: Assessment of enzyme induction and enzyme inhibition in humans: toxicological implications. *Xenobiotica* 20:1171-1185, 1990
27. Higgins LG, Cavin C, Itoh K, Yamamoto M, Hayes JD: Induction of cancer chemopreventive enzymes by coffee is mediated by transcription factor Nrf2. Evidence that the coffee-specific diterpenes cafestol and kahweol confer protection against acrolein. *Toxicol Appl Pharmacol* 226:328-337, 2008
28. Huber WW, Rossmanith W, Grusch M, Haslinger E, Prustomersky S, Peter-Vorosmarty B, Parzefall W, Scharf G, Schulte-Hermann R: Effects of coffee and its chemopreventive components kahweol and cafestol on cytochrome P450 and sulfotransferase in rat liver. *Food Chem Toxicol* 46:1230-1238, 2008
29. Huber WW, Scharf G, Rossmanith W, Prustomersky S, Grasl-Kraupp B, Peter B, Turesky RJ, Schulte-Hermann R: The coffee components kahweol and cafestol induce gamma-glutamylcysteine synthetase, the rate limiting enzyme of chemoprotective glutathione synthesis, in several organs of the rat. *Arch Toxicol* 75:685-694, 2002

FIGURE LEGEND

Figure 1: Estimated Plasma SHBG and Risk of Type 2 Diabetes in Women, According to Caffeinated-Coffee Consumption

Panel A shows 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% confidence limits (dashed curves).
Panel B shows the odds ratio 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).
Panel C shows the multivariate-adjusted odds ratio of type 2 diabetes from quadratic conditional logistic spline model with further adjustment for plasma SHBG.

Table 1. Baseline Characteristics between Participants with Incident Cases of Type 2 Diabetes and Control Participants among 718 Women

Characteristics	Cases	Controls	P-values *
No.	359	359	
Age (yr)	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
Smoking (% current)	14.5	13.7	0.74
Physical activity (% ≥ once/wk)	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	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
Marital status (% 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

Abbreviation: SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone.
Data are means ± SD.

* Baseline characteristics were compared between case patients and controls using the paired t-test for continuous variables and the McNemar's test for categorical variables.

Table 2. Geometric Mean Levels of Sex Hormone-binding Globulin according to Caffeinated-Coffee, Decaffeinated-Coffee, Tea, and Caffeine Consumption

Plasma SHBG levels (nmol/L)	Categories of intake				*P* for trend *
	No cups per day	Less than one cup or one cup per day	Two to three cups per day	Four or more cups per 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, Median (mg/day) (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 expressed as geometric means (95% CI). Abbreviation: SHBG, sex hormone-binding globulin; CI, confidence interval.

* *P* 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.

Table 3. Geometric Mean Levels of total Estradiol, total Testosterone, and DHEAS according to Caffeinated-Coffee, Decaffeinated-Coffee, Tea, and Caffeine Consumption

	Categories of intake				*P* for trend *
	No cups per day	Less than one cup or one cup per day	Two to three cups per day	Four or more cups per day	
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 expressed as geometric mean (95% CI). Abbreviation: DHEAS, dehydroepiandrosterone sulfate; CI, confidence interval.

* *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.

Table 4. Odds Ratios for Type 2 Diabetes according to Caffeinated-Coffee, Decaffeinated-Coffee, Tea, and Caffeine Consumption

	Categories of intake				
	No cups per day	**Less than one cup to one cup per day**	**Two to three cups per day**	**Four or more cups per day**	***P* for trend ***
Caffeinated-coffee (cases/controls)	99/86	103/84	105/107	49/73	
Median (cups per 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 (cases/controls)	211/190	94/94	37/47	8/15	
Median (cups per 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 (cases/controls)	114/128	180/171	37/39	18/10	
Median (cups per 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, Median (mg/day)	13	140	366	656	
(cases/controls)	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 ORs (95% CI). Abbreviation: SHBG, sex hormone-binding globulin; ORs, odds ratios; CI, confidence interval

* *P*-values for trend 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: odds ratios 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 plus plasma SHBG: further adjusted for plasma SHBG.

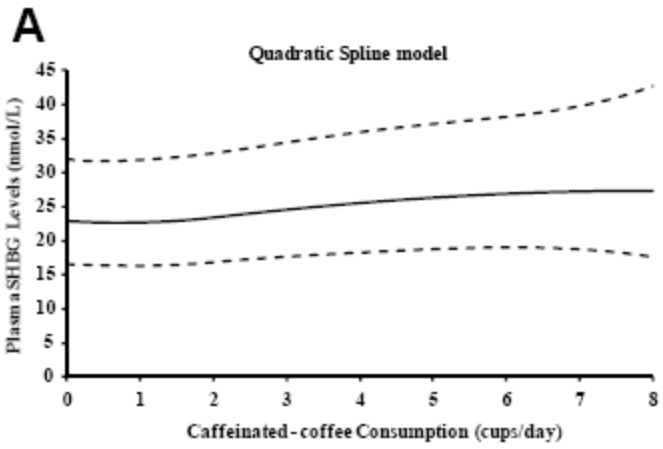
Table 5. Caffeinated-Coffee Consumption in Relation to Plasma SHBG and Type 2 Diabetes Stratified by SHBG SNPs

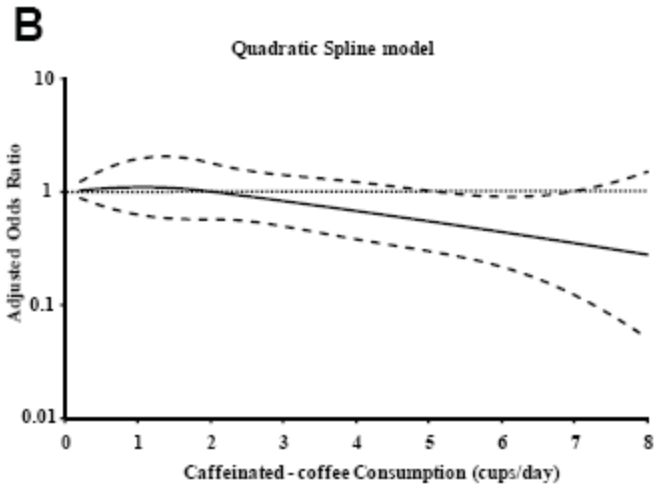
* The multivariate-adjusted geometric mean SHBG levels with 95% confidence intervals for combinations of SHBG genotypes and caffeinated-coffee intake levels (2 cups/day or more vs. less than 2 cups/day) adjusted for matching factors, smoking status, physical activity, family history of diabetes, alcohol use, total calories, and BMI.

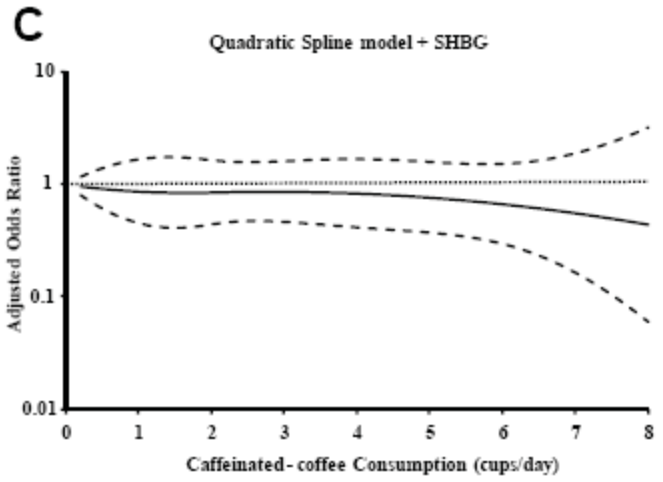
† Wald tests were used to test for statistical interaction by entering product terms into the regression models.

‡ The multivariable adjusted odds ratios and 95% confidence intervals of type 2 diabetes risk for combinations of SHBG genotypes and caffeinated-coffee intake levels (2 cups/day or more vs. less than 2 cups/day) adjusted for matching factors, smoking status, physical activity, family history of diabetes, alcohol use, total calories, and BMI.

	SHBG Genotype							
	rs6259				rs6257			
	GG (wild type)		AG or AA (variant)		CT or CC (variant)		TT (wild type)	
Plasma SHBG Levels (nmol/L) *	No.		No.		No.		No.	
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		
Odds Ratios ‡	**(cases/ controls)**		**(cases/ controls)**		**(cases/ controls)**		**(cases/ controls)**	
Caffeinated-Coffee Intake								
Low (< 2 cups per 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 per 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		


A
Quadratic Spline model
Plasma SHBG Levels (nmol/L)
45
40
35
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8
Caffeinated - coffee Consumption (cups/day)


B
Quadratic Spline model
Adjusted Odds Ratio
10
1
0.1
0.01
0 1 2 3 4 5 6 7 8
Caffeinated - coffee Consumption (cups/day)


C
Quadratic Spline model + SHBG
Adjusted Odds Ratio
10
1
0.1
0.01
0 1 2 3 4 5 6 7 8
Caffeinated - coffee Consumption (cups/day)