

RESEARCH

Longitudinal associations between sex hormone-binding globulin and insulin resistance

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Abstract

Purpose: We aimed to investigate the association between SHBG and the homeostatic model assessment of insulin resistance (HOMA-Ir) in men and women in a prospective observational study.

Methods: The Vara-Skövde cohort is a random population of 2816 participants living in southwestern Sweden, aged 30–74. It was recruited between 2002 and 2005, and followed up in 2012–2014. After excluding participants on insulin therapy or hormone replacement therapy, 1193 individuals (649 men, 544 women) were included in the present study. Fasting blood samples were collected at both visits and stored in biobank. All participants were physically examined by a trained nurse. SHBG was measured with immunoassay technique. Linear regressions were computed to investigate the association between SHBG and HOMA-Ir both in cross-sectional and longitudinal analyses, adjusting for confounding factors.

Results: The mean follow-up time was 9.7 ± 1.4 years. Concentrations of SHBG were significantly inversely associated with log transformed HOMA-Ir in all groups with estimated standardized slopes (95% CI): men: -0.20 (-0.3 ; -0.1), premenopausal women: -0.26 (-0.4 ; -0.2), postmenopausal women: -0.13 (-0.3 ; -0.0) at visit 1. At visit 2 the results were similar. When comparing the groups, a statistically significant difference was found between men and post-menopausal women (0.12 (0.0 ; 0.2) P value = 0.04). In the fully adjusted model, SHBG at visit 1 was also associated with HOMA-Ir at visit 2, and the estimated slopes were -0.16 (-0.2 ; -0.1), -0.16 (-0.3 ; -0.1) and -0.07 (-0.2 ; 0.0) for men, premenopausal and postmenopausal women, respectively.

Main conclusion: Levels of SHBG predicted the development of insulin resistance in both men and women, regardless of menopausal state.

Key Words

- ▶ sex hormone-binding globulin
- ▶ cohort studies
- ▶ insulin resistance
- ▶ pre-diabetes
- ▶ prospective studies
- ▶ sex hormones

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Introduction

Sex hormone-binding globulin is a glycoprotein traditionally known as a carrier protein for sex hormones in both men and women. Cross-sectional and prospective epidemiological cohort studies have demonstrated that low levels of sex hormone-binding globulin (SHBG) are associated with increased risk to develop type 2 diabetes

(1, 2, 3). Furthermore, Ding *et al.* showed an association between polymorphism of the SHBG gene and risk of type 2 diabetes (4), in two different cohorts including men and women, respectively, suggesting a causal effect. The mechanism behind the association between SHBG and type 2 diabetes is not fully understood, but may

be mediated through insulin resistance (5). In a meta-analysis conducted in 2014 (6), low SHBG in men was a strong predictor for both prevalent and incident metabolic syndrome, a state that includes alterations in the glucose metabolism and is associated with a five-fold increase in risk for type 2 diabetes mellitus (7). Another meta-analysis from 2011 showed an inverse association between SHBG and the metabolic syndrome in both men and women; however, no significant sex-specific associations between SHBG and the metabolic syndrome were found (8). Cross-sectional studies have found an inverse association between levels of SHBG and insulin resistance in men (9, 10) and women (11, 12). Only few studies have investigated whether levels of SHBG can predict the development of insulin resistance, and there are no studies investigating this association simultaneously in men and women, and whether these associations change after menopause. The association with insulin resistance might be a link to better understand why levels of SHBG are associated with increase in risk for type 2 diabetes. Therefore, we aim to investigate the association between levels of SHBG and the homeostatic model assessment of insulin resistance (HOMA-Ir) in a longitudinal study including men and women, both pre-menopausal and post-menopausal. We will also assess the difference in the association between these groups.

Subjects and methods

The Vara-Skövde cohort is a cohort study of a gender balanced random sample of 2816 individuals living in southwestern Sweden 2002–2005. The aim of the study

was to investigate the development of hypertension and type 2 diabetes in a longitudinal design (13). Due to limitations in resources, only 1954 representative participants were consecutively summoned to follow-up survey in 2012–2014. Of those, 1327 (participation rate 68%; M=657) participants completed the study protocol at visit 2 accordingly. Eligible for the present study were those without insulin therapy or hormone replacement therapy. Only subjects that could provide full information at visit 1 and visit 2 were included in the analyses. No self-reported information regarding menopause state was available at visit 1. We stratified the women cohort at 50 years of age at visit 1 as a proxy of menopause. At visit 2, the female participants answered questions regarding menopause status and were accordingly grouped in pre-menopause and post-menopause groups.

Finally, 1193 subjects participated at visit 1 analyses and 1110 subjects at visit 2 (Fig. 1).

Physical examination

Specially trained nurses assessed study participants, measuring waist circumference and blood pressure in supine and sitting position at visit 2, supine and standing at visit 1. Validated questionnaires were used to obtain information on lifestyle including current smoking habits, alcohol intake, and leisure time physical activity (14).

Laboratory analyses

Fasting venous blood samples were drawn in the morning and 2 h after a 75 g oral glucose load. Serum concentrations

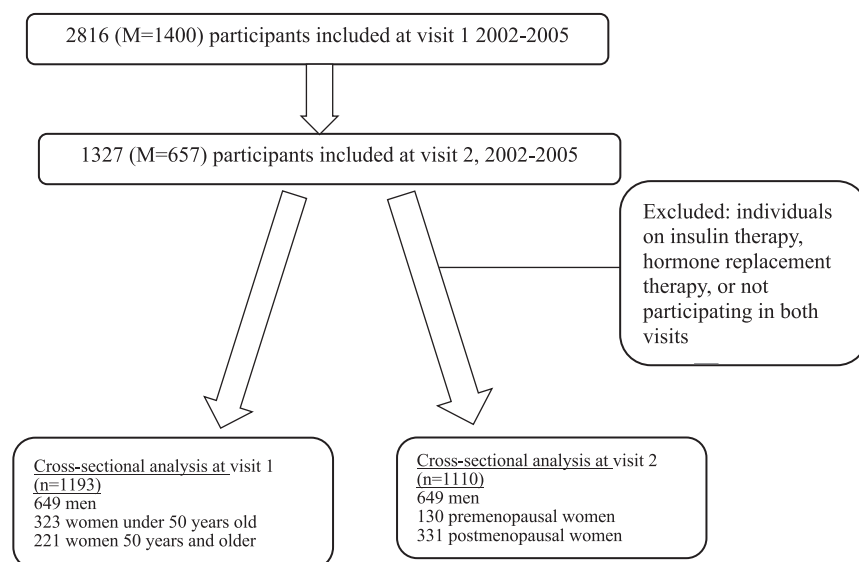


Figure 1
Flowchart illustrating the study design.

of sex hormone-binding globulin were obtained using RIA at visit 1 and at visit 2 (15). All blood samples were immediately frozen at -82°C . Diabetes and hypertension were defined based on WHO and JNC7 recommendations (16, 17), respectively, according to information obtained from medical history and clinical assessment. Concentrations of insulin and glucose at fasting were measured, and HOMA-Ir (homeostatic model assessment of insulin resistance) (18) was calculated by using the formula: $(\text{fasting insulin} \times \text{fasting glucose}) / 22.5$, in subjects without insulin therapy. Due to changes in insulin measurement methodology (from Roche Cobas to DxI Beckman) in 2011, the insulin values at visit 2 were about 35% higher than at visit 1. The insulin values therefore had to be re-calculated with the following formula: $\text{new method} = 1.3544 \times \text{old method} + 0.3237$. The correlation between methods was $r^2 = 0.9974$. The correlation between methods was investigated within the laboratory that provided the formula for the re-calculation.

Statistical analyses

Descriptive statistics were used to characterize the study population at visit 1 and visit 2. Due to skewness in HOMA-Ir, this variable was log-transformed. To assess the aim of examining the relationship between SHBG and HOMA-Ir, in the three groups (men, premenopausal and postmenopausal women) the functional form of the relationship was first investigated graphically using splines. It was concluded that a log-linear relationship was a reasonable assumption and HOMA-Ir was consequently log-transformed prior to analysis.

A linear regression model was used with logHOMA-Ir as dependent variable, and SHBG, group and the interaction term SHBG*group as independent variables. The relationship was quantified by the estimated slopes and group differences in slopes was evaluated by the interaction term. The analyses were adjusted for potential confounders including age, current smoking habits, physical activity, alcohol intake, WHR, LDL, CRP, hypertension and type 2 diabetes mellitus. WHR was chosen instead of BMI since WHR is more representative for visceral adiposity. Prior to analysis all continuous variables including logHOMA-Ir and SHBG were standardized to zero mean and unit standard deviation such that results were presented as standardized regression coefficients with 95% confidence intervals (95%CI) and *P* values. Cross-sectional analyses at visit 1 and visit 2 were analyzed separately. To evaluate the predictive relationship between SHBG at baseline and HOMA-Ir at follow-up on a group (average) level, a similar

regression model was used with logHOMA-Ir at follow-up as the dependent variable and SHBG, group belonging as independent variables. Baseline logHOMA-Ir as well as adjustment variables at baseline were included as a covariate. Results were reported as the predicted change (with 95% CI) in logHOMA-Ir on a group (average) level at follow-up for a unit standard deviation change in baseline SHBG.

Based on (19, 20) previous studies, we defined insulin resistance as the highest quartile of HOMA-Ir in each group. To investigate the correlation between SHBG level at visit 1 (explanatory variable) and risk of developing insulin resistance at visit 2 (dependent variable), a logistic regression model using the same covariates as above was used.

Analyses were stratified for sex and menopausal state based on age (visit 1) and self-reported data (visit 2). Results were presented as odds ratios with 95% CI and *P* values. Analyses were conducted using IBM SPSS Statistics version 24 and SAS software (SAS Institute Inc.).

Ethics

The Regional Ethical Review Board in Gothenburg, Sweden approved the study (D-nr 161.92 and 036-12), and all participants gave their written consent to participation.

Results

The mean follow-up time was 9.7 ± 1.4 years. Mean age at visit 1 was 49.2 ± 11.6 years for men, 40.9 ± 5.4 years for women <50 years of age, and 60.8 ± 6.9 years for women >50 years of age. After exclusion of individuals with insulin therapy or hormone replacement therapy, 1193 (649 men; 323 women under 50 years of age; 221 women over 50 years of age) participants were included in the analyses at visit 1, and 1110 (649 men, 130 premenopausal women, 331 postmenopausal women) participants at visit 2. Sensitivity analyses were conducted to assess the association between SHBG and HOMA-Ir among participants and non-participants at baseline, and the associations were similar in the two groups.

Characteristics of the study population at visit 1 and visit 2 are presented in Table 1. Concentrations of SHBG were significantly inversely associated with log transformed HOMA-Ir in all groups with estimated standardized slopes (95%CI): men: -0.20 ($-0.3; -0.1$), premenopausal women: -0.26 ($-0.4; -0.2$), postmenopausal women: -0.13 ($-0.3; 0.0$) at visit 1 in a model adjusting for confounding factors. At visit 2

Table 1 Characteristics of the study population.

	Visit 1			Visit 2		
	Men (n = 649)	Women <50 years of age (n = 323)	Women ≥50 years of age (n = 221)	Men (n = 649)	Premenopausal women (n = 130)	Postmenopausal women (n = 331)
Age (years)	49.2 ± 11.6	40.9 ± 5.4	60.8 ± 6.9	59.0 ± 11.9	47.0 ± 3.7	63.5 ± 9.9
Waist-hip-ratio	0.94 ± 0.1	0.82 ± 0.1	0.86 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	0.9 ± 0.1
Systolic blood pressure (mmHg)	124 ± 16	111 ± 12	131 ± 18	126 ± 13	115 ± 12	127 ± 15
HOMA-Ir	1.6 ± 1.2	1.3 ± 0.9	1.7 ± 1.4	2.1 ± 2.3	1.4 ± 1.1	2.0 ± 2.4
Smoker %	13.1	14.9	12.2	9.2	13.1	10.9
SHBG (nmol/L)	32.8 ± 13.6	50.7 ± 23.3	50.2 ± 22.2	46.7 ± 20.3	70.9 ± 31.6	68.0 ± 31.0
Diabetes %	5.2	1.5	9.0	12.0	1.5	12.7
LDL (mmol/L)	3.4 ± 0.9	2.9 ± 0.7	3.7 ± 0.9	3.5 ± 1.0	3.2 ± 0.7	3.6 ± 1.0
Hypertension %	15.4	4.6	29.0	15.3	5.4	14.5
Fasting glucose (mmol/L)	5.5 ± 0.8	5.1 ± 0.5	5.7 ± 1.2	5.9 ± 1.2	5.3 ± 1.3	5.8 ± 1.1
Body mass index	26.9 ± 3.3	25.7 ± 4.8	28.0 ± 4.9	27.5 ± 3.6	26.5 ± 5.2	27.4 ± 5.0

HOMA-Ir, homeostatic model assessment of insulin resistance; LDL, low-density lipids; SHBG, sex hormone-binding globulin.

the corresponding slopes were -0.29 (-0.4 ; -0.2), -0.22 (-0.4 ; -0.1) and -0.17 (-0.3 ; -0.1), respectively (Table 2). There was a statistically significant difference between the slopes in men and post-menopausal women (0.12 (0.0;0.2) P value=0.04). No significant differences in the slopes were observed when we compared the associations in these three groups at visit 1 or when we compared the differences between premenopausal women and the other two groups at follow-up (Supplementary Table 1, see section on [supplementary materials](#) given at the end of this article). Analyses were also made adjusting for BMI instead of WHR with similar results, and therefore, we chose WHR due to its relation to visceral adiposity.

In the predictive relationship between SHBG at visit 1 and HOMA-Ir at visit 2, the estimated slopes were -0.16 (-0.2 ; -0.1), -0.16 (-0.3 ; -0.1) and -0.07 (-0.2 ; 0.0) in the fully adjusted model for men, premenopausal women (women <50 years of age at visit 1) and postmenopausal women (women >50 years of age at visit 1), respectively (Table 3).

In the analysis investigating the correlation between SHBG at visit 1 and risk of developing insulin resistance defined as the highest quartile of HOMA-Ir, we found that an increase in SHBG by 10 nmol/L decreased the odds of developing insulin resistance by 18% in premenopausal women (OR 0.82; 95% CI: 0.71–0.94; $P=0.006$) and 22%

Table 2 Association between logHOMA-Ir and SHBG at visit 1 and visit 2, respectively.

Visit 1					
Men (n = 649)		Women <50 years of age (n = 323)		Women ≥50 years of age (n = 221)	
β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>
Model 1, adjusted for age					
-0.31 (-0.39 ; -0.24)	<0.001	-0.37 (-0.47 ; -0.26)	<0.001	-0.38 (-0.51 ; -0.25)	<0.001
Model 2, adjusted for age, smoking, alcohol intake, PA, WHR					
-0.21 (-0.29 ; -0.13)	<0.001	-0.26 (-0.37 ; -0.16)	<0.001	-0.24 (-0.37 ; -0.11)	<0.001
Model 3, adjusted as in model 2 + LDL, CRP, DM, HT					
-0.20 (-0.27 ; -0.12)	<0.001	-0.26 (-0.36 ; -0.16)	<0.001	-0.13 (-0.26 ; -0.00)	0.046
Visit 2					
Men (n = 649)		Pre-menopausal women (n = 130)		Post-menopausal women (n = 331)	
Model 1, adjusted for age					
-0.38 (-0.46 ; -0.31)	<0.001	-0.38 (-0.54 ; -0.23)	<0.001	-0.37 (-0.47 ; -0.27)	<0.001
Model 2, adjusted for age, smoking, alcohol intake, PA, WHR					
-0.29 (-0.36 ; -0.22)	<0.001	-0.24 (-0.39 ; -0.10)	<0.001	-0.24 (-0.34 ; -0.15)	<0.001
Model 3, adjusted as in model 2 + LDL, CRP, DM, HT					
-0.29 (-0.36 ; -0.22)	<0.001	-0.22 (-0.35 ; -0.09)	0.001	-0.17 (-0.26 ; -0.08)	<0.001

Dependent variable: logHOMA-Ir. Due to the skewness of the variable, log-transformed HOMA-Ir was used in these analyses. Menopause was defined per self-reported data at visit 2.

Adj, adjusted; CRP, C-reactive protein; DM, diabetes mellitus; HT, hypertension; LDL, low density lipoprotein; PA, physical activity; SHBG, sex hormone-binding globulin; WHR, waist-hip-ratio.

Table 3 Association between logHOMA-Ir at visit 2 and SHBG at visit 1.

Men (<i>n</i> = 649)	Women <50 years of age at visit 1 (<i>n</i> = 130)		Women ≥50 years of age at visit 1 (<i>n</i> = 331)			
	β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>
Model 1, adjusted for age						
−0.17 (−0.24; −0.11)	<0.001	−0.19 (−0.28; −0.11)	<0.001	−0.20 (−0.31; −0.10)	<0.001	
Model 2, adjusted for age, smoking, alcohol intake, PA, WHR						
−0.16 (−0.23; −0.09)	<0.001	−0.15 (−0.24; −0.06)	0.001	−0.17 (−0.28; −0.05)	0.005	
Model 3, adjusted as in model 2 + LDL, CRP, DM, HT						
−0.16 (−0.23; −0.09)	<0.001	−0.16 (−0.25; −0.08)	<0.001	−0.07 (−0.18; 0.04)	0.197	

Dependent variable: LogHOMA-Ir at visit 2. Due to the skewness of the variable, log-transformed HOMA-Ir was used in these analyses. Adj, adjusted; CRP, C-reactive protein; DM, diabetes mellitus; HT, hypertension; LDL, low-density lipoprotein; PA, physical activity; SHBG, sex hormone-binding globulin; WHR, waist-hip-ratio.

(OR 0.78; CI: 0.64–0.94; *P*=0.009) in postmenopausal women, in the fully adjusted model. In men, the decrease was 19% (OR 0.81; CI 0.65–0.99; *P*=0.042) in the fully adjusted model (Table 4).

Discussion

In this study we observed a strong association between levels of SHBG at baseline and the development of insulin resistance at follow-up that was independent of the levels of the insulin resistance at baseline. These associations were statistically significant, in both men and women, regardless of menopausal state and remained so after adjustments for possible confounders in both the longitudinal and the cross-sectional analyses. Our data suggested that there might be differences in this association when comparing men and post-menopausal women, revealing a slightly stronger association in men, probably without clinical significance. Other comparisons between groups were not statistically significant.

There is evidence that there is an association between levels of SHBG and the incidence of type 2 diabetes (4,

21, 22, 23, 24, 25). Although there is reason to believe that these associations might be mediated through insulin resistance, little is known about the mechanism behind the association. We found an independent association between low SHBG and higher HOMA-Ir at both timepoints for examination. This finding is in line with other cross-sectional studies in men (9, 10). This has been found previously in obese post-menopausal women as well, as in the study conducted by Akin *et al.* (11); however, in that study adjustments were made only for age, BMI and estradiol. There is less evidence regarding the association between SHBG and insulin resistance in pre-menopausal women. Our study shows a statistically significant negative association between SHBG and HOMA-Ir also in this group too. In the same study by Akin *et al.* (11) the association was weaker and not significant in premenopausal obese women. In our study, however, the associations were significant even after further adjustments for confounding factors in both groups and no significant differences in the slopes were observed when pre- and postmenopausal women were compared. Although our study was larger and capable to identify even weaker associations, even larger samples

Table 4 Logistic regression showing the association between concentrations of SHBG at visit 1 and the highest quartile of logHOMA-Ir at visit 2.

Men	Women <50 years of age (at visit 1)		Women ≥50 years of age (at visit 1)			
	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
Model 1, adjusted for age						
0.73 (0.60–0.89)	0.002	0.76 (0.66–0.87)	<0.001	0.71 (0.60–0.85)	<0.001	
Model 2, adjusted as in model 1 + smoking, alcohol intake, PA, WHR						
0.80 (0.65–0.98)	0.034	0.81 (0.71–0.94)	0.004	0.77 (0.64–0.93)	0.006	
Model 3, adjusted as in model 2 + LDL, CRP, hypertension and diabetes mellitus						
0.81 (0.65–0.99)	0.042	0.82 (0.71–0.94)	0.006	0.78 (0.64–0.94)	0.009	

Dependent variable: Insulin resistance defined as the highest quartile of logHOMA-Ir at visit 2. Odds ratio are for change in 10 nmol/L of SHBG. Adj, adjusted; CRP, C-reactive protein; DM, diabetes mellitus type 2; HT, hypertension; LDL, low-density lipoprotein; PA, physical activity; SHBG, sex hormone-binding globulin; WHR, waist-hip-ratio.

might be needed to investigate the question about effect modification of menopause status.

In our longitudinal analyses, the association between SHBG and insulin resistance remained, regardless of adjustments for confounding factors. Although there are prospective studies investigating the association between SHBG and type 2 diabetes and the metabolic syndrome as outcome (1, 3, 4, 26, 27, 28), only few studies have had HOMA-Ir as outcome (29, 30). The study by Wang *et al.* (30), however, included younger participants aged 24–39 ($n=1377$), and adjusted for fewer confounding factors, but found a similar association as in our study. Our results are also in line with the prospective study conducted by Joyce *et al.* in 2017 (29), showing that SHBG was inversely associated with HOMA-Ir in a 852 men, mean age 77 years. To our best knowledge, no study has investigated the association between SHBG and HOMA-Ir in men, premenopausal women and postmenopausal women within the same cohort, as is the case in our present study. These findings suggest an independent pathway of SHBG on the development of insulin resistance. In fact, SHBG regulates the bioavailability of sex hormones in tissues. It has been suggested that the association between SHBG and diabetes might at least partially be explained by SHBG's effect on the bioavailability of sex steroids (5, 26). However, these associations between SHBG and type 2 diabetes remain significant even after adjustments for sex hormones, both in men and women, and the association is stronger for SHBG than for sex hormones (5, 26), suggesting an independent effect of SHBG on the risk for developing type 2 diabetes. Furthermore, studies on polymorphism of the SHBG gene have showed that genetic variants associated with low levels of SHBG are also associated with higher risk for developing type 2 diabetes, suggesting that SHBG might be involved *per se* in the pathophysiology of diabetes and insulin resistance (4, 31).

There is reason to believe that SHBG has its own biological effect on a cellular level. Studies in breast cancer cells exposed to estrogen have observed that SHBG, via intracellular cross talk, interferes with the pro-cancerous estrogen effect (32, 33). Furthermore, there is evidence that SHBG has anti-inflammatory effects on the molecular level, by suppressing mRNA levels for inflammatory cytokines such as IL-6 and TNF α in macrophages and adipocytes (34). However, after adjustments for hsCRP the associations remained significant suggesting other mechanisms are involved in this association. Another mechanism that needs to be acknowledged is the link between SHBG and non-alcohol fatty liver disease (NAFLD). NAFLD seems to be an important determinant

of SHBG levels while strongly associated with metabolic syndrome and insulin resistance (35, 36). We were not able to adjust for liver fat steatosis status or transaminases, since we did not have access to those variables, which is a limitation in the study.

To our knowledge no previous studies have investigated sex differences in the associations between SHBG and insulin resistance. In this study we had the opportunity to investigate men and women simultaneously and observed a tendency toward stronger associations in men. The difference between groups was, however, only significant in one comparison, i.e. between postmenopausal women versus men in the fully adjusted cross-sectional model at visit 2. These differences in associations seem to have no clinical relevance.

The strengths of our study are the prospective design, with a large representative sample size, long follow-up time, high participation rates, and detailed characterization of participants that permitted adequate adjustments for possible confounders. Our study cohort consisted of both men and women and included both pre- and postmenopausal women, which provided the opportunity to compare the strength of the associations between groups. Morning blood tests were used for this study, avoiding diurnal variation of the concentration for hormones.

Some limitations of the study should be mentioned. First, information on menopausal status was not available at visit 1. Therefore, the group of female participants at visit 1 were divided according to age below and over 50, as a proxy for menopausal state. This, indeed is a limitation, since some women may be misclassified. The question about menopause was later added in the questionnaire at visit 2. Second, the method of analyzing insulin changed during the observation period, making it impossible to study the change of HOMA-Ir over time. However, the new and previous method of measuring insulin had a high correlation ($R^2=0.9974$) and instead of the change we were able to adjust for the baseline value in all longitudinal analyses. The long follow-up time is mainly a strength, but can also be regarded as a limitation, due to the lack of control on the exposure during the time between measurements. This may lead to residual confounding, which can either underestimate or overestimate actual correlations.

In conclusion, SHBG has a strong inverse association with HOMA-Ir, both cross-sectionally and longitudinally, in men and in women, regardless of menopausal state. Although not explaining mechanisms on the molecular level, this study adds knowledge about the association

between SHBG and insulin resistance. There is a need of further studies acknowledging the interesting possibility that the SHBG molecule, together with its receptor, may have own properties in regulating glucose metabolism.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/EC-20-0141>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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