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ORIGINAL ARTICLE

Follicle stimulating hormone, its novel association with sex hormone binding globulin in men and postmenopausal women

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Abstract
Purpose Follicle stimulating hormone plays direct roles in a variety of nongonadal tissues and sex hormone binding globulin is becoming the convergence of the crosstalk among metabolic diseases. However, no studies have explored the association between follicle stimulating hormone and sex hormone binding globulin. We aimed to study this association among men and women.
Methods SPECT-China is a population-based study conducted since 2014. This study included 4206 men and 2842 postmenopausal women. Collected serum was assayed for gonadotropins, sex hormone binding globulin, sex hormones etc. Regression analyses were performed to assess the relationship between sex hormone binding globulin and follicle stimulating hormone and other variables including metabolic factors, thyroid function and sex hormones. Treatment with follicle stimulating hormone at different concentrations of 0, 5, 50 and 100 IU/L for 24 h was performed in HepG2 cells.
Results In Spearman correlation, sex hormone binding globulin was significantly correlated with FSH, triglycerides, thyroxins, body mass index and blood pressure in men and postmenopausal women (all $P < 0.05$). In regression analyses, follicle stimulating hormone was a significant predictor of sex hormone binding globulin in men and postmenopausal women ($P < 0.05$), independent of above variables. Follicle stimulating hormone induced sex hormone binding globulin expression in a dose-dependent fashion in HepG2 cells.
Conclusion Serum follicle stimulating hormone levels were positively associated with circulating sex hormone binding globulin levels in men and postmenopausal women. This association is independent of age, insulin resistance, hepatic function, lipid profile, thyroid function, adiposity, blood pressure, and endogenous sex hormones.

Keywords Follicle stimulating hormone · Sex hormone binding globulin · Men · Postmenopausal women

Introduction

Follicle-stimulating hormone (FSH), one of the gonadotropins, is necessary for follicular and sperm maturation and regulation of estrogen synthesis. However, besides in above reproductive tissues, FSH receptor is found to be expressed in non-reproductive tissues such as liver [1], adipose tissue [2], osteoclasts [3], and blood vessel [4]. Thus, recent studies including ours found that FSH was associated with obesity [5], fatty liver [6], diabetes [7], and metabolic syndrome [8]. However, we still know very little about the role of FSH in metabolic disorders.

Sex hormone-binding globulin (SHBG) is a glycoprotein, produced mostly by the liver and released into the bloodstream [9]. Testosterone (T) and estradiol (E2) in the bloodstream bound mostly to SHBG, and a lesser extent to

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serum albumin and corticosteroid-binding globulin. Thus, the level of SHBG regulates the bioavailability of sex hormones [10]. During recent decades, however, emerging evidence indicates that serum SHBG could be a biomarker or predictor of obesity [11], diabetes [12, 13], metabolic syndrome [13], and cardiovascular diseases [14]. Furthermore, SHBG could be regulated by liver fat [15], thyroid hormones [16], proinflammatory cytokines and anti-inflammatory cytokines [17, 18], carbohydrates [19] and olive oil [20] through affecting hepatocyte nuclear factor 4 alpha (HNF-4α) or peroxisome proliferator-activated receptor gamma (PPARγ) [9].

Considering FSH receptor expressed in liver and the association of both FSH and SHBG with metabolic diseases mentioned above, whether they are independently related has not been studied. Exploration of this relationship may help reveal the function of gonadotropins on non-reproductive tissues and metabolic diseases.

The data used were from a large investigation, the Survey on Prevalence in East China for Metabolic Diseases and Risk Factors (SPECT-China), which was performed in 2014–2015. Based on this study, we aimed to explore whether FSH and SHBG was associated and whether its association was independent of potential predictors of SHBG and whether FSH could regulate SHBG expression in HepG2 cells.

Research design and methods

Participants

The data are from the participants of SPECT-China, a cross-sectional survey in East China (ChiCTR-ECS-14005052, www.chictr.org.cn). Recruitment and enrollment have been described in detail [21–23]. Chinese citizens ≥ 18 years old who had lived in their current area for ≥6 months were selected. We also excluded subjected with severe communication problems, acute illness or who were unwilling to participate. From 2014 January to 2015 December, 10,441 subjects who were 18–93 years old were recruited in the SPECT-China study from 22 sites in Shanghai, Zhejiang, Jiangsu, Anhui and Jiangxi Province. The study protocol was approved by the Ethics Committee of Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients included in the study.

There were 3226 postmenopausal women. Postmenopausal women were defined as subjects who reported that they had stopped menstruating for a minimum of 12 months ($n = 1431$), who were 55 years of age or older ($n = 2872$), or who had previous hysterectomy or oophorectomy ($n = 139$). Exclusion criteria included missing values of SHBG or FSH ($n = 244$), and FSH < 25.0 IU/L (according to the 2011 Stages of Reproductive Aging Workshop + 10 recommendation, late perimenopausal state is characterized as FSH level ≥ 25 IU/L) ($n = 140$) [24]. Finally, 2842 postmenopausal women were included in this study. There were also 4309 men recruited. Men were excluded who had missing values of SHBG or FSH ($n = 103$). Thus, 4206 men were in included in the final study (Fig. 1).

Measurements

Interview and collection of biological specimens at each site was undertaken with a single assessment protocol. Blood

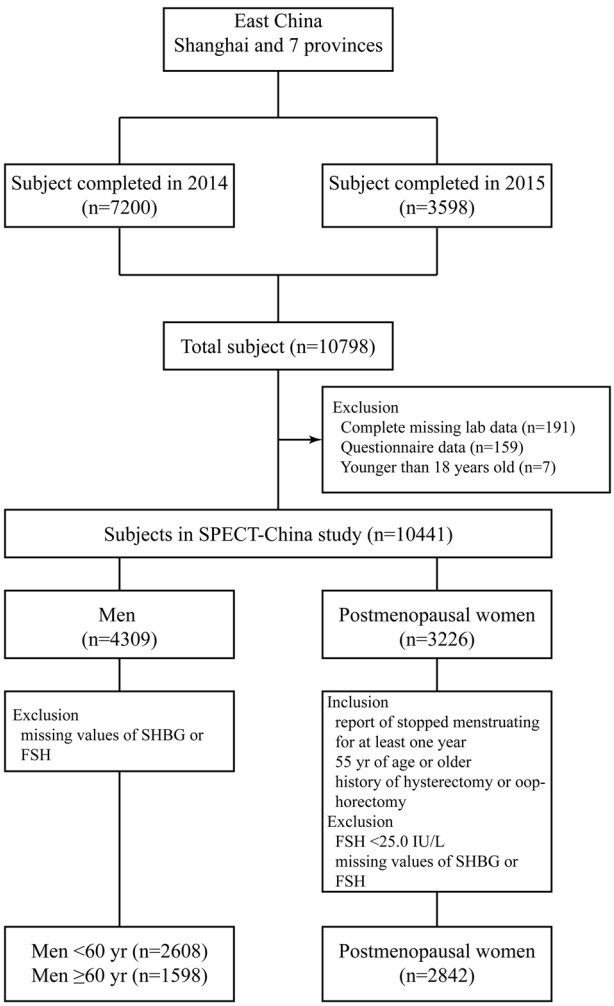

Fig. 1 Flowchart of the participants in this study selected from SPECT-China

samples were obtained between 7:00 a.m. and 10:00 a.m. after fasting for at least 8 h. Blood was refrigerated immediately after phlebotomy, and in 2–4 h it was shipped to a central laboratory, which was certified by the College of American Pathologists. After immediate centrifugation, the blood, serum, and plasma were frozen in a central laboratory. Serum thyroid function, 25(OH)-vitamin D (Siemens ADVIA Centaur XP, Germany), total T, E2, luteinizing hormone (LH), FSH (Siemens, immulite 2000, Erlangen, Germany) and SHBG levels (Roche Cobas E601, Basel, Switzerland) were detected using a chemiluminescence assay. Glycated hemoglobin (HbA1c) was measured by high-performance liquid chromatography (MQ-2000PT, Medconn, Shanghai, China). Plasma glucose, serum alanine aminotransferase (ALT), triglycerides, total cholesterol were measured by a Beckman Coulter AU 680 (Brea, USA). Samples with values below the minimal detectable limit were given a value midway between zero and the minimal detectable limit for the analyses [25]. The inter-assay and intra-assay coefficients of variation were 6.6 and 5.7% for total T, 7.5 and 6.2% for E2, 4.5 and 3.8% for FSH, and 7.0% for SHBG.

Weight (kilograms) and height (centimeters) were measured using a stadiometer and a vertical ruler when subjects wore light clothing without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured at a level midway between the lowest rib and the iliac crest. Blood pressure was measured using standard methods as described previously [26]. Current smoking was defined as having smoked at least 100 cigarettes in one's lifetime and currently smoking cigarettes [26]. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting serum insulin (mIU/L) × FPG (mmol/L)/22.5. Free androgen index was calculated as 100 × (total T/SHBG).

Quantitative real-time PCR analysis of SHBG treated by FSH in HepG2 cells

HepG2 cells were cultured in 12-well plates at a density of 1×10^4 and in the regular medium containing Dulbecco's modified eagle's medium (GIBCO, USA), 15% fetal calf serum (FCS; GIBCO, USA), 1% penicillin-streptomycin (P-S; Sigma, USA) and 1% beta-glutamine (Sigma, USA). When cells reached to approximately 80% confluence, the fresh medium was changed and then treated with FSH at different concentrations of 0, 5, 50 and 100 IU/L for 24 h. 0.9% NaCl solution was used as a vehicle control. At the end of the treatment, HepG2 cells were used for real-time PCR to determine the effect of FSH on the expression of SHBG. The mRNA expressions of SHBG were normalized to that of β-actin. The primers for SHBG detection were as follows: forward 5-GTTGCT ACTACT GCGTCA CAC-3, reverse 5-GCCATC TCCCAT CATCCA GCCG-3. The primers for β-actin detection were as follows: forward 5-AAGGTG ACAGCA GTCGGT T-3, reverse 5-TGTGTG GACTTG GGAGAG G-3.

Statistical analysis

Data analyses were performed using IBM SPSS Statistics, Version 22 (IBM Corporation, Armonk, NY, USA). All analyses were two-sided. A P-value $< .05$ indicated significance. Continuous variables were expressed as the mean ± standard deviation (SD) or median (interquartile range) and categorical variables as a percentage (%), respectively. To test for differences of characteristics among men under 60, men over 60 and postmenopausal women, Kruskal–Wallis and one-way ANOVA test was used for non-normally and normally distributed continuous data, respectively, and Pearson χ^2 tests was used for categorical variables.

Spearman correlation analyses were used to observe the correlation between SHBG and each potentially associated factor including fasting plasma glucose (FPG), HbA1c, insulin, HOMA-IR, ALT, lipid profile, thyroid hormones, 25(OH)-vitamin D, BMI, waist circumference, blood pressure, total T, E2, FSH, and LH.

The associations between SHBG and multiple factors were assessed by multiple linear regression. Statistical and biologic factors were considered when selecting which variables to include in adjusted models. Age, HOMA-IR, ALT, triglycerides, total triiodothyronine, total thyroxine, BMI, systolic blood pressure, total T, E2, FSH, and LH were considered as independent variables in the model. SHBG were transformed using the natural logarithm. The stepwise procedure was used. Data were expressed as standardized coefficients (standard error).

Stratified analyses were conducted to determine the association between SHBG and FSH in different glucose, weight and smoking statuses. The covariates were the same as in the above multiple linear regression.

Finally, one-way ANOVA and least significant difference post-hoc tests were used to evaluate the statistical significance of the differences between different FSH-treating groups of HepG2 cells.

Results

Characteristics of the study population

An overview of the study characteristics, including laboratory results, anthropometric measures as well as sex steroids and SHBG, can be found in Table 1. Of the study

Table 1 Characteristics of men (<60 years and ≥60 years) and postmenopausal women

	Men		Postmenopausal women
	<60 years	≥60 years	
N	2608	1598	2842
Age, year	46 ± 9	67 ± 6*	63 ± 7*,**
Laboratory results			
Fasting blood glucose, mmol/L	5.59 ± 1.47	5.91 ± 1.58*	5.83 ± 1.56*
HbA1c, %	5.5 ± 1.0	5.8 ± 1.1*	5.7 ± 1.0*
Insulin, pmol/L#	31.7(21.8–46.9)	27.0(17.9–40.1)*	35.2(25.5–51.0)*,**
HOMA-IR	1.09(0.73–1.69)	0.98(0.65–1.53)*	1.26(0.89–1.89)*,**
Triglycerides, mmol/L	1.54(1.07–2.31)	1.25(0.92–1.83)*	1.45(1.08–2.02)*,**
Alanine aminotransferase, IU/L	23(17–33)	19(15–25)*	17(13–23)*,**
Total cholesterol, mmol/L	5.15 ± 1.05	5.14 ± 1.04	5.49 ± 1.07*,**
Total triiodothyronine, nmol/L	1.79 ± 0.53	1.78 ± 0.44	1.79 ± 0.39
Total thyroxine, nmol/L	112.4 ± 21.6	116.2 ± 24.6*	118.5 ± 21.5*,**
25(OH)-vitamin D, nmol/L	42.6 ± 11.3	48.5 ± 13.7*	41.7 ± 11.4*,**
Anthropometric measures			
Body mass index, kg/m^2	25.0 ± 3.3	24.6 ± 3.4*	25.0 ± 3.6**
Waist circumference, cm	83.9 ± 9.6	85.0 ± 9.7*	81.7 ± 9.7*,**
Systolic blood pressure, mmHg	130.0 ± 19.1	142.4 ± 21.1*	139.8 ± 21.8*,**
Diastolic blood pressure, mmHg	82.0 ± 13.0	82.6 ± 13.1*	80.4 ± 12.8*,**
Current smoking, %	49.6	45.8	3.5*,**
Lipid lowering drugs, %	0.7	1.7*	2.5*
Glucose lowering drugs, %	3.9	5.4	6.9*
Blood pressure lowering drugs, %	9.7	18.7*	18.5*
Sex hormones			
Total T, nmol/L	15.15(12.30–18.70)	17.10(13.14–22.01)*	0.35(0.35–0.83)*,**
E2, pmol/L#	95.0(42.9–126.0)	119.0(87.9–163.3*	36.7(33.8–86.4)*,**
FSH, IU/L	5.8(4.1–8.1)	9.9(7.2–14.3)*	60.7(46.7–77.5)*,**
LH, IU/L	4.2(3.1–5.9)	6.5(4.7–9.1)*	24.6(18.5–32.6)*,**
SHBG, nmol/L	34.5(25.0–47.5)	56.2(40.3–76.4)*	61.3(42.8–85.7)*,**
Free androgen index	43.6(34.4–54.1)	31.0(25.4–37.7)*	0.8(0.5–1.5)*,**

Data were summarized as median (interquartile range) or mean ± standard deviation for continuous variables or as number with a proportion for categorical variables

E2 estradiol, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *HbA1c* glycated hemoglobin, *HOMA-IR* homeostasis model assessment of insulin resistance, *SHBG*, sex hormone binding globulin, *T* testosterone

*vs. men <60 years, $P < 0.05$

**vs. men ≥60 years, $P < 0.05$

#insulin(pmol/L) ÷ 6.945 = insulin(μIU/mL); E2(pmol/L) ÷ 3.67 = E2(pg/mL)

population, all 7048 subjects were Asian population and 40.3% were postmenopausal women. The mean age of the study population was 54 years (SD 13) in men and 63 years (SD 7) in postmenopausal women. SHBG levels in men < 60 years, men ≥ 60 years and postmenopausal women were 34.5(25.0–47.5), 56.2(40.3–76.4), and 61.3(42.8–85.7), respectively. FSH levels were 5.8(4.1–8.1), 9.9(7.2–14.3), and 60.7(46.7–77.5), respectively in these groups. FSH and SHBG levels were significantly different among the three groups (all $P < 0.05$).

Correlation between SHBG and potential associated factors

Table 2 summarizes the results of the Spearman correlation analysis between SHBG and potential associated factors in all subjects. No matter in men or postmenopausal women, SHBG was negatively or positively correlated with FPG, HOMA-IR, ALT, triglycerides, thyroxins, BMI, waist circumference and blood pressure (all $P < 0.05$). Regarding the sex hormones, SHBG was significantly correlated with

Table 2 Spearman correlation between SHBG and potential associated factors

	Men		Postmenopausal women
	<60 years	≥60 years	
Age	0.324*	0.280*	0.181*
Laboratory results			
Fasting blood glucose	−0.081*	−0.216*	−0.175*
HbA1c	0.014	−0.151*	−0.243*
Insulin	−0.332*	−0.358*	−0.410*
HOMA-IR	−0.339*	−0.386*	−0.429*
Alanine aminotransferase	−0.229*	−0.189*	−0.200*
Triglycerides	−0.351*	−0.399*	−0.362*
Total cholesterol	−0.008	−0.037	0.005
Total triiodothyronine	0.143*	0.119*	0.010
Total thyroxine	0.148*	0.200*	0.104*
25(OH)-vitamin D	0.082*	0.139*	0.047*
Anthropometric measures			
Body mass index	−0.338*	−0.451*	−0.441*
Waist circumference	−0.297*	−0.336*	−0.357*
Systolic blood pressure	−0.061*	−0.054*	−0.126*
Diastolic blood pressure	−0.111*	−0.118*	−0.170*
Sex hormones			
Total T	0.623*	0.709*	−0.096*
E2	0.159*	0.226*	0.018
FSH, IU/L	0.208*	0.196*	0.219*
LH, IU/L	0.237*	0.273*	0.129*

Data are Spearman correlation coefficients

E2 estradiol, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *HbA1c* glycated hemoglobin, *HOMA-IR* homeostasis model assessment of insulin resistance, *SHBG* sex hormone binding globulin, *T* testosterone

*indicates $P < 0.05$

FSH, LH, and total T in both men and postmenopausal women.

Association between SHBG and potential predictors

Table 3 summarizes the results of the associations between lnSHBG and potential predictors. To consider the impact of multiple correlated factors on SHBG levels simultaneously, all sex hormones and other covariates (age, HOMA-IR, ALT, triglycerides, total triiodothyronine, BMI, and systolic blood pressure) were considered together in linear regression models for SHBG. In the final linear regression models, FSH remained as a predictor of lnSHBG in men and postmenopausal women with β coefficients varying from 0.112–0.159 ($P < 0.05$). Interestingly, LH did not enter the final model. Regarding other metabolic and hormonal factors, lnSHBG was significantly associated with age, triglycerides, total triiodothyronine, BMI, and total T in men (all $P < 0.05$). In postmenopausal women, additional variables including HOMA-IR, ALT, and systolic blood pressure also entered the regression model (all $P < 0.05$).

Because previous studies found FSH was probably associated with glucose, weight, and smoking status [5, 7, 27], we conducted stratified analyses to determine the association between FSH and lnSHBG in subgroups of the strata variables in men and post-menopausal women (Table 4). According to the stratified analyses, the associations between FSH and lnSHBG were significant in both glucose strata (diabetes and non-diabetes), both BMI strata (<25 and ≥25 kg/m^2) and both current smoking strata (yes and no) in all men and post-menopausal women. The sample of smoking women is too small to perform multiple regression analyses.

FSH decreased SHBG expression in HepG2

To prove if FSH regulated directly SHBG production, treatment with FSH at different concentrations of 0, 5, 50, and 100 IU/L for 24 h was performed in HepG2 cells

Table 3 Association between lnSHBG (dependent variable) and potential predictors (independent variables) by linear regression

	Men		Postmenopausal women
	<60 years	≥60 years	
Age	0.288 (0.001)	0.149 (0.001)	0.216 (0.001)
HOMA-IR	/	/	−0.083 (0.003)
Alanine aminotransferase	/	/	−0.084 (0.001)
Triglycerides	−0.079 (0.003)	−0.099 (0.006)	−0.178 (0.007)
Total triiodothyronine	0.062 (0.013)	0.047 (0.018)	0.143 (0.021)
Body mass index	−0.215 (0.002)	−0.203 (0.002)	−0.338 (0.003)
Systolic blood pressure	/	/	−0.061 (0.000)
Total T	0.504 (0.001)	0.577 (0.001)	/
E2	0.031 (0.000)	/	0.052 (0.000)
FSH	0.112 (0.002)	0.159 (0.001)	0.119 (0.000)
LH	/	/	/

Data are expressed as standardized coefficients (standard error). Multiple linear regression was performed. SHBG were transformed using the natural logarithm. The stepwise procedure was used. The variables entering the model show significant association ($P < 0.05$)

E2 estradiol, *FSH* follicle-stimulating hormone, *HOMA-IR* homeostasis model assessment of insulin resistance, *LH* luteinizing hormone, *SHBG* sex hormone binding globulin, *T* testosterone

"/" represents the variable does not enter the model

Table 4 Subgroup analyses of the association between lnSHBG and FSH

	lnSHBG (dependent variable)	
FSH (independent variable)	Men	Postmenopausal women
Glucose status		
Non-diabetes	0.125 (0.001)*	0.098 (0.000)*
Diabetes	0.097 (0.002)*	0.125 (0.001)*
Weight status		
BMI < 25	0.154 (0.001)*	0.112 (0.000)*
BMI ≥ 25	0.101 (0.001)*	0.197 (0.001)*
Current smoking		
Yes	0.107 (0.001)*	N/A
No	0.118 (0.001)*	0.126 (0.000)*

Data are expressed as standardized coefficients (standard error).

SHBG sex hormone binding globulin

*represents $P < 0.05$. N/A means the subgroup sample is too small to perform multiple regression analyses. Stepwise linear regression was performed. SHBG were transformed using the natural logarithm. The model was adjusted for age, HOMA-IR, alanine aminotransferase, triglycerides, total triiodothyronine, body mass index, systolic blood pressure, total testosterone, estradiol, luteinizing hormone

(Fig. 2). We found FSH induced SHBG expression in a dose-dependent fashion in HepG2 cells. Treatment with 50 IU/L FSH induced significantly higher SHBG expression than 5 IU/L FSH ($P < 0.05$). Cells treated with 100 IU/L FSH also had higher SHBG expression than the other three groups (all $P < 0.05$).

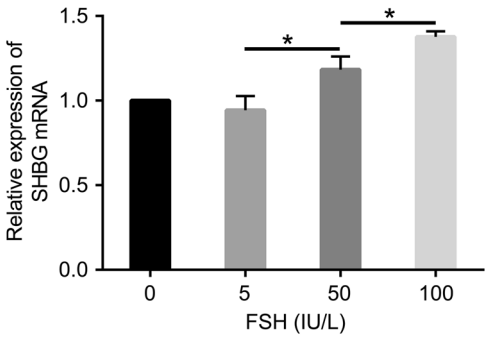

Fig. 2 FSH dose-dependent suppression of SHBG expression. Concentration-dependent effects of FSH (0–100 IU/L) on the expression of SHBG mRNA in HepG2 cells after 24 h of FSH treatment. The mRNA expressions of SHBG were normalized to that of β-actin. *indicates $P < 0.05$

Discussion

Overall, this study explored the associations between SHBG as the dependent variable and its multiple potential predictors, in men and postmenopausal women. For the first time, our study revealed the positive association of FSH with SHBG in men and postmenopausal women, independent of age, insulin resistance, hepatic function, lipid profile, thyroid function, adiposity, blood pressure, and endogenous sex hormones. Moreover, we found FSH could induce SHBG expression in a dose-dependent fashion in vitro.

This study contained multiple traditional SHBG influencers. Total and visceral adiposity was strongly associated

with low SHBG in previous studies [28, 29], which had the highest standardized coefficient among the metabolic parameters in our results. Moreover, subjects with severe obesity had an increase of plasma SHBG levels after bariatric surgery, which showed that SHBG was closely correlated with weight loss [30]. Thyroid hormones could also impact SHBG levels, probably through altering hepatic SHBG production [16]. Thus, patients with hyperthyroidism had markedly increased SHBG levels [31] and in subjects with severe hypothyroidism, low SHBG levels increased significantly to physiologic values after thyroid state correction [32]. Other factors such as age, insulin resistance state, blood pressure, and lipid profile may also be associated with SHBG levels, which was determined in this and previous studies [33].

With role of FSH found beyond reproductive system [1–4], no study has ever explored its association with SHBG as far as we know. This study indicated FSH was positively associated with SHBG levels in men and postmenopausal women. Weight, diabetes, and smoking status did not change this association. Previous studies found higher circulating FSH was related with lower BMI and prevalence of diabetes, fatty liver, and metabolic syndrome [5–8], most of which were also negatively associated with SHBG, thus the observed association may be partly mediated by the above metabolic diseases. However, because independent association was observed, we hypothesized that circulating FSH might have direct relation with SHBG synthesis and secretion. To further prove this hypothesis, we did an in vitro study using HepG2 cells treated with FSH at different concentrations, and a dose-dependent manner of SHBG expression was found. What is the possible underlying mechanism? Previous study found the FSH receptor protein was expressed in cell membranes of human hepatic tissues [1]. In granulosa cells, FSH could regulate the functions of PPARγ through proteinkinase A, extracellular signal-regulated kinase 1/2, and p38 mitogen-activated protein kinase signaling pathways [34]. The human SHBG gene has several transcription binding sites in a TATA-less proximal promoter that PPARγ can bind to regulate SHBG gene expression [9, 10, 20]. Thus, the hypothesis whether circulating FSH could induce SHBG levels through regulation of PPARγ in liver needs further explorations (Fig. 3).

We found FSH may regulate directly hepatic SHBG production. However, we also found that the young and middle-aged men had ten times less FSH levels than postmenopausal women but roughly only half plasma SHBG. The fact is SHBG is regulated by various factors other than FSH [35]. Table 3 showed the main predictors of SHBG were total T, age, BMI, and FSH in men. Men over 60 had higher total T and FSH but lower BMI than men under 60. Moreover, it is known that SHGB concentrations tend to increase with aging that is associated with lower concentrations of free T in men [36]. The finding in Table 2 that the positive correlation between SHGB and total T increased with aging may also indicate that there is more T combined with SHBG and less circulating free T when men get older [37]. Although there were no data of measured free testosterone, free androgen index was calculated and men under 60 had lower free androgen index. These may partly explain why men over 60 had higher SHBG than men under 60 in our study. And also because of multiple regulators of SHBG other than FSH, men had 6–10 times less FSH plasma levels than postmenopausal women but just 8–50% less SHBG levels. Interestingly, postmenopausal women have a marked reduction of estradiol levels that is the principal SHBG stimulatory hormones in women [35] but why they still had very high SHBG. SHBG level is the result of a balanced effect of stimulatory and inhibitory factors. We suspected that FSH partly replaced the role of estradiol as the SHBG stimulatory factor in postmenopausal women. However, what's the usage of maintaining SHBG at such a high level in postmenopausal women needs further investigation.

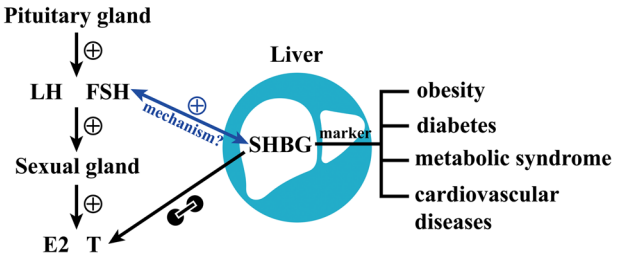

Fig. 3 FSH is necessary for follicular and sperm maturation and regulation of estrogen synthesis. Moreover, serum SHBG could be a biomarker of metabolic and cardiovascular diseases. For the first time, this study indicated FSH was positively associated with SHBG in men and postmenopausal women, independent of multiple variables and FSH could induce SHBG expression in liver cells, though the mechanism is unknown. Weight, diabetes, and smoking status also did not change this association

Our study had some strengths. An important one is having a large population-based sample with detailed information on the potential SHBG-associated factors for each individual. This study is also the first to focus on the association of SHBG with gonadotropins, instead of merely the traditional factors such as adiposity, insulin resistance and thyroid function. Our data source is from a general population as opposed to a clinic-based population, so the results may be more reflective.

However, our study limitations also need attention. Firstly, the temporality of the observed associations cannot be addressed because of the cross-sectional design. Thus, we cannot draw the causal relationship between SHBG and FSH. However, the finding in vitro study indicated FSH

might be the causal side. Secondly, some of postmenopausal women were defined based on the age proxy as previous studies [38, 39]. Whereas in China, the overall median age at natural menopause is 50 years and at the age of 55, about 97% of women are postmenopausal [40]. Finally, we have not measured the albumin, so the calculated free testosterone could not be obtained.

In conclusion, serum FSH levels were positively associated with circulating SHBG levels in men and postmenopausal women regardless of glycemic, weight, and smoking status. This association was independent of age, insulin resistance, hepatic function, lipid profile, thyroid function, adiposity, blood pressure, and endogenous sex hormones. FSH could induce SHBG expression in a dose-dependent fashion in vitro. Given FSH playing direct roles in a variety of nongonadal tissues [11–14] and SHBG being the convergence of the crosstalk among obesity, diabetes, and insulin resistance [1], further explorations on whether FSH could impact SHBG levels through regulating PPARγ or other factors in liver may have important implications for new knowledge on the pathophysiology of metabolic disturbances.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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