Aging, cardiovascular risk and SHBG levels in men and women from the general population

Elif Aribas, MD¹; Maryam Kavousi, PhD¹; Joop S.E. Laven, PhD²; M. Arfan Ikram, PhD¹; Jeanine E. Roeters van Lennep, PhD³

¹Department of Epidemiology, Erasmus Medical Center, University Medical Center, Rotterdam, the Netherlands

²Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, the Netherlands

³Department of Internal Medicine, Erasmus Medical Center, University Medical Center, Rotterdam, the Netherlands

Corresponding author: Dr. Jeanine E. Roeters van Lennep, MD, PhD

Department of Internal Medicine, Erasmus Medical Center, Rotterdam 3015 GD, the Netherlands. Email: j.roetersvanlennep@erasmusmc.nl. Telephone number: (+31)107035193, fax number: (+31)107033639.
Abstract

Aims: Prior studies have reported inconsistent results for the association between sex hormone-binding globulin (SHBG) and cardiovascular disease among men and women. Although it is suggested that SHBG levels change with aging, the exact trend of SHBG across age and cardiovascular risk, and the underlying mechanisms of these changes remain to be elucidated.

Methods: Using data of 3264 men and women from a large population-based cohort study, we visualized the distribution of serum SHBG levels across age. Second, we computed a cardiovascular risk factor sum score and investigated the mean SHBG levels across categories of the risk factor sum score and stratified per age-category. Next, linear regression models were used to investigate the associations between serum SHBG levels and age and potential regulators of SHBG, including body mass index (BMI), fasting insulin, sex steroids, thyroxine and triglycerides.

Results: Among men, a linear increase in SHBG levels with age and among women a U-shaped pattern was observed. Participants with larger number of cardiovascular risk factors had lower SHBG levels. When stratified by age, older participants had higher SHBG levels. A multivariate model including total testosterone and triglyceride levels in men and total testosterone, triglycerides, BMI, and fasting insulin in women explained, respectively, 46.2% and 31.8% of the variance in SHBG levels.
Conclusion: We observed a clear sex-specific pattern for SHBG levels with age. Our findings highlight the importance of taking into account the age-related changes in SHBG levels to avoid controversial results in the assessment of the cardiovascular risk associated with SHBG.

Key words: SHBG, aging, cardiovascular risk, epidemiology
Background

Population-based and clinical studies have provided robust evidence regarding sex-specific variations in cardiovascular risk, in particular during the reproductive period (1,2). This has led to an interest in examining the contribution of sex steroids to cardiovascular risk.

Sex hormone-binding globulin (SHBG) is a protein produced by the liver that binds to and transports the androgens and oestrogens in blood and regulates their bioavailability. As such, the possible role of SHBG in cardiometabolic disorders has increasingly gained attention. Various studies, however, have reported inconsistent results for the association between SHBG with cardiovascular disease (CVD). While numerous studies have shown an inverse association between SHBG levels and cardiovascular risk (3,4), others have reported a positive association between increase in SHBG levels and increased CVD risk (5-7). The underlying mechanism behind these controversial results remains largely unknown.

Prior studies have suggested age-related changes in SHBG levels (8-10). Cardiovascular risk also shows a strong age-related pattern, particularly in women starting during the perimenopausal period (2). From the perimenopausal period onwards, a gradual increase in the rate of ischemic events (together with a rise in traditional cardiovascular risk factors) is observed, reaching significant levels 5-10 years after menopause (11). However, robust epidemiological data on the age-related change of SHBG, in both men and women, are lacking. So far, several potential regulators of SHBG have been suggested, including dietary composition, BMI, fasting insulin, sex steroids, thyroxine (12) and triglycerides (12-16). However, the contribution of these potential regulators to the variance in SHBG levels remains unknown as well. We hypothesized that age-related changes in SHBG and cardiovascular disease risk may contribute to the reported controversial results between
SHBG and CVD risk in the literature.

We, therefore, aimed to explore the distribution of serum SHBG levels across age and cardiovascular risk among middle-aged and elderly men and women from general population. Furthermore, we examined the association between SHBG and the main physiological regulators of SHBG levels.

**Methods**

*Study population*

This cross-sectional study was embedded within the Rotterdam Study, an ongoing prospective, population-based cohort study among individuals of 55 years and older living in the Ommoord district of Rotterdam, The Netherlands. The rationale and study design have been described in detail elsewhere (17). Nearly all participants (> 97%) were of Caucasian descent. The baseline examination of the Rotterdam Study included 7983 individuals between 1989-1993 (Rotterdam Study-I) and has been extended twice (3011 individuals, Rotterdam Study-II in 2000 and 3932 individuals, Rotterdam Study-III in 2006) to include participants who were 45 years or older or who had moved to the study research area. The overall response for all three Rotterdam Study cycles at study entry was 72.0% (14926 of 20744). The Rotterdam Study has been approved by the Medical Ethical Review Board of Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare, and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl) and into the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/network/primary/en/) under shared catalog number NTR6831.

All participants provided written informed consent to participate in the study and to have
their information obtained from their treating physicians.

This study included participants from the first visit of the extended cohort (Rotterdam Study-III-1, 2006-2008) from whom measurements of SHBG was available (N=3449). Among these participants, 115 with current hormone medication use were excluded. Furthermore, 70 participants aged > 79 years were excluded, as CVD risk algorithms are mainly validated up to the age of 79 years (18). In total, 1481 men and 1783 women were included in this study. (Figure 1)

**Measurements of SHBG and sex steroids**

All blood samples were drawn in the morning (≤ 11 am) and were fasting. SHBG levels were measured with the Immulite platform (Diagnostics Products Corporation), total estradiol levels with COBAS 8000 Modular Analyzer (Roche Diagnostics GmbH) and serum levels of total testosterone with liquid chromatography-tandem mass spectrometry (LC-MS/MS). The corresponding interassay coefficients of variations for SHBG, total estradiol and total testosterone are < 5%, < 7% and < 5%. The minimum detection limit for total estradiol was 18.35 pmol/L. Undetectable total estradiol was scored as 18.35.

Storage: Serum was immediately frozen (within 2 hours) at -150°C and afterwards stored at -80°C.

**Assessment of covariates**

At study entry, an interview was performed to obtain information on current health status, medical history, medication use, menopausal status and smoking. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m²). Smoking status was assessed asking participants whether they were current smokers of cigarettes, cigars, or pipe. Blood pressure was measured in the sitting position on the right upper arm with a random-zero
sphygmomanometer. All biochemical parameters were assessed in fasting serum. Total cholesterol, high-density lipoprotein cholesterol (HDL) and triglycerides were measured on the COBAS 8000 Modular Analyzer (Roche Diagnostics GmbH). LDL was calculated with a Roche Modular P800. The corresponding inter assay coefficients of variations are the following: lipids < 4%. Fasting insulin was determined by metric assay (Biosource Diagnostics, Camarillo, CA). Thyroxine was measured using the electrochemiluminescence immunoassay “ECLIA” (Roche Diagnostics GmbH). Prevalent diabetes type 2 was based on using antidiabetic medication, fasting serum glucose levels > 7.0 mmol/L or, in case a non-fasting status, random serum glucose level ≥ 11.1 mmol/L. Prevalent coronary heart disease (CHD) was defined as a history of myocardial infarction or coronary revascularization including percutaneous coronary intervention or coronary artery bypass grafting. Postmenopausal women were defined as women who reported absence of menstrual periods for 12 months. The retrospective data on self-reported use of antihypertensive was collected by a questionnaire during the home interview. Current hormone use was defined as use of hormone medication within 90 days before the date of blood collection. Medication with Anatomical Therapeutic Chemical-code g03 were included.

**Statistical analysis**

Characteristics of men and women were presented as mean (standard deviation – SD) or median (25th–75th quartile) for continuous variables and number (percentage) for categorical variables.

All analysis was performed separately for men and women. First, we visualized the distribution of serum SHBG levels across age.

Next, we investigated the mean serum SHBG levels across cardiovascular risk. As the
currently available algorithms to calculate cardiovascular risk are highly age-driven, we calculated a cardiovascular risk factor sum score as an alternative. We computed a cardiovascular risk factor sum score using the following variables: BMI, hypertension (defined as systolic blood pressure $\geq$ 140 mmHg or diastolic blood pressure $\geq$ 90 mmHg or use of blood pressure lowering medication), current smoking, prevalent diabetes and dyslipidemia (defined as LDL $>$ 4.0 mmol/L or use of lipid lowering medication). We then investigated the mean SHBG levels across categories of the cardiovascular risk factor sum score (categorized as 0-1 risk factors, 2 risk factors and $\geq$ 3 risk factors), stratified per age-category (45-55 years, 55-60 years, 60-65 years and 65-79 years). The non-parametric Kruskall-Wallis test was used to indicate statistical significance of the differences in SHBG levels across different categories of the cardiovascular risk factor sum score for each age category.

To evaluate the validity of the use of the risk factor sum score, we also investigated the proportion of participants with prevalent CVD across different age and risk factor sum score categories.

Moreover, as an additional analysis we used cox-regression models to explore the association between SHBG levels and incident clinical CVD outcomes in this relatively young cohort. Models were adjusted for: age, diastolic and systolic blood pressure, blood pressure lowering medication, total cholesterol, HDL, lipid lowering medication, waist-to-hip ratio, prevalent diabetes, CRP, smoking and years since menopause (in women).

Furthermore, linear regression models were used to investigate the associations between serum SHBG levels, age and potential regulators of SHBG suggested in current literature, including BMI, fasting insulin, sex steroids, thyroxine and triglycerides. For this analysis SHBG, fasting insulin, total estradiol, total testosterone and triglycerides were natural-log
transformed to approximate normal distribution. We first investigated the univariate models, next age-adjusted models and finally multi-variate adjusted models (including all investigated determinants). To investigate and adjust for non-linear relationship between age and SHBG, age was modeled using linear, quadratic terms and two or three (natural) splines. The model with the best fit was chosen using lowest Akaike information criterion (AIC). Determinants with statistically significant association in the multi-variate adjusted model were selected for the final model. A Bonferroni corrected P-value of 0.007 (corrected for the number of regulators: 0.05/7) was used to take into account multiple testing and select the regulators with statistically significant associations.

Statistical analysis was performed with IBM SPSS Statistics software version 24.0 (SPSS, Chicago, Illinois) and R version 3.4.4 (R Foundation for Statistical Computing, Vienna, Austria). R package "ggplot2" was used to construct the plots. Generalized additive mode smoothing method was used to visualize the data using smoothed lines.

Results

Characteristics of the study population

Table 1 depicts the characteristics of the study population for men and women.

The mean age in men was 56.6 years and in women 56.8 years. Women were on average 10.3 years (SD 7.6) after menopause. The prevalence of CVD was 8.9% in men and 3.1% in women and the prevalence of diabetes was 12.9% in men and 7.2% in women. Among men 31.3% and in women 23.2% were current smokers.
**Serum SHBG levels with aging and cardiovascular risk**

Among men individuals older than 46 years had higher SHBG levels. Among women a U-shaped pattern was observed. Women younger than 65 years had overall lower and women older than 65 years had higher SHBG levels. (Figure 2)

After stratification by the cardiovascular risk factors sum score and age, overall SHBG levels were lower with increasing number of risk factors across different age categories in both sexes. Among men, SHBG levels were 18%, 12%, 6% and 18% lower in participants with \( \geq 3 \) risk factors compared to participants with 0-1 risk factors in age-category 45-55 years, 55-60 years, 60-65 years and 65-79 years, respectively. In women, the U-shaped trend was also reflected in this plot in the age categories 45-55 years and 55-60 years. SHBG levels were 24%, 21%, 18% and 13% lower in women with \( \geq 3 \) risk factors compared to participants with 0-1 risk factors in age-category 45-55 years, 55-60 years, 60-65 years and 65-79 years, respectively.

However, within each category of the cardiovascular risk factor sum score (including 0-1, 2 or \( \geq 3 \) risk factors) SHBG levels were higher among both older men and women. (Figure 3) Among men, SHBG levels were 39%, 23% and 40% higher in the oldest age-category (60-79 years) in participants with 0-1 risk factors, 2 risk factors and \( \geq 3 \) risk factors, respectively compared to the youngest age-category (45-55 years). In women, SHBG levels were 21% and 5% higher in the oldest age-category (60-79 years) compared to younger participants in age-category 55-60 years, among participants with 2 risk factors and \( \geq 3 \) risk factors, respectively.
Overall, in both men and women statistically significant differences in SHBG levels between the different risk factor sum score categories across different age-categories were observed, except for the last age-categories in women (due to low numbers).

**Clinical outcomes**

Similarly, in each age category, the largest proportion for prevalent CVD was observed in the highest risk factor sum score category in both women and in men. (Supplementary table 1(19)) For incident CVD, due to the relatively young baseline age of the population and the short follow-up time after assessment of the sex steroids, the number of events was limited. Overall, we saw a trend towards an increase in risk with increasing SHBG levels in total population. Due to small number of events, in particular among women, the stratified analysis per age-category should be interpreted with caution. (Supplementary table 2(19))

**SHBG, BMI, fasting insulin, sex steroids and triglycerides**

The associations of SHBG levels with age, BMI, fasting insulin, sex steroids and triglycerides are shown in Table 2.

BMI, fasting insulin and triglycerides explained a larger variance in SHBG levels among women compared to men (the amount of explained variance was 18.7% and 7.3% for BMI, 18.1% and 5.3% for fasting insulin, and 13.2% and 9.6% for triglycerides among women and men respectively). Total testosterone explained 38.1% of the variance in SHBG levels for men but only 3.8% for women. (Table 2)

In the final model for men age, total testosterone and triglycerides were included, which resulted in an (adjusted) explained variance in SHBG of 45.6%. In women, the model
additionally included BMI and fasting insulin and yielded an (adjusted) explained variance of 31.4% for SHBG levels.

Discussion

We showed that SHBG levels increased from mid-forties and mid-fifties onwards until older ages, with a different pattern for men and women. Among men, we observed a linear increase in SHBG levels with age while among women SHBG levels followed a U-shaped pattern with a decrease between 45 to 65 years followed by a linear increase afterwards. Moreover, participants with larger number of cardiovascular risk factors had lower SHBG levels. However, when stratified by age, older participants had higher SHBG levels. Finally, total testosterone and triglyceride levels explained 46.2% of the variance in SHBG levels among men. In women, total testosterone and triglyceride levels, BMI and fasting insulin explained 31.8% of the variance in SHBG.

SHBG, aging and cardiovascular risk

Distribution of SHBG levels showed marked sex differences with age. While SHBG levels were consistently higher with increasing age among men, a U-shaped trend with age was observed among women. Although several studies among adult and elderly Australian(9) and American(10) populations have reported increasing SHBG levels with age in men, data in women is limited. One previous study among 616 Italian women free of diabetes showed a similar U-shaped trend for SHBG, with a similar increasing trend after 60 years of age(8). Another small study in 317 women observed similarly a U-shaped trend. However, the increase of SHBG levels started later and after the age of 70 years(20). In our study, we observed higher levels of SHBG in women after the age of 65 years. The lower levels of SHBG in women between 45 to 65 years are in line with the parallel decrease
of the estradiol concentrations during the menopausal transition. This hormonal change in women could at least partly, explain the observed sex-differences among younger men and women in our study. However, the underlying mechanisms for the observed increase of SHBG levels after the age of 65 years in both men and women remains to be elucidated. We speculate that relative androgen excess during the menopausal transition may also partly explain the U-shaped trend of SHBG. Perimenopausal androgen production in the ovaries in response to strong stimulation by gonadotropins (luteinizing hormone and follicle-stimulating hormone) produced in the pituitary, disappears after the menopause (and thus after the drop in estrogen). Furthermore, postmenopausal increase in BMI and fatmass(21), leading to more conversion of androgens in estrogens in peripheral fat, may contribute to the increasing trend of SHBG.

We also observed lower levels of SHBG among participants with larger number of cardiovascular risk factors across all age categories in men and women. For example, in men, the mean SHBG in the youngest age category (45-55 years) with 0 or 1 risk factors was almost similar to the mean SHBG levels in the older age category (60-65 years) but with >= 3 factors. This shows the mixing and confusing impact of age and cardiovascular risk factors when assessing the association of SHBG with CVD risk. Our results, therefore, underscore the importance of taking into account the age-related changes in SHBG levels in assessment of the cardiovascular risk associated with SHBG. As such, the controversial cardiovascular associations reported by prior studies may be due to inadequate consideration of the impact of age, particularly in women.

Nevertheless, besides age, other factors such as use of hormone medication(22), ethnicity(23) and genetics(24) could additionally account for, at least part of the, controversial results between SHBG levels and CVD in the literature.
Prior studies have similarly shown that higher SHBG levels were associated with a favorable cardiovascular risk profile among young and middle-aged men\(^\text{25,26}\) and postmenopausal women\(^\text{27}\). One study amongst 253 women aged 54-60 years, observed a U-shaped trend of SHBG plotted against 12-year myocardial infarction incidence, which is in line with our study\(^\text{28}\). To the best of our knowledge, no prior study investigated the distribution of SHBG across both age and cardiovascular risk. Therefore, it remains unknown if and how the occurrence and timing of the shift in CVD risk during and after the menopausal transition precedes, parallels or lags the age-related changes in serum SHBG levels. As such, future studies should elucidate to what extent SHBG is an independent predictor of CVD risk or a marker of common underlying factors and/or disorders (e.g. cardiometabolic disorders or normal aging) that underlie both the age-related changes in SHBG levels and CVD risk.

**Regulators of SHBG levels among men and women**

Although the exact underlying mechanisms of the regulation of SHBG levels remains unknown, one of the most important determinants that have been suggested in the literature are sex steroids such as testosterone and estradiol. It is well known that SHBG has a high binding affinity to sex steroids with a higher binding affinity for testosterone compared to estradiol\(^\text{29}\). As such, it has been suggested that in men testosterone levels and in premenopausal women estradiol levels may play a more important role in the regulation of SHBG, due to the abundance of testosterone levels in men and estradiol levels in women.

Second, BMI is suggested as a strong determinant of SHBG. Two studies reported an inverse relation between BMI and SHBG levels. We speculated that BMI and other markers of (abdominal) obesity, e.g. fasting insulin and triglycerides, may be more important determinants in postmenopausal women compared to men, due to the deprivation of estradiol
levels in women after the menopause. As in men no such sharp decline in hormone levels
occur comparable to the menopausal transition in women, testosterone levels remain the
largest determinant.

In the final model for men age, total testosterone and triglycerides were included, which
resulted in an (adjusted) explained variance in SHBG of 45.6%. In women, the model
additionally included BMI and fasting insulin and yielded an (adjusted) explained variance of
31.4% for SHBG levels. In our study, the included possible regulators explained a larger
variance of SHBG levels among men compared to women. This difference was mainly
derived by the relatively large contribution of total testosterone levels in explained variance
in men. This could be explained by the abundance of testosterone in men and that two-thirds
of testosterone is specifically bound to SHBG in the circulation(27). Other factors had a
larger contribution to the explained variance in SHBG in women compared to men, including
BMI and fasting insulin and triglycerides. As such, our results suggest that the regulation of
SHBG levels can be sex-specific. In line with our findings, several studies have reported
larger associations between SHBG and for instance diabetes type 2 among women compared
to men(30). Similarly, SHBG was inversely associated with triglycerides and positively
associated with age, total testosterone and thyroxine in another study among 2563
community-dwelling predominantly Caucasian men aged 35-80 years(14). Also in line with
our study, in the multi-adjusted model no significant association was found for insulin levels
in men in this study(14). In line with our study, a study among 329 women found no
correlation between estradiol and SHBG among postmenopausal women(31). Another study
in men reported that a multi-adjusted model including anthropometric measures, body
composition, insulin growth factors and leptin, yielded 40.5% of explained variance of
SHBG, which was to a similar extent as in our study(32). In this study, fat mass and insulin-
like growth factor-binding protein 3 (IGFBP3) provided the most significant contribution (32). This study also suggested that determinants of SHBG may change during the menopausal transition, as in the group with premenopausal women estradiol was significantly associated with SHBG levels (31). Moreover, a study using mouse models expressing human SHBG transgene, showed that SHBG in vivo resulted in an increase of total testosterone and estradiol levels (33).

Prior studies have suggested thyroxine alters SHBG production in the hepatocytes and that SHBG levels vary by thyroid function (12). However, in our study thyroxine was not significantly associated with SHBG levels in both sex after adjusting for other covariates, including BMI. However, we included a sample from the general population, whereas prior studies have shown that in patients with hyperthyroidism, thyroxine was significantly associated with SHBG after controlling for BMI (34).

Although previous studies have suggested androgens may decrease SHBG levels, (35,36) we found that higher total testosterone levels were positively associated with SHBG levels. This may indicate that SHBG levels may drive higher testosterone levels, rather than the other way round. Nevertheless, the exact role of sex steroids and their interplay with SHBG, which is known to be complex, remains also to be elucidated. Moreover, this may indicate that the relationship between total testosterone levels and CVD risk, as shown by prior studies (3,4), may be also affected by (change in) SHBG levels.

Whether the direct effect of these regulators or a common underlying factor (age) drives the changes in SHBG levels remains unknown. Interestingly, the association between age and SHBG and the associations between BMI, fasting insulin, and triglycerides with SHBG were in the opposite direction. As such, considering also the rise in SHBG levels after the age of 65 years, one could speculate that SHBG could also be a marker of aging (37). Nevertheless, an SHBG-receptor has been identified which also suggests a direct effect of SHBG itself.
This has highlighted the role of SHBG as a potential biomarker for several comorbid conditions(38).

Moreover, our study did not investigate all potential regulators of SHBG. The remaining variance of SHBG may be explained by other factors, which were not assessed in this study, including dietary factors(13), liver fat(13,39) and genetics(23).

Furthermore, it remains to be elucidated whether determinants of SHBG are also age-specific and if the relative contribution of these potential regulatory factors changes with ageing.

**Strength and limitations**

To the best of our knowledge, this is the first study investigating the underlying factors of the controversial relationship between SHBG levels and CVD risk. We have provided significant insight into the relationship between SHBG and CVD risk, for both research and clinical practice, important for clinical disorders accompanied with altered SHBG levels (such as PCOS and sexual dysfunction) and increased CVD risk.

The major strengths of this study include the large community-dwelling study sample, availability of SHBG and other sex steroids in both men and women across a broad age spectrum, and the availability of detailed assessment of a broad range of cardiovascular risk factors. Additionally, androgens were measured using the golden standard method.

Limitations of this study include the cross-sectional design that does not allow for conclusions regarding causality.

Also, although follow-up data was available for CVD events, SHBG levels were only available at baseline of this cohort study.

Additionally, due to low number of events and short follow-up period (as the study population consisted of a relatively young cohort), detailed stratified analyses for different age-categories were not possible. The validity of the use of the risk factors sum score was
confirmed by additional analysis showing that the highest proportions of CVD were observed in the highest age categories and risk factor sum score categories.

Moreover, this study population consisted of mainly Caucasian participants. As such, our results may not be directly generalizable to other ethnic populations, as prior studies have suggested that SHBG and sex steroid levels may show ethnic differences. (24) Furthermore, in this study no longitudinal data or repeated measurements for SHBG were available. Therefore, future studies with longitudinal repeated measurements for SHBG, investigating the within-person change, are warranted. Finally, the immunoassay to measure total estradiol had a minimum detection limit of 18.35 pmol/L.

Conclusion

We observed a clear sex-specific pattern for SHBG levels with age. Among men, a linear increase in SHBG levels with age and among women a U-shaped pattern was observed. Participants with a larger number of cardiovascular risk factors had lower SHBG levels. However, when stratified by age, older participants had higher SHBG levels. This highlights the importance of taking into account the age-related changes in SHBG levels (e.g. using stratified analysis by age categories or using age-adjusted reference values for SHBG) to avoid controversial results in the assessment of the cardiovascular risk associated with SHBG. Finally, total testosterone and triglyceride levels in men and total testosterone, triglycerides, BMI, and fasting insulin in women explained 46.2% and 31.8% of the variance in SHBG levels in men and women respectively.
Authors’ contributions: EA, MK and JLRL contributed to the conception or design of the work. EA, MK, JSEL, MAI and JLRL contributed to the acquisition, analysis, or interpretation of data for the work. EA drafted the manuscript. All critically revised the manuscript and gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

Data availability: Data can be obtained upon request. Requests should be directed towards the management team of the Rotterdam Study (secretariat.epi@erasusmc.nl), which has a protocol for approving data requests. Because of restrictions based on privacy regulations and informed consent of the participants, data cannot be made freely available in a public repository.

All supplementary materials and figures are located in a digital research materials repository listed in References.
References

1. Humphries KH, Izadnegahdar M, Sedlak T, Saw J, Johnston N, Schenck-Gustafsson K, Shah RU, Regitz-Zagrosek V, Grewal J, Vaccarino V, Wei J, Bairey Merz CN. Sex differences in cardiovascular disease - Impact on care and outcomes. *Front Neuroendocrinol*. 2017;46:46-70.

2. Garcia M, Mulvagh SL, Merz CN, Buring JE, Manson JE. Cardiovascular Disease in Women: Clinical Perspectives. *Circulation research*. 2016;118(8):1273-1293.

3. Brand JS, van der Schouw YT. Testosterone, SHBG and cardiovascular health in postmenopausal women. *International Journal Of Impotence Research*. 2010;22:91.

4. Chen Y, Zeleniuch-Jacquotte A, Arslan AA, Wojcik O, Toniolo P, Shore RE, Levitz M, Koenig KL. Endogenous hormones and coronary heart disease in postmenopausal women. *Atherosclerosis*. 2011;216(2):414-419.

5. Jaspers L, Dhana K, Muka T, Meun C, Kiefte-de Jong JC, Hofman A, Laven JS, Franco OH, Kavousi M. Sex Steroids, Sex Hormone-Binding Globulin and Cardiovascular Health in Men and Postmenopausal Women: The Rotterdam Study. *The Journal of clinical endocrinology and metabolism*. 2016;101(7):2844-2852.

6. Ouyang P, Vaidya D, Dobs A, Golden SH, Szklo M, Heckbert SR, Kopp P, Gapstur SM. Sex hormone levels and subclinical atherosclerosis in postmenopausal women: the Multi-Ethnic Study of Atherosclerosis. *Atherosclerosis*. 2009;204(1):255-261.

7. Khatibi A, Agardh CD, Shakir YA, Nerbrand C, Nyberg P, Lidfeldt J, Samsioe G. Could androgens protect middle-aged women from cardiovascular events? A population-based study of Swedish women: The Women's Health in the Lund Area (WHILA) Study. *Climacteric: the journal of the International Menopause Society*. 2007;10(5):386-392.
8. Maggio M, Lauretani F, Basaria S, Ceda GP, Bandinelli S, Metter EJ, Bos AJ, Ruggiero C, Ceresini G, Paolisso G, Artoni A, Valenti G, Guralnik JM, Ferrucci L. Sex hormone binding globulin levels across the adult lifespan in women— the role of body mass index and fasting insulin. *J Endocrinol Invest*. 2008;31(7):597-601.

9. Liu PY, Beilin J, Meier C, Nguyen TV, Center JR, Leedman PJ, Seibel MJ, Eisman JA, Handelsman DJ. Age-Related Changes in Serum Testosterone and Sex Hormone Binding Globulin in Australian Men: Longitudinal Analyses of Two Geographically Separate Regional Cohorts. *The Journal of Clinical Endocrinology & Metabolism*. 2007;92(9):3599-3603.

10. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. *The Journal of clinical endocrinology and metabolism*. 2001;86(2):724-731.

11. Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease: the Framingham study. *Annals of internal medicine*. 1976;85(4):447-452.

12. David MS, Geoffrey LH. Thyroid hormones act indirectly to increase sex hormone-binding globulin production by liver via hepatocyte nuclear factor-4α. *Journal of Molecular Endocrinology*. 2009;43(1):19-27.

13. Simo R, Saez-Lopez C, Barbosa-Desongles A, Hernandez C, Selva DM. Novel insights in SHBG regulation and clinical implications. *Trends Endocrinol Metab*. 2015;26(7):376-383.

14. Gyawali P, Martin SA, Heilbronn LK, Vincent AD, Jenkins AJ, Januszewski AS, Taylor AW, Adams RJT, O’Loughlin PD, Wittert GA. Cross-sectional and
longitudinal determinants of serum sex hormone binding globulin (SHBG) in a cohort of community-dwelling men. *PLoS One.* 2018;13(7):e0200078-e0200078.

15. Pasquali R, Vicennati V, Bertazzo D, Casimirri F, Pascal G, Tortelli O, Labate AMM. Determinants of sex hormone—binding globulin blood concentrations in premenopausal and postmenopausal women with different estrogen status. *Metabolism.* 1997;46(1):5-9.

16. Wallace IR, McKinley MC, Bell PM, Hunter SJ. Sex hormone binding globulin and insulin resistance. *Clinical Endocrinology.* 2013;78(3):321-329.

17. Ikram MA, Brusselle GGO, Murad SD, van Duijn CM, Franco OH, Goedegebuure A, Klaver CCW, Nijsten TEC, Peeters RP, Stricker BH, Tiemeier H, Uitterlinden AG, Vernooij MW, Hofman A. The Rotterdam Study: 2018 update on objectives, design and main results. *European journal of epidemiology.* 2017;32(9):807-850.

18. Lui GK, Rogers IS, Ding VY, Hedlin HK, MacMillen K, Maron DJ, Sillman C, Romfh A, Dade TC, Haeffele C, Grady SR, McElhinney DB, Murphy DJ, Fernandes SM. Risk Estimates for Atherosclerotic Cardiovascular Disease in Adults With Congenital Heart Disease. *The American journal of cardiology.* 2017;119(1):112-118.

19. Aribas E, Kavousi M, Laven JSE, Ikram MA, Roeters van Lennep J. Supplementary data for: Aging, cardiovascular risk and SHBG levels in men and women from the general population (2021). DANS https://doi.org/10.17026/dans-xg7-28jv.

20. Elmlinger MW, Kühnel W, Wormstall H, Döller PC. Reference intervals for testosterone, androstenedione and SHBG levels in healthy females and males from birth until old age. *Clinical laboratory.* 2005;51(11-12):625-632.

21. Ambikairajah A, Walsh E, Tabatabaei-Jafari H, Cherbuin N. Fat mass changes during menopause: a metaanalysis. *Am J Obstet Gynecol.* 2019;221(5):393-409 e350.
22. Zimmerman Y, Eijkemans MJC, Coelingh Bennink HJT, Blankenstein MA, Fauser BCJM. The effect of combined oral contraception on testosterone levels in healthy women: a systematic review and meta-analysis. *Hum Reprod Update*. 2014;20(1):76-105.

23. Laurent MR, Vanderschueren D. Functional effects of sex hormone-binding globulin variants. *Nature Reviews Endocrinology*. 2014;10(9):516-517.

24. Kim C, Golden SH, Mather KJ, Laughlin GA, Kong S, Nan B, Barrett-Connor E, Randolph JF, Jr., for The Diabetes Prevention Program Research G. Racial/Ethnic Differences in Sex Hormone Levels among Postmenopausal Women in the Diabetes Prevention Program. *The Journal of Clinical Endocrinology & Metabolism*. 2012;97(11):4051-4060.

25. Canoy D, Barber TM, Pouta A, Hartikainen AL, McCarthy MI, Franks S, Jarvelin MR, Tapanainen JS, Ruokonen A, Huhtaniemi IT, Martikainen H. Serum sex hormone-binding globulin and testosterone in relation to cardiovascular disease risk factors in young men: a population-based study. *Eur J Endocrinol*. 2014;170(6):863-872.

26. Firtser S, Juonala M, Magnussen CG, Jula A, Loo BM, Marniemi J, Viikari JS, Toppari J, Perheentupa A, Hutri-Kahonen N, Raitakari OT. Relation of total and free testosterone and sex hormone-binding globulin with cardiovascular risk factors in men aged 24-45 years. The Cardiovascular Risk in Young Finns Study. *Atherosclerosis*. 2012;222(1):257-262.

27. Brand JS, van der Schouw YT. Testosterone, SHBG and cardiovascular health in postmenopausal women. *Int J Impot Res*. 2010;22(2):91-104.

28. Lapidus L, Lindstedt G, Lundberg PA, Bengtsson C, Gredmark T. Concentrations of sex-hormone binding globulin and corticosteroid binding globulin in serum in relation...
to cardiovascular risk factors and to 12-year incidence of cardiovascular disease and overall mortality in postmenopausal women. *Clinical chemistry*. 1986;32(1 Pt 1):146-152.

29. Mean F, Pellaton M, Magrini G. Study of the binding of dihydrotestosterone, testosterone and oestradiol with sex hormone binding globulin. *Clinica Chimica Acta*. 1977;80:171-180.

30. Muka T, Nano J, Jaspers L, Meun C, Bramer WM, Hofman A, Dehghan A, Kavousi M, Laven JS, Franco OH. Associations of Steroid Sex Hormones and Sex Hormone-Binding Globulin With the Risk of Type 2 Diabetes in Women: A Population-Based Cohort Study and Meta-analysis. *Diabetes*. 2017;66(3):577-586.

31. Pasquali R, Vicennati V, Bertazzo D, Casimirri F, Pascal G, Tortelli O, Labate AM. Determinants of sex hormone-binding globulin blood concentrations in premenopausal and postmenopausal women with different estrogen status. Virgilio-Menopause-Health Group. *Metabolism*. 1997;46(1):5-9.

32. Gomez JM, Maravall FJ, Gomez N, Navarro MA, Soler J. Determinants of sex hormone-binding globulin concentrations in a cross-sectional study of healthy men randomly selected. *The journal of nutrition, health & aging*. 2007;11(1):60-64.

33. Laurent MR, Hammond GL, Blokland M, Jardí F, Antonio L, Dubois V, Khalil R, Sterk SS, Gielen E, Decallonne B, Carmeliet G, Kaufman JM, Fiers T, Huhtaniemi IT, Vanderschueren D, Claessens F. Sex hormone-binding globulin regulation of androgen bioactivity in vivo: validation of the free hormone hypothesis. *Scientific reports*. 2016;6:35539.

34. Brenta G, Schnitman M, Gurfinkel M, Damilano S, Pierini A, Sinay I, Pisarev MA. Variations of sex hormone-binding globulin in thyroid dysfunction. *Thyroid: official journal of the American Thyroid Association*. 1999;9(3):273-277.
35. Kalme T, Loukovaara M, Koistinen R, Koistinen H, Angervo M, Leinonen P, Seppälä M. Estradiol increases the production of sex hormone-binding globulin but not insulin-like growth factor binding protein-1 in cultured human hepatoma cells. *Fertility and sterility.* 1999;72(2):325-329.

36. Ruokonen A, Alén M, Bolton N, Vihko R. Response of serum testosterone and its precursor steroids, SHBG and CBG to anabolic steroid and testosterone self-administration in man. *Journal of steroid biochemistry.* 1985;23(1):33-38.

37. Caldwell JD, Jirikowski GF. Sex hormone binding globulin and aging. *Horm Metab Res.* 2009;41(3):173-182.

38. Fortunati N. Sex hormone-binding globulin: not only a transport protein. What news is around the corner? *J Endocrinol Invest.* 1999;22(3):223-234.

39. Jaruvongvanich V, Sanguankeo A, Riangwiwat T, Upala S. Testosterone, Sex Hormone-Binding Globulin and Nonalcoholic Fatty Liver Disease: a Systematic Review and Meta-Analysis. *Ann Hepatol.* 2017;16(3):382-394.
Figure legends

Figure 1. Study flowchart
N; Number

Figure 2. The relationship between serum sex-hormone binding globulin levels (nmol/L) and age

Figure 3. Mean serum sex-hormone binding globulin levels across age and the cardiovascular risk factors sum score categories

The cardiovascular risk factor sum score was composed of the following variables: BMI, hypertension (defined as systolic blood pressure $\geq 140$ mmHg or diastolic blood pressure $\geq 90$ mmHg or use of blood pressure lowering medication), current smoking, prevalent diabetes and dyslipidemia (defined as LDL $> 4.0$ mmol/L or use of lipid lowering medication). The difference in (median) SHBG levels between the different risk factor sum score categories was significant in men aged 45-55 years ($\chi^2 53.5$, $p < 0.001$), 55-60 years ($\chi^2 59.9$, $p < 0.001$), 60-65 years ($\chi^2 48.4$, $p < 0.001$) and 65-79 years ($\chi^2 69.6$, $p < 0.001$) and women aged 45-55 years ($\chi^2 20.5$, $p < 0.001$), 55-60 years ($\chi^2 9.7$, $p < 0.001$), except for in the age-categories with lower number 60-65 years ($\chi^2 5.5$, $p=0.06$) and 65-79 years ($\chi^2 1.3$, $p=0.52$).
Table 1. Characteristics of the study population

|                                      | Men       | Women     |
|--------------------------------------|-----------|-----------|
|                                      | (N=1481)  | (N=1783)  |
| Age, years                           | 56.6 (5.9)| 56.8 (5.9)|
| Body mass index, kg/m²               | 27.9 (3.9)| 27.7 (5.1)|
| Current smoking, N (%)               | 426 (31.3)| 398 (23.2)|
| Diastolic blood pressure, mmHg       | 83.7 (10.7)| 82.2 (11.1)|
| Systolic blood pressure, mmHg        | 135.7 (17.8)| 130.0 (19.2)|
| Antihypertensive therapy, N (%)      | 326 (24.5)| 404 (23.8)|
| Total cholesterol, mmol/L            | 5.4 (1.02)| 5.8 (1.05)|
| High-density lipoprotein, mmol/L     | 1.2 (0.4) | 1.6 (0.4) |
| Lipid lowering medication, N (%)     | 289 (21.7)| 320 (18.9)|
| Triglycerides, mmol/L                | 1.4 [1.04, 2.0] | 1.2 [0.9, 1.7] |
| Fasting insulin, pmol/L              | 84.0 [60.0, 126.0] | 75.0 [53.0, 109.0] |
| Thyroxine, pmol/L                    | 15.9 (2.2) | 15.4 (2.2) |
| Prevalent diabetes, N                | 191 (12.9) | 128 (7.2) |
| Prevalent CVD, N (%)                 | 132 (8.9) | 56 (3.1) |
| Incident CVD, N (%)                  | 35 (2.4) | 63 (3.5) |
| Sex-hormone binding globulin, nmol/L | 40.3 [30.8, 50.9] | 56.6 [40.7, 78.1] |
| Total estradiol, pmol/L              | 93.9 [73.8, 118.5] | 30.6 [18.4, 66.3] |
| Total testosterone, nmol/L           | 16.9 [13.4, 21.3] | 0.8 [0.6, 1.1] |
| Age at menopause, years              | NA        | 48.1 (6.3) |
| Time since menopause, years          | NA        | 10.3 (7.6) |
| Natural menopause, N (%)             | NA        | 974 (76.1) |

Values are reported as number (percentage) for categorical variables and mean (SD) or median [25th–75th quartile] for continuous variables. N; number, CVD; cardiovascular disease, NA; not applicable.
Table 2. The relationship between SHBG, age, BMI, fasting insulin, sex steroids and triglycerides

|                | Univariate models | Age-adjusted model | Multi-variate model | Final model |
|----------------|-------------------|--------------------|---------------------|-------------|
| **A. Men**     |                   |                    |                     |             |
| Mean difference (95%CI) | P-value | Mean difference (95%CI) | P-value | Mean difference (95%CI) | P-value | Mean difference (95%CI) | P-value |
| ns(age, 2), 1  | 0.13 (0.08;0.18)  | \(<0.001\)         | 0.15 (0.10;0.20)   | \(<0.001\)   | 0.20 (0.16;0.24)   | \(<0.001\)   | 0.20 (0.16;0.24)   | \(<0.001\)   |
| ns(age, 2), 2  | 0.13 (0.08;0.18)  | \(<0.001\)         | 0.14 (0.09;0.19)   | \(<0.001\)   | 0.14 (0.10;0.18)   | \(<0.001\)   | 0.14 (0.10;0.18)   | \(<0.001\)   |
| Body mass index | -0.27 (-0.32;-0.22) | \(<0.001\)         | -0.28 (-0.33;-0.23) | \(<0.001\)   | 0.04 (-0.09;0.01)  | 0.08         |
| Total testosterone | 0.62 (0.58;0.66)  | \(<0.001\)         | 0.64 (0.60;0.68)   | \(<0.001\)   | 0.58 (0.53;0.63)   | \(<0.001\)   | 0.60 (0.56;0.64)   | \(<0.001\)   |
| Fasting insulin | -0.23 (-0.28;-0.18) | \(<0.001\)         | -0.24 (-0.29;-0.20) | \(<0.001\)   | -0.02 (-0.07;0.03) | 0.46         |
| Total estradiol | 0.20 (0.15;0.25)  | \(<0.001\)         | 0.18 (0.13;0.23)   | \(<0.001\)   | 0.001 (-0.04;0.04) | 0.96         |
| Triglycerides  | -0.31 (-0.36;-0.26) | \(<0.001\)         | -0.31 (-0.36;-0.26) | \(<0.001\)   | -0.16 (-0.20;-0.11) | \(<0.001\)  |
| Thyroxine      | 0.08 (0.03;0.13)  | \(0.002\)          | 0.08 (0.03;0.13)   | \(0.001\)   | 0.01 (-0.03;0.05)  | 0.79         |
| **Total explained variance** | 45.7%      |                    |                     |             | 45.6%                   |             |
|                      | Mean difference (95% CI) | Standardized Mean Difference (95% CI) | p-value | 95th Percentile Difference |
|----------------------|--------------------------|--------------------------------------|---------|---------------------------|
| **Testosterone**     | -0.43 (-0.47; -0.38)    | -0.42 (-0.46; -0.38)                 | <0.001  | -0.19 (-0.24; -0.14)      |
| **Fasting insulin**  | 0.07 (0.02; 0.11)        | 0.01 - (-0.07; 0.04)                 | 0.005   | 0.04 (0.08; 0.01)         |
| **Total estradiol**  | -0.36 (-0.41; -0.32)    | -0.35 (-0.40; -0.31)                 | <0.001  | -0.18 (-0.22; -0.13)      |
| **Triglycerides**    | 0.05 (0.01; 0.10)        | 0.06 (0.01; 0.11)                    | 0.01    | 0.02 (-0.02; 0.06)        |
| **Thyroxine**        |                          |                                      |         |                           |

**Total explained variance**: 31.4%

Values represent standardized mean difference (95% confidence interval) in z-score of SHBG per standard deviation increase in determinants of SHBG. SHBG, fasting insulin, total testosterone, total estradiol and triglycerides were natural-log transformed. Significant results (P-value < 0.007) are in bold. SHBG; sex-hormone binding globulin, CI; confidence interval, R2; adjusted explained variance, ns; natural splines.
Figure 1

All participants of the Rotterdam Study-III-1 (N=3932)

Excluded participants with missing data for sex-hormone binding globulin (N=43)
Excluded participants with current hormone medication use (N=115)
Excluded participants with age above 79 years (N=79)

Eligible participants with data for sex-hormone binding globulin (N=3264)
Figure 3

A. Men

B. Women