Lower levels sex hormone-binding globulin independently associated with metabolic syndrome in pre-elderly and elderly men in China

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Abstract

Objective To examine the relationship between sex hormone–binding globulin (SHBG) and the metabolic syndrome (MetS) in pre-elderly and elderly men in China. Methods A cross-sectional study was done among 437 men, aged 45 to 94 years old. Early morning fasting sera were assayed for total testosterone (TT), SHBG and other biochemical markers. Free testosterone (FT) was calculated. Results The SHBG level of the MetS group was significantly lower than those without MetS 35.70 (25.18, 47.10) nmol/L vs. 41.90 (31.80, 55.20) nmol/L; P < 0.001). As the number of MetS components increases, SHBG and TT levels became lower. SHBG correlated with age, as did TT and most of metabolic components. Body mass index (BMI), high density lipoprotein-cholesterol (HDL-C), triglyceride (TG), and TT remained independently associated with SHBG by multivariate regression analysis. In a logistic regression taking MetS as the dependent variable, SHBG (95% confidence interval (95% CI): 0.975–0.994, P = 0.018) and homeostasis model assessment for insulin resistance (HOMA-IR) (95%CI: 1.535–2.647, P < 0.001) were included in the final model. Conclusions Lower SHBG is independently associated with MetS among pre-elderly and elderly men. SHBG may be an independent predictor of MetS, but the mechanism of how SHBG is involved in MetS requires further studied.

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1 Introduction

Metabolic syndrome (MetS) and reduced circulating testosterone are common in older men. Obesity, insulin resistance, and dyslipidemia are significant risk factors for atherosclerosis and the most common earlier stage for cardiovascular disease. A number of studies have shown that serum testosterone levels are related to insulin resistance[1-3] and other features of MetS,[4-6] and cardiovascular disease.[7,8] In the circulating system, total testosterone (TT) has four major fractions: sex hormone-binding globulin (SHBG)-bound testosterone (44%), albumin-bound testosterone (50%), cortisol-binding globulin-bound testosterone (4%), and free testosterone (FT) (2%).[9] SHBG, produced by the liver, is a circulating steroid-transporting protein. Free and albumin-bound testosterones are thought to be readily available to the tissues of the body. During aging, total and free testosterone levels decline, while SHBG levels increase,[10-13] even though TT and SHBG concentrations are correlated.[12,12] The relationships between testosterone, SHBG, insulin resistance and cardiovascular risk may change during male aging as sex hormone profiles mature. Clarifying the interaction between SHBG and TT and FT levels on insulin resistance could help us better understand the association between hormone and MetS in men. In this study, we evaluated the strength and independence of associations between testosterone, SHBG and MetS in pre-elderly and elderly men in China, which has no similar report thus far in a Chinese population.

2 Methods

2.1 Subjects

A cross-sectional study was conducted among 437 men, aged 45 to 94, from the health checkup population of Zhongshan Hospital Fudan University. Patients who were under treatment with hormone replacement therapy, or diagnosed with thyroid disease, chronic renal failure, chronic hepatopathy, or cancer, were excluded from the study.
Complete medical history was taken and reviewed for each patient. The study protocol was approved by the Ethics Committee of Zhongshan Hospital of Fudan University. All participants gave informed written consent to participate in the study, which was carried out in accordance with the Helsinki Declaration.

2.2 Measurements

All the subjects underwent measurements of weight, height, waist, and hip circumferences. Body mass index (BMI) was calculated as weight divided by the square of height (kg/m²). Waist hip rate (WHR) was calculated as waist circumference divided by hip circumference. Blood pressure was taken twice with the subject in the sitting position at a 2 min to 3 min intervals after resting for at least 15 min. The average of the two blood pressure measurements was recorded.

Serum levels of fasting plasma glucose (FPG), total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and uric acid were analyzed (Hitachi 7600), while the levels of serum insulin, TT and SHBG were determined using the chemiluminescence method (Beckman Coulter DxI 800).

Insulin resistance was estimated by the omeostatic model assessment ratio formula. Homeostasis model assessment for insulin resistance (HOMA-IR) index = (Fasting plasma insulin (mIU/L) × fasting plasma glucose (mmol/L))/22.5.[14] FT was calculated using the Vermeulen equation.[15]

Participants who had smoked fewer than 100 cigarettes in the past five years were defined as non-smokers and the others as smokers. Participants who had consumed alcoholic beverages at least once per week for at least one year in the past five years were categorized as alcohol drinkers and the others as non-alcohol drinkers.

2.3 Criteria of metabolic syndrome

MetS was defined using the criteria of American Heart Association and the National Heart, Lung Blood Institute (AHA/NHLBI).[16]

2.4 Statistical analysis

Descriptive statistics, proportions for categorical variables, means and standard deviations for continuous variables, median (interquartile range; i.e., the range of values lying between the 25th and 75th centiles) were used to describe the study groups for abnormally distributed variables. The statistical differences between groups were calculated with the Student’s t-test, Mann Whitney-U test or Pearson X² test, depending on the normality distribution of the data. The correlation between variables was measured by Spearman correlation analysis. The multi-variant regression analysis was performed using the stepwise method to analyze the relationship between SHBG and other factors. Binary logistic regression analyses were undertaken to explore associations with the MetS and other factors. All tests were two-sided, and values of $P < 0.05$ were considered statistically significant.

3 Results

3.1 Characteristics of study participants with and without MetS

The characteristics of the 437 subjects, stratified as to their MetS status, are shown in Table 1. The prevalence of MetS was 34.9%. There was no significant difference in the TC, LDL-C, calculated FT, smoke status or alcohol intake of the men with and without MetS. Men with MetS had significantly lower mean serum HDL-C, TT, SHBG and higher BMI, waist circumference, WHR, systolic blood pressure (SBP), diastolic blood pressure (DBP), FPG, TG, insulin, HOMA-IR than those without MetS ($P < 0.001$ for each).

3.2 The relationships between SHBG, testosterone and the MetS score

The relationships between the SHBG, TT, FT, and the MetS score (the number of MetS components) are shown in Figure 1. As the score of MetS increased, the levels of SHBG became lower. The trend of TT changing with MetS score was similar to SHBG. The differences between the score groups were significant ($P < 0.05$). However, the level of FT showed no regular variation as the score increased.

3.3 The correlations between SHBG and each variable

Table 2 shows the results of univariate analyses between SHBG and each variable. SHBG correlated inversely with BMI, waist circumference, WHR, systolic blood pressure (SBP), diastolic blood pressure (DBP), FPG, TG, insulin, HOMA-IR, and FT ($P < 0.05$). There were positive correlations between age, HDL-C, TT, and SHBG ($P < 0.05$).

3.4 Multivariate analysis

We used multiple linear regression analysis to determine the correlations of the SHBG with metabolic parameters. As shown in Table 3, BMI, HDL-C, TT, and TT were independently associated with SHBG level.

Binary logistic regression analyses were undertaken to explore study parameters with the MetS. SHBG was associated with the MetS, and was entered into a multivariate
Table 1. Baseline characteristics of MetS group and non-MetS group.

|                  | Non-MetS (n = 270) | MetS (n = 167) | P value |
|------------------|--------------------|----------------|---------|
| Age (yrs)        | 71 (14)            | 69 (16)        | 0.044   |
| BMI (kg/m²)      | 22.94 ± 2.64       | 25.52 ± 2.50   | < 0.001 |
| Waist (cm)       | 87.06 ± 7.40       | 95.26 ± 6.07   | < 0.001 |
| WHR              | 0.89 ± 0.05        | 0.93 ± 0.04    | < 0.001 |
| SBP (mmHg)       | 120 (19)           | 130 (20)       | < 0.001 |
| DBP (mmHg)       | 80 (10)            | 85 (15)        | < 0.001 |
| FPG (mmol/L)     | 5.3 (0.53)         | 5.8 (1.19)     | < 0.001 |
| TC (mmol/L)      | 4.71 ± 0.81        | 4.65 ± 0.84    | 0.432   |
| HDL-C (mmol/L)   | 1.34 (0.37)        | 1.06 (0.28)    | < 0.001 |
| LDL-C (mmol/L)   | 2.88 ± 0.72        | 2.93 ± 0.74    | 0.576   |
| TG (mmol/L)      | 1.28 (0.60)        | 2.09 (1.08)    | < 0.001 |
| Insulin (µIU/L)  | 4.37 (2.84)        | 5.40 (3.64)    | < 0.001 |
| HOMA-IR          | 1.05 (0.69)        | 1.43 (1.17)    | < 0.001 |
| SHBG (nmol/L)    | 41.90 (23.40)      | 35.70 (21.93)  | < 0.001 |
| TT (nmol/L)      | 14.85 ± 4.91       | 13.56 ± 4.31   | 0.009   |
| FT (pmol/L)      | 256.81 ± 81.28     | 257.97 ± 80.22 | 0.884   |
| Smoke (%)        | 35 (12.96%)        | 27 (34.13%)    | 0.351   |
| Drink (%)        | 6 (2.22%)          | 5 (2.99%)      | 0.617   |

Data are presented as mean ± SD for normally distributed or median (interquartile range; i.e., the range of values lying between the 25th and 75th centiles) for not normally distributed variables unless otherwise stated. Categorical variables were compared using χ² test. Differences in continuous variables were tested with the unpaired t test or the nonparametric Mann-Whitney U test. BMI: body mass index; DBP: diastolic blood pressure; FT: free testosterone; FPG: fasting plasma glucose; HDL-C: high density lipoprotein-cholesterol; HOMA-IR: homeostasis model assessment for insulin resistance; LDL-C: low density lipoprotein-cholesterol; MetS: metabolic syndrome; SBP: systolic blood pressure; SHBG: sex hormone-binding globulin; TC: total cholesterol; TG: triglyceride; TT: total testosterone; WHR: waist hip ratio.

Figure 1. Levels of SHBG, TT and FT in each MetS score group. As the score of MetS (the number of MetS components) increases, the levels of SHBG and TT became lower. However, the level of FT showed no regular variation with score increasing. FT: free testosterone; SHBG: sex hormone–binding globulin; TT: total testosterone; MetS: metabolic syndrome.

Discussion

Whether SHBG is a causal factor of the MetS, or merely represents a marker for primary endocrine abnormalities...
leading to these metabolic alterations, remains unclear at present. We investigated the relationship among a variety of clinical and laboratory parameters characterizing the MetS. We focused on men above 45 years old in China to include men not only from the geriatric period, but also pre-elderly period. Most of the previous studies were on postmenopausal women or older men.

In this study, we found that the levels of TT and SHBG for the group with MetS were lower than those without MetS. As the scores of MetS increased, the levels of SHBG and TT became lower. Additionally, SHBG correlated with age, TT and most of the metabolic components, while BMI, TT, and TG remained associated with SHBG in the multivariate model. Because most patients with hypertension or hyperglycemia were undergoing treatment, their blood pressures and glucose levels were influenced by drugs. This might explain why FPG, SBP and DBP were not accepted as significant by multiple regression analysis. In our multivariate analyses, SHBG and HOMA-IR were associated with MetS, however, there was no association with TT or FT and MetS.

Our findings are, in part, consistent with previous studies that included different age periods. Li, et al.\textsuperscript{[17]} studied men aged 20 years who participated in the Third National Health and Nutrition Examination Survey and found low concentrations of SHBG were strongly associated with an increased likelihood of having MetS, independent of traditional cardiovascular risk factors and insulin resistance. No significant associations of calculated FT and MetS were detected after adjustment for all possible confounders. Rodriguez, et al.\textsuperscript{[18]} found lower TT and SHBG predicted a higher incidence of the MetS while SHBG levels exerted the greatest influence on the development of MetS in community-dwelling, healthy, adult men. Chubb, et al.\textsuperscript{[19]} conducted a cross-sectional study of 2502 community-dwelling men aged ≥ 70 years without known diabetes and found lower SHBG was more strongly associated with MetS than lower TT. However, FT showed no significant difference between MetS and non-MetS group, and was not independently as-

### Table 2. Correlations between SHBG and each variable (univariate analyses).

| Variable        | R    | P value  |
|-----------------|------|----------|
| Age (yrs)       | 0.444| < 0.001  |
| BMI (kg/m\(^2\))| -0.369| < 0.001  |
| Waist (cm)      | -0.208| < 0.001  |
| WHR             | -0.104| 0.002    |
| SBP (mmHg)      | 0.036| 0.283    |
| DBP (mmHg)      | -0.135| < 0.001  |
| FPG (mmol/L)    | -0.137| < 0.001  |
| TC (mmol/L)     | -0.008| 0.821    |
| HDL-C (mmol/L)  | 0.266| < 0.001  |
| LDL-C (mmol/L)  | -0.043| 0.205    |
| TG (mmol/L)     | -0.277| < 0.001  |
| Insulin (μIU/L)| -0.146| < 0.001  |
| HOMA-IR         | -0.162| < 0.001  |
| TT (nmol/L)     | 0.478| 0.002    |
| FT (pmol/L)     | -0.307| < 0.001  |

BMI: body mass index; DBP: diastolic blood pressure; FT: free testosterone; FPG: fasting plasma glucose; HDL-C: high density lipoprotein-cholesterol; HOMA-IR: homeostasis model assessment for insulin resistance; LDL-C: low density lipoprotein-cholesterol; SBP: systolic blood pressure; SHBG: sex hormone-binding globulin; TC: total cholesterol; TG: triglyceride; TT: total testosterone; WHR: waist hip rate.

### Table 3. Multivariable linear regression analysis of variables with SHBG level (SHBG level as dependent variable).

| Variable        | β (unstandardized) | 95% CI          | P    |
|-----------------|---------------------|-----------------|------|
| Adjusted \(\hat{r}^2\) | 0.330               |                 |      |
| BMI (kg/m\(^2\)) | -1.981              | (-2.579, -1.382) | < 0.001 |
| SBP (mmHg)      | NA                  |                 |      |
| DBP (mmHg)      | NA                  |                 |      |
| FPG (mmol/L)    | NA                  |                 |      |
| TC (mmol/L)     | -2.345              | (-4.338, -0.369) | 0.020 |
| HDL-C (mmol/L)  | 7.625               | (2.157, 13.093)  | 0.006 |
| LDL-C (mmol/L)  | NA                  |                 |      |
| TG (mmol/L)     | NA                  |                 |      |
| TT (nmol/L)     | 1.755               | (1.415, 2.095)   | < 0.001 |

BMI: body mass index; DBP: diastolic blood pressure; FPG: fasting plasma glucose; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; NA: not accepted as significant in stepwise multiple regression analysis; SBP: systolic blood pressure; TC: total cholesterol; TG: triglyceride; TT: total testosterone.
Table 4. Multivariate logistic regression analysis of factors associated with MetS (MetS as dependent variable).

| Models | Coefficient | Odds ratio | 95% CI       | P value |
|--------|-------------|------------|--------------|---------|
| 1      | Age         | -0.17      | 0.983        | (0.963, 1.004) | 0.119   |
|        | Smoke       | 0.209      | 1.232        | (0.681, 2.228) | 0.490   |
|        | Alcohol     | 0.189      | 1.208        | (0.329, 4.428) | 0.776   |
|        | HOMA-IR     | 0.701      | 2.016        | (1.535, 2.647) | < 0.001 |
|        | SHBG        | -0.014     | 0.986        | (0.975, 0.994) | 0.018   |
|        | TT          | -0.031     | 0.969        | (0.919, 1.022) | 0.248   |
| 2      | Age         | -0.022     | 0.979        | (0.961, 0.997) | 0.021   |
|        | Smoke       | 0.215      | 1.240        | (0.687, 2.239) | 0.476   |
|        | Alcohol     | 0.219      | 1.244        | (0.339, 4.569) | 0.742   |
|        | HOMA-IR     | 0.769      | 2.158        | (1.642, 2.837) | < 0.001 |
|        | TT          | -0.002     | 0.998        | (0.996, 1.001) | 0.248   |

FT: free testosterone; HOMA-IR: homeostasis model assessment for insulin resistance; MetS: metabolic syndrome; SHBG: sex hormone–binding globulin; TT: total testosterone.

associated with MetS in our study. By contrast, previous studies in which lower calculated FT were associated with MetS involved middle-aged men.[2,5] Other authors have found that FT was not associated with MetS in older non-diabetic men.[20,21] The inconformity may be attributed to race and age differences of subjects.

SHBG and testosterone play a role in the development of cardiovascular disease (CVD). Men with the MetS and/or diabetes (insulin-resistant patients) have a high incidence of erectile dysfunction (ED) which may be due, at least partially, to a decrease in testosterone level.[22] In addition, ED is a strong predictor for CVD.[23] The association between ED and CVD may be partially mediated by SHBG and testosterone.

There was no clear mechanism on how SHBG influences metabolic components. The peroxisome-proliferator receptors (PPARs) are nuclear fatty acid receptors that bind fatty acids and eicosanoids, and act as metabolic sensors and regulators of lipid and glucose homeostasis in many cell types, including the liver. All three PPAR family members (PPARα, PPARγ, and PPARδ) are present in the liver. PPARα potentiates fatty acid catabolism in the liver and is the molecular target of the lipid-lowering fibrates (e.g., fenofibrate and gemfibrozil). However, PPARγ is essential for adipocyte differentiation and mediates the activity of the insulin-sensitizing thiazolidinediones (e.g., rosiglitazone and pioglitazone), while PPARδ may be important in controlling TG levels by sensing very low-density lipoprotein.[24] The human SHBG promoter contains a PPAR-response element (PPAR-RE). Selva & Hammond[25] used human HepG2 hepatoblastoma cells in vitro and found PPARγ repressed human SHBG expression in liver cells, and that differences in PPARγ levels and activity contributed directly to variations in plasma SHBG levels. Gamage-Yared, et al.[26] speculated SHBG was regulated by adiponectin and that there was an inhibitory effect of testosterone on the adiponectin gene. Further studies are needed to fully elucidate these mechanisms.

Limitations of our study: it is a cross-sectional study and we only use a single blood sample for testing. Although single point plasma androgen measurements reflect fairly reliably the annual mean androgen level in healthy middle-aged and elderly men,[27] the scope of the study did not extend to repeat blood sampling, or to assays of other hormones, such as estrogen. The drug treatments of subjects were not considered in the statistic analysis.

In conclusion, lower SHBG is strongly associated with MetS in pre-elderly and elderly men in China, independently of traditional cardiovascular risk factors and surrogate measures of insulin resistance. SHBG may be an independent predictor of MetS, but the mechanism of how SHBG is involved in the MetS needs to be further studied.

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