Association of dietary factors and selected plasma variables with sex hormone-binding globulin in rural Chinese women\textsuperscript{1–3}

Jeffrey R Gates, Banoo Parpia, T Colin Campbell, and Chen Junshi

ABSTRACT Sex hormone-binding globulin (SHBG) is an important regulator of plasma sex steroids as well as a sensitive indicator of insulin resistance. SHBG may be an important diagnostic measure of risk for pathologies associated with insulin resistance syndrome (IRS) such as non-insulin-dependent diabetes mellitus (NIDDM), obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. In women, SHBG is also implicated in diverse pathologies such as cancers of steroid-sensitive tissues and hirsutism. Data from an ongoing ecological study linking diet and health in rural China were analyzed to determine the relation of selected plasma variables and diet to plasma concentrations of SHBG. All data represent county mean values, pooled by age and sex, to assess the relation between biochemical and lifestyle characteristics and disease-specific mortality rates at the county level. The study sample consisted of 3250 Chinese women between the ages of 35 and 64 y living in 65 widely dispersed rural counties. Consumption patterns for 21 different food groups were derived from a food-frequency questionnaire and a 3-d dietary survey and subsequently compared. Correlation analyses of county mean values demonstrated a significant association between SHBG and insulin, testosterone, triacylglycerols, body mass index, age at menarche, and several foods. In regression analyses, after adjustments, the strongest predictors of SHBG concentrations were the dietary intakes of rice (β = 0.42, \( P < 0.01 \)), fish (β = 0.34, \( P < 0.05 \)), millet (β = −0.27, \( P < 0.01 \)), and wheat (β = −0.34, \( P < 0.01 \)). When insulin, testosterone, and triacylglycerols were added to the model only triacylglycerols (β = −0.26, \( P < 0.05 \)) remained a significant independent predictor of SHBG. Additional analyses suggested that the consumption of green vegetables was modestly positively correlated with SHBG and negatively with insulin values. Consumption of rice and fish in particular appeared to favorably influence the principle plasma variables associated with a reduction in the risk for IRS pathologies. Am J Clin Nutr 1996;63:22–31.

KEY WORDS Sex hormone-binding globulin, insulin, diet, China, fish, rice, wheat

INTRODUCTION

Sex hormone-binding globulin (SHBG), also referred to in other studies as sex steroid-binding protein (SBP), testosterone-estradiol-binding globulin (TeBG) or gonadal steroid-binding globulin (GBG), is a plasma glycoprotein mainly synthesized in hepatocytes and possibly by the placenta during pregnancy (1, 2). Mean serum concentrations of SHBG are higher in women than in men by as much as twofold (3), decreasing in puberty but increasing during pregnancy (4).

In women, SHBG is clinically implicated in several conditions, ranging from cancer and cardiovascular disease to polycystic ovarian disease and hirsutism (5–8). Lapidus et al (9) found that a decrease in the concentration of SHBG was a significant risk factor for 12-y overall mortality, concluding that SHBG, androgens, and estrogens should be evaluated as risk factors for cardiovascular disease and death. Suggested positive physiologic regulators of SHBG include testosterone, prolactin, growth hormone, somatotrem-C, and insulin, whereas enterolactone, oral estrogen, and thyroid hormone are potential negative regulators (10).

Recently however, a growing body of research supports insulin as the primary regulator of SHBG (11–14). Furthermore, SHBG has been shown to be an independent predictor of insulin resistance (15), which is itself suggested to be the common denominator in non-insulin-dependent diabetes mellitus (NIDDM), obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease, otherwise known in the aggregate as insulin resistance syndrome (IRS) or syndrome X (16, 17). Thus, plasma SHBG itself may be an important indicator for assessing IRS risk.

The relative influence of various foods or diets on the SHBG-disease relation has yet to be fully addressed. There is good evidence that diet is an important modifier of SHBG (10, 18–21). In this communication we examine the relations of SHBG, selected plasma variables, and the intakes of 21 foods for women aged 35–64 y in 65 rural Chinese counties.

SUBJECTS AND METHODS

A detailed description of the experimental sampling procedures, methods, and ethics approval is available in the mono-

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DIET AND SHBG IN CHINESE WOMEN

23

graph by Chen et al (22). For the purpose of this communica-
tion, relevant procedures are noted briefly below. Average

elevation, latitude, and longitude data for the geographic area
(county) were included from the Times Atlas of the World (23).

Subjects

In 1983 an ecologic survey was conducted in 130 Chinese
rural communes located in 65 widely dispersed counties (sam-
pred from a total of 2392 counties). The counties were chosen
to represent the full range of mortality rates for seven of the
most prevalent cancers. Two communes were randomly chosen
from within each county and within each of these communes
25 women (aged 35–64 y) were chosen for study. The informa-
tion collected included the intakes of foods and nutrients in
the household, individual concentrations of various blood and
urinary constituents in pooled samples, questionnaire-based
information on lifestyle, and frequency of intakes of selected
food categories for individual subjects. Data on demographic,
social, and selected geographic characteristics were included
from a secondary source (23). Between September and Decem-
ber of 1983 each subject donated blood and completed a
questionnaire. Data were subsequently compiled for analysis
and published in a monograph (22).

A blood pooling scheme was devised to assess the relation
between characteristics (biochemical, lifestyle, and dietary)
and disease-specific mortality rates at the county level. Thus,
all data presented herein (dietary survey, blood and urine,
questionnaire, and food intake frequency) represent county
mean values. Plasma samples were pooled by sex, age (35–44
y, 45–54 y, and 55–64 y), and commune to yield a total of 780
samples.

Methods

Among the 367 items of information available in the data set,
this study retains only those variables of relevance to the
research question: plasma SHBG, selected plasma variables,
food intake frequencies, 3-d household dietary survey re-
sponses for various foods, height and weight, and age at men-
arche. Additionally, the gross value of industrial and agricul-
tural output (GVIAO), a measure of relative economic
development, was examined to adjust for the potential influ-
ence of affluence on SHBG.

The methods of blood sample collection and preparation
were reported in greater detail elsewhere (22). Briefly, between
September and December 1983 a 10-mL sample of fasting
venous blood was drawn from 50 individuals in 130 Chinese
communes between 0600 and 1200 by using trace-mineral-free
evacuated containers with heparin. Approximately equal num-ers of subjects of each sex and each of the three age groups
were included. Samples were immediately placed on ice in
light-free insulated jars and transported to county laboratories
within 4 h. On arrival at the county laboratory, the blood
samples were immediately divided into fractions and stored at
−15 °C to −20 °C. Seven hundred eighty age- and sex-specific
plasma pools were sent on dry ice in transporter boxes to
Cornell University where all sample temperatures were found
to be between 18 and 30 °C. Subsequent storage at Cornell
University was at −80 °C. Thus, between Beijing and Cornell,
a total of ∼24,000 pooled aliquots and 39,000 individual
sample aliquots were eventually prepared. All analyses de-
scribed below were conducted on blood specimen pools at
Cornell University except when otherwise specified.

Plasma triacylglycerols and cholesterol

Triacylglycerol concentrations were determined according to
the method of Biggs et al (24). Triacylglycerols were hydro-
dyzed to produce glycerol, which was then oxidized to form
formaldehyde, which was reacted with acetylacetone to pro-
duce a colored adduct as first described by Nash (25). Choles-
terol analyses were done in China by the method of Parekh and
Jung according to the modification of Feng et al (26).

Plasma sex steroids and insulin

A solid-phase 125I-radioimmunoassay kit procedure was
used for male samples (Diagnostic Products Corporation, Los
Angeles) to quantify plasma testosterone. This method is capa-
ble of detecting 110 ng/L. A more sensitive (60 ng/L) double
antibody assay using a rabbit primary antiserum was used for
samples from females (27).

A radioimmunassay kit was used (28) with 125I-labeled pro-
lactin and prolactin-specific antibody coincubated with the
sample plasma. The range of native prolactin routinely assayed
by this method is 0–200 μg/L.

Estradiol was determined by a nonextraction, solid-phase
125I-radioimmunoassay kit procedure developed by Diagnostic
Products Corporation. Values below the lowest estradiol stan-
dard (20 ng/L) were estimated by extrapolation of the standard
curve (29).

SHBG was measured by the noncompetitive liquid-phase
immunoradiometric assay of Hammond et al (30) as provided
in kit form by Farmos Diagnostica (Oulunsalo, Finland; 31).
Samples were collected and analyzed by age category—35–44
y, 45–54 y, and 55–64 y—for females only. Results for the
aggregated age category (ages 35–64 y) are presented in anal-
yses reported here because the county mean values for SHBG
did not vary significantly across the three age categories.

Insulin concentrations were determined in 1994 at the
Cornell University School of Veterinary Medicine by using a
radioimmunoassay kit (Diagnostic Products Corporation).

Questionnaire

All 6500 persons in this study (ie, 50 persons per commune
or 100 per county) answered a questionnaire administered by a
trained interviewer who elicited information on marital status,
reproductive history, age, sex, health status, smoking history,
food consumption (frequency), and household rations for 30
different foods (g/d). In this report, only the answers given by
the women were retained for analysis.

Household 3-d dietary survey

The pattern of food consumption among the Chinese house-
holds was determined by weighing and recording all raw and
processed foods before and after a meal period for 3 consecu-
tive days. In each of the 65 counties, 30 households (15
households per commune) were selected for the dietary survey,
with one trained surveyor being responsible for four to six
households. This method, although expensive and time-con-
suming, is considered to be the “gold standard” for determi-
ning the actual dietary intakes of individuals (32). The number of
persons present for each meal was recorded for each day.
Pregnant or lactating women were also noted as such. Children < 2 y of age were excluded from the survey. Five grades of physical activity (very light to very heavy) were used to assess the exercise level of the survey subjects. The number of person-days was calculated by using proportions of 0.2, 0.4, and 0.4 as the daily energy consumed during breakfast, lunch, and supper, respectively. This number was then standardized per reference man, defined as an adult male 19–59 y of age with a body weight of 65 kg, and undertaking very light physical work on the basis of an energy conversion factor derived from the Chinese recommended dietary allowances. By using the Chinese Food Composition Tables (33), 14 nutrients derived from 25 individual food categories were estimated. This method is described in further detail elsewhere (34).

The food intake data represent the average intake per reference man for the 30 households in each of the 65 counties. A subsequent study validated the ability of this method to predict individual intake from the reference-man calculations with correlation coefficients ranging from 0.53 to 0.78 (P < 0.001) (34).

**Statistical analysis**

A computerized statistical software package (SAS) was used for all the statistical analyses (including all descriptive and analytical measures) reported here (35). The independent variables initially retained for correlation analyses were chosen because of either demonstrated biological relevance (ie, plasma biomarkers, anthropometric data, and smoking habits) or hypothesized relevance (ie, general income index, dietary variables). Additionally, each variable needed to meet the criterion of a significantly high degree of reliability (P < 0.05) before being included in the correlation analyses. This measure of reliability (r) was used to evaluate between-commune or within-county homogeneity for any given variable (data were available for two communes per pooled county sample). Thus, any biologically or hypothetically relevant variable with low reliability (high within-county heterogeneity) was excluded from the list of possible inclusion variables, with the exception of testosterone, which was judged to have marginally acceptable reliability (r = 26% and t test statistic of 1.91) and to be biologically relevant to include in the overall model. Mean county SHBG concentrations for women between the ages of 35–64 y in each of the 65 counties was used as the dependent variable in the analyses. Primary predictors of SHBG modulation and possible confounding factors were identified on the basis of biologically plausible relations.

Correlation analyses were used to examine the magnitude and significance of the association of selected dietary, plasma, and anthropometric variables to SHBG. Because of space limitations, the correlation tables in this communication present only those variables that demonstrated a significant association with SHBG (P < 0.05).

From the 21 food groupings originally examined in the 3-d dietary survey and food-frequency questionnaire, 16 were found to be significantly correlated (P < 0.0005) with either SHBG or insulin and were thus retained for further analysis. Of the 12 nondietary predictor variables, 6 were found to be significantly correlated (P < 0.05) with either SHBG or insulin and were retained for further analysis.

Data from the 3-d dietary survey, reporting intakes per reference man (see Methods), were compared with those from the food-frequency questionnaire to assess the relation between cross-sectional means of intake and intake over a longer time.

Multiple-regression analyses were used to examine the effects of various foods on SHBG, with potential confounding factors adjusted for. Multicollinearity was assessed by using the collinearity diagnostic in the REG procedure of SAS. This diagnostic procedure adopted by SAS determines whether a combination of regressors in a given model are collinear, thereby generating biased estimators and/or high SEs. This diagnostic attempt to identify this problem for the analyst by following the approach of Belsley et al (36). As a result of high multicollinearity and thus redundancy or over-correction, a few food groups (green vegetables and corn) were not included in the food-frequency regression analysis. Standardized β coefficients were estimated to assess the relative importance of a given factor within each model specification. Two separate regression analyses were done to compare food data derived from the 3-d dietary survey (cross-sectional) measured as intakes per reference man and the data from the food-frequency questionnaire (longitudinal retrospective recall) administered to all the women in the study. The final model specification reported here was chosen on the basis of the highest proportion of variation explained in the dependent variable (R²) for the most parsimonious list of explanatory factors included.

**RESULTS**

Two counties—Shun and Lean—from the provinces of Guangdong and Jiangxi were not included in the analyses because of missing data for the dependent variable (SHBG). Tuoli county from the province of Xinjiang was not included in the analyses because of unreliable data due to measurement problems for several of the covariates of interest.

County means, SDs, and ranges for selected foods and other factors of interest are presented in Table 1, together with the respective reliability coefficient as a measure of the within-county homogeneity. The average age of this sample of women was 48.7 y, ranging from 35 to 64 y. None of the women surveyed were obese: mean body mass index was 20.7, mean weight and height were 48.5 kg and 153.5 cm, respectively. Approximately 12% of women in this group reported smoking at the time of the survey. The average age at which women began menstruation was 17 y, with a range of 15.8–18.9 y.

The average county SHBG concentration for rural Chinese women was 73.0 nmol/L, ranging from 51.4 to 102.6 nmol/L. The average county insulin concentrations ranged from 27.26 to 68.16 pmol/L with a mean of 36.6 pmol/L, whereas triacylglycerols ranged from 0.7 to 1.9 mmol/L, with a mean of 1.2 mmol/L. Three categories of cholesterol are reported here: total cholesterol ranged from 2.2 to 4.4 mmol/L (x̄: 3.3 mmol/L), non-high-density-lipoprotein (HDL) cholesterol ranged from 1.3 to 3.2 mmol/L, and HDL cholesterol, ranged from 0.8 to 1.4 mmol/L (x̄: 1.1 mmol/L).

On the basis of both the food-frequency and food consumption data, considerable variation was demonstrated between counties for the intakes of virtually all foods. The population means for the frequency of meat, milk, and fish consumption were highly variable with meat (x̄: 53.1 times/y) being the most consumed of the animal foods, ranging from a minimum county mean consumption of 0.8 times/y up to 295 times/y.
TABLE 1
Selected plasma indicators of hormonal and lipid status, anthropometric measures, and dietary intakes for 65 mostly rural Chinese counties

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>( \bar{x} \pm S )</th>
<th>Range</th>
<th>r%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex hormone-binding globulin (nmol/L)</td>
<td>62</td>
<td>73.0 ± 9.9</td>
<td>51.4–102.6</td>
<td>47$^2$</td>
</tr>
<tr>
<td>Insulin (mmol/L)</td>
<td>59</td>
<td>36.6 ± 8.6</td>
<td>27.3–68.4</td>
<td>52$^2$</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/L)</td>
<td>64</td>
<td>1.2 ± 0.2</td>
<td>0.7–1.9</td>
<td>56$^2$</td>
</tr>
<tr>
<td>Testosterone (pmol/L)</td>
<td>62</td>
<td>1.1 ± 0.2</td>
<td>0.8–2.0</td>
<td>26</td>
</tr>
<tr>
<td>Estradiol (mmol/L)</td>
<td>61</td>
<td>224.3 ± 73.8</td>
<td>103.5–580.4</td>
<td>29$^5$</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>64</td>
<td>3.3 ± 0.4</td>
<td>2.2–4.4</td>
<td>71$^2$</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mmol/L)</td>
<td>64</td>
<td>2.2 ± 0.4</td>
<td>1.3–3.2</td>
<td>62$^2$</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>64</td>
<td>1.1 ± 0.1</td>
<td>0.8–1.4</td>
<td>32$^2$</td>
</tr>
<tr>
<td><strong>Anthropometric data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63</td>
<td>48.5 ± 3.1</td>
<td>43.1–55.9</td>
<td>79$^2$</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>63</td>
<td>153.5 ± 2.4</td>
<td>148.8–157.9</td>
<td>64$^3$</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>63</td>
<td>20.6 ± 0.9</td>
<td>18.5–22.9</td>
<td>NA$^4$</td>
</tr>
<tr>
<td><strong>General</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent smoking now (%)</td>
<td>63</td>
<td>11.7 ± 14.8</td>
<td>0–68.3</td>
<td>75$^5$</td>
</tr>
<tr>
<td>Age at menarche (y)</td>
<td>63</td>
<td>17.0 ± 0.7</td>
<td>15.8–18.9</td>
<td>66$^5$</td>
</tr>
<tr>
<td>GVIAO$^3$ (yuan)</td>
<td>63</td>
<td>642.0 ± 666.1</td>
<td>134.0–4706.0</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Questionnaire data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green vegetables</td>
<td>63</td>
<td>199.2 ± 94.5</td>
<td>25.6–335</td>
<td>89$^2$</td>
</tr>
<tr>
<td>Carrots</td>
<td>63</td>
<td>13.3 ± 27.3</td>
<td>0–138.8</td>
<td>87$^2$</td>
</tr>
<tr>
<td>Potatoes</td>
<td>63</td>
<td>45.8 ± 80.5</td>
<td>0–326.4</td>
<td>95$^2$</td>
</tr>
<tr>
<td>Fish</td>
<td>63</td>
<td>36.7 ± 65.0</td>
<td>0–276.3</td>
<td>93$^3$</td>
</tr>
<tr>
<td>Meat</td>
<td>63</td>
<td>53.1 ± 55.9</td>
<td>0.8–295.1</td>
<td>84$^2$</td>
</tr>
<tr>
<td>Fruit</td>
<td>63</td>
<td>16.0 ± 16.9</td>
<td>0.3–108.2</td>
<td>94$^4$</td>
</tr>
<tr>
<td>Sweet potatoes</td>
<td>63</td>
<td>40.5 ± 41.3</td>
<td>0–175.1</td>
<td>93$^5$</td>
</tr>
<tr>
<td>Egg</td>
<td>63</td>
<td>31.9 ± 27.7</td>
<td>0.9–121.2</td>
<td>84$^3$</td>
</tr>
<tr>
<td>Milk</td>
<td>63</td>
<td>2.2 ± 5.6</td>
<td>0–30.0</td>
<td>99$^4$</td>
</tr>
<tr>
<td>Daily amount (g/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>63</td>
<td>324.3 ± 246.1</td>
<td>0–724.5</td>
<td>98$^5$</td>
</tr>
<tr>
<td>Wheat</td>
<td>63</td>
<td>106.2 ± 131.3</td>
<td>0–476.4</td>
<td>94$^5$</td>
</tr>
<tr>
<td>Corn</td>
<td>63</td>
<td>71.8 ± 108.3</td>
<td>0–551.1</td>
<td>89$^2$</td>
</tr>
<tr>
<td>Sorghum</td>
<td>63</td>
<td>5.2 ± 17.1</td>
<td>0–100.0</td>
<td>38$^3$</td>
</tr>
<tr>
<td>Millet</td>
<td>63</td>
<td>13.9 ± 34.6</td>
<td>0–201.9</td>
<td>94$^2$</td>
</tr>
<tr>
<td>Legumes</td>
<td>63</td>
<td>9.5 ± 8.9</td>
<td>0–403.0</td>
<td>69$^2$</td>
</tr>
<tr>
<td>3-d Dietary survey (g/d)$^7$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>64</td>
<td>319.9 ± 226.9</td>
<td>0–785.6</td>
<td>NA</td>
</tr>
<tr>
<td>Wheat</td>
<td>64</td>
<td>117.4 ± 164.1</td>
<td>0–628.6</td>
<td>NA</td>
</tr>
<tr>
<td>Other cereals</td>
<td>64</td>
<td>82.9 ± 131.9</td>
<td>0–538.4</td>
<td>NA</td>
</tr>
<tr>
<td>Light-colored vegetables</td>
<td>64</td>
<td>210.9 ± 132.1</td>
<td>10.7–616.7</td>
<td>NA</td>
</tr>
<tr>
<td>Meat</td>
<td>64</td>
<td>24.9 ± 20.6</td>
<td>0–94.6</td>
<td>NA</td>
</tr>
<tr>
<td>Fish</td>
<td>64</td>
<td>17.2 ± 28.1</td>
<td>0–119.0</td>
<td>NA</td>
</tr>
</tbody>
</table>

$^1$ Reliability coefficient, which assesses the between-commune or within-county homogeneity of the variable.

$^2$ P < 0.0001.

$^3$ P < 0.05.

$^4$ Not available.

$^5$ Gross value of industrial and agricultural output, a measure of a county’s economic status.

$^6$ P < 0.001.

$^7$ 3-d (Cross-sectional) household dietary survey data was derived by using a food-weighing method that was standardized to a “reference man” (see Methods).

The most dominant non-animal food sources in the rural Chinese diet nationwide were rice (324 g/d), wheat (106 g/d), and light-colored vegetables (210 g/d). Noteworthy is the remarkably low frequency of consumption of fruit, eggs, and milk in rural China where the county means were 16.0, 31.9, and 2.2 times eaten per year, respectively.

Univariate correlation coefficients (Table 2) were computed to assess the association between SHBG and selected dietary, plasma, and anthropometric variables. Nonsignificant correlates—such as GVIAO, number of years smoking, and estradiol and cholesterol concentrations—were eliminated from subsequent analyses. Because of space constraints, only those variables with a significant association with SHBG are presented in the table. Interestingly, insulin and SHBG concentrations were consistently associated with essentially the same foods but in opposite directions (data not shown). More frequent consumption of rice (0.61, P < 0.0001), green vegetables (0.49, P < 0.001), fish (0.42, P < 0.001), and meat
TABLE 2
Pearson correlation coefficients for sex hormone–binding globulin (SHBG) and selected dietary, plasma, and anthropometric variables

<table>
<thead>
<tr>
<th></th>
<th>SHBG</th>
<th>Insulin</th>
<th>Triacylglycerols</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>-0.37*</td>
<td>-0.07</td>
<td>0.07</td>
<td>0.25</td>
</tr>
<tr>
<td>Testosterone</td>
<td>-0.34*</td>
<td>0.25</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>-0.52*</td>
<td>0.07</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>Anthropometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>-0.33*</td>
<td>0.21</td>
<td>0.11</td>
<td>0.22</td>
</tr>
<tr>
<td>Weight</td>
<td>-0.46*</td>
<td>0.30*</td>
<td>0.32*</td>
<td>0.41*</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.41*</td>
<td>0.25*</td>
<td>0.35*</td>
<td>0.40*</td>
</tr>
<tr>
<td>General</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at menarche</td>
<td>0.30*</td>
<td>-0.25*</td>
<td>-0.11</td>
<td>-0.10</td>
</tr>
<tr>
<td>Questionnaire*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>0.61*</td>
<td>-0.46*</td>
<td>-0.49*</td>
<td>-0.37*</td>
</tr>
<tr>
<td>Wheat</td>
<td>-0.57*</td>
<td>0.21</td>
<td>0.49*</td>
<td>0.34*</td>
</tr>
<tr>
<td>Millet</td>
<td>-0.37*</td>
<td>0.33*</td>
<td>0.14</td>
<td>0.16</td>
</tr>
<tr>
<td>Sorghum</td>
<td>-0.28*</td>
<td>0.55*</td>
<td>-0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Legume (mostly soy beans)</td>
<td>-0.35*</td>
<td>0.46*</td>
<td>0.11</td>
<td>0.17</td>
</tr>
<tr>
<td>Green vegetables</td>
<td>0.49*</td>
<td>-0.42*</td>
<td>-0.41*</td>
<td>-0.29*</td>
</tr>
<tr>
<td>Meat</td>
<td>0.38*</td>
<td>-0.27*</td>
<td>-0.48*</td>
<td>-0.01</td>
</tr>
<tr>
<td>Fish</td>
<td>0.42*</td>
<td>0.27*</td>
<td>-0.45*</td>
<td>-0.07</td>
</tr>
<tr>
<td>Corn</td>
<td>-0.46*</td>
<td>0.64*</td>
<td>0.29*</td>
<td>0.23</td>
</tr>
<tr>
<td>Carrots</td>
<td>-0.37*</td>
<td>0.27*</td>
<td>0.29*</td>
<td>0.09</td>
</tr>
<tr>
<td>3-d Dietary survey*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>0.57*</td>
<td>-0.49*</td>
<td>-0.42*</td>
<td>-0.36*</td>
</tr>
<tr>
<td>Wheat</td>
<td>-0.59*</td>
<td>0.38*</td>
<td>0.47*</td>
<td>0.42*</td>
</tr>
<tr>
<td>Other cereals</td>
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<td>0.38*</td>
<td>0.07</td>
</tr>
<tr>
<td>Light-colored vegetables</td>
<td>-0.43*</td>
<td>0.19</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Meat</td>
<td>0.32*</td>
<td>-0.09</td>
<td>-0.37*</td>
<td>0.05</td>
</tr>
<tr>
<td>Fish</td>
<td>0.32*</td>
<td>-0.24</td>
<td>-0.39*</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* P < 0.05.
* P < 0.001.
* Questionnaire data was derived from a longitudinal retrospective recall survey (see Methods).
* P < 0.0001.
* 3-d (Cross-sectional) household dietary survey data was derived by using a food-weighing method that was standardized to a “reference man” (see Methods).

(0.38, P < 0.01) was positively associated with SHBG concentrations. On the other hand, SHBG showed significant negative associations with other foods: legumes (−0.35, P < 0.01), corn (−0.46, P < 0.001), and wheat (−0.57, P < 0.0001). Analyses of the plasma variable correlations with SHBG revealed that SHBG was significantly negatively correlated with insulin (−0.37, P < 0.05), triacylglycerols (−0.52, P < 0.0001), and testosterone (−0.34, P < 0.05). No significant correlation was found between SHBG and estradiol, prolactin, low-density lipoprotein, or total cholesterol. The other nondietary variables significantly correlated with SHBG were weight (−0.46, P < 0.00), body mass index (−0.41, P < 0.001), and age at menarche (0.30, P < 0.05).

The regression models included in Table 3 are the result of an extensive exploration of different specifications for different subsets of data (not shown). The seven models presented represent two distinct groupings of foods (models 1–4 and models 5–7). Separate analyses (data not shown) reveal that rice and wheat are highly inversely correlated with each other (r = 0.75, P < 0.001) and could not logically be included in the same model. Millet had a similar strong inverse relation with rice, but not with wheat. Both green vegetables and corn were identified by a SAS diagnostic (see Methods) as being collinear in the models and were thus subsequently analyzed separately. The resulting R² values for each of the models were remarkably high (R² = 0.58). The strongest predictors of SHBG were rice (β = 0.42) and wheat (β = 0.34). Interestingly, the strong and significant predictive strength of insulin (β = −0.24), testosterone (β = −0.21), and triacylglycerols (β = −0.44) was considerably reduced (−0.04, −0.13, and −0.20, respectively) when the dietary variables were added. The inclusion of plasma covariates (triacylglycerols in particular) in model 7 resulted in an important reduction in the predictive strength of both meat (β = 0.21 to β = 0.00) and wheat (β = −0.45 to β = −0.34). Less of an effect was seen on rice (model 4). With plasma triacylglycerol concentrations adjusted for, fish consumption was an important positive independent predictor of SHBG (β = 0.34, P < 0.05).

Age at menarche and body mass index were determined to be collinear (SAS diagnostic) in the various models analyzed; consequently, they were analyzed separately to determine their relative contribution to explaining SHBG variation. In comparing models it can be seen that the effect of body mass index is not significant. Age at menarche, on the other hand, was positively related to SHBG, as seen in models 2 and 6. However, the significance of the relation was attenuated after selected plasma variables were adjusted for.

Neither meat nor fish intake showed any particular strength or significant influence on SHBG after other foods were adjusted for. The remaining foods (carrots and legumes) were ultimately subtracted from the full model because they did not increase the overall R² value. Millet, though eaten relatively little in China compared with wheat (see Table 1), retained significant predictive strength in a variety of models tested (models 4–6).

Green vegetables demonstrated similar positive predictive strength for SHBG variation as did rice after excluding rice from the regression model due to collinearity. The principal vegetables included in this category are green bok choy, green onion, peppers, spinach, Chinese kale, radishes, mustard greens, lettuce, bean sprouts, and asparagus (22).

Comparison of the analyses including dietary intakes measured in the 3-d dietary survey and the questionnaire suggests similar results in both significance level of the estimate and R² values for the final model. The regression analysis using the questionnaire data was chosen for this communication because it reported responses by women, whereas the 3-d dietary survey data represented intakes per reference man. Nevertheless, the strongest positive (rice) and negative (wheat) predictors of SHBG remained the same in the final model for each set of regression analyses.

DISCUSSION

This study examined the relation of foods and selected plasma variables on plasma concentrations of SHBG in women aged 35–64 y. Disaggregation of the dependent variable by age (35–44 y, 45–54 y, or 55–64 y), showed no differences in means or SDs by age group. This finding agrees with some studies but not others. Pasquali et al (37) found that although
TABLE 3
Standardized regression coefficients: consumption frequency of specific foods from the questionnaire data as predictors of sex hormone-binding globulin concentrations in rural Chinese women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.45 †</td>
</tr>
<tr>
<td>Fish</td>
<td>0.17</td>
</tr>
<tr>
<td>Meat</td>
<td>0.13</td>
</tr>
<tr>
<td>Carrots</td>
<td>-0.09</td>
</tr>
<tr>
<td>Legumes</td>
<td>-0.04</td>
</tr>
<tr>
<td>Millet</td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td></td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
</tr>
<tr>
<td>Age at menarche</td>
<td></td>
</tr>
<tr>
<td>( R^2 )</td>
<td>0.46</td>
</tr>
</tbody>
</table>

\[ † P < 0.001. \]
\[ ‡ P < 0.01. \]
\[ † † P < 0.05. \]

estradiol decreased from before to after menopause, there were no significant differences in SHBG concentrations by age in a population of 952 women. Some reports have found increases in SHBG concentrations after menopause whereas others have reported a decline in SHBG concentrations for the same age groups (38).

The geographic distribution of plasma SHBG concentration by quartile is shown in Figure 1. The range of county mean values for SHBG (52–103 nmol/L) of rural Chinese women is similar to that reported for other studies (15, 39, 40). In fact, the mean county values tend to approximate vegetarian concentrations reported by some researchers in the West (19). However, differences in methods used to measure SHBG do present a potential problem for comparison across studies. The range of county mean insulin values (27.3–68.2 pmol/L) in rural Chinese women was at the lower end of the range of values reported for populations in France (31.6–58.8 pmol/L), England (25.8–121.9 pmol/L), and South Asia (30.8–140.6 pmol/L) (41, 42).

A particularly advantageous characteristic of the data variables analyzed here was a several-fold difference across counties for the consumption of different foods and the various plasma variables. For example, the county average of rice consumed by women ranged from 0 to 700 g/d, total fat intake as a percentage of energy ranged from 6% to 25%, and the amount of meat consumed per day ranged from 0 to 94 g on average across the 65 rural counties surveyed. It is remarkable that the range of county mean values is as large or larger than the range of values found between individuals. One would normally expect an attenuation effect, resulting in a much narrower range between counties. Thus, we are confident that the several-fold differences reported are in fact conservative estimates of true differences between counties. Further, the results of validation assays indicate good agreement between pool values and averages of individual values (22).

Our study population was genetically (primarily Han) and culturally (mostly rural) homogeneous. Genetic influences on SHBG are not likely because the extreme values observed in the distribution of SHBG concentration were from those counties where the majority ethnic group is Han. Only 6% of the counties studied in this communication have a non-Han ethnic group as the majority population; and the SHBG values for these provinces approximately equal the mean of the total sample. The principal remaining differences are those of geography, socioeconomic status, and diet.

Socioeconomic status did not demonstrate any effect on the magnitude or direction of the significant relations in our analyses. Although there is no biological reason to suspect that geography itself has any influence on SHBG biochemistry, we questioned whether the significantly higher SHBG concentrations found in the north and the significantly lower SHBG concentrations in the south were not due, in fact, to some other unmeasured variable that covaried with the geographic differences between the north and south. To assess whether or not the significant association of rice and wheat with SHBG was artifactual because of unmeasured variables, two different geographic partitioning schemes (data not shown) were used: a north-south partition based on distinctive agricultural practices, and a coast-center-periphery partition based on zones of economic development (43). Despite such partitioning and broad adjustments for variables associated with geography, wheat and rice still retained significance and direction similar to that seen in the other analyses. Thus, the evidence suggests that the significant differences seen in the SHBG concentrations of the 65 Chinese counties may be largely attributed to differences in diet.

Correlation of SHBG with plasma and anthropometric variables

Pearson product correlation coefficients between SHBG, selected plasma variables, and other selected covariates were used to determine the strength and magnitude of the relations. This analysis indicated that SHBG was significantly negatively correlated with the following plasma variables: insulin (\( -0.37, P < 0.05 \)), testosterone (\( -0.34, P < 0.05 \)), and triacylglycerols (\( -0.52, P < 0.001 \)). Particularly striking was that insulin was
consistently inversely related to almost every dietary and plasma variable that was positively related to SHBG (Table 2). For example, insulin was inversely correlated with HDL cholesterol \((-0.35, P < 0.05)\) and positively correlated with testosterone \((0.25, P < 0.05)\). The consistency of these relations between HDL, insulin, testosterone, triacylglycerol, and SHBG agree with previously published research and are associated with a group of chronic degenerative diseases known as Reaven’s syndrome \((44-46)\).

SHBG was significantly negatively correlated with weight \((-0.46, P < 0.001)\) and body mass index \((-0.41, P < 0.001)\). Again, insulin was significantly positively correlated with weight \((0.30, P < 0.05)\) and body mass index \((0.25, P < 0.05)\). From the parent survey, a separate analysis of body mass index at the individual level revealed the mean body mass index to be 20.6 (median: 20.4), with only 5.3\% of the female population being obese (ie, body mass index > 25). Body mass index did not show any predictive strength or significance in any of the regression models. Caution should be used in interpreting these relations because much of the data used in deriving body mass index comes from the weight-height relations in white adults, which have been shown to have significant limitations \((47)\), especially when applied to Asians, who have a significantly greater proportion of subcutaneous fat in the arms and trunk when compared with whites \((48)\). Nevertheless, the leanness of this population is remarkable when compared with the body mass index of the average American woman (mean body mass index = 26.2) during the same period \((49)\).

Also noteworthy is the late age at menarche in this population, \(\sim 17\) y on average compared with the US and western European average age at menarche of \(13\) y on average \((22)\). Both leanness and late age at menarche have been associated with poor nutrition, genetics, and vegetarianism \((50)\). The data analyzed as part of the parent study \((22)\) suggest that the county mean values for both the degree of leanness and the late age of menarche can be explained in part by the relative poverty and

**FIGURE 1.** County means of plasma sex hormone-binding globulin by quartile in 65 mostly rural Chinese counties. First quartile: 51.4–66.6 nmol/L, ○; second quartile: 66.8–72.3 nmol/L, ●; third quartile: 72.5–78.6 nmol/L, ◊; fourth quartile: 79.3–102.6 nmol/L, ◔.
possible nutritional inadequacy of some of the rural counties considered. Regression analyses (Table 3) demonstrated that in the absence of body mass index, the age at menarche covariate remained a strong independent predictor of SHBG in each model. This suggests that SHBG concentrations may be influenced by the overall nutritional adequacy of the diet.

In the population of women studied, only 12% reported a history of smoking. We found no association between smoking and SHBG. This agrees with some reports (51) but not with others in which serum concentrations of SHBG were found to be either positively (52) or negatively (53) correlated with smoking. Insulin, on the other hand, was significantly correlated with smoking (0.47, \( P < 0.001 \)), corroborating other researchers’ findings (54).

Relation of SHBG to specific foods

The principal positive food-SHBG correlates in order of magnitude were rice (0.61, \( P < 0.0001 \)), green vegetables (0.49, \( P < 0.001 \)), fish (0.42, \( P < 0.001 \)), and meat (0.38, \( P < 0.05 \)). The strongest negative food correlate with SHBG (positively correlated with insulin) was wheat (\((-0.57, P < 0.0001)\).

The majority of individuals in our study population ate both meat and fish in varying amounts. Culturally, it should be noted that meat is a luxury food in China. Whenever finances permit it, or celebration demands it, meat (principally pork) is consumed. Small amounts, by Western standards, were eaten occasionally throughout the year in many of the counties. In contrast, counties reporting a higher frequency of fish consumption also ate relatively higher amounts per day. An inverse relation of fish to insulin in particular was reported previously (55–57).

Intakes of rice and wheat contribute significantly to the overall nutritional profile of the Chinese people. In the northern region, where wheat consumption dominates, wheat provides 44% of energy, 45% of protein, 50% of carbohydrate, and 13% of fat consumed. In the southern regions, rice dominates. It contributes 61% of energy, 51% of protein, 78% of carbohydrate, and 13% of fat consumed. The absolute amount of a particular food, coupled with its nutrient density, determine to a large extent its individual contribution and influence on the by-products of digestion. Therefore, the amount of rice and wheat consumed in the different regions of China could be expected to significantly impact nutrient profiles and subsequently biochemical physiology.

Regression analyses are shown in Table 3. The percentage of the variation in SHBG that is explained by the model is remarkably high (\( R^2 = 0.58 \)). The principal independent effects of certain foods on SHBG seen from these analyses were those of rice and wheat, which were also the greatest contributors to the \( R^2 \) values in each model. The effect of rice and wheat on SHBG was remarkable and unexpected. Whether this effect was due to an unmeasured covariate remains to be investigated. Nevertheless, there is some evidence to suggest that rice and wheat can have significantly different effects on the biochemical variables we measured. Panlasigui et al (58) found that the high-amylose rice varieties had blood glucose responses that were lower than those of wheat bread. Other varieties, particularly “converted” rice, gave considerably higher values. Miller et al (59) in comparing rice and wheat varieties found that the insulin index (II) was unusually low on the relative scale compared with the glycemic index (GI) of the same foods. For example, Calrose brown rice had a GI = 83 but an II = 51. White bread was used as the reference food (GI = 100, II = 100). Wheat may be unique in its relative capacity to stimulate insulin. Most recently, Behall and Howe (60) reported a significantly lower insulin response curve area in both normal and hyperinsulinemic men consuming a high-amylose diet. The relative differences in the fatty acid proportions and/or amylose content for wheat and rice may thus be responsible for modulating serum SHBG, triacylglycerols, and insulin. Our study did not find insulin to be predictive of SHBG variation after the effect of the specific foods were adjusted for (particularly rice and wheat).

In general, there seems to be good evidence to suggest that a plant-based diet and lower body fat are positively correlated with SHBG concentrations, and negatively correlated with insulin and triacylglycerols. Several reports have found that the vegetarian diet is positively associated with SHBG (5, 61) although others showed no association (7, 19). On the other hand, obesity or weight gain has consistently shown a significant inverse association with SHBG and a positive association with insulin blood concentrations (11, 19, 61–64).

Implications for insulin resistance syndrome

Any dietary effect on SHBG and plasma variables such as insulin or triacylglycerol could have important implications in the pathogenesis of the IRS pentad: NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease.

Taken together—low SHBG, high insulin, low HDL-cholesterol, and high plasma triacylglycerol concentrations—this cluster of biochemical relations is suggestive of the pattern seen in IRS (also called syndrome X or Reaven syndrome). The nature of these biochemical relations to the IRS diseases (NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease) is beyond the scope of this communication and will be addressed in a future paper. Nevertheless, there are important implications of these results. Habitual consumed foods or food combinations that elicit a high insulin response may be directly responsible for the variations seen in SHBG concentrations across different diets. Dietary fatty acids too have already been shown to influence the pool of specific fatty acids from which plasma membranes derive their phospholipid structures and can subsequently significantly affect insulin binding, triacylglycerols, cholesterol, and SHBG (19, 55). We believe that further research is warranted to determine the relative effect of specific foods or food combinations on insulin resistance.

Study limitations

This study provided a unique opportunity to analyze a remarkable variety of dietary variables in an ethnically homogeneous population with a remarkable heterogeneity in diet and plasma variables. However, several limitations must be recognized.

The dependent variable SHBG may have been affected by other dietary or lifestyle elements not measured in the study (including herbal tea, traditional medicines, and intakes of mushroom) and may thus represent potentially important covariates. Additionally, the use of oral contraceptives was not
measured in this survey. The most common contraceptive in China is the IUD, although it has been found that educated women (rural or urban) do use contraceptive pills. There is anecdotal evidence to suggest that contraceptive use is evenly distributed across the counties studied and therefore does not significantly influence the county mean values of SHBG. Additionally, physical activity was not effectively assessed in this population and may contribute to the modulation of SHBG and other plasma variables that may influence SHBG concentrations (17).

The small sample size \((n = 65)\) leaves this study vulnerable to the effect of outliers. The study population is essentially rural, and inclusion of the urban population would have likely yielded greater dietary variation that would have increased statistical power.

The correlations in this study are cross-sectional at the county level and should not be interpreted as demonstrating cause-and-effect relations. In addressing the issue of ecological fallacy, however, we believe that the large number of variables measured in this study reduces the risk of specifying a model without accounting for the effects of various confounding variables. Further, the regression coefficients, which are less sensitive to aggregation bias than are correlation coefficients, describe the associations of rice and wheat with SHBG with significance and direction similar to that of the correlation analyses.

Summary

In summary, the data presented in this paper confirm the findings of other research suggesting that the interplay of dietary habits and anthropometric characteristics in a population may contribute to the variation in SHBG concentrations. Significant differences in the diet of rural Chinese populations studied suggest that wheat consumption may promote higher insulin, higher triacylglycerol, and lower SHBG values. Such a profile is consistent with that commonly associated with obesity, dyslipidemia, diabetes, hypertension, and heart disease. On the other hand, the intake of rice, fish, and possibly green vegetables may elevate SHBG concentrations independent of weight or smoking habits.

The complex relations between food constituents, metabolites, and disease processes are problematic for researchers attempting to interpret nutrition-disease studies. This study provides an alternative answer to the clinically important question of the effect of diet on the IRS in general and to the question of the diet-SHBG interaction in particular.

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