Serum sex hormone-binding globulin and testosterone in relation to cardiovascular disease risk factors in young men: a population-based study

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**Key finding:** Lower sex hormone-binding globulin but higher testosterone concentrations were independently associated with adverse cardiovascular risk profile in young men.
Abstract

Objective: Reduced sex hormone-binding globulin (SHBG) concentration predicts insulin resistance and type 2 diabetes but its association with cardiovascular disease (CVD) risk is unclear. We examined the association between SHBG and cardiovascular risk factors, independently of total testosterone (TT), in young men.

Design: Observational, cross-sectional study

Setting: General community

Participants: 2,716 31-year old men in the 1966 Northern Finland Birth Cohort with clinical examination data and fasting blood samples.

Outcome variables: Blood pressure (BP), lipids and C-reactive protein (CRP) as biological CVD risk markers.

Results: SHBG concentration was significantly and inversely related to systolic and diastolic BP, triglycerides and CRP but positively to high-density lipoprotein (HDL) cholesterol after adjusting for insulin, body mass index, waist circumference, smoking, education and physical activity (all P <0.05). These linearly graded associations persisted with additional adjustment for TT. SHBG was significantly associated with total cholesterol only with adjustment for covariates and TT (P <0.05). The direction and magnitude of associations between TT and risk factors were variable but further adjustment for insulin, adiposity and SHBG showed positive associations between TT and blood pressure, total and low-density lipoprotein cholesterol and triglycerides and an inverse association with CRP (all P<0.05), but its relation with HDL-cholesterol was no longer significant.

Conclusions: In this cohort of young adult men, higher SHBG concentration was associated with a more favourable CVD risk profile, independently of TT. SHBG concentration modified the associations of TT with CVD risk factors.
Introduction

First reported in 1967, sex hormone-binding globulin (SHBG) is the specific binding protein for sex steroids in the blood plasma and it regulates the availability of free testosterone (FT) and oestradiol to hormone-responsive tissues and their metabolic clearance rate. In men, over 40% of total testosterone (TT) in circulation is bound to SHBG. Although SHBG has traditionally been considered a passive carrier protein, it could have an important biological function of its own. It is known to modify local steroid hormone effects by interacting specifically with high-affinity receptors on cell membranes and participate in steroid hormone signal transduction at the cell membrane independently of the classical intracellular androgen receptors. However, the wider clinical implications of this function remain to be elucidated.

Numerous studies have shown that reduced blood concentrations of SHBG are associated with increased risk of insulin resistance, type 2 diabetes, obesity and the metabolic syndrome but the role of SHBG in the aetiology of cardiovascular disease (CVD) remains unclear. Studies investigating associations between SHBG and cardiovascular risk have focused only on a few biological risk markers, such as dyslipidaemia, were based on relatively small sample sizes, or did not explore associations independently of adiposity and insulin. As insulin is known to suppress hepatic production of SHBG and its elevated concentration has been closely associated with dyslipidaemia, hypertension and incident coronary heart disease, it may play a role in explaining the relation between low SHBG and increased CVD risk. Further, testosterone (T) is closely correlated with, and may influence, SHBG concentration. As low T is associated, albeit inconsistently, with insulin resistance, type 2 diabetes, and atherosclerosis, it is therefore unclear if the observed associations between SHBG and CVD risk factors are simply explained by T. We examined the relation between SHBG and risk factors of CVD, independently of T levels, in a population-based cohort of young adult men.
Subjects and Methods

Study population

The Northern Finland Birth Cohort (NFBC) consists of 12,231 unselected births (representing 96.3% of all births) of women from the Finnish provinces of Oulu and Lapland in 1966. The number of children born alive was 12,058 (52% boys). In 1997, we sent a health and lifestyle questionnaire to 97% of the birth cohort (at age 31 years), and invited those living in Northern Finland and in the Helsinki area (n=8,463) for a clinical examination, of whom 6,033 men and women attended and gave fasting blood samples. The current study sample is limited to men (n=2,716) aged 31 years who had given informed consent, had attended clinical examination and had a blood sample taken for measurement of SHBG and testosterone concentrations. The questionnaires provided health and lifestyle data including information on smoking, educational qualification and physical activity. Of those who were invited to attend the clinical examination, we found no statistically significant difference in birth weight (P=0.99) nor in educational attainment (P=0.06) or cigarette smoking habit (P=0.39) at age 31 years between men who attended the clinical examination and those who did not.

The University of Oulu Ethics committee approved the conduct of the study.

Anthropometric and biochemical measurements

We measured blood pressure (average of two separate measurements) taken with a mercury sphygmomanometer in a sitting position after 15 minutes of rest. We obtained anthropometric measurements including weight, height and waist circumference, and calculated body mass index (BMI) as weight/height$^2$. Participants gave blood samples between 0800 and 1100 hours following an overnight fast which were centrifuged immediately and the serum stored at -20°C. Assays were performed within 7 days of the samples being taken. We analysed serum SHBG by immunofluorometric assay (Perkin Elmer-Wallac, Ltd., Turku, Finland) and serum testosterone by an automated chemiluminescence system (ACS-180, Ciba-Corning, Inc., Medfield, MA), and calculated free testosterone (cFT) using Vermeulen’s equation.
analysed fasting insulin by radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden), fasting glucose by glucose dehydrogenase method (Granutest 250, Diagnostica Merck), and high sensitivity C-reactive protein (CRP) by an immunoenzymometric assay (Medix Biochemica, Espoo, Finland). We determined cholesterol (total, high-density lipoprotein [HDL] and low-density lipoprotein [LDL]) and triglycerides using a Hitachi 911 automatic analyser and commercial reagents (Boehringer Mannheim, Germany). Intra- and inter-assay coefficients of variation (CVs) were 1.3% and 5.1% for SHBG, respectively, and 4% and 5.6% for TT, respectively. For insulin, glucose, lipids and CRP, intra-assay CVs did not exceed 5.3% and inter-assay CVs did not exceed 7.6%. The sensitivities of TT and insulin assays were 0.35 nmol/L and 14.4 pmol/L, respectively.

We defined those with metabolic syndrome as having any 3 of the following characteristics: waist circumference ≥94 cm, triglycerides ≥1.7 mmol/L, HDL-cholesterol <1.0 mmol/L, systolic BP ≥130 or diastolic BP ≥85 mmHg, and plasma glucose ≥5.5 mmol/L.

**Statistical methods**

We determined the separate relations between SHBG and an intermediate risk marker of CVD using regression models. We log-transformed triglycerides, insulin and CRP values prior to linear regression analyses. We conducted similar analyses for TT and cFT, as well as analyses that used metabolic syndrome as the outcome variable. To examine systematically the impact of potential confounders, we analysed data by adjusting sequentially for smoking, education and physical activity, then additionally for fasting insulin and adiposity (BMI and waist circumference) in separate models. We also calculated the homeostatic assessment model for insulin resistance (HOMA-IR) and examined the impact of using this parameter, rather than insulin, in our regression models.

To show the relative independent associations of SHBG and TT on cardiovascular risk outcomes, we adjusted these two exposure variables for each other in the multivariate regression models. We also examined the distribution of cardiovascular risk factors across SHBG and TT quartiles based on multivariate-adjusted models to demonstrate the magnitude of cardiovascular risk across the distribution of SHBG and
TT in our study population. Estimates were presented with their standard error or 95% confidence interval (CI), and a P value <0.05 was considered statistically significant. All analyses were conducted using the statistics package Stata 11 MP (College Station, Texas, USA).
Results

In this cohort, mean (standard deviation [SD]) SHBG, TT and calculated cFT were 33.3 (SD=13.3) nmol/L, 21.6 (SD=6.5) nmol/L, and 481 (SD=144) pmol/L, respectively. Mean BMI was 25.2 (SD=3.6) kg/m² and 8.4% of men were obese (BMI ≥30 kg/m²). Table 1 shows the distribution of characteristics across quartiles of SHBG. Higher SHBG quartile was associated higher HDL-cholesterol but with lower blood pressure, total cholesterol, LDL-cholesterol, triglycerides, fasting levels of plasma glucose and insulin, HOMA-IR index, and CRP. There was no clear variation in smoking, educational attainment and level of physical activity, but those at the top quartile of SHBG had the highest proportions of current smokers whereas those at the bottom quartile had the highest proportion with low physical activity level. The correlation of SHBG with TT and cFT were 0.59 (P<0.001) and -0.06 (P=0.004), respectively and the BMI-adjusted partial correlations were 0.53 (P<0.001) and -0.09 (p<0.001), respectively.

Sex hormone-binding globulin, testosterone and the metabolic syndrome

We first examined the relation of SHBG and TT with metabolic syndrome as the outcome variable (Table 2). The odds ratio for metabolic syndrome decreased with increasing SHBG independently of other factors including TT, with over 46% reduced risk associated with 10.6 nmol/L increase in SHBG concentration. While the risk for metabolic syndrome decreased with increasing TT, this relation did not persist when taking BMI into account. However, with additional adjustment for SHBG, the risk for metabolic syndrome increased with higher TT, with over 38% excess risk associated with 5.2 nmol/L increase in TT concentration. Thus, when mutually adjusting for each other (and taking into account other potential confounding factors), SHBG and TT showed independent and opposing associations with metabolic syndrome. Adjusting for smoking, education, physical activity and body mass index, the odds ratios (95% CI) from the second to the fourth quartile of cFT, as compared to the lowest quartile, were 1.31 (0.89 to 1.92), 1.51 (1.04 to 2.20) and 2.47 (1.71 to 3.55), respectively, with 34% excess risk associated with 110 pmol/L increase in cFT concentration.
Sex hormone-binding globulin and cardiovascular risk factors

Table 3 shows the association between SHBG and cardiovascular risk factors. Adjusting for smoking, education, physical activity and fasting insulin, increasing SHBG concentration was significantly associated with decreasing blood pressure, triglycerides and CRP as well as with increasing HDL-cholesterol (all P for trend <0.001). These associations were attenuated with additional adjustment for BMI and waist circumference but the relation remained significant except for total and LDL-cholesterol and systolic blood pressure. With further adjustment for TT, the association persisted, with increasing SHBG associated with decreasing blood pressure, total cholesterol, triglycerides and CRP, and increasing HDL-cholesterol (all P for trend <0.05). SHBG was unrelated to LDL-cholesterol in models that adjusted for insulin, adiposity or TT.

Findings were similar when we used HOMA-IR, instead of insulin, as a covariate in the regression model for each risk factor being studied.

Figures 1 and 2 show the relative difference in blood pressure and lipids across the whole SHBG gradient taking into account TT and other covariates (full values are shown in the online supplementary Table S1). The relation of SHBG with these cardiovascular risk factors, independently of TT, tended to be graded and linear (except for LDL-cholesterol). The online supplementary Table S1 also shows the magnitude of the difference in risk. For example, those in the top fourths of SHBG distribution had lower systolic blood pressure by 2.8 (95% CI 1.0 to 4.5) mmHg, diastolic blood pressure by 3.7 (2.1 to 5.2) mmHg, and total cholesterol by 0.16 (95% CI 0.03 to 0.30) mmol/L as compared to those at the bottom fourths.

Testosterone and cardiovascular risk factors

After adjusting for smoking, education, physical activity and fasting insulin, increasing TT was significantly associated with decreasing blood pressure, triglycerides and CRP, and increasing HDL-cholesterol as shown on Table 4 (all P for trend <0.05). Adjusting for insulin and adiposity attenuated the associations, but further adjustment for SHBG changed the direction of the slope of the association between TT and the outcome measures (except for CRP). With adjustment for SHBG, increasing TT was
associated with increasing blood pressure, total and LDL-cholesterol, and triglycerides as well as with decreasing CRP (all P for trend <0.05); but the relation with HDL-cholesterol was no longer significant. Findings were similar after substituting insulin with HOMA-IR in the regression model for all cardiovascular risk factors considered in this study.

When fasting insulin was used as an outcome variable, higher TT was associated with lower fasting insulin levels even after adjusting for adiposity and other covariates (P=0.007), but this association did not persist when SHBG was taken into account (P>0.05).

Figures 1 and 2 show the relative difference in blood pressure and lipids across the whole gradient of TT after adjusting for SHBG and other covariates (full values are shown in the online supplementary Table S2). The relation of TT with these cardiovascular risk factors tended to be graded and linear (except for HDL-cholesterol). The online supplementary Table S2 also shows the magnitude of the difference in risk. For example, those in the top fourths of TT distribution had higher systolic blood pressure by 0.7 (95% CI 0.2 to 1.2) mmHg, diastolic blood pressure by 0.8 (95% CI 0.3 to 1.2) mmHg, and total cholesterol by 0.12 (95% CI 0.08 to 0.16) mmol/L as compared to those in the bottom fourths.

After adjusting for smoking, education, physical activity, fasting insulin, body mass index and waist circumference, increasing cFT was significantly and positively related to blood pressure, total cholesterol and triglycerides (online supplementary Table S3). The direction and magnitude of the associations with the various risk factors were similar to those of covariate- and SHBG-adjusted associations for TT, except for CRP wherein the magnitude of the reduction in CRP was twice bigger than the association seen for cFT. For example, for one quartile increase in TT (5.2 nmol/L) and cFT (110 pmol/L) were associated with an increase in systolic blood pressure by 0.7 (95% CI 0.2 to 1.2) and 0.6 (0.2 to 1.1), respectively; and an increase in cholesterol by 0.12 (95% CI 0.08 to 0.16) and 0.10 (0.07 to 0.14), respectively.
Discussion

In this cohort of young adult men, increasing serum SHBG concentration was generally associated with a better cardiovascular risk profile. Higher SHBG concentration was associated with lower blood pressure, cholesterol, triglycerides and CRP as well as higher HDL-cholesterol, independently of TT, insulin, adiposity and other potential confounding factors. The magnitude of the association varied across CVD risk factors but the associations were largely linear across a wide range of SHBG concentration in this population. Our findings suggest that SHBG may primarily play a role in the cardiovascular health of young men through a number of mediating biological risk factors.

Many studies have previously investigated the separate relation of SHBG with different CVD risk factors ([online supplementary Tables S4 and S5](#)) but findings in men, particularly of sufficient sample size, remain relatively limited (few studies had N>1,000). Considering that CVD can involve a number of biological pathways, few studies considered examining a number of risk factors from within a single cohort.\(^{15, 19, 35-37}\) Many of the earlier studies have also not accounted for the potential confounding effects of adiposity and insulin. Considering that SHBG and TT concentrations co-vary with one other, it is unclear if findings for SHBG are simply explained by TT. Our study is based on a relatively large sample of young men with a very narrow age range (same year of birth). We examined associations across a number of classical and non-classical risk factors of CVD, and systematically took into account important covariates, such as adiposity and insulin, as well as TT. Our results persisted even when we substituted insulin with HOMA-IR as a covariate in our analyses.

Over 40% of TT in the circulation is bound with strong affinity to SHBG in men and most of the remaining T is loosely bound to albumin, with only 1 to 2% of circulating as biologically active FT.\(^{38}\) However, direct measures of FT from blood samples for large cohorts is not feasible to do, so it is only estimated from simple, quantitative methods based on the law of mass action.\(^{32}\) Thus, cFT has been suggested to provide an estimate of the biologically active form of T with reasonable accuracy.\(^{38}\) Because SHBG has been traditionally seen to only have a passive role as a carrier protein, and that TT correlates with
and explains over 80% of the variance of FT,\textsuperscript{39} TT is widely used in many studies that examine the
importance of T on health in population-based studies.

However, SHBG could have a biological function on its own other than simply to regulate levels of
the biologically active fraction of T.\textsuperscript{4} As our findings suggest, it may be involved in determining
cardiovascular risk although the underlying mechanism is unclear. As the relation of SHBG with CVD risk
factors was independent of T, other pathways could be involved. A study that identified genetic variants
associated with SHBG concentration indicates that these genes are likely to be involved in various
metabolic pathways, such as lipid and carbohydrate metabolism, liver function and type 2 diabetes.\textsuperscript{40} It is
possible that the association between SHBG and CVD risk factors may have nothing to do with the role of
SHBG as T carrier; it responds to a number of metabolic alterations and its concentration may reflect these
metabolic perturbations.

Our study has also shown interesting findings for T. Although it has been suggested to play direct
and indirect role in lipid metabolism, blood pressure regulation and immunomodulation,\textsuperscript{41-47} reports on the
relation between T and CVD or its risk factors have been inconsistent,\textsuperscript{41, 48-50} and possibly confounded.\textsuperscript{51} The
conflicting findings particularly between endogenous T and CVD risk factors could stem from the fact that
many studies used the biologically inactive rather than the bioavailable fraction of T (without accounting
for the potential influence of SHBG and other potential confounders). In our study, we demonstrated the
change in the magnitude and direction of the association of TT with cardiovascular risk factors (except for
HDL-cholesterol and CRP) by systematically taking into account various lifestyle factors, insulin, adiposity
and SHBG. Because SHBG regulates the availability of the active fraction of T, the SHBG-adjusted
associations for TT could be indicative of the effects of biologically active FT (as shown by the results for
cFT). Nevertheless, age might modify the association between T and CVD risk, which seems to suggest that
higher T could be associated with increased CVD risk in younger men, as we have observed in our cohort,
but potentially the reverse is true in older men.\textsuperscript{48} It has been shown that ageing and subclinical chronic
diseases that are prevalent in older men are associated with reduced T levels.\textsuperscript{52} Thus, low T may indicate
poor underlying health status and may be predictive of adverse health risks in older men, but the reverse may be the case in young men.

Interestingly, TT was independently but inversely related with CRP, an association which differed from that of other biological risk markers. This relation might reflect the effect of androgens in inhibiting the synthesis of pro-inflammatory cytokines, including tumour necrosis factor-α and interleukin-6, which may consequently attenuate the production of downstream markers of inflammation such as CRP.

The recent identification of genetic markers associated with circulating levels of TT found in the SHBG locus could shed light on the nature of the association between TT and CVD risk factors. In a recent study, the association between lower TT and CVD risk factors have been investigated using two different analytical approaches. Based on multivariate regression models (but without adjusting for SHBG, adiposity and insulin as we did in our study), low TT was associated with a more adverse CVD risk profile. In contrast, when using genetic variants of TT as instruments in an instrumental variable analysis (Mendelian randomization), most of the associations weakened in magnitude and were no longer significant, with the slope of the associations possibly changing in their direction. For example, the regression analysis results showed a significant inverse relation between TT and systolic blood pressure; in contrast, the instrumental variable analyses suggested a significant positive relation between TT and systolic blood pressure. Such observations were similar to our findings when we adjusted for various covariates including SHBG (although in their analyses they did not adjust for insulin and adiposity).

The cross-sectional design of this study does not allow us to demonstrate the temporal nature of the associations, and a longitudinal study is warranted. Our findings need to be evaluated across a wider age range as ageing is related to an increase in SHBG concentration, a fall in androgen levels and a rise in CVD absolute rates. It has been suggested that specific genotypes might influence binding affinity of SHBG with T and thereby affect calculation of FT based on the law of mass action, as used in the Vermeulen equation (which is essentially the same as statistically adjusting for SHBG in a regression model).

However, the frequency of the allele that has been shown to influence the SHBG binding with T is rare (around 2%). Within a population perspective, our findings for endogenous TT, when adjusted for SHBG,
could still reflect the effects of the biologically active fraction of T. Nevertheless, we could not rule out another factor that might underlie the modulation of both SHBG and TT levels and impact on cardiovascular risk at the same time. Future work may require using the principles of Mendelian inheritance by using genetic variants of SHBG as randomization instruments\textsuperscript{10, 59} to enable an unbiased examination of the nature of the relation of SHBG with CVD risk factors and with the development of CVD endpoints.

In this cohort of young men, increasing SHBG concentration was associated with improving CVD risk profile independently of TT and other factors, suggesting that SHBG may play a role in the cardiovascular health of young men through a number of mediating biological risk factors. Further, SHBG modified the relation between TT and CVD risk factors. Understanding the role of SHBG in these different pathways might provide clues to the biological basis for explaining the relation of SHBG and CVD risk factors.

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Disclosure statement: All authors have nothing to declare.

Author contribution:
MRJ, ALH, AP, HM and AR were involved in the conception, design and data acquisition for the Northern Finland Birth Cohort 1966 study. DC analysed the data, and drafted the initial version of the manuscript. HM, ITH, TMB, JST, SF, MIM, MRJ, AP and ALH gave critical intellectual input and contributed in revising subsequent versions of the manuscript. All authors gave their final approval of the version to be published.
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539  **Legend:**
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541  **Figure 1.** Relative difference (β coefficient [95% confidence interval]) in systolic and diastolic blood pressure
542 according to quartiles of sex hormone-binding globulin (SHBG) or total testosterone in 2,716 men in the
543 Northern Finland Birth Cohort 1966 study. Values are adjusted for smoking, education, physical activity,
544 fasting insulin, body mass index, waist circumference, and either total testosterone or SHBG. P values
545 indicate trends across quartile categories. Coefficients indicate that blood pressure for that group could be
546 higher (positive values) or lower (negative values) as compared to the mean blood pressure of the
547 reference group (quartile 1).
Figure 2. Relative difference (β coefficient [95% confidence interval]) in total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol according to quartiles of sex hormone-binding globulin (SHBG) or total testosterone in 2,716 men in the Northern Finland Birth Cohort 1966 study. Values are adjusted for smoking, education, physical activity, fasting insulin, body mass index, waist circumference, and either total testosterone or SHBG. P values indicate trends across quartile categories. Coefficients indicate that cholesterol for that group could be higher (positive values) or lower (negative values) as compared to the mean cholesterol of the reference group (quartile 1).
Table 1. Characteristics of men aged 31 years in the NFBC 1966 study by sex hormone-binding globulin concentration.

<table>
<thead>
<tr>
<th>Variables</th>
<th>&lt;24.1</th>
<th>24.1 – 31.5</th>
<th>31.6 – 40.4</th>
<th>&gt;40.4</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHBG (nmol/L)</td>
<td>18.6 (3.9)</td>
<td>27.8 (2.2)</td>
<td>35.7 (2.6)</td>
<td>51.4 (10.2)</td>
<td>33.3 (13.3)</td>
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<tr>
<td>Total testosterone (nmol/L)</td>
<td>16.8 (5.4)</td>
<td>20.2 (4.6)</td>
<td>22.6 (5.0)</td>
<td>26.8 (6.3)</td>
<td>21.6 (6.5)</td>
</tr>
<tr>
<td>Free testosterone (pmol/L)</td>
<td>478 (182)</td>
<td>490 (130)</td>
<td>486 (128)</td>
<td>469 (130)</td>
<td>481 (144)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.7 (4.0)</td>
<td>25.5 (3.0)</td>
<td>24.2 (2.9)</td>
<td>23.5 (2.9)</td>
<td>25.2 (3.6)</td>
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<tr>
<td>Waist circumference (cm)</td>
<td>95.9 (10.4)</td>
<td>89.8 (8.3)</td>
<td>86.2 (8.1)</td>
<td>83.9 (7.9)</td>
<td>88.9 (9.8)</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>134.6 (13.2)</td>
<td>131.5 (12.9)</td>
<td>129.0 (12.5)</td>
<td>128.5 (12.9)</td>
<td>130.9 (13.1)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>85.3 (12.3)</td>
<td>80.9 (10.8)</td>
<td>79.4 (11.4)</td>
<td>78.1 (11.5)</td>
<td>80.9 (11.8)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.41 (1.04)</td>
<td>5.19 (1.02)</td>
<td>5.12 (1.00)</td>
<td>5.15 (0.97)</td>
<td>5.22 (1.01)</td>
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<td>LDL-cholesterol (mmol/L)</td>
<td>3.37 (0.89)</td>
<td>3.23 (0.89)</td>
<td>3.17 (0.89)</td>
<td>3.17 (0.91)</td>
<td>3.23 (0.90)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.28 (0.30)</td>
<td>1.39 (0.28)</td>
<td>1.45 (0.33)</td>
<td>1.53 (0.35)</td>
<td>1.41 (0.33)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.52 (1.18)</td>
<td>1.14 (0.69)</td>
<td>0.98 (0.60)</td>
<td>0.91 (0.53)</td>
<td>1.11 (1.77)</td>
</tr>
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<td>Plasma glucose (mmol/L)</td>
<td>5.24 (0.59)</td>
<td>5.17 (0.44)</td>
<td>5.16 (0.70)</td>
<td>5.12 (0.63)</td>
<td>5.17 (0.60)</td>
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<td>Serum insulin (pmol/L)</td>
<td>70.3 (34.4)</td>
<td>56.9 (23.0)</td>
<td>51.7 (20.8)</td>
<td>48.8 (17.9)</td>
<td>56.0 (25.1)</td>
</tr>
<tr>
<td>HOMA-IR index</td>
<td>2.69 (1.65)</td>
<td>1.99 (0.91)</td>
<td>1.77 (0.69)</td>
<td>1.70 (1.02)</td>
<td>2.04 (1.19)</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>1.00 (1.70)</td>
<td>0.70 (1.10)</td>
<td>0.60 (0.90)</td>
<td>0.50 (0.80)</td>
<td>0.70 (1.2)</td>
</tr>
<tr>
<td>% Current smokers (n)</td>
<td>30.0 (202)</td>
<td>27.0 (181)</td>
<td>33.9 (226)</td>
<td>40.4 (272)</td>
<td>32.8 (881)</td>
</tr>
<tr>
<td>% Low educational attainment* (n)</td>
<td>17.6 (120)</td>
<td>16.6 (113)</td>
<td>17.6 (119)</td>
<td>18.4 (125)</td>
<td>17.6 (477)</td>
</tr>
<tr>
<td>% Low physical activity level†(n)</td>
<td>63.3 (432)</td>
<td>55.1 (374)</td>
<td>56.1 (380)</td>
<td>54.0 (366)</td>
<td>57.1 (1,552)</td>
</tr>
<tr>
<td>% Metabolic syndrome‡ (n)</td>
<td>36.4 (248)</td>
<td>12.5 (85)</td>
<td>8.4 (57)</td>
<td>5.3 (36)</td>
<td>15.7 (426)</td>
</tr>
</tbody>
</table>

NFBC – Northern Finland Birth Cohort; SHBG – sex hormone-binding globulin; LDL – low-density lipoprotein; HDL – high-density lipoprotein; HOMA-IR – homeostasis model assessment-estimated insulin resistance; Data presented as mean (standard deviation) for continuous variables except for triglycerides, insulin and C-reactive protein which are presented as median (25th to 75th percentile range), and percentage for categorical variables; *No degree (university, secondary/polytechnic, vocational) or unfinished education; †Less than twice a week of brisk physical activity of at least 30 minutes; ‡With any three of the following features: waist circumference ≥94 cm; triglycerides ≥1.7 mmol/L, HDL-cholesterol <1.0 mmol/L, systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg, and plasma glucose ≥5.56 mmol/L.
<table>
<thead>
<tr>
<th>SHBG (nmol/L)</th>
<th>% with metabolic syndrome* (N)</th>
<th>Adjusted for smoking, education &amp; physical activity</th>
<th>Additionally adjusted for body mass index</th>
<th>Additionally adjusted for SHBG or total testosterone†</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;24.1 (N=682)</td>
<td>36.4 (248)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>24.1 to 31.5 (N=679)</td>
<td>12.5 (85)</td>
<td>0.25 (0.19 to 0.33)</td>
<td>0.41 (0.30 to 0.56)</td>
<td>0.33 (0.24 to 0.47)</td>
</tr>
<tr>
<td>31.6 to 40.4 (N=677)</td>
<td>8.4 (57)</td>
<td>0.15 (0.11 to 0.21)</td>
<td>0.39 (0.28 to 0.57)</td>
<td>0.28 (0.19 to 0.42)</td>
</tr>
<tr>
<td>&gt;40.4 (N=678)</td>
<td>5.3 (36)</td>
<td>0.09 (0.06 to 0.13)</td>
<td>0.29 (0.19 to 0.45)</td>
<td>0.16 (0.10 to 0.27)</td>
</tr>
<tr>
<td>All (per 10.6 nmol/L)</td>
<td>15.7 (426)</td>
<td>0.42 (0.37 to 0.47)</td>
<td>0.65 (0.57 to 0.74)</td>
<td>0.54 (0.46 to 0.63)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total testosterone (nmol/L)</th>
<th>% with metabolic syndrome* (N)</th>
<th>Adjusted for smoking, education &amp; physical activity</th>
<th>Additionally adjusted for body mass index</th>
<th>Additionally adjusted for SHBG or total testosterone†</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;17.4 (N=687)</td>
<td>23.3 (160)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>17.4 to 21.9 (N=678)</td>
<td>18.0 (122)</td>
<td>0.70 (0.53 to 0.92)</td>
<td>1.46 (1.05 to 2.03)</td>
<td>2.01 (1.42 to 2.83)</td>
</tr>
<tr>
<td>21.1 to 25.6 (N=674)</td>
<td>11.3 (76)</td>
<td>0.40 (0.30 to 0.55)</td>
<td>0.93 (0.65 to 1.34)</td>
<td>1.67 (1.12 to 2.48)</td>
</tr>
<tr>
<td>&gt;25.6 (N=677)</td>
<td>10.0 (68)</td>
<td>0.34 (0.25 to 0.46)</td>
<td>1.13 (0.77 to 1.66)</td>
<td>3.17 (2.06 to 5.03)</td>
</tr>
<tr>
<td>All (per 5.2 nmol/L)</td>
<td>15.7 (426)</td>
<td>0.68 (0.62 to 0.77)</td>
<td>1.00 (0.88 to 1.12)‡</td>
<td>1.38 (1.20 to 1.60)‡</td>
</tr>
</tbody>
</table>

All P for trend <0.001 except ‡P>0.05; *With any three of the following features: waist circumference ≥94 cm; triglycerides ≥1.7 mmol/L, HDL-cholesterol <1.0 mmol/L, systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg, and plasma glucose ≥5.56 mmol/L; †SHBG and total testosterone mutually adjusted for each other in the model.
**Table 3.** Relative difference (β coefficient [95% confidence interval]) in cardiovascular risk markers per 10.6 nmol/L higher sex hormone-binding globulin in 2,716 men aged 31 years in the NFBC 1966 study.

<table>
<thead>
<tr>
<th>Cardiovascular risk markers</th>
<th>Adjusted for smoking, education &amp; physical activity</th>
<th>Additionally adjusted for fasting insulin</th>
<th>Additionally adjusted for BMI &amp; waist circumference</th>
<th>Additionally adjusted for total testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>-2.1 (-2.6 to -1.7)†</td>
<td>-1.4 (-1.9 to -1.0)†</td>
<td>-0.5 (-0.9 to 0.0)</td>
<td>-1.0 (-1.5 to -0.4)‡</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>-2.3 (-2.7 to -1.9)†</td>
<td>-1.5 (-2.0 to -1.1)†</td>
<td>-0.6 (-1.1 to -0.2)‡</td>
<td>-1.1 (-1.6 to -0.6)†</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>-0.09 (-0.12 to -0.05)†</td>
<td>-0.03 (-0.07 to 0.00)</td>
<td>0.02 (-0.02 to 0.05)</td>
<td>-0.05 (-0.09 to -0.01)‡</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>-0.06 (-0.10 to -0.03)†</td>
<td>-0.03 (-0.06 to 0.00)</td>
<td>0.02 (-0.01 to 0.06)</td>
<td>0.00 (-0.04 to 0.04)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>0.08 (0.07 to 0.09)†</td>
<td>0.07 (0.06 to 0.08)†</td>
<td>0.05 (0.04 to 0.06)†</td>
<td>0.04 (0.03 to 0.05)†</td>
</tr>
<tr>
<td>Log-triglycerides (mmol/L)</td>
<td>-0.17 (-0.18 to -0.15)†</td>
<td>-0.12 (-0.14 to -0.11)†</td>
<td>-0.09 (-0.11 to -0.07)†</td>
<td>-0.15 (-0.17 to -0.13)†</td>
</tr>
<tr>
<td>Log-C-reactive protein (mg/L)</td>
<td>-0.32 (-0.38 to -0.27)†</td>
<td>-0.25 (-0.30 to -0.19)†</td>
<td>-0.13 (-0.20 to -0.07)†</td>
<td>-0.08 (-0.16 to -0.01)‡</td>
</tr>
</tbody>
</table>

CI – confidence interval; NFBC – Northern Finland Birth Cohort; LDL – low-density lipoprotein; HDL – high-density lipoprotein; Log – natural logarithmic transformation; All P>0.05 except: †P<0.001, ‡P<0.05.
Table 4. Relative difference (β coefficient [95% confidence interval]) in cardiovascular risk markers per 5.2 nmol/L higher total testosterone in 2,716 men aged 31 years in the NFBC 1966 study.

<table>
<thead>
<tr>
<th>Cardiovascular risk markers</th>
<th>Adjusted for smoking, education &amp; physical activity</th>
<th>Additionally adjusted for fasting insulin</th>
<th>Additionally adjusted for BMI &amp; waist circumference</th>
<th>Additionally adjusted for SHBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>-0.8 (-1.3 to -0.4)†</td>
<td>-0.3 (-0.8 to 0.1)</td>
<td>0.4 (-0.1 to 0.9)</td>
<td>0.7 (0.2 to 1.2)‡</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>-0.8 (-1.2 to -0.4)†</td>
<td>-0.3 (-0.7 to 0.1)</td>
<td>0.4 (-0.0 to 0.8)</td>
<td>0.8 (0.3 to 1.2)‡</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>0.03 (-0.01 to 0.06)</td>
<td>0.06 (0.03 to 0.10)†</td>
<td>0.10 (0.07 to 0.14)†</td>
<td>0.12 (0.08 to 0.16)†</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>-0.01 (-0.04 to 0.02)</td>
<td>0.01 (-0.02 to 0.04)</td>
<td>0.05 (0.02 to 0.08)†</td>
<td>0.04 (0.01 to 0.08)‡</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>0.06 (0.05 to 0.07)†</td>
<td>0.05 (0.04 to 0.06)†</td>
<td>0.03 (0.02 to 0.04)†</td>
<td>0.01 (-0.00 to 0.02)</td>
</tr>
<tr>
<td>Log-triglycerides (mmol/L)</td>
<td>-0.03 (-0.05 to -0.01)‡</td>
<td>0.01 (-0.01 to 0.02)</td>
<td>0.04 (0.03 to 0.06)†</td>
<td>0.11 (0.09 to 0.12)†</td>
</tr>
<tr>
<td>Log-C-reactive protein (mg/L)</td>
<td>-0.28 (-0.33 to -0.22)‡</td>
<td>-0.22 (-0.28 to -0.16)†</td>
<td>-0.13 (-0.19 to -0.07)†</td>
<td>-0.10 (-0.17 to -0.03)‡</td>
</tr>
</tbody>
</table>

NFBC – Northern Finland Birth Cohort; SHBG – sex hormone-binding globulin; LDL – low-density lipoprotein; HDL – high-density lipoprotein; Log – natural logarithmic transformation; All P>0.05 except: †P<0.001, ‡P<0.05.
Figure 1
Figure 2

- Total testosterone
- SHBG

Lipid concentration (mmol/L)

- Total cholesterol
- LDL-cholesterol
- HDL-cholesterol

P-values:
- P > 0.05
- P < 0.001
- P = 0.019
- P = 0.029