Relation of Dietary Carbohydrates Intake to Circulating Sex Hormone-binding Globulin Levels in Postmenopausal Women

Running Title: dietary carbohydrates and circulating SHBG

Mengna HUANG*[,1,2], Jinjie LIU*[,1], Xiaochen LIN[1,2], Atsushi GOTO[3], Yiqing SONG[4], Lesley F. TINKER[5], Kei-hang Katie CHAN[1,2,6], Simin LIU[1,2,7]

* Co-first authors

[1] Department of Epidemiology, Brown University School of Public Health, Providence, RI

[2] Center for Global Cardio-metabolic Health, Brown University, Providence, RI

[3] Metabolic Epidemiology Section, Division of Epidemiology, Center for Public Health Sciences, National Cancer Center, Japan, Tokyo

[4] Department of Epidemiology, Indiana University Richard M. Fairbanks School of Public Health, Indianapolis, IN

[5] Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA

[6] Hong Kong Institute of Diabetes and Obesity, the Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong Special Administrative Region, China

[7] Division of Endocrinology, Department of Medicine, Alpert School of Medicine and Rhode Island Hospital, Providence, RI

Corresponding Author: Simin LIU

Postal address: Box G-S121-2, Brown University, Providence, RI 02912

Phone: (401) 863-5247

Fax: None

E-mail address: simin_liu@brown.edu

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Abstract

Background: Circulating sex hormone-binding globulin (SHBG) concentrations have been suggested to be a protective factor for type 2 diabetes, cardiovascular diseases, and hormone-dependent cancers. However, the relation between various aspects of dietary carbohydrates and circulating SHBG concentrations remains unclear.

Methods: We analyzed the baseline data from postmenopausal women with available SHBG measurements (n=11,159) who participated in the Women's Health Initiative (WHI). Associations of total dietary carbohydrates, glycemic load (GL), glycemic index (GI), fiber, sugar and intake of various carbohydrate-abundant foods with circulating SHBG were assessed using linear regression models with adjustment for multiple covariates. Linear trend was tested across quartiles of the dietary variables. Benjamini and Hochberg's procedure for controlling the false discovery rate (FDR) was used to account for multiple comparisons.

Results: Higher dietary GL based on total and available carbohydrates, dietary GI based on total and available carbohydrates, and higher intake of sugar and sugar sweetened beverages were associated with lower concentrations of circulating SHBG (all $P_{trend} < 0.05$; q-value after FDR correction = 0.035, 0.013, 0.067, 0.103, 0.008, <.001, respectively). Higher intake of fiber was associated with increased SHBG concentrations ($P_{trend} = 0.011$, q-value after FDR correction = 0.037). There was no significant association of total carbohydrates or other carbohydrate-abundant foods with SHBG concentrations.

Conclusions: These findings suggest that low GL/GI diets with low sugar and high fiber content may be associated with higher serum SHBG concentrations among postmenopausal women. Future studies investigating whether lower GL/GI diets increase SHBG concentrations are warranted.

Highlights

Our study suggested that low GL/GI diets with low sugar and high fiber content may be associated with higher serum SHBG concentrations among postmenopausal women. This supports a role of diet in influencing circulating SHBG concentrations, which is in turn an important and probable protective factor of type 2 diabetes, cardiovascular disease, and hormone-dependent cancers.

Keywords: dietary carbohydrates, glycemic load, glycemic index, sex hormone binding globulin (SHBG), type 2 diabetes

Introduction

Sex hormone-binding globulin (SHBG) is a serum protein synthesized by the liver that binds to both androgens and estrogens, with higher affinity to androgens.[1,2] SHBG was originally thought to primarily regulate the amount of sex hormones that are bioavailable to the cells. However, recent epidemiological studies consistently show that low SHBG concentrations are strongly associated with the development of insulin resistance, type 2 diabetes, cardiovascular diseases (CVD), hormone-dependent cancers, as well as hip fractures, either indirectly by modulating the biologic effects of testosterone or exert more direct effects through its own SHBG receptor.[1,3-10] Mendelian randomization analyses using single nucleotide polymorphisms within or near the SHBG gene as instrumental variables for blood SHBG concentrations also provided supporting evidence to the causal relationship between SHBG and the risk of type 2 diabetes.[3,11]

Given the role SHBG may play in the etiologies of type 2 diabetes, CVD, and hormone-dependent cancers, investigating the determinants of blood SHBG concentrations is of great importance. In addition to several common variants identified within or near the SHBG gene,[3,11] lifestyle factors, especially dietary factor, may have a direct effect on circulating concentrations of free endogenous sex hormones through the regulation of SHBG concentrations.[12] Physical activity, regular coffee consumption, as well as weight loss by exercise and/or caloric restriction has been found to increase SHBG concentrations in postmenopausal women.[13,14] Emerging evidence also shows that different types of dietary carbohydrates may have heterogeneous associations with SHBG concentrations. In a dietary intervention study, lower serum SHBG concentrations were observed among participants on a conventional high glycemic load diet, while the SHBG concentrations increased among those on a high-protein low glycemic load diet.[15] Fiber intake was found to be positively correlated with SHBG concentrations in a previous study in men,[16] but another study failed to observe a similar correlation in postmenopausal women.[17] Moreover, although an inverse association between monosaccharides and SHBG

production was reported previously in transgenic mice and hepatic cell models,[18] a more recent study found that sweets intake may positively correlate with SHBG concentrations, although the result was not significant.[2] Increasing attention has been attracted to the impact of dietary factors on circulating SHBG concentrations. Nevertheless, studies examining the effect of quality and quantity of dietary carbohydrates on SHBG are still scarce and inconclusive, and very few have studied foods that are abundant in carbohydrates. Therefore, we conducted a comprehensive examination of the relations between various measures of dietary carbohydrates and concentrations of circulating SHBG among a subsample from the large-scale national Women's Health Initiative study.[19]

Methods

Study Subjects

The Women's Health Initiative (WHI) is a long-term national health study that focused on strategies for preventing heart diseases, breast and colorectal cancer, and osteoporotic fractures in postmenopausal women. The original WHI study included 161,808 postmenopausal women enrolled between 1993 and 1998 in two major parts: a partial factorial randomized Clinical Trial (CT) and an Observational Study (OS); both were conducted at 40 Clinical Centers nationwide. The CT enrolled 68,132 postmenopausal women between the ages of 50 to 79 into trials testing three prevention strategies. The OS examined the relationship between lifestyle, environmental, medical and molecular risk factors and specific measures of health or disease outcomes. This component involved tracking the medical history and health habits of 93,676 women not participating in the CT. The current analysis included an initial total of 13,955 unique participants from either the WHI-CT or the WHI-OS whose blood samples from baseline had been measured for serum SHBG in the following ancillary studies: AS90 (400 hip fracture cases and 400 controls), AS110 (385 coronary heart disease cases and 385 controls), AS167 (311 breast cancer cases and 592 controls), AS238 (700 type 2 diabetes cases and 1,400 controls),

BA7 (422 venous thromboembolism, 534 stroke, 753 CHD, 204 spine fracture, 830 non-hip-or-spine fracture cases, and 1,576 controls), BA9 (1,132 fracture cases and 1,132 controls), BA21 (400 colorectal cancer cases and 800 controls), W5 (300 controls), W9 (750 hip fracture cases and 750 controls), W10 (755 breast cancer cases and 755 controls), and W18 (240 controls). Participants were excluded if they self-reported diabetes at baseline or had implausible total energy intake (< 600 or > 5000 kcal/day) as determined by the food frequency questionnaire, or if they had missing information in important covariates such as age, race/ethnicity, body mass index, smoking status, physical activity, and hormone therapy use. No missing in dietary measurements were observed after applying the above exclusion criteria.

Measurement of Serum SHBG Concentrations

For each study participant, blood was collected at the baseline visit after at least a 12-hour fast and then stored at −80 °C to -70 °C. Samples used for the hormone measurements were taken from these baseline specimens. The serum SHBG concentrations were measured using an electrochemiluminescence immunoassay (ECL) in AS238, a solid-phase, two-site chemiluminescent immunoassay (solid-phase, two-site CIA) in AS90, AS110, AS167, BA7, BA9, BA21, W9, W10, and W18), or an immunoradiometric assay (IRMA) in W5. The inter-assay coefficients of variation ranged between 3.7% and 17.7%.[2]

Dietary Measurements

The methods of data collection and validation have been reported previously.[19,20] Participants completed at baseline a 122-item standardized food frequency questionnaire (FFQ) developed for the WHI to estimate average daily dietary intake over the past 3 months.[21] The FFQ was based on instruments used in the WHI feasibility studies and the original National Cancer Institute/Block FFQ.[22-24] The dietary database, linked to the University of Minnesota Nutrition Coordinating Center Nutrition Data System for Research (Nutrition Coordinating Center,

Minneapolis, MN, USA), is based on the U.S. Department of Agriculture standard reference releases and manufacturer information.[25] The detailed description of the methods used to calculate GI and GL values can be found elsewhere.[26] In summary, GI values based on food consumption or expert judgment were assigned to each food items that contained at least five grams of carbohydrates, and then for each FFQ line item GL values were calculated by multiplying GI by intake frequencies and portion sizes. Both total carbohydrates and available carbohydrates (total carbohydrates minus total fiber) were used to calculate GI and GL values. In addition, dietary intakes of total carbohydrates, total sugar, and total fiber were included in the analyses in separate models. As a secondary analysis, the associations of different carbohydrates abundant food items (daily servings of white bread, dark bread, rice grains and noodles, potato, cereal, fruits, beans, sugar sweetened beverages, pasta, and whole grains) with serum SHBG concentrations were also examined. The potato variable included French fries, potato salad, sweet potatoes and yams, and other potato/cassava/yucca. The cereal variable included cold and cooked cereals. The beans variable included green or string beans, English peas, refried beans, all other beans, and bean soup. The sugar sweetened beverage variable included regular soft drinks (not diet), orange or grapefruit juice, other fruit juice, and fruit drinks. The pasta variable included macaroni and cheese, lasagna, or noodles with a cream sauce, spaghetti with meat sauce, and spaghetti with tomato sauce. All other variables were pre-calculated by the WHI. This FFQ has demonstrated reasonably good validity as a measurement of dietary intake compared with 24-hour dietary recalls and food records.[21]

Statistical Analysis

Baseline characteristics were summarized according to SHBG quartiles. Continuous variables were presented as means ± standard deviations, and categorical variables were presented in percentages. The statistical significances of differences among SHBG quartiles were tested by

ANOVA for continuous variables and by chi-square test for categorical variables. Individual level data from different ancillary studies were pooled and analyzed to assess the associations between measures of carbohydrate intakes and natural-log-transformed SHBG concentrations using linear multivariable models. Dietary carbohydrate intakes were each analyzed in quartiles. We adjusted for potential confounding factors including total energy intake, total carbohydrates intake (except when total carbohydrates intake was exposure of interest), ancillary study indicators, case/control status in each ancillary study, age (continuous), ethnicity, body mass index (BMI, continuous), cigarette smoking (never, past, or current), alcohol consumption (continuous), physical activity (metabolic equivalent of tasks per week, continuous), and hormone therapy use (never, past, or current user of unopposed estrogen and/or estrogen plus progesterone). From this model, we calculated the adjusted geometric means of SHBG concentrations for each quartile of the carbohydrate of interest by exponentiating the estimated mean log SHBG concentrations evaluated at the mean of each continuous variable and averaged over the groups of each categorical variable in the model. We also performed a linear trend analysis for each measure of carbohydrate by assigning the median of each quartile to each observation and using the resulting continuous variable as the independent variable in the model. In order to address multiple testing issue, Benjamini and Hochberg's procedure for controlling the false discovery rate (FDR) was performed with the results of the trend analysis.[27] Measures of carbohydrate intake with *q*-value below 0.05 were considered statistically significant, which corresponded to less than one false positive result per 20 comparisons.

Sensitivity analyses were performed by (a) restricting to only controls of each ancillary study, and (b) using linear mixed effects models to pool the estimates from each ancillary study. Furthermore, since SHBG concentrations has been inversely linked to the risk of type 2 diabetes previously,[1,3,5] we hypothesized that dietary carbohydrates may influence the risk of type 2 diabetes through affecting serum SHBG concentrations. Thus, we performed exploratory

mediation analyses within the type 2 diabetes case control study in our sample (AS238, n = 1,586 after applying exclusion criteria), with quartiles of dietary carbohydrate measures as the exposure (contrasting the highest and the lowest quartile), SHBG concentrations as the mediator, and case control status as the outcome.[28-30] The average causal mediation effects, average direct effects, the proportion mediated, and their respective confidence intervals were quantified. All statistical analyses were conducted using R version 3.3.1 (The R Foundation for Statistical Computing, Vienna, Austria).[31]

Results

We included a total of 11,159 postmenopausal women in the current analysis, with a median serum SHBG concentration of 47.3 nmol/L and interquartile range of 33.0 – 68.8 nmol/L. This group of participants were on average 65.3 years old (SD = 7.5), had an average BMI of 28.6 kg/m^2 (SD = 6.1), an average total energy intake of 1,617.5 kcal per day (SD = 660.6), and an average total carbohydrates intake of 201.4 grams per day (SD = 80.9). Sixty-eight percent of them were white and 8.6% were smokers at study baseline. When comparing women across SHBG quartiles, those within higher quartiles of SHBG concentrations tended to be older, less likely to be white and more likely to be black or Asian, had lower BMI and lower alcohol intake, and were more likely to be current smokers. Women with higher concentrations of serum SHBG also had lower intake of total energy, total carbohydrates, sugar, GL, and GI, while they had similar intake of fiber compared to women with lower concentrations of serum SHBG (Table 1).

Since the original continuous SHBG variable was skewed to the right, we performed natural logarithm transformation and used the log-transformed SHBG variable as the dependent variable in the subsequent linear regression analyses. After adjusting for total energy intake, total carbohydrates (except when total carbohydrates was the exposure of interest), age, race, BMI, smoking status, physical activity, alcohol intake, hormone therapy use, ancillary study

indicator, and case-control status in each ancillary study, higher dietary GL based on both total carbohydrates and available carbohydrates were significantly associated with lower concentrations of serum SHBG (*P*-value for trend = 0.008 and 0.002, *q*-value = 0.035 and 0.013, respectively). Women within the lowest quartile of dietary GL based on available carbohydrates had an adjusted average SHBG of 56.7 nmol/L (95% CI: 54.6, 58.9), while women within the highest quartile of dietary GL had an adjusted average SHBG of 52.6 nmol/L (95% CI: 50.5, 54.7), and results were very similar for GL based on total carbohydrates. Similar trend was observed for dietary GI based on total and available carbohydrates (*P*-value for trend = 0.024 and 0.042, *q*-value = 0.067 and 0.103, respectively) and dietary sugar intake (*P*-value for trend < 0.001, *q*-value = 0.008), for which the lowest intake quartile had an average SHBG concentration of 56.2 nmol/L (95% CI: 54.3, 58.1), while the highest intake quartile had an average of 52.1 nmol/L (95% CI: 50.2, 54.1). We also found a positive trend for fiber, where higher intake of fiber was associated with higher SHBG concentrations (*P*-value for trend = 0.011, *q*-value = 0.037). No significant findings were observed for total carbohydrates intake (Table 2).

For analyses regarding carbohydrate-abundant food items, we found a significant inverse relationship between quartiles of sugar sweetened beverages and circulating SHBG concentrations (*P*-value for trend < 0.001, *q*-value < 0.001). The lowest intake quartile corresponded to an adjusted average SHBG concentration of 56.7 nmol/L (95% CI: 55.0, 58.6), while the highest intake quartile corresponded to an average of 52.7 nmol/L (95% CI: 51.1, 54.4). Interestingly, we also observed borderline inverse associations for potatoes intake (*P*-value for trend = 0.074, *q*-value = 0.157) and beans intake (*P*-value for trend = 0.101, *q*-value = 0.191). Other food items were not significantly associated with circulating SHBG concentrations (Table 3).

When restricting to just controls from ancillary studies, this subgroup included 5,457 participants. Without correction for multiple comparison, results were similar to those obtained from the primary analyses in the whole group (Table 4 and 5). Dietary GL based on total and available carbohydrates, fiber, sugar, and sugar sweetened beverages remained significantly associated with SHBG concentrations after the FDR procedure, and the effect sizes were also similar. The discrepancy was that the *P*-values for trend for dietary GI based on total and available carbohydrates were significant before and after the FDR procedure in the controls, while in the whole sample they were only significant before multiple testing correction. From the second sensitivity analyses we performed, the linear mixed effects models where ancillary study indicators were treated as random effects yielded very similar results to the primary analyses (data not shown). The exploratory mediation analyses did not find significant average causal mediation effects or average direct effects, possibly due to the fact that one single case control study was not powered enough to detect significant mediation effects.

Discussion

In this cross-sectional sample of 11,159 non-diabetic postmenopausal women that enrolled in the WHI, positive associations with SHBG concentrations were observed for total dietary fiber intake. Total dietary sugar intake and dietary GL based on total and available carbohydrates were observed to be significantly associated with reduced serum concentrations of SHBG, before and after correction for multiple comparisons. Dietary GI based on total and available carbohydrates were also associated with lower levels of SHBG before multiple testing correction. In addition, significant association between sugar-sweetened beverages and decreased concentrations of serum SHBG was demonstrated based on analyses regarding carbohydrate-abundant food items, corroborating our results for total sugar intake.

Our finding of the negative relationship between dietary GL based on total and available carbohydrates and serum SHBG concentrations was consistent with results from a previous dietary intervention trial where SHBG was considerably lowered after a 12-week high GL diet compared to the baseline, and also significantly decreased compared to those on a low GL diet,[15] although another study contrasting low GI and high GI diet did not find significant difference in SHBG after an 8-week intervention.[32] This somewhat contradicted the fact that in our analysis dietary GI based on total or available carbohydrates were significantly associated with SHBG concentrations, although only before correction for multiple comparisons. Mechanistically, it has been suggested that high GL/GI diet induced greater insulin production, and insulin could act as an inhibitor of hepatic synthesis of SHBG.[18,33-35] Dietary sugar, which is usually high in glycemic index and glycemic load, was found to be significantly and inversely associated with serum SHBG. This result was in line with biological evidence from human-SHBG-transgenic mice and human hepatic cells, where glucose and fructose reduced human SHBG production by hepatocytes via the downregulation of hepatocyte nuclear factor-4α, which was independent of the actions of insulin.[18] Sugar also likely contributed to the relation between dietary GL/GI and SHBG concentrations. The null relationship we observed between total carbohydrates intake and SHBG was consistent with observations from another previous study.[16] Collectively, these results suggested that the quality of dietary carbohydrates might be of greater importance than quantity in affecting circulating SHBG levels, given that our analyses with respect to GL and GI were adjusted for the total amount of dietary carbohydrates.

Dietary fiber, which did not contribute to GL or GI based on available carbohydrates, was positively associated with serum SHBG in our analysis. Previous findings from the WHI Dietary Modification trial associated a low fat dietary pattern with significant reduction in SHBG after 1 year of intervention, which were thought to be partially contributed by the concurrent increase in fiber intake as well as weight loss.[36] Even though an early study found no correlation between

dietary fiber and SHBG,[17] a more recent investigation with regression modelling did reveal significant positive relations between the two, accounting for age, BMI, and other covariates.[16] The biomarker of lignans intake was also found to be positively related to SHBG levels, albeit the null association between dietary fiber and SHBG in the same study.[37] The mechanism by which fiber intake may be a controlling factor on SHBG is not yet well-understood, but it is possible that it acts through modulating glucagon-like peptide-1 and insulin secretion.[38]

We also systematically examined the primary carbohydrate-abundant food items that might be responsible for the dietary effects on SHBG concentrations in these data. A significant inverse relationship between sugar sweetened beverages and circulating SHBG concentrations was discovered, and this mirrored our findings in the relationship between total sugar intake and SHBG concentrations. The significant negative associations between total sugar intakes, sugar sweetened beverages intakes and plasma SHBG concentrations illustrated the negative effect of excessive sugar consumptions, which indicated that cutting down sugar intake may be an important intervention to increase SHBG concentrations. We also identified 2 categories of foods, potatoes and beans, which were borderline significantly inversely associated with SHBG concentrations, albeit the complete null association after correction for multiple comparison or in controls only. Physiological studies show that most potatoes are of high GI regardless of cooking method, which over the long term may increase the risk of obesity and chronic diseases such as type-2 diabetes and cardiovascular disease.[39]

The cross-sectional nature of the current investigation raises concern over the temporality of the associations that we observed. However, the WHI food frequency questionnaire inquired dietary intakes during the period of 3 months prior to study baseline, while the blood samples from which SHBG was measured were taken at baseline. Thus, the temporality between dietary carbohydrates intake and serum SHBG concentrations can be established to a certain extent.

Another limitation of this study is that measurements of SHBG from different ancillary studies of the WHI were included in order to boost power in detecting the associations, especially with the moderately large number of testings in the current analyses. Heterogeneity among these studies in measuring serum SHBG, as well as different criteria for choosing study participants may introduce bias into our results. We attempted to address this issue by including both the indicators of ancillary studies and indicators of case or control status in each ancillary study in our statistical modelling. To evaluate the extent of bias, we also performed sensitivity analyses in controls only, as well as using linear mixed effects models, and our results were largely robust to the different methods used. Adiposity could also potentially influence both dietary intake and blood SHBG concentrations.[2] Although we controlled for BMI in our analyses, we could not rule out the possibility of residual confounding as it is not a perfect measure of adiposity. Finally, while a large national sample of postmenopausal women participated in the WHI studies, which was broader and more representative than those in studies based on samples of convenience, the findings presented here can only be generalized to postmenopausal women, which is another limitation of this investigation.

In conclusion, our study found that dietary fiber intake, sugar intake and GL/GI based on total and available carbohydrates have significant associations with serum SHBG concentrations, thus supporting a role of diet in influencing blood levels of SHBG, which is in turn an important protective factor probably associated with insulin resistance, type 2 diabetes, cardiovascular disease, and hormone-dependent cancers. Further studies are needed to better elucidate the biological mechanisms underlying the associations between dietary carbohydrates and circulating SHBG concentrations, and mediation analyses with sufficient power are also needed to evaluate whether these possible effects of dietary carbohydrates on SHBG extend to the ultimate cardio-metabolic disease risk, which will contribute to a better understanding of the

mechanisms of action underlying the effect of diets, particularly of high GL/GL diets rich in refined carbohydrates.

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Figure legends

None.

Tables (each table complete with title and footnotes)

Table 1. Baseline characteristics by quartiles of untransformed plasma SHBG concentrations in a subpopulation of the postmenopausal women from the Women's Health Initiative (n = 11,159)

	SHBG				*P*-value
	Q1	Q2	Q3	Q4	
Number of participants	2801	2794	2776	2788	
Median (nmol/L)	25.7	40.0	56.3	92.0	
(Interquartile range)	(21.0, 29.7)	(36.5, 43.5)	(51.7, 62.0)	(78.0, 125.0)	
Age (years) [mean (SD)]	63.7 (7.1)	65.6 (7.3)	66.5 (7.5)	65.6 (7.8)	<.001
Race/ethnicity [n (%)]	31.6 (5.9)	29.5 (6.0)	27.3 (5.5)	26.0 (5.5)	
White					
Black/African American	1930 (68.9)	1997 (71.5)	1972 (71.0)	1734 (62.2)	<.001
Hispanic/Latino	502 (17.9)	456 (16.3)	443 (16.0)	592 (21.2)	<.001
Asian/Pacific Islander	198 (7.1)	190 (6.8)	183 (6.6)	227 (8.1)	
Other	124 (4.4)	102 (3.7)	137 (4.9)	196 (7.0)	
BMI (kg/m^2) [mean (SD)]	47 (1.7)	49 (1.8)	41 (1.5)	39 (1.4)	<.001
Smoking status [n (%)]					
Never	1444 (51.6)	1495 (53.5)	1457 (52.5)	1471 (52.8)	0.019
Former	1144 (40.8)	1067 (38.2)	1076 (38.8)	1040 (37.3)	
Current	213 (7.6)	232 (8.3)	243 (8.8)	277 (9.9)	
Total energy (kcal/d) [mean (SD)]	1715.1 (694.6)	1625.0 (665.1)	1573.7 (627.5)	1555.4 (641.5)	<.001
Alcohol intake (g/d) [mean (SD)]	4.5 (11.0)	5.1 (11.6)	5.1 (10.5)	4.4 (11.2)	<.001
GL (total CHO) [mean (SD)]	110.4 (46.8)	104.9 (43.5)	104.0 (43.7)	103.2 (43.3)	<.001
GL (available CHO) [mean (SD)]	102.9 (44.3)	97.5 (41.0)	96.5 (41.1)	95.8 (40.8)	<.001
GI (total CHO) [mean (SD)]	52.6 (3.7)	52.1 (3.9)	52.0 (3.9)	52.1 (3.8)	<.001
GI (available CHO) [mean (SD)]	53.0 (3.7)	52.5 (3.9)	52.4 (3.9)	52.5 (3.7)	<.001
Total fiber (g/d) [mean (SD)]	15.4 (6.9)	15.4 (6.7)	15.7 (7.0)	15.7 (6.9)	0.133
Total sugar (g/d) [mean (SD)]	103.2 (51.0)	98.7 (47.1)	97.9 (45.6)	96.9 (46.2)	<.001
Total carbohydrates (g/d) [mean (SD)]	208.9 (84.6)	200.4 (79.7)	199.0 (79.4)	197.4 (79.4)	<.001

Abbreviations: SHBG: sex hormone-binding globulin; Q: quartile; SD: standard deviation; BMI: body mass index; GL: glycemic load; CHO: carbohydrates; GI: glycemic index.

Table 2. Adjusted means of serum SHBG concentrations according to quartiles of dietary glycemic load, glycemic index, and intakes of fiber, sugar, and total carbohydrates

	Adjusted mean*	95% CI	P_{trend}
GL (total CHO)			
Q1	56.4	(54.3, 58.6)	
Q2	55.6	(53.8, 57.4)	0.008
Q3	53.0	(51.3, 54.7)	
Q4	52.9	(50.8, 55.1)	
GL (available CHO)			
Q1	56.7	(54.6, 58.9)	
Q2	55.5	(53.7, 57.3)	0.002
Q3	53.1	(51.4, 54.8)	
Q4	52.6	(50.5, 54.7)	
GI (total CHO)			
Q1	55.5	(53.7, 57.3)	
Q2	54.8	(53.1, 56.5)	0.024
Q3	53.9	(52.3, 55.7)	
Q4	53.9	(52.2, 55.6)	
GI (available CHO)			
Q1	55.3	(53.5, 57.1)	
Q2	54.9	(53.2, 56.6)	0.042
Q3	54.1	(52.4, 55.8)	
Q4	53.9	(52.2, 55.6)	
Total fiber (g/d)			
Q1	53.6	(51.9, 55.4)	
Q2	54.1	(52.5, 55.9)	0.011
Q3	54.0	(52.3, 55.8)	
Q4	56.4	(54.5, 58.5)	
Total sugar (g/d)			
Q1	56.2	(54.3, 58.1)	
Q2	55.1	(53.3, 56.8)	<.001
Q3	54.1	(52.4, 55.9)	
Q4	52.1	(50.2, 54.1)	
Total carbohydrates (g/d)			
Q1	54.9	(53.0, 56.8)	
Q2	55.2	(53.4, 57.0)	0.368
Q3	53.4	(51.7, 55.1)	
Q4	54.3	(52.3, 56.3)	

Abbreviations: SHBG: sex hormone-binding globulin; CI: confidence interval; P_{trend}: *P*-value for trend; Q: quartile; GL: glycemic load; CHO: carbohydrates; GI: glycemic index.

* Adjusted means were computed by exponentiating the least squares means of estimated log-transformed SHBG concentrations from model including the exposure of interest, total carbohydrates (except when total carbohydrates was the exposure of interest), total energy intake, age, race, BMI, smoking status, physical activity, alcohol intake, hormone therapy use, ancillary study indicator, and case-control status in each ancillary study. Linear mixed model with ancillary study indicator as random effect yielded very similar results.

Table 3. Adjusted means of serum SHBG concentrations according to the intake quartiles of carbohydrates abundant food items

	Adjusted mean*	95% CI	P_{trend}
White bread (servings/d)			
Q1	54.5	(52.8, 56.2)	0.128
Q2	55.2	(53.5, 56.9)	
Q3	54.5	(52.8, 56.2)	
Q4	53.7	(52.0, 55.5)	
Dark bread (servings/d)			
Q1	54.0	(52.3, 55.6)	0.643
Q2	54.8	(53.1, 56.6)	
Q3	54.8	(53.1, 56.6)	
Q4	54.7	(52.9, 56.5)	
Rice, grains and plain noodles (servings/d)			
Q1	54.0	(52.3, 55.6)	0.117
Q2	54.5	(52.7, 56.4)	
Q3	53.9	(52.1, 55.7)	
Q4	55.2	(53.6, 57.0)	
Potato (servings/d)			
Q1	55.4	(53.7, 57.1)	0.074
Q2	54.6	(52.9, 56.3)	
Q3	53.7	(52.1, 55.5)	
Q4	53.9	(52.2, 55.7)	
Cereal (servings/d)			
Q1	54.5	(52.9, 56.2)	0.917
Q2	54.8	(53.1, 56.6)	
Q3	54.0	(52.3, 55.7)	
Q4	54.8	(53.0, 56.7)	
Fruits (servings/d)			
Q1	54.3	(52.6, 56.0)	0.205
Q2	54.1	(52.4, 55.9)	
Q3	54.4	(52.7, 56.2)	
Q4	55.3	(53.5, 57.1)	
Beans (servings/d)			
Q1	54.8	(53.1, 56.5)	0.101
Q2	54.8	(53.1, 56.5)	
Q3	54.9	(53.2, 56.7)	
Q4	53.6	(51.9, 55.3)	
Sugar sweetened beverages (servings/d)			
Q1	56.7	(55.0, 58.6)	<.001
Q2	55.2	(53.4, 56.9)	
Q3	54.0	(52.3, 55.7)	
Q4	52.7	(51.1, 54.4)	
Pasta (servings/d)			
Q1	55.0	(53.3, 56.8)	0.198
Q2	53.7	(52.1, 55.4)	
Q3	53.8	(52.1, 55.5)	

Q4	55.5	(53.7, 57.4)	
Whole grains (servings/d)			
Q1	54.2	(52.5, 56.0)	0.223
Q2	54.4	(52.7, 56.1)	
Q3	54.2	(52.5, 55.9)	
Q4	55.3	(53.5, 57.1)	

Abbreviations: SHBG: sex hormone-binding globulin; CI: confidence interval; P_{trend}: *P*-value for trend; Q: quartile.

* Adjusted means were computed by exponentiating the least squares means of estimated log-transformed SHBG concentrations from model including the exposure of interest, total carbohydrates, total energy intake, age, race, BMI, smoking status, physical activity, alcohol intake, hormone therapy use, ancillary study indicator, and case-control status in each ancillary study. Linear mixed model with ancillary study indicator as random effect yielded very similar results.

Table 4. Adjusted means of serum SHBG concentrations according to quartiles of dietary glycemic load, glycemic index, and intakes of fiber, sugar, and total carbohydrates **in controls of the ancillary studies**

	Adjusted mean*	95% CI	P_{trend}
GL (total CHO)			
Q1	55.9	(53.0, 58.9)	
Q2	55.8	(53.3, 58.4)	0.025
Q3	52.8	(50.5, 55.2)	
Q4	51.7	(48.8, 54.7)	
GL (available CHO)			
Q1	56.3	(53.4, 59.3)	
Q2	55.5	(53.0, 58.1)	0.014
Q3	52.8	(50.5, 55.2)	
Q4	51.5	(48.6, 54.5)	
GI (total CHO)			
Q1	55.7	(53.3, 58.2)	
Q2	54.7	(52.4, 57.1)	0.001
Q3	53.3	(51.0, 55.7)	
Q4	52.5	(50.2, 54.8)	
GI (available CHO)			
Q1	55.5	(53.1, 58.0)	
Q2	54.5	(52.2, 56.9)	0.005
Q3	53.5	(51.2, 55.9)	
Q4	52.6	(50.4, 55.0)	
Total fiber (g/d)			
Q1	52.0	(49.7, 54.5)	
Q2	53.5	(51.1, 55.9)	<.001
Q3	53.6	(51.3, 56.0)	
Q4	57.5	(54.8, 60.4)	
Total sugar (g/d)			
Q1	55.5	(52.9, 58.2)	
Q2	54.8	(52.4, 57.3)	0.018
Q3	53.9	(51.5, 56.3)	
Q4	51.5	(48.9, 54.3)	
Total carbohydrates (g/d)			
Q1	55.0	(52.4, 57.7)	
Q2	55.7	(53.2, 58.2)	0.052
Q3	53.2	(50.9, 55.6)	
Q4	52.2	(49.6, 55.1)	

Abbreviations: SHBG: sex hormone-binding globulin; CI: confidence interval; P_{trend}: *P*-value for trend; Q: quartile; GL: glycemic load; CHO: carbohydrates; GI: glycemic index.

* Adjusted means were computed by exponentiating the least squares means of estimated log-transformed SHBG concentrations from model including the exposure of interest, total carbohydrates (except when total carbohydrates was the exposure of interest), total energy intake, age, race, BMI, smoking status, physical activity, alcohol intake, hormone therapy use, and ancillary study indicator. Linear mixed model with ancillary study indicator as random effect yielded very similar results.

Table 5. Adjusted means of serum SHBG concentrations according to the intake quartiles of carbohydrates abundant food items **in controls of the ancillary studies**

	Adjusted mean*	95% CI	P_{trend}
White bread (servings/d)			
Q1	54.3	(52.0, 56.7)	0.907
Q2	53.5	(51.2, 55.9)	
Q3	54.3	(52.0, 56.7)	
Q4	53.8	(51.4, 56.4)	
Dark bread (servings/d)			
Q1	53.7	(51.5, 56.1)	0.681
Q2	54.0	(51.7, 56.5)	
Q3	54.2	(51.8, 56.6)	
Q4	54.3	(51.8, 56.9)	
Rice, grains and plain noodles (servings/d)			
Q1	53.0	(50.7, 55.4)	0．103
Q2	54.0	(51.5, 56.7)	
Q3	53.8	(51.5, 56.1)	
Q4	55.3	(52.7, 58.1)	
Potato (servings/d)			
Q1	55.5	(53.2, 58.0)	0.127
Q2	52.9	(50.7, 55.3)	
Q3	54.1	(51.7, 56.5)	
Q4	53.1	(50.7, 55.6)	
Cereal (servings/d)			
Q1	53.7	(51.4, 56.0)	0.527
Q2	54.4	(52.1, 56.8)	
Q3	52.9	(50.6, 55.4)	
Q4	55.0	(52.5, 57.6)	
Fruits (servings/d)			
Q1	53.7	(51.4, 56.2)	0.129
Q2	52.6	(50.4, 55.0)	
Q3	55.1	(52.7, 57.6)	
Q4	54.9	(52.5, 57.5)	
Beans (servings/d)			
Q1	53.3	(51.0, 55.7)	0.675
Q2	54.1	(51.8, 56.5)	
Q3	54.8	(52.4, 57.3)	
Q4	53.9	(51.6, 56.4)	
Sugar sweetened beverages (servings/d)			
Q1	56.6	(54.1, 59.1)	<.001
Q2	54.6	(52.3, 57.1)	
Q3	53.3	(51.0, 55.7)	
Q4	52.2	(49.9, 54.6)	
Pasta (servings/d)			
Q1	54.6	(52.3, 57.1)	0.658
Q2	53.6	(51.4, 56.0)	
Q3	53.1	(50.8, 55.5)	

Q4	54.8	(52.4, 57.4)	
Whole grains (servings/d)			
Q1	54.5	(52.1, 56.9)	0.923
Q2	53.2	(51.0, 55.6)	
Q3	54.1	(51.8, 56.5)	
Q4	54.2	(51.8, 56.7)	

Abbreviations: SHBG: sex hormone-binding globulin; CI: confidence interval; P_{trend}: *P*-value for trend; Q: quartile.

* Adjusted means were computed by exponentiating the least squares means of estimated log-transformed SHBG concentrations from model including the exposure of interest, total carbohydrates, total energy intake, age, race, BMI, smoking status, physical activity, alcohol intake, hormone therapy use, and ancillary study indicator. Linear mixed model with ancillary study indicator as random effect yielded very similar results.