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Testosterone, sex hormone-binding globulin and risk of cardiovascular events: A report from the Outcome Reduction with an Initial Glargine Intervention trial

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Abstract

Aims: Testosterone and its binding protein sex hormone-binding globulin have been associated with cardiovascular disease and dysglycaemia. However, information on the prognostic implication in patients at high cardiovascular risk with dysglycaemia is inconsistent. The study objective was to determine whether testosterone and/or sex hormone-binding globulin predict cardiovascular events or death in dysglycaemic patients.

Methods: Dysglycaemic males at high cardiovascular risk ($n = 5553$) who participated in the Outcome Reduction with an Initial Glargine Intervention (ORIGIN) trial and provided baseline blood samples were studied. Testosterone and sex hormone-binding globulin were measured at baseline and used to estimate free testosterone. Low levels of total and free testosterone were defined as $\leq$300 ng/dl and $\leq$7 ng/dl, respectively. Patients were followed for six years for cardiovascular events (defined as the composite of cardiovascular death, non-fatal myocardial infarction or stroke) and all-cause mortality.

Results: The mean total and free testosterone levels were 416.6 ng/dl and 8.4 ng/dl, and low levels were present in 13% and 37% of the patients. The median sex hormone-binding globulin level was 35 nmol/l. In Cox regression models adjusted for age, previous diseases and pharmacological treatment, neither total nor free testosterone predicted cardiovascular events. However, a one-standard-deviation increase in sex hormone-binding globulin predicted both cardiovascular events (hazard ratio 1.07; 95% confidence interval 1.00–1.14; $p = 0.03$) and all-cause mortality (hazard ratio 1.13; 95% confidence interval 1.06–1.21; $p < 0.01$).

Conclusion: Sex hormone-binding globulin, but not total testosterone, predicts cardiovascular disease and all-cause mortality in dysglycaemic males at high cardiovascular risk.

Keywords

Testosterone, sex hormone-binding globulin, cardiovascular, prognosis, diabetes, glucose intolerance

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Introduction

People with type 2 diabetes mellitus (T2DM) are more prone to develop cardiovascular disease (CVD) and their prognosis is more dismal than that of people without T2DM.[1,2] The reasons for this increased risk are not fully understood. In men, a subnormal testosterone level has been suggested as a potential explanation.

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Indeed, low free and total testosterone levels have been reported in up to 40% of men with diabetes,[3,4] and sex hormone-binding globulin (SHBG), which binds testosterone in plasma, predicts diabetes and the metabolic syndrome in men.[5] Moreover, men with angiographically confirmed coronary artery disease have lower testosterone levels compared to those with normal coronary arteries.[6] These observations together with an inverse correlation between testosterone levels and cardiovascular outcomes in several population-based, observational male cohorts with or without diabetes[7–9] suggest that low testosterone may be a particularly important cardiovascular risk marker in men with dysglycaemia.

The Outcome Reduction with an Initial Glargine Intervention (ORIGIN) trial[10] recruited and followed 12,537 dysglycaemic people for the development of cardiovascular outcomes over a period of six years. The availability of baseline testosterone and SHBG levels in a subset of men in this study made it possible to assess the relationship between total testosterone, free testosterone and SHBG on incident cardiovascular events in dysglycaemic men.

Methods

The design, patient population and main results of the ORIGIN trial have been described in detail elsewhere.[10,11] In brief the participants were men (65%) and women (35%) at high cardiovascular risk with either impaired fasting glucose, impaired glucose tolerance, newly detected diabetes or established diabetes on stable therapy with 0–1 oral agents. Exclusion criteria for study participation were: serious comorbid conditions e.g. active cancer, hepatic cirrhosis, chronic or recurrent treatment with systemic corticosteroids or an expected survival of <3 years for non-cardiovascular causes. Other conditions that may influence testosterone and/or SHBG were uncommon. The proportion of participants in the biomarker subpopulation who ever had been diagnosed or treated for obstructive sleep apnoea was 4.4% and 3.0% were on thyroid-hormone treatment at baseline. Only 0.9% had a thyroid-stimulating hormone level of 0.1–0.5 mIU/l, i.e. indicating mild hyperthyroidism. Only a small proportion reported consumption of >2 drinks of alcohol/week (22.7%).

The mean age of the patient population was 63.5 years. CVD risk was defined as confirmation of at least one of (a) prior myocardial infarction, stroke or revascularization; (b) angina pectoris with documented myocardial ischaemia; (c) a morning urinary albumin/creatinine ratio >30 μg/mg; (d) evidence of left ventricular hypertrophy; (e) angiographically confirmed ≥50% stenosis of a coronary, carotid or lower extremity artery; or (f) an ankle/brachial index <0.9.[11] Participants were randomly assigned to either insulin glargine (Gla-100) targeting a fasting plasma glucose ≤95 mg/dl (5.3 mmol/l) or standard care and, in a 2 × 2 factorial design, omega 3 fatty acids or placebo. During a median follow-up of 6.2 years, the incidence of the primary outcome (described below) and all-cause mortality did not differ between the groups.

A subset of the ORIGIN participants including both male and females ($n = 8494$) consented to the storage of blood samples for future analyses.[12] The present study comprises all males from this subset ($n = 5553$) in whom blood samples were available and assayed for total testosterone and SHBG.

The ORIGIN trial was approved by the ethics committee of each site and all participants provided written informed consent.

Definitions

Free testosterone was calculated based on the Vermeulen formula, using total testosterone and SHBG levels together with a fixed albumin concentration of 43 g/l.[13] Low levels of testosterone were defined as total testosterone ≤300 ng/dl (≈10.4 nmol/l) and free testosterone ≤7 ng/dl, respectively.[14] Low SHBG was defined as those with levels at or below the median (35 nmol/l).

Outcomes

The primary outcome for these analyses was a composite of cardiovascular events, including death from cardiovascular causes, non-fatal myocardial infarction or non-fatal stroke and an expanded primary outcome also included a revascularization process and heart failure hospitalization. The secondary endpoint was all-cause mortality. Outcomes were determined by an adjudication committee based on information provided at every visit together with supporting documentation.[11]

Laboratory analyses

Blood samples obtained after an overnight fast from the baseline visit were divided into aliquots and stored in nitrogen vapour-cooled tanks at –160°C. None of the participants were on insulin when the baseline samples were obtained. As previously reported, following completion of the ORIGIN trial, coded aliquots of serum from each study participant with no other identifying information were transported to Myriad RBM Inc. (Austin, Texas, USA) for blinded multiplex analysis of a prespecified panel of 284 biomarkers. After careful, blinded scrutiny of the results, a total of 237 biomarkers from 8401 study subjects were available for analysis.[12] These included total testosterone

and SHBG, which had inter-run coefficients of variation of 7% and 14%, respectively.[12]

Statistical analyses

Continuous variables were presented as mean and standard deviation (SD) or median and interquartile range (IQR) and categorical variables were presented as numbers and percentages. Comparisons between the SHBG groups for the total and free testosterones were assessed using *t*-tests. Kaplan-Meier curves were constructed to depict the relation between the categorical levels of testosterone and SHBG with cardiovascular events and all-cause mortality. Hazard ratios (HRs) and their 95% confidence intervals (CIs) for the relationship between the two endpoints and either total testosterone, free testosterone and SHBG were estimated using Cox proportional hazard regression models. These models were adjusted for age (Model A) and age, luteinizing hormone (LH) levels, previous CVD, previous diabetes, use of metformin, use of statins, systolic blood pressure, glycosylated haemoglobin (HbA1c), low-density lipoprotein (LDL) cholesterol, body mass index and smoking (Model B). Whether the estimates differed according to allocated treatment group was assessed by testing for interactions. Hazard ratios for an increase by one SD were estimated for continuous variables and for higher vs lower levels for categorical variables. The nominal level of significance for all analyses was a two-sided p-value < 0.05. All statistical analyses were carried out using SAS version 9.2 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Clinical characteristics

In total, 5553 men were included. Clinical and biochemical characteristics for the study cohort are outlined in Table 1. The mean age was 63.5 years, 80% had previously established diabetes and there was a high prevalence of previous CVD (68%), prior myocardial infarction (43%) and hypertension (76%). Most frequently prescribed pharmacological treatments were statins, ACE/ARB (angiotensin-converting enzyme/angiotensin receptor II blocker) inhibitors and aspirin.

The mean (SD) of total and free testosterone levels were 416.6 (109.2) ng/dl and 8.4 (3.2) ng/dl, respectively, with 13% and 37% having low levels. The median (IQR) SHBG was 35 (25–47) nmol/l and individuals with SHBG levels above the median value had a lower free testosterone level (6.7 vs 10.0 ng/dl; $p < 0.001$) and higher LH level (3.0 vs 2.5 mIU/ml; $p < 0.001$) (data not shown).

Table 1. Baseline characteristics of the study participants. Data are presented as either *n* (%) or mean (standard deviation) unless otherwise indicated.

	All participants $n = 5553$
Clinical characteristics	
Age (years)	63.5 (7.9)
Known diabetes	4441 (80.0)
Newly detected diabetes	400 (7.2)
Newly detected IGT/IFG	710 (12.8)
Diabetes duration (years)	5.2 (5.8)
Prior cardiovascular disease	3763 (67.8)
Myocardial infarction	2397 (43.2)
Hypertension	4219 (76.0)
Smoker	754 (13.6)
Thyroid hormone treatment	167 (3%)
Current alcohol consumption >2 drinks/week	1260 (22.7)
Pharmacological treatment	
Metformin	1431 (25.8)
Sulfonylurea	1613 (29.0)
Other glucose lowering drug	118 (2.1)
No glucose lowering drug	2394 (43.1)
Statin	3375 (60.8)
ACE/ARB inhibitors	3836 (69.1)
Beta-blockers	3160 (56.9)
Thiazide diuretics	853 (15.4)
Aspirin	3941 (71.0)
Other antiplatelet drugs	855 (15.4)
Laboratory findings at baseline	
eGFR	79.0 (21.4)
Body weight (kg)	87.7 (16.2)
BMI (kg/m^2)	29.6 (4.7)
Systolic blood pressure (mm Hg)	145.2 (21.2)
HbA1C (%)	6.5 (0.9)
Cholesterol (mmol/l)	4.7 (1.1)
HDL cholesterol (mmol/l)	1.1 (0.3)
LDL cholesterol (mmol/l)	2.8 (1.00)
Triglycerides (mmol/l)	1.9 (1.3)
Total testosterone (ng/dl)	416.6 (109.2)
Low total testosterone	701(12.6)
Free testosterone (ng/dl)	8.4 (3.2)
Low free testosterone	2035 (36.6)
LH (mIU/ml)	2.8 (1.6)
SHBG (nmol/l)[a]	35 (25–47)

ACE/ARB: angiotensin-converting enzyme/angiotensin receptor II blocker; BMI: body mass index; eGFR: estimated glomerular filtration rate; HbA1c: glycated haemoglobin; HDL: high-density lipoprotein; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; LDL: low-density lipoprotein; LH: luteinizing hormone; SHBG: sex hormone-binding globulin.

[a]The median (interquartile range) is shown.

Testosterone, SHBG and prognosis

During a median follow-up of 6.2 years (IQR: 5.8–6.7) 1028 patients had a CVD event and 951 patients died from all causes. In age-adjusted analyses, a one-SD higher total testosterone level predicted an 8% higher hazard ratio (HR) of 1.08 (95% CI 1.02–1.16; $p = 0.01$) of CVD events and a one-SD higher free testosterone level predicted a 10% lower hazard (HR 0.90, 95% CI 0.84–0.97; $p < 0.01$) of all-cause mortality (Model A in Figure 1). These relationships did not remain significant after additional adjustment (Model B in Figure 1).

Conversely, a one-SD higher SHBG level predicted cardiovascular events in models adjusted for age (HR 1.10, 95% CI 1.04–1.17; $p < 0.01$) as well as additional risk factors (HR 1.07, 95% CI 1.00–1.14; $p = 0.03$). SHBG also predicted all-cause mortality in models adjusted for age (HR 1.19; 95% CI 1.12–1.26; $p < 0.01$) as well as additional risk factors (HR 1.13; 95% CI 1.06–1.21; $p < 0.01$). There were no significant interactions between study allocation and total testosterone, free testosterone, or SHBG levels (data not shown). In analyses of the expanded primary outcome, including revascularization and heart failure hospitalizations, none of total testosterone, free testosterone or SHBG were significant predictors (data not shown).

When analysed as categorical variables, total testosterone remained insignificant as a predictor (Figures 2 and 3) but normal vs low levels of free testosterone predicted a lower all-cause mortality in age-adjusted models and when adjusted for additional risk factors (Model B in Figure 2 and Figure 4). As noted in Figures 2 and 5, after multiple adjustments, SHBG above the median predicted an 18% higher risk for cardiovascular events (HR 1.18; 95% CI 1.03–1.34; $p = 0.02$) and 26% higher risk for all-cause mortality (HR 1.26; 95% CI 1.10–1.45; $p < 0.01$) than levels at or below the median.

Discussion

In this six-year-long follow-up of dysglycaemic men at high cardiovascular risk, SHBG levels independently predicted the primary composite outcome of cardiovascular death, non-fatal myocardial infarction and non-fatal stroke as well as the secondary outcome all-cause mortality. Neither total nor free testosterone levels were related to cardiovascular events.

The relationship between SHBG and these outcomes could be explained in part by its close link with free testosterone levels.[15,16] As >98% of circulating testosterone is more or less biologically inactive because of binding to SHBG, albumin or other binding proteins,[16,17] high SHBG concentrations may simply reflect low levels of the bioactive free testosterone. The

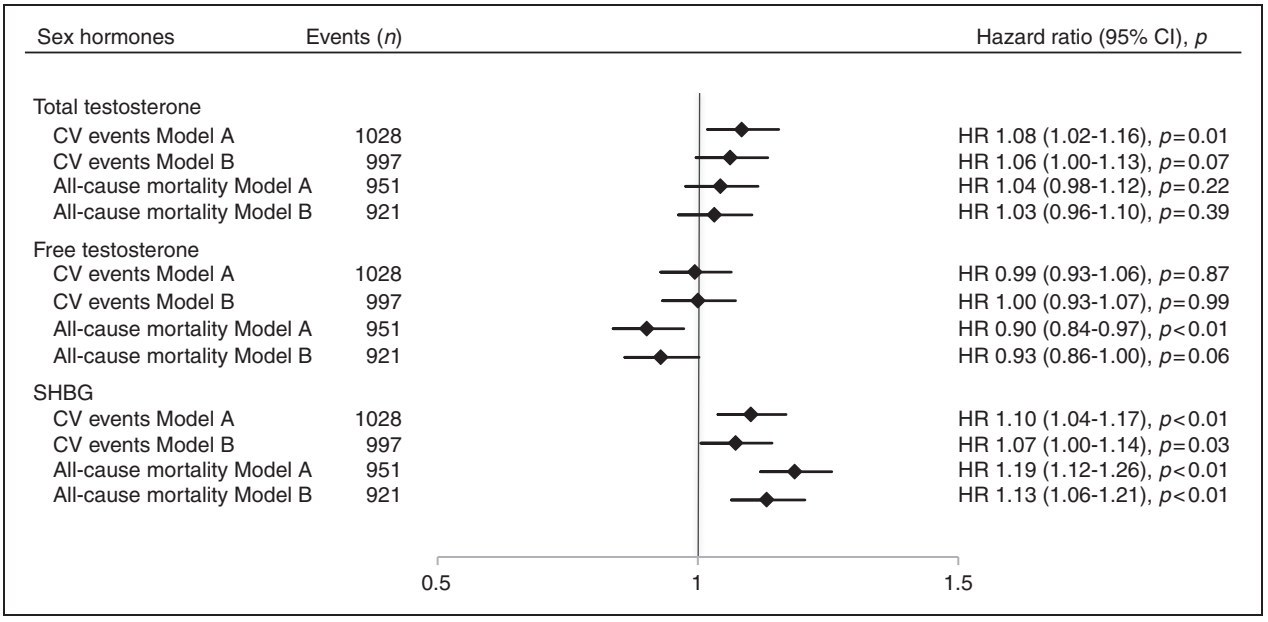

Figure 1. Prognostic predictability (Cox regression analyses) of testosterone (total and free) and sex hormone-binding globulin (SHBG) as continuous variables by increment of one standard deviation (TT: 109 ng/dl, FT: 3 ng/dl, SHBG: 18 nmol/l) for variable of interest with regards to cardiovascular (CV) events and all-cause mortality.
Model A: adjusted for only age. Model B: adjusted for age, luteinizing hormone levels, previous cardiovascular disease, previous diabetes diagnosis, use of metformin, use of statins, systolic blood pressure, glycated haemoglobin, low-density lipoprotein, body mass index and smoking. CI: confidence interval; HR: hazard ratio.

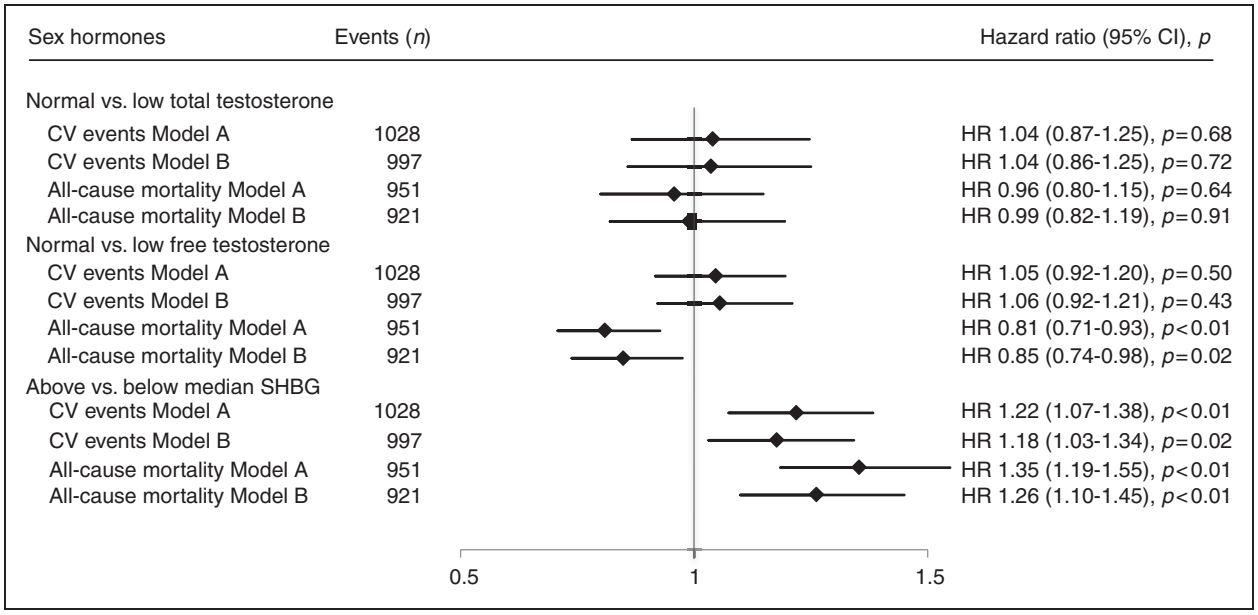

Figure 2. Prognostic predictability (Cox regression analyses) of testosterone (total and free) and sex hormone-binding globulin (SHBG) as categorical variables, stratified as normal vs low testosterone levels (total ≤300 ng/dl and free ≤7 ng/dl) and SHBG levels above or below the median level (≤35 nmol/l) with regards to cardiovascular (CV) events and all-cause mortality.
Model A: adjusted for only age. Model B: adjusted for age, luteinizing hormone levels, previous cardiovascular disease, previous diabetes diagnosis, use of metformin, use of statins, systolic blood pressure, glycated haemoglobin, low-density lipoprotein, body mass index and smoking. CI: confidence interval; HR: hazard ratio.

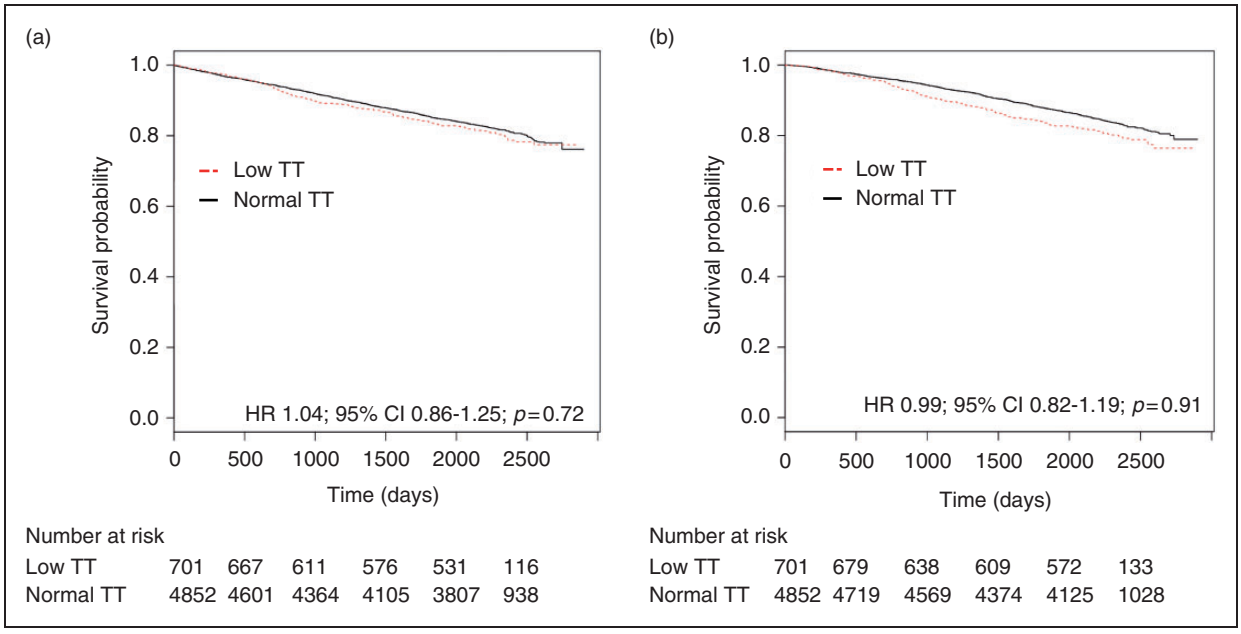

Figure 3. Kaplan-Meier curves for categorical variables of total testosterone (TT≤300 ng/dl) with regards to cardiovascular events (a) and all-cause mortality (b). Hazard ratio (HR) (95% confidence interval (CI)) from the multivariable Cox regression model.

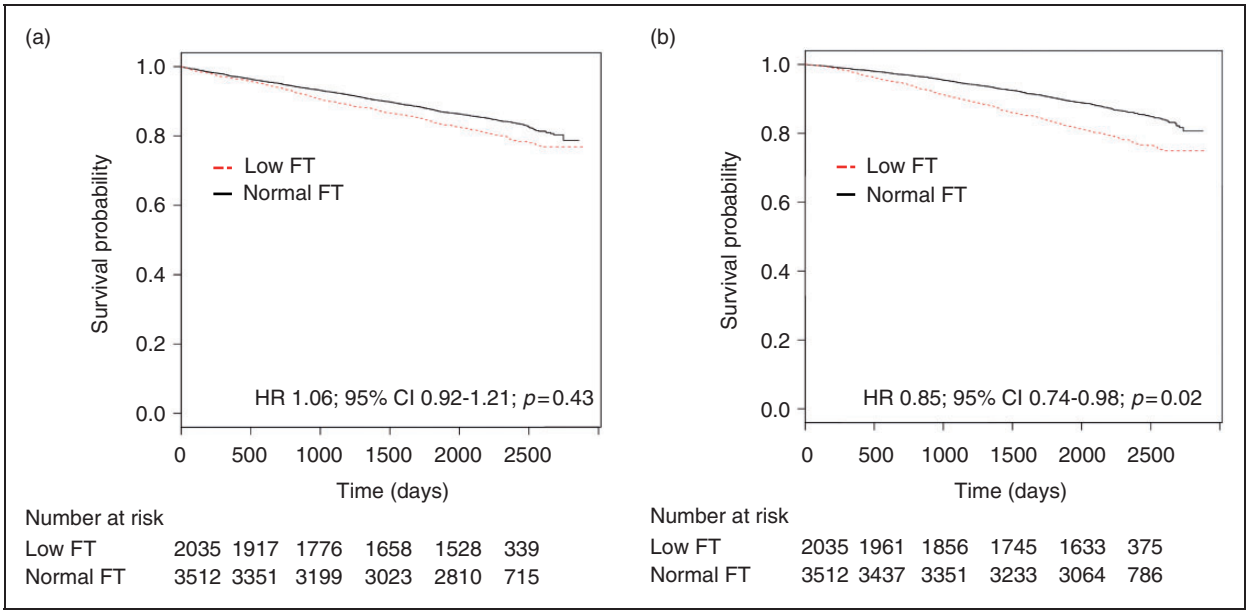

Figure 4. Kaplan-Meier curves for categorical variables of free testosterone (FT≤7 ng/dl) with regards to cardiovascular events (a) and all-cause mortality (b). Hazard ratio (HR) (95% confidence interval (CI)) from the multivariable Cox regression model.

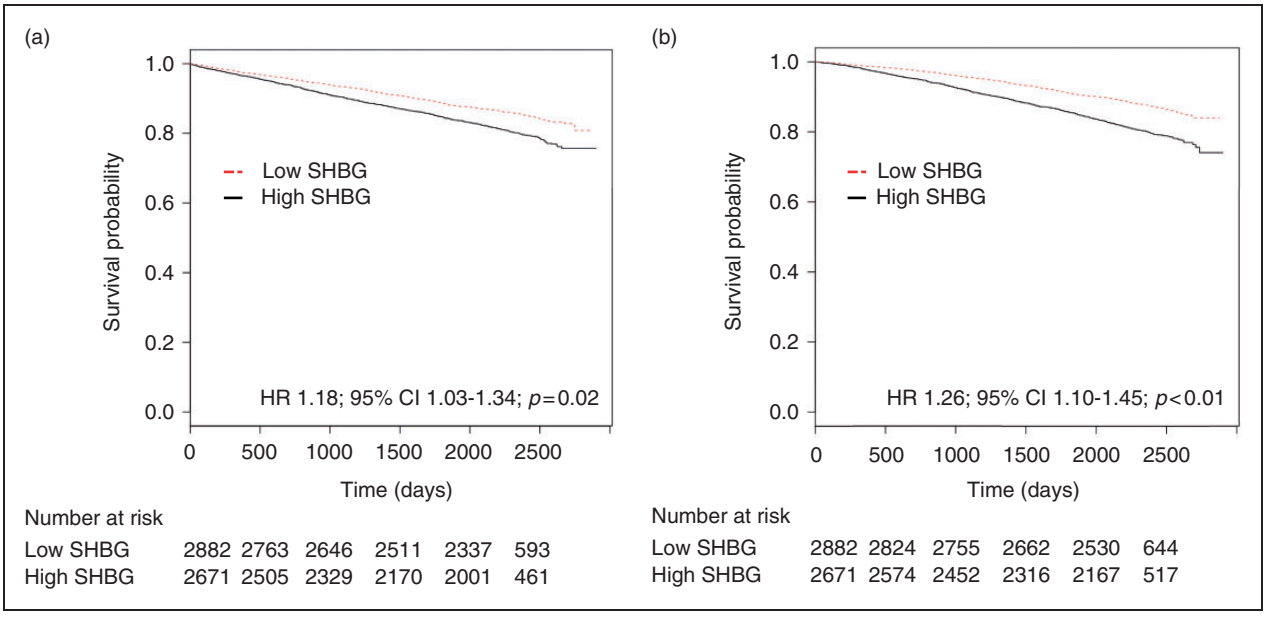

Figure 5. Kaplan-Meier curves for categorical variables of sex hormone-binding globulin (SHBG) (≤35 nmol/l) with regards to cardiovascular events (a) and all-cause mortality (b). Hazard ratio (HR) (95% confidence interval (CI)) from the multivariable Cox regression model.

observation that low free testosterone levels were marginally related to all-cause mortality is consistent with that possibility and with previous data.[15,18,19] Conversely, the observation that the relationship between SHBG and outcomes was only modestly attenuated after adjustment for many factors including LH levels, which are closely correlated to low free testosterone,[20] suggests that the SHBG relationship is not solely mediated through testosterone-related effects.[21] SHBG is produced in the liver and indirectly inhibited by insulin.[16] Low SHBG has previously been associated with diabetes.[22] In the present study, low SHBG was associated with a better prognosis but patients on insulin treatment did not differ from those free from such glucose-lowering therapy in this respect. This indicates that the effect of SHBG in the present cohort is not

mediated through its role in dysglycaemia and that the mechanism through which SHBG may act as a direct, independent predictor is not yet fully understood.

These results are in line with, and expand, previous data derived from males with T2DM or heart failure.[15,23,24] However, they are not supported by other studies in populations with different baseline comorbidities[8,25] and these differences may explain the discordant results.[26] While low total testosterone levels predict CVD in relatively healthy cohorts drawn from the general population,[7,8,27] the link in sicker study populations like the present study is unclear with some studies suggesting free testosterone as a more important predictor of mortality.[15,28–30] The fact that testosterone levels are lowered as a result of chronic illness such as CVD may clearly obscure any potentially causal relationship.[31] Moreover in elderly men, total testosterone levels remain rather stable with increasing age whereas free testosterone decreases and SHBG increases,[32] suggesting that modulation of androgen activity in elderly men occurs primarily due to shifts in free testosterone and SHBG. This may, at least to some extent, explain why total testosterone was not a significant predictor of outcomes in this study population. Clearly the relationship between SHBG, testosterone and prognosis is complex and it is possible that SHBG acts through several different pathways as both a direct and indirect predictor.

Strengths and weaknesses

The large number of participants and a long period of follow-up provides a solid basis for the present observations. Moreover all cardiovascular events were prospectively collected and determined by an adjudication committee. While free testosterone levels were calculated and not directly measured with equilibrium dialysis, the estimation by algorithms is considered adequate[33] and the Vermeulen formula has been validated against equilibrium dialysis.[34] Testosterone samples were only obtained on one occasion, however, the large sample number limits the influence of outliers. Since the patients of the present study were at high cardiovascular risk, the results cannot be extrapolated to low or intermediate risk populations. However, very few study participants were under the influence of medical conditions or pharmacological treatment with a known influence on the testosterone and/or SHBG levels.

In conclusion, SHBG, but not testosterone, was an independent predictor of cardiovascular events and all-cause mortality in a large population of dysglycaemic men at high cardiovascular risk. Whether this action is mediated through the role of SHBG on testosterone distribution or as a direct cardiovascular risk marker remains to be clarified.

Author contributions

AW, SA, HG, LR and LM contributed to the conception and design of the study. AW, SA, KB, HG, SL, SH, LR and LM contributed to the acquisition, analyses and interpretation of data. AW drafted the manuscript together with HG, LR and LM and all authors critically revised it. All authors gave final approval and agree to be accountable for all aspects of the work ensuring integrity and accuracy.

Declaration of conflicting interests

The authors declared the following potential conflicts of interest with respect to the research, authorship and/or publication of this article: AW has nothing to declare. SA reports speakers' honoraria from AstraZeneca and Ferring; and consulting fees from Pfizer. KB reports grants from Visare Norr. HG holds the McMaster-Sanofi Population Health Institute Chair in Diabetes Research and Care. He has received research grant support from AstraZeneca, Eli Lilly, Merck and Sanofi; honoraria for speaking from AstraZeneca, Boehringer Ingelheim, Novo Nordisk and Sanofi; and consulting fees from Abbott, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Merck, Novo Nordisk, Jannsen and Sanofi. SL has nothing to declare. SH is an employee and shareholder of Sanofi. LR reports grants from The Swedish Heart-Lung Foundation, The European Society of Cardiology, Amgen, Boehringer-Ingelheim, Merck and Novo Nordisk and honoraria from Boehringer-Ingelheim, Merck and Novo Nordisk all outside the present work. LM reports grants and consulting fees from Bayer AG and Sanofi-Aventis.

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