Definition, prevalence, and risk factors of low sex hormone-binding globulin in US adults

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ABSTRACT

Context Lower sex hormone-binding globulin (SHBG) is associated with many diseases including cardiovascular disease, cancer, polycystic ovarian syndrome, arthritis, and liver disease. However, the definition of low SHBG and its prevalence in US adults are unknown.

OBJECTIVE To define low SHBG and to determine its prevalence and risk factors in US adults.

DESIGN, SETTING, AND PARTICIPANTS This cohort study included adults ≥ 20 years from the US National Health and Nutrition Examination Survey (NHANES) from 2013 to 2016 who had fasting serum SHBG. EXPOSURES NHANES coverage during 2013-2016.

MAIN OUTCOMES MEASURES Definition, prevalence, and risk factors of low SHBG.

RESULTS This study included 4,093 adults (weighted sample size of 204,789,616) with a mean (SD) age of 47.5 (17.0) years. In a “healthy” reference sub-cohort of 1,477 adults, low SHBG was defined as SHBG< 12.3 nmol/L in men <50 years, or < 23.5 nmol/L in men ≥50 years, or < 14.5 nmol/L in women <30 years, or < 21.9 nmol/L in women ≥30 years. The estimated US national prevalence of low SHBG was 3.3% in men, 2.7% in women, and 3.0% overall. Risk factors for this condition in both men and women included higher body mass index, diabetes, ethnicity (being other than Hispanic, non-Hispanic black, or non-Hispanic white), chronic obstructive pulmonary disease, coronary heart disease, and smoking.

CONCLUSIONS This study established the criteria for low SHBG among US adults. The estimated US national prevalence of low SHBG was 3.3% in men and 2.7% in women.

Key words: sex hormone-binding globulin, prevalence, risk factor
INTRODUCTION

Sex hormone-binding globulin (SHBG) is a plasma glycoprotein that can bind sex hormones. SHBG is important in transporting sex hormones and regulating their bioavailability (1). SHBG can also bind and activate its receptors on the cell surface to exert direct effects on cellular function (2,3). Recent studies indicate that SHBG may be involved in many diseases. In particular, lower levels of SHBG are reported to be associated with higher prevalence or incidence of hypertension (4), arterial stiffness (5), insulin resistance (3), type 2 diabetes (3), coronary heart disease (6), stroke (7), cancer (8,9), polycystic ovarian syndrome (10), arthritis (11), liver disease (12), and inflammation (13), highlighting the importance of investigating the condition of low SHBG. The mechanisms by which lower SHBG levels are related to an increased risk of these diseases are not well understood. It has been proposed that potential mechanisms include a combination of direct effects of SHBG on cellular function and indirect effects of SHBG through alterations in the balance between testosterone and estradiol (14).

However, low SHBG has not been defined for US adults. Previous SHBG-related studies conducted their analyses treating SHBG as either a continuous variable or a categorical variable with multiple categories (e.g. quartiles). Due to the lack of a definition, the prevalence of low SHBG in the general population is unknown.

Using a representative cohort of US adults who attended the US National Health and Nutrition Examination Survey (NHANES) from 2013 to 2016, this study aimed to define low SHBG among US adults through the determination of the reference interval of SHBG in a “healthy” sub-cohort. Subsequently, this study investigated the prevalence and risk factors of low SHBG.
METHODS

Study participants

The National Center for Health Statistics ethics review board approved the NHANES protocols and written informed consent was obtained from all participants. This analysis of deidentified data did not involve direct interaction with participants and was not subject to institutional review board review. NHANES provides data from a representative sample of noninstitutionalized US population. From 2013 to 2016, a total of 4 093 adults aged 20 years or older had fasting serum SHBG levels and were included in the analyses of this study.

SHBG determination

SHBG data were obtained from the NHANES website. Fasting serum SHBG was measured at the National Center for Environmental Health. It was quantified based on the reaction of SHBG with immuno-antibodies and chemo-luminescence measurements of the reaction products by a photomultiplier tube. The limit of detection was 0.80 nmol/L, well below the lowest SHBG reading of the cohort, i.e., 6.70 nmol/L. The accuracy of the detection method was 97.6%.

Selection of a “health” reference sub-cohort

A sub-cohort of “healthy” participants were selected based on the following exclusion criteria. The numbers of excluded participants were the number of participants who were excluded progressively. 1) those having a history of taking sex hormone medication including testosterone, progesterone, estrogen, or unspecified sex hormones (N=400); 2) those having polycystic ovary syndrome, or undergoing hysterectomy or ovary removal (N=225); 3) those pregnant or breastfeeding (N=46); 4) those having a history of the following diseases: (a) cardiovascular diseases including heart attack, angina, congestive heart failure, coronary heart disease, or stroke (N=334), (b) lung diseases including chronic bronchitis, chronic obstructive pulmonary disease, or emphysema
(N=162), (c) liver disease (N=100), (d) kidney disease (N=65), (e) thyroid disease (N=183), (f) cancer (N=139), (g) HIV (N=7), (h) arthritis (N=345), (i) diabetes (N=237), or (j) hypertension (N=373). The resulting 1,477 individuals were included in the “healthy” reference sub-cohort.

**Determination of reference intervals**

Reference intervals of SHBG were determined using the method outlined by the Clinical and Laboratory Standards Institute for appropriate statistical determination of reference intervals (15,16). In brief, a cohort of healthy reference individuals (N=1,477) were selected as detailed in the above section; the SHBG data were then normalized by natural log-transformation; the natural log-transformed SHBG data were truncated by excluding outliers (i.e., outside of the range of mean ± 3SD); the reference interval of natural log-transformed SHBG was established by calculating the 2.5th (mean - 2SD) and 97.5th (mean + 2SD) percentiles of the truncated natural log-transformed SHBG distribution (15); finally, inverse transformation of natural log-transformed SHBG values corresponding to mean ± 2 SD yielded the reference intervals for SHBG.

**Statistical Analysis**

Data from two NHANES cycles (2013-14 and 2015-16) were combined using the appropriate weighting methods (17). Four-year weights were calculated by dividing the fasting subsample 2-year weights by two (18) and used in all analyses to adjust for unequal selection probability and non-response bias following NHANES analytical guidelines (17). Estimated population means, medians, and proportions were reported. Descriptive statistics were presented as weighted median and interquartile range (non-normally distributed continuous data), or weighted mean and SD (approximately normally distributed continuous data), or weighted percentages (categorical data). The differences in the prevalence of low SHBG between those with and without a specific condition or disease were analyzed using the weighted Pearson Chi-square test. The differences in circulating
SHBG concentrations between men and women were analyzed using the weighted Mann-Whitney U test.

The analyses of risk factors for low SHBG were conducted using weighted multivariable binary logistic regression analysis. Risk factors included age (continuous variable), ethnicity (Hispanic, non-Hispanic white, non-Hispanic black, or other), body mass index (natural log-transformed, continuous variable), physically active (yes, no, or unknown), past or current smoker (yes, no, or unknown), and past or current alcohol drinker (yes, no, or unknown). Self-reported comorbidities with three categories (yes, no, or unknown) included heart attack, angina, congestive heart failure, coronary heart disease, stroke, chronic bronchitis, chronic obstructive pulmonary disease, emphysema, liver disease, kidney disease, thyroid disease, cancer, HIV, and arthritis. Potential risk factors for low SHBG also included diabetes (yes or no) and hypertension (yes, no, or unknown). Diabetes was defined as fasting plasma glucose ≥126 mg/dl, taking hypoglycemic drugs, or self-reported diagnosis (19). Hypertension was defined as systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg, or prior diagnosis and treatment of hypertension (20). Female-specific risk factors included self-reported pregnancy, breastfeeding, parity (≥2 deliveries), and oophorectomy, with three categories of yes, no, or unknown. Hysterectomy did not affect SHBG (21,22) and therefore was not treated as a confounder for low SHBG. Thirty-two participants had missing body mass index and excluded from this analysis. Therefore, the risk factor analysis was conducted in the remaining 4061 participants (a weighted sample size of 203 635 433).

Correlation between low SHBG and low testosterone (total testosterone <200 ng/dL) (23) was analyzed using weighted multivariable binary logistic regression, with or without adjustment for demographic factors, lifestyle confounders, and comorbidities.

All tests were two-sided and a P value of < 0.05 was regarded as statistically significant. All statistical analyses were performed using SPSS version 27.0 (IBM SPSS Statistics for Windows, Armonk, NY, International Business Machines Corporation).
RESULTS

The characteristics of the cohort

This study included a total of 4 093 participants (weighted sample size of 204 789 616) aged 20-80 years, with a mean (SD) age of 47.5 (17.0) years. Table 1 describes the characteristics of the cohort. Out of these 4 093 participants, 1 477 were included in the “healthy” reference sub-cohort (weighted sample size of 80 718 623).

Reference intervals of SHBG

Serum levels of SHBG were higher in women than those in men in both the whole cohort and the reference sub-cohort (Figure 1). Therefore, the reference interval of SHBG was determined separately for men and women. SHBG in the reference sub-cohort (N=815 men and 662 women) showed a clear right-skewed shift from the Gaussian distribution for either sex (Figures 2A-B).

Natural log-transformed SHBG (Ln SHBG) appeared approximately normally distributed (Figures 2C-D). After outlier rejection (two cases were deemed as outliers with one from each sex, Figures 2C-D), mean ± 2 SD of Ln SHBG values were calculated (Figures 2E-F). Inverse transformation of Ln SHBG values corresponding to mean ± 2 SD yielded the reference intervals for SHBG: 12.6-92.4 nmol/L in men and 18.4-211.5 nmol/L in women (Reference Interval #1, Table 2).

SHBG concentrations changed over decades of human life (Figure 3). Therefore, age-specific reference intervals were determined for each decade of age (Reference Interval #2, Table 2). The results obtained (Figure 3 & Table 2) suggested that men may be re-grouped as those aged 20-49 years and those aged 50-80 years, and women may be re-grouped as those aged 20-29 years and those aged 30-80 years. Thus, these simplified sex- and age-specific reference intervals were determined (Reference Interval # 3, Table 2).
The lower boundary values of the SHBG reference intervals were used as criteria to define low SHBG.

To choose the best criteria for low SHBG, the prevalence of low SHBG in the reference group (N=1,477) was calculated according to three criteria derived from those three reference intervals (Table 2). The results suggested that the optimal criteria were those from the simplified sex- and age-specific criteria (Criteria #3, Table 3), as this set of criteria resulted in a prevalence of low SHBG close to the expected 2.5% in the reference sub-cohort. Therefore, Criteria #3 was the chosen criteria for low SHBG by this study.

**Prevalence of low SHBG**

According to Criteria #3 (Table 3), the estimated US national prevalence of low SHBG was 3.3% in men, 2.7% in women, and 3.0% overall. Prevalences of low SHBG in sub-cohorts with various conditions, status, or diseases are listed in Table 4. Of note, participants with diabetes or undergoing oophorectomy had a high prevalence of low SHBG, 7.1% and 4.8%, respectively (Table 4).

**Risk factors for low SHBG**

There were some similarities and differences in risk factor profiles between men and women (Table 5). Risk factors for low SHBG in both sexes included higher body mass index, diabetes, ethnicity (being other than Hispanic, non-Hispanic black, or non-Hispanic white), chronic obstructive pulmonary disease, coronary heart disease, and smoking. Female-specific risk factors for low SHBG included oophorectomy, thyroid disease, angina, breastfeeding, and hypertension. Male-specific risk factors for low SHBG included cancer, alcohol drinking, and heart attack (Table 5).
Association of low testosterone with low SHBG

Low testosterone was strongly associated with low SHBG with an OR [95%CI] of 5.28 [5.25-5.29], after adjustment for all the tested confounders (Table 6).

DISCUSSION

This study defined criteria for low SHBG in US adults, which were SHBG < 12.3 nmol/L in men < 50 years, or < 23.5 nmol/L in men ≥ 50 years, or < 14.5 nmol/L in women < 30 years, or < 21.9 nmol/L in women ≥ 30 years. In addition, this study showed that the estimated US national prevalence of low SHBG was 3.3% in men, 2.7% in women, and 3.0% overall. Moreover, risk factors for this condition in both men and women included higher body mass index, diabetes, ethnicity (being other than Hispanic, non-Hispanic black, or non-Hispanic white), chronic obstructive pulmonary disease, coronary heart disease, and smoking.

To my knowledge, there are no prior studies reporting the definition of low SHBG for US adults. This deficiency becomes more problematic given that accumulating studies have shown that a lower level of SHBG (which was treated as a continuous variable or a multi-level categorical variable) is associated with many diseases including cardiovascular diseases (3-7), cancer (8, 9), liver disease (12), arthritis (11), and polycystic ovarian syndrome (10). Therefore, the availability of the definition of low SHBG, provided by the current study, could facilitate future research to investigate the role of low SHBG in the pathogenesis of other diseases as well as the suitability of using low SHBG as a potential therapeutic target.

A few studies investigated the reference interval of SHBG with participants from other countries, e.g. Chinese men with a low SHBG cut-off of 11.5 nmol/L (24) and Kuwait men with a low SHBG cut-off of 6.5 nmol/L (25). The study with Kuwait men (25) also supported the current study’s approach of re-grouping men to < 50 years and ≥ 50 years as that study similarly showed that the mean SHBG in men ≥ 50 years was higher than that in those < 50 years. The cut-offs of low SHBG in Chinese and Kuwait
men were lower than those for US men reported in the current study. This may be due to differences in ethnicity, location, lifestyle, or other factors.

In the US, each laboratory or hospital uses its in-house criteria for low SHBG. For example, Mayo Clinic Laboratories use a low SHBG cut-off of 13.3 nmol/L for men and 18.2 nmol/L for women ≤46 years and 16.8 nmol/L for women >47 years; whereas the Department of Pathology from the University of Iowa use a cut-off of 10 nmol/L for men and 20 nmol/L for women.

The current study has a few advantages in establishing the reference interval of SHBG for US adults. First, it used a representative US adult cohort selected by the National Center for Health Statistics from 15 different counties. Second, it used an extensive list of exclusion criteria to select the “healthy” reference group. Third, it investigated the reference intervals for different age groups, simplified the age-specific reference intervals, and compared and finally selected the best criteria for low SHBG.

SHBG is commonly measured by immunological and mass spectrometric assays (26). The detection limits of most assays range from 0.1-2 nmol/L (26-28), well below the cut-offs for low SHBG established by the current study. Immunological assays are indirect detection methods and employ a standard curve that has been set up with the use of a standard preparation. The accuracy of the assays may be affected by non-specificity bias, as the preparation of the standard may differ from the individual serum composition in which SHBG locates (29). The mass spectrometric assays directly measure SHBG; however, the accuracy of the assays may be affected by various procedures such as sample purification.

Different methods or platforms for SHBG detection have different variability. For example, the mass spectrometry-IAL assay had a coefficient of variation (CV) of 9.0% at the control SHBG level of 10.7 nmol/L, whereas the mass spectrometry-SISCAPA assay had a CV of 15.3% at the control SHBG level.
of 10.9 nmol/L. Immunological assays seemed to have a lower CV in general (26). For example, COBAS e411 had an intraassay CV of 2.1% and an interassay CV of 2.7% at the control SHBG level of 14 nmol/L; and Immulite 2000 had an intraassay CV of 2.7% and an interassay CV of 4.0% at the control SHBG level of 5.4-5.5 nmol/L (26).

Different assay methods or platforms can produce different SHBG readings (26,27). For example, a recent study compared four commonly used immunoassay platforms, i.e., Abbott Architect, Roche, Beckman, and Siemens, and found that the Roche platform produced the highest readings whereas the Abbott Architect platform produced the lowest (27). That study also showed that the major difference in SHBG readings was from the high end of SHBG concentrations (27). For example, different platforms could produce results that were 30% off from each other when SHBG was >100 nmol/L; however, when SHBG was <25 nmol/L, all the four platforms produced strikingly similar results, with ~3% variation among them (27). These results suggest that variation in cut-off values of low SHBG may be less likely due to the platforms used, and rather may be due to differences in selection of the “healthy” reference group, composition of the reference group, thoroughness of the investigation, or other factors.

This study revealed that the prevalence of low SHBG was higher in people with diabetes (7.1%) compared to that in non-diabetic counterparts (2.3%). In addition, diabetes posted a 2.65-fold and 3.52-fold higher risk for low SHBG in men and women, respectively, after adjustment for all the tested confounders. These observations are consistent with the previous finding that lower levels of SHBG were associated with insulin resistance and type 2 diabetes (3). Genetic studies, which are less likely to be confounded, biased, or influenced by disease processes, showed that SHBG-raising alleles were associated with a reduced risk of type 2 diabetes (30), suggesting that SHBG may be involved in the etiology of diabetes. However, the causal relationship between low SHBG and diabetes cannot be established by the current study due to its cross-sectional nature. Indeed, diabetes may also lead
to low SHBG, as insulin and carbohydrate can decrease SHBG production (3). The importance of low SHBG in the pathogenesis of diabetes warrants further investigation.

The study also found that women undergoing oophorectomy had a higher prevalence of low SHBG of 4.8%, compared to the prevalence of 2.5% in women without oophorectomy. The causal relationship between oophorectomy and low SHBG needs to be clarified in the future. SHBG is produced by the liver (31) and its concentration, as demonstrated by the current study, was relatively consistent over decades of age in women aged 30-80 years. Why ovary removal might affect SHBG production is unclear. Given that the estimated US national percentage of oophorectomy among women was high (10.7%), whether the association between oophorectomy and low SHBG has any clinical implication needs to be investigated in the future.

This study also showed that a higher body mass index was the biggest risk factor for low SHBG, with a 1-SD change in log-transformed value representing a 13-fold and 20-fold higher risk of low SHBG in men and women, respectively, after adjustment for all tested confounders. This is consistent with literature reports that obese people had low plasma SHBG levels compared to non-obese counterparts (32) and weight loss was associated with an increase in SHBG (33). The current study also found smoking was a risk factor for low SHBG in both men and women. These results suggest that weight loss and smoking cessation may be effective means to treat low SHBG.

This study confirmed that low SHBG was positively associated with many diseases in both men and women such as diabetes, chronic obstructive pulmonary disease, and coronary heart disease. However, there are sex differences in the associations between low SHBG and other diseases. For example, thyroid disease and hypertension were positively associated with low SHBG in women, whereas these associations were negative ones in men. On the other hand, cancer was positively associated with low SHBG in men, whereas the association was a negative one in women. The reasons underlying these sex differences are not clear and need to be investigated in the future.
Strengths and limitations

A strength of this study is the large sample size which is representative of the US general population (a weighted sample size of 204,789,616). This study has a number of limitations. First, most diseases except for diabetes and hypertension were based on self-reported data. Second, this study defined diabetes as fasting plasma glucose ≥126 mg/dL, taking hypoglycemic drugs, or self-reported diagnosis. However, according to the ADA guidelines (19), assessment of diabetes was defined according to the fulfilment of one of the following criteria: fasting plasma glucose ≥ 126 mg/dL, or 2-h plasma glucose ≥ 200 mg/dL, or HbA1c ≥ 6.5 %, or in a patient with classic symptoms of hyperglycaemia a random plasma glucose ≥ 200 mg/dL. Therefore, the diabetes status in the current study could be underestimated. Third, this study is based on cross-sectional data; therefore, the causal relationship between low SHBG and other diseases cannot be established.

In conclusion, this study provided data on the definition, prevalence, and risk factors of low SHBG for US adults. The availability of these data would facilitate future research investigating the role of low SHBG in the pathogenesis of many other diseases.

Author Contributions: Dr Wang had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Dr Wang solely contributed to all aspects of this study.

Data availability: All data in the current analysis are publicly available on the NHANES website.
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FIGURE LEGENDS

Figure 1. Serum levels of SHBG. Weighted levels of SHBG in the whole cohort (N=4 093, unweighted number, A) and the “healthy” sub-cohort (N=1 477, unweighted number, B). Error bars=standard deviation. SHBG: sex hormone-binding globulin. The difference between men and women was analyzed by the weighted Mann-Whitney U test.

Figure 2. Determination of reference intervals of SHBG in men and women. A- B: weighted data distribution of SHBG in the sub-cohort of “healthy” men (N=815, unweighted number, A) and women (N=662, unweighted number, B). C-D: weighted data distribution of natural log-transformed SHBG (Ln SHBG) in the “healthy” men (C) and women (D). E-F: after truncation of Ln SHBG values which were outside of mean ± 3 SD, mean ± 2 SD of Ln SHBG was calculated in the “healthy” men (N=814, unweighted number, E) and women (N=661, unweighted number, F). The resulting reference interval of SHBG in men was 12.6-92.4 nmol/L and it was 18.4-211.5 nmol/L in women. SHBG: sex hormone-binding globulin.

Figure 3. Serum levels of SHBG in the “healthy” sub-cohort of men (N=814, unweighted number) and women (N=661, unweighted number) over the years of human life. Box plots of natural log-transformed SHBG in “healthy” men (A) and women (B) in each decades of human life. SHBG: sex hormone-binding globulin.
Table 1. Characteristics of the cohort

|                      | Men          | Women         | Overall       |
|----------------------|--------------|---------------|---------------|
| No., unweighted      | 1,977        | 2,116         | 4,093         |
| No., weighted        | 99,576,283   | 105,213,333   | 204,789,616   |
| SHBG, median (IQR), nmol/L | 38.9 (26.9-55.5) | 62.2 (41.4-96.4) | 48.9 (32.6-75.5) |
| Age, mean (SD), y    | 46.9 (16.7)  | 48.1 (17.1)   | 47.5 (17.0)   |
| BMI, median (IQR), kg/m² | 28.1 (24.9-32.0) | 28.3 (23.7-33.9) | 28.2 (24.3-33.0) |
| Ethnicity, %         |              |               |               |
| Hispanic             | 15.8         | 15.1          | 15.5          |
| Non-Hispanic white   | 65.8         | 64.2          | 65.0          |
| Non-Hispanic black   | 9.8          | 12.0          | 10.9          |
| Other                | 8.6          | 8.7           | 8.7           |
| Physical active, %   | 54.0         | 50.2          | 52.0          |
| Smoker, %            | 51.2         | 36.9          | 43.8          |
| Alcohol drinker, %   | 86.2         | 76.8          | 81.4          |
| Hysterectomy, %      | NA           | 20.1          | NA            |
| Oophorectomy, %      | NA           | 10.7          | NA            |
| Pregnant, %          | NA           | 0.8           | NA            |
| Breastfeeding, %     | NA           | 1.8           | NA            |
| Parity (≥2 deliveries), % | NA         | 55.8          | NA            |
| Heart attack, %      | 4.2          | 2.6           | 3.4           |
| Angina, %            | 2.1          | 1.7           | 1.9           |
| Congestive HF, %     | 2.5          | 2.4           | 2.4           |
| CHD, %               | 4.1          | 2.8           | 3.4           |
| Stroke, %            | 2.6          | 3.1           | 2.9           |
| Chronic bronchitis, %| 3.4          | 6.8           | 5.2           |
| COPD, %              | 3.7          | 2.9           | 3.3           |
| Emphysema, %         | 2.1          | 1.6           | 1.8           |
| Liver disease, %     | 4.4          | 3.4           | 3.9           |
| Kidney disease, %    | 2.3          | 3.0           | 2.7           |
| Thyroid disease, %   | 3.8          | 18.3          | 11.3          |
| Cancer, %            | 9.3          | 10.1          | 9.7           |
| HIV, %               | 0.5          | 0.0           | 0.2           |
| Arthritis, %         | 20.7         | 30.9          | 25.9          |
| HTN, %               | 34.0         | 34.8          | 34.4          |
| DM, %                | 16.2         | 13.7          | 14.9          |

Abbreviations: BMI, body mass index; CHD, coronary heart disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; HF, heart failure; HIV, human immunodeficiency virus disease; HTN, hypertension; IQR, interquartile range; NA, not applicable; No., number; SD, standard deviation; SHBG, sex hormone-binding globulin.
Table 2. Reference intervals of SHBG in the “healthy” sub-cohort of men and women

| Reference Interval # 1: Sex-specific | Number, unweighted | Mean (nmol/L) | Reference intervals (nmol/L) |
|-------------------------------------|--------------------|---------------|----------------------------|
|                                     |                    |               | Lower boundary | Upper boundary |
| Men                                 | 814                | 34.1          | 12.6           | 92.4           |
| Women                               | 661                | 62.4          | 18.4           | 211.5          |

| Reference Interval # 2: Sex- and age-specific | Number, unweighted | Mean (nmol/L) | Reference intervals (nmol/L) |
|-----------------------------------------------|--------------------|---------------|----------------------------|
|                                             |                    |               | Lower boundary | Upper boundary |
| Men                                           |                     |               |               |                 |
| 20-29 y                                      | 257                | 30.2          | 12.2           | 74.6           |
| 30-39 y                                      | 223                | 30.7          | 11.3           | 83.3           |
| 40-49 y                                      | 164                | 34.1          | 14.4           | 81.2           |
| 50-59 y                                      | 95                 | 49.7          | 21.8           | 113.3          |
| 60-80 y                                      | 74                 | 55.9          | 27.4           | 114.1          |
| Women                                         |                     |               |               |                 |
| 20-29 y                                      | 229                | 60.2          | 14.5           | 249.8          |
| 30-39 y                                      | 192                | 64.0          | 21.4           | 191.5          |
| 40-49 y                                      | 139                | 68.2          | 23.8           | 195.5          |
| 50-59 y                                      | 62                 | 54.9          | 20.8           | 144.9          |
| 60-80 y                                      | 38                 | 62.6          | 22.6           | 173.5          |
## Reference Interval # 3: Simplified sex- and age-specific

|        |         |         |         |         |
|--------|---------|---------|---------|---------|
|        | Men     | Women   | Men     | Women   |
| 20-49 y| 644     | 229     | 60.2    | 63.9    |
| 50-80 y| 169     |         | 52.0    | 21.9    |

Abbreviations: SHBG, sex hormone-binding globulin.
Table 3. Criteria and prevalence of low SHBG in the “healthy” reference sub-cohort (N=1,477, unweighted)

| Criteria | Criteria for low SHBG | Prevalence of low SHBG |
|----------|-----------------------|------------------------|
|          |                       | Men | Women | Overall |
| Criteria # 1: |                       |     |       |         |
| Sex- specific | Men: <12.6 nmol/L | 2.1% | 1.6% | 1.9% |
|              | Women: <18.4 nmol/L |     |       |         |
| Criteria # 2: |                       |     |       |         |
| Sex- and age-specific | Men | 3.6% | 1.7% | 2.8% |
|              | 20-29 y: < 12.2 nmol/L |     |       |         |
|              | 30-39 y: < 11.3 nmol/L |     |       |         |
|              | 40-49 y: < 14.4 nmol/L |     |       |         |
|              | 50-59 y: < 21.8 nmol/L |     |       |         |
|              | 60-80 y: < 27.4 nmol/L |     |       |         |
|              | Women |     |       |         |
|              | 20-29 y: < 14.5 nmol/L |     |       |         |
|              | 30-39 y: < 21.4 nmol/L |     |       |         |
|              | 40-49 y: < 23.8 nmol/L |     |       |         |
|              | 50-59 y: < 20.8 nmol/L |     |       |         |
|              | 60-80 y: < 22.6 nmol/L |     |       |         |
| Criteria # 3: |                       |     |       |         |
| Simplified sex- and age-specific | Men | 2.9% | 1.8% | 2.4% |
|              | 20-49 y: < 12.3 nmol/L |     |       |         |
|              | 50-80 y: < 23.5 nmol/L |     |       |         |
|              | Women |     |       |         |
|              | 20-29 y: < 14.5 nmol/L |     |       |         |
|              | 30-80 y: < 21.9 nmol/L |     |       |         |
Table 4. Prevalence of the condition of low SHBG in sub-cohorts with various conditions, status, or diseases

| Condition                        | Number, unweighted | Weighted prevalence | Participants without the specified condition, status, or disease (control) | Number, unweighted | Weighted prevalence | Participants with the specified condition, status, or disease | Change from control | P value |
|----------------------------------|--------------------|---------------------|---------------------------------------------------------------------------|--------------------|---------------------|----------------------------------------------------------------|---------------------|---------|
| Hysterectomy                     | 1 508              | 2.7%                | 420                                                                       | 3.2%                | 19%                 | <0.001                                                         |                     |         |
| Oophorectomy                     | 1 702              | 2.5%                | 207                                                                       | 4.8%                | 92%                 | <0.001                                                         |                     |         |
| Pregnant                         | 497                | 1.7%                | 15                                                                        | 0.0%                | -100%               | <0.001                                                         |                     |         |
| Breastfeeding                    | 90                 | 1.2%                | 33                                                                        | 1.4%                | 17%                 | <0.001                                                         |                     |         |
| Hispanic                         | 2 964              | 2.9%                | 1 129                                                                     | 3.7%                | 28%                 | <0.001                                                         |                     |         |
| Non-Hispanic white               | 2 502              | 3.3%                | 1 591                                                                     | 2.9%                | -12%                | <0.001                                                         |                     |         |
| Non-Hispanic black               | 3 335              | 3.1%                | 758                                                                       | 2.2%                | -29%                | <0.001                                                         |                     |         |
| Non-Hispanic other               | 3 478              | 2.8%                | 615                                                                       | 3.9%                | 39%                 | <0.001                                                         |                     |         |
| Heart attack                     | 3 921              | 3.0%                | 169                                                                       | 3.3%                | 10%                 | <0.001                                                         |                     |         |
| Angina                            | 3 994              | 3.0%                | 94                                                                        | 1.8%                | -40%                | <0.001                                                         |                     |         |
| Congestive HF                    | 3 947              | 3.1%                | 142                                                                       | 1.8%                | -42%                | <0.001                                                         |                     |         |
| CHD                              | 3 911              | 3.0%                | 171                                                                       | 3.2%                | 7%                  | <0.001                                                         |                     |         |
| Stroke                           | 3 944              | 3.0%                | 142                                                                       | 3.0%                | 0%                  | <0.001                                                         |                     |         |
| Chronic bronchitis               | 3 847              | 3.0%                | 232                                                                       | 3.4%                | 13%                 | <0.001                                                         |                     |         |
| COPD                             | 3 947              | 3.0%                | 143                                                                       | 3.3%                | 10%                 | <0.001                                                         |                     |         |
| Emphysema                        | 4 005              | 3.1%                | 85                                                                        | 1.4%                | -55%                | <0.001                                                         |                     |         |
| Liver disease                    | 3 903              | 3.0%                | 184                                                                       | 3.6%                | 20%                 | <0.001                                                         |                     |         |
| Kidney disease                   | 3 936              | 3.0%                | 151                                                                       | 1.8%                | -40%                | <0.001                                                         |                     |         |
| Thyroid disease                  | 3 633              | 2.9%                | 456                                                                       | 4.1%                | 41%                 | <0.001                                                         |                     |         |
| Cancer                           | 3 722              | 3.0%                | 369                                                                       | 3.4%                | 13%                 | <0.001                                                         |                     |         |
| HIV                              | 2 700              | 3.3%                | 14                                                                        | 0.0%                | -100%               | <0.001                                                         |                     |         |
| Condition   | Count | Low SHBG (%) | Reference Value | p-Value |
|-------------|-------|--------------|-----------------|---------|
| Arthritis   | 3013  | 2.9%         | 12.3 nmol/L     | <0.001  |
| HTN         | 2431  | 2.7%         | 15.9 nmol/L     | <0.001  |
| DM          | 3325  | 2.3%         | 14.5 nmol/L     | <0.001  |

Abbreviations: CHD, coronary heart disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; HF, heart failure; HIV, human immunodeficiency virus disease; HTN, hypertension.

\(^a\)Low SHBG was defined as SHBG < 12.3 nmol/L in men <50 years, or < 15.9 nmol/L in men ≥50 years, or < 14.5 nmol/L in women <30 years, or < 21.9 nmol/L in women ≥30 years.

\(^b\)Differences in the prevalence of low SHBG between those with and without the specified condition, status, or disease were analyzed using the weighted Pearson Chi-square test.
Table 5. OR (95% CI) of various conditions, status, or diseases for low SHBG in 4 061 participants analyzed by weighted multivariable binary logistic regression

| Condition                        | Men OR | 95% CI    | P value | Men OR | 95% CI    | P value |
|----------------------------------|--------|-----------|---------|--------|-----------|---------|
| Pregnancy                        | NA     | NA        | NA      | 0.00   | 0-1.4E+27 | 0.677   |
| Breastfeeding                    | NA     | NA        | NA      | 1.94   | 1.91-1.97 | <0.001  |
| Parity (≥2 deliveries)           | NA     | NA        | NA      | 0.74   | 0.74-0.75 | <0.001  |
| Oophorectomy                     | NA     | NA        | NA      | 2.13   | 2.13-2.14 | <0.001  |
| Age, y                           | 1.02   | 1.02-1.02 | <0.001  | 0.98   | 0.98-0.98 | <0.001  |
| Ethnicity                        |        |           |         |        |           |         |
| Hispanic                         | 1.00   |           |         | 1.00   |           |         |
| Non-Hispanic white               | 0.65   | 0.64-0.65 | <0.001  | 1.08   | 1.08-1.08 | <0.001  |
| Non-Hispanic black               | 0.76   | 0.76-0.76 | <0.001  | 0.43   | 0.43-0.44 | <0.001  |
| Non-Hispanic other               | 1.59   | 1.58-1.60 | <0.001  | 2.19   | 2.18-2.20 | <0.001  |
| Ln [BMI (kg/m²)]                 | 13.75  | 13.67-13.83 | <0.001 | 21.11  | 20.99-21.22 | <0.001 |
| Physically active                | 0.68   | 0.68-0.68 | <0.001  | 1.07   | 1.06-1.07 | <0.001  |
| Smoker                           | 1.02   | 1.02-1.02 | <0.001  | 1.43   | 1.43-1.44 | <0.001  |
| Alcohol drinker                  | 1.90   | 1.89-1.91 | <0.001  | 0.92   | 0.91-0.92 | <0.001  |
| Heart attack                     | 1.63   | 1.62-1.64 | <0.001  | 0.00   | 0-1.4E+11 | 0.425   |
| Angina                           | 0.47   | 0.46-0.47 | <0.001  | 1.99   | 1.97-2.02 | <0.001  |
| Congestive HF                    | 0.28   | 0.28-0.28 | <0.001  | 0.64   | 0.63-0.65 | <0.001  |
| CHD                              | 1.68   | 1.67-1.69 | <0.001  | 1.27   | 1.26-1.28 | <0.001  |
| Stroke                           | 0.94   | 0.93-0.95 | <0.001  | 1.01   | 1.00-1.02 | 0.038   |
| Chronic bronchitis               | 0.36   | 0.36-0.36 | <0.001  | 1.05   | 1.04-1.05 | <0.001  |
| COPD                             | 1.77   | 1.76-1.78 | <0.001  | 1.23   | 1.22-1.24 | <0.001  |
| Emphysema                        | 0.29   | 0.28-0.29 | <0.001  | 0.28   | 0.27-0.28 | <0.001  |
| Liver condition | 1.06  | 1.05-1.06 | <0.001 | 0.44  | 0.44-0.44 | <0.001 |
|-----------------|-------|-----------|--------|-------|-----------|--------|
| Kidney disease  | 0.81  | 0.81-0.82 | <0.001 | 0.12  | 0.11-0.12 | <0.001 |
| Thyroid disease | 0.6   | 0.6-0.61  | <0.001 | 2.41  | 2.40-2.41 | <0.001 |
| Cancer          | 3.22  | 3.21-3.23 | <0.001 | 0.53  | 0.53-0.53 | <0.001 |
| HIV             | 0.00  | 0-2.4E+39 | 0.749  | 0.00  | 0-2.9E+167 | 0.938 |
| Arthritis       | 0.79  | 0.79-0.79 | <0.001 | 0.71  | 0.71-0.71 | <0.001 |
| DM              | 2.65  | 2.64-2.65 | <0.001 | 3.49  | 3.49-3.51 | <0.001 |
| HTN             | 0.46  | 0.46-0.46 | <0.001 | 1.40  | 1.39-1.40 | <0.001 |

Abbreviations: CI, confidence interval; CHD, coronary heart disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; HF, heart failure; HIV, human immunodeficiency virus disease; HTN, hypertension; Ln [BMI (kg/m^2)], natural log-transformed body mass index (kg/m^2); NA, not applicable; OR, odds ratio.

^OR, odds ratio of the absence of the specified condition, status, or disease for low SHBG was regarded as 1.
Table 6. Association of low testosterone (independent variable) with low SHBG (dependent variable) in 4,061 participants analyzed by weighted multivariable binary logistic regression

| Model   | Odds ratio | 95% CI  | P value |
|---------|------------|---------|---------|
| Model 1\(^a\) | 1.34       | 1.34-1.34 | <0.001  |
| Model 2\(^b\) | 9.85       | 9.82-9.87 | <0.001  |
| Model 3\(^c\) | 5.44       | 5.43-5.46 | <0.001  |
| Model 4\(^d\) | 5.28       | 5.25-5.29 | <0.001  |

\(^a\)Model 1: unadjusted.

\(^b\)Model 2: adjusted for age, sex, and race/ethnicity.

\(^c\)Model 3: adjusted for all the factors in Model 2 plus body mass index (natural log-transformed), physical activity, smoking status, and alcohol drinking status.

\(^d\)Model 4: adjusted for all the factors in Model 3 plus comorbidities including heart attack, angina, congestive heart failure, coronary heart disease, stroke, chronic bronchitis, chronic obstructive pulmonary disease, emphysema, liver condition, kidney disease, thyroid disease, cancer, HIV, arthritis, diabetes, and hypertension.
Figure 2
Figure 3