The association of sex hormone binding globulin with mortality is mediated by age and testosterone in men with type 2 diabetes.

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Abstract

**Background**

Serum sex hormone binding globulin (SHBG) levels have been associated with mortality in adult men with type 2 diabetes (T2DM).

**Objectives**

To confirm that serum SHBG is associated with mortality and then determine if this association is mediated by age and total testosterone (TT) concentration.

**Materials and Methods**

We studied 364 men (mean age: xx) with T2DM over a mean follow up of 4.2 years using Cox’s regression to study associations between SHBG, age, TT and mortality

**Results**

Mortality was significantly and independently associated with SHBG, age and TT. In pairwise combinations of age and SHBG dichotomised by median values, the association of SHBG with mortality was age-dependent. Relative to the combination of age >66 years/SHBG >35 nmol/l (mortality 22.5%), the other combinations were associated with significantly less mortality (mortality in men≤66 years/SHBG ≤35 nmol/l was 3.23%). In men >66 years, SHBG ≤35 nmol/l was associated with decreased mortality (HR: 0.41, p=0,037) compared with SHBG >35 nmol/l. In men≤66 years there was no significant difference between those with SHBG above or below the median (HR:1.73, p=0.56, reference: SHBG ≤ 35 nmol/l). TT<12 nmol/l was associated with increased mortality in both age categories. Men > 66 years with the reference combination of SHBG >35 nmol/l and TT<12 nmol/l (36.84%) nmol/l had significantly higher mortality than those with SHBG >35 nmol/l and TT≥12 (18.06%) and those with SHBG ≤35 nmol/l and TT <12 nmol/l (13.79%).

**Discussion**

Our data suggest SHBG and TT have particular impact on mortality in men aged over 66 years. Further, in older men, the combination of high SHBG levels and low TT is associated with greater risk than either high SHBG or low TT individually.

**Conclusions**

Our findings are compatible with data suggesting the importance of SHBG lies in mediating free testosterone levels.

Introduction

Men with type 2 diabetes (T2DM) suffer a mean reduction in life expectancy of 7 years [Morgan et al. 2000, Hansen et al 2009, Mulnier et al. 2006]. A number of pathologies contribute to this phenomenon including hypogonadism (HG) which is found in about 70% of males with T2DM [Hackett et al. 2009]. HG is characterised by low serum testosterone (TT) and sexual dysfunction [Hackett et al. 2009, Kapoor et al. 2007] with both these factors being independent determinants of mortality [Pye et al. 2014]. Recognition of the importance of HG has led to the American Association of Clinical Endocrinologists / American College of Endocrinology guidelines (<https://www.aace.com/files/guidelines/ObesityExecutiveSummary.pdf> - accessed on 04/04/2018) on obesity recommending that testosterone deficiency is excluded in all males with T2DM. Importantly, testosterone replacement therapy (TRT) has wide ranging beneficial effects; for example, Hackett et al [2016a, 2017a] and Corona et al [2017] reported improvement in erectile function, sexual desire and other sexual parameters. Further, Corona et al [2016] found significant reduction in body fat and increased lean mass with reduced fasting glycaemia and insulin resistance. Importantly, TRT has also been associated with reduced all-cause mortality in men with T2DM [Hackett et al. 2016b, Hackett et al. 2017b, Muraleedaran et al. 2013].

The recognition of the clinical importance of the association of testosterone with mortality has lead to interest in the putative role of sex hormone binding globulin (SHBG). Thus, while SHBG was considered a passive carrier of testosterone and other steroids, studies now indicate a more complex relationship between the peptide, testosterone and health. For example, while high serum levels of SHBG are associated with increased all-cause mortality [Tint et al. 2016], levels of the peptide are inversely associated with insulin resistance and may mediate the association, between low serum testosterone and increased mortality in men with T2DM [Hackett et al. 2016b, Khaw et al. 2007, Muraleedaran et al. 2013, Shores et al. 2006, Shores et al. 2012]. Further, Rastrelli et al [2017] recently reported that higher serum levels of SHBG, independently of TT, are associated with markers of androgen deficiency.

Both SHBG and mortality increase markedly with age [Tint et al. 2016] while testosterone levels fall (<https://uroweb.org/wp-content/uploads/EAU-Guidelines-Male-Hypogonadism-2015.pdf> - accessed on 04/04/2018). Accordingly, we now describe studies on the relationship between mortality, age and SHBG. While our focus was on SHBG, we also examined its associations with TT and calculated free testosterone (cFT) as well as other variables associated with SHBG and mortality to determine if these mediated the relationship between SHBG and mortality. Statin use was included as it is associated with reductions in CVD related mortality [Scandinavian Simvastatin Survival Study Group.1994], serum testosterone [Stanworth et al. 2009] and SHBG levels [de Keyser et al. 2015].Accordingly, **w**e first determined if SHBG is associated with all cause mortality in men with T2DM not treated with testosterone. Secondly, we studied the independence of this association by including SHBG and adjusting for variables associated with SHBG. As SHBG, age and TT were independently associated with mortality, we thirdly, examined the association between mortality and pairwise combinations of age with SHBG or TT to determine if these influenced mortality similarly in men of different ages. Fourthly, in men aged over the median age we studied associations between pairwise combinations of SHBG and TT with mortality.

Patients and Methods

Patients
We recruited our study group from 857 men with T2DM listed in the registers of 5 English Midlands primary care practices screened for TT and cFT levels during April 2007- April 2009 as part of recruitment for the BLAST randomised controlled study investigating the effects of Testosterone Undecanoate (TU) (1000 mg) on sexual symptoms and metabolic parameters [Hackett et al. 2014]. The 364 men (320 men alive at study end; 44 men died) included in this study were selected because they had not been treated with TRT and, had available SHBG measurements (362 men had both SHBG and TT data).
Each man was assessed by a clinician and considered fit for the trial. Normal or low levels of testosterone were defined as TT> or ≤12 nmol/l and cFT was > or ≤0.25 nmol/l, respectively. The study was approved by the Multicentre Research Ethics Committee (reference: 08/H1208/30), the National Institute for Health Research (Birmingham and the Black Country Comprehensive Local Research Park – RM&G reference: 1268) and Warwickshire Primary Care Trust (reference: WAR230909), included in the European Union Clinical Trials Register (EudraCT 2008-000931-16) and conducted in accordance with the revised guidelines of the World Medical Association Declaration of Helsinki (<https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/> - accessed on 04/04/2018)[.](https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/) Mortality data were collected from practice databases, hospital letters and death certificates, along with READ codes (coded clinical terms used in the National Health Service in England: <https://digital.nhs.uk/article/1104/Read-Codes> - accessed on 04/04/2018).

Laboratory Methods
Blood samples were taken from fasting men at baseline. All analyses were carried out in a Pathology Accredited NHS Laboratory and were subjected to daily internal and regular external blind assessment of accuracy and precision. TT was measured using the validated Roche immunoassay. The reference range for adult males for this method was 210–810 ng/dl (7.3–28.1 nmol/l) and between-run CV averaged 4.6%. cFT was estimated using the equation of Vermeulen et al [1999]. Serum SHBG, albumin and lipids were analysed using a Roche Modular automated analyzer (Roche Diagnostics, Burgess Hill, UK). HbA1c was measured in whole blood using a Tosoh G7 ion exchange high performance liquid chromatography analyzer (Tosoh Bioscience Ltd., Redditch, UK); the method standardized using International Federation of Clinical Chemistry reference material and the results were adjusted to give derived National Glycohaemoglobin Standardisation Programme units (%).

Statistical Methods

Stata version 8 (College Station, TX) was used for statistical analyses. Associations between SHBG and other variables were established by multiple regression analysis while Cox regression analysis with all-cause mortality as the primary end point was used to identify associations between SHBG, other related variables and mortality.

Results

Factors associated with all-cause mortality

Table 1 shows in the 364 T2DM men, mean baseline levels of SHBG, TT, cFT and variables recorded that were considered associated with SHBG and mortality. The mean age of the total group was 64.8 years though the range was wide; 22.8 - 88.9 years. Similarly, the range of SHBG concentrations (7.9 - 185.5 nmol/l) was wider than in published reference ranges for adult males (e.g. 10 - 57 nmol/l, Mayo Medical Laboratories:  [http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/9285](http://www.mayomedicallaboratories.com/test-catalog/Clinical%2Band%2BInterpretive/9285) - accessed on 04/04/2018), though the 95% range was 14 - 85.6 nmol/l. Stratified baseline data recorded from 320 men alive at study end and 44 deceased men (first death: 0.37 years from study start) and statin treatment details at last visit are also shown. As expected mean follow-up was significantly (p<0.001) longer in survivors (4.4±1.1 years) compared to those who died (2.8±1.5 years). Mean body mass index (BMI) was 31.0Kg/m2.

**Association of SHBG and age with mortality**

In a model comprising only SHBG as an independent variable, Cox regression analysis showed SHBG levels were significantly associated with mortality in the 364 men; HR 1.02, 95% CI 1.01, 1.03, p<0.001. In a separate model, age was also significantly associated with mortality; HR 1.09, 95% CI 1.05, 1.12, p<0.001. As SHBG and age are positively correlated with mortality as seen above and each other (linear regression, p<0.001), we further examined the relationship between these variables by stratifying SHBG serum concentrations into quartiles (≤26.8, >26.8-≤35, >35-≤50, >50-85 nmol/l) and examining the association of each quartile with mortality (Figure 1). Mean follow-up (range 4.1-4.2 years) was similar in the quartiles. The data show that with each increase in SHBG, the mean age of each group increased as did mortality, though in quartiles >26.8-≤35 and >35-≤50nmol/l changes in mortality (11.0%,12.1%) and age (65.5 years, 66.2 years) were modest.

Other factors associated with SHBG

We examined the association of SHBG levels with variables that have been reported to be associated with SHBG and/or mortality. In linear regression analyses, models (SHBG as dependent variable) comprising each variable individually, age, TT, BMI, HbA1c, triglycerides (TG), high density lipoprotein- cholesterol (HDL-c) at baseline and statin therapy at the end of follow-up were significantly associated with SHBG (Table 2). Neither blood pressure (systolic nor diastolic) nor total cholesterol (TC) were associated with SHBG.

Survival analysis of factors associated with SHBG and mortality

We entered all the factors significantly associated with SHBG (Table 2) into a single Cox’s regression model and showed that only age, SHBG, TT and TG were significantly associated with all-cause mortality (Table 3). These significant factors were then entered into a single Cox’s regression model (Table 4a) and while age, SHBG and TT were significantly and independently associated with mortality, TG only approached significance. We repeated the Cox’s regression analysis using a model comprising age, SHBG and TT in 362 men (Table 4b) and all three variables remained significantly associated with all cause mortality. We then used Cox regression analysis to show that in a model comprising age, SHBG and cFT (instead of TT) all variables remained significantly associated with mortality; age, HR 1.10 (95% CI: 1.05 / 1.15) p<0.001; SHBG, HR 1.02 (95% CI: 1.00 / 1.03) p=0.013; HR 1.10 (95% CI: 1.05 / 1.15) p<0.001; cFT, HR 0.01 (95% CI: 0.00 / 0.76) p=0.036.
Thus, it is interesting that age, SHBG and TT whilst strongly associated with each other (p<0.001) were also independently associated with all-cause mortality. We wished to explore this further and carried out associations between pairwise combinations of these factors and mortality.

Association between mortality and pairwise combinations of SHBG and age

We initially investigated the relationship between mortality, age and SHBG by categorising age and SHBG by their respective median values (age: 66 years, SHBG: 35 nmol/l) and examining the associations of different pairwise combinations of these two variables on mortality using Cox regression analysis. The model also included TT dichotomised using a clinical HG cut-off <12 nmol/l vs. ≥12 nmol/l. Table 5a shows results using age ≤66 years / SHBG ≤35 nmol/l as reference category. The data show that no difference in mortality was observed between SHBG categories (HR 1.73, 95% CI: 0.27 / 11.08) in men aged ≤66 years. Using age ≤66 years / SHBG ≤35 nmol/l as reference category once again, we found mortality was significantly greater in the older age group; age >66 years / SHBG ≤35 nmol/l, (HR 3.90, 95% CI: 1.04 / 14.70) and age >66 years / SHBG >35 nmol/l (HR 9.37, 95% CI: 2.68 / 32.79). Secondly, when using age >66 years/SHBG >35 nmol/l as the reference category, the other three combinations were associated with significantly lower mortality (Table 5a). Thus, SHBG levels ≤35 nmol/l are associated with lower mortality in men > 66 years (HR 0.41, 95% CI: 0.18 / 0.95) compared to the reference group (age >66 years / SHBG >35 nmol/l) which was associated with the highest mortality (22.45%). The dichotomised TT included, TT<12nmol/l was also associated with increased mortality.

Associations between mortality and pairwise combinations of TT and age

Similarly Cox regression analysis was used to compare the associations of mortality with combinations of TT and age (Table 5b). The model also included SHBG (reference category ≤35 nmol/l). TT levels <12 nmol/l were significantly associated with increased mortality in both age categories. Table 5b also suggested the combination of age >66 years with TT <12 nmol/l is associated with the highest mortality of 25.0%. SHBG >35 nmol/l remained significantly associated with mortality in the model. Kaplan-Meier survival plots are also shown in Figure 2a and b to graphically demonstrate the associations between age and SHBG and TT and survival during follow up.

Associations between mortality and pairwise combinations of SHBG and TT

The above analyses demonstrate that whilst elevated SHBG appeared to be associated with increased mortality in men > 66 years, low testosterone was associated with mortality in men above and below 66 years. Thus, when examining the effects of combinations of SHBG and TT, we restricted our cohort to men > 66 years. Table 6 shows in men age >66 years, that those with the combination SHBG>35 nmol/l and TT≥12 nmol/l had significantly lower mortality (18.06%) than the corresponding men in the reference category with TT<12 nmol/l (36.84%). Men with the other two combinations of SHBG and TT also demonstrated lower mortality than those in the reference category (Figure 3).

Discussion

In serum, SHBG and albumin mediate levels of free testosterone. Accumulating data show associations between mortality and testosterone, TRT and SHBG. However, the mechanism whereby SHBG mediates mortality is unclear; thus, while increased levels of SHBG are associated with increased mortality, the metabolic syndrome (MetS), a condition itself linked with increased mortality [Gami et al. 2007] is associated with reduced serum levels of the protein [Holmboe et al. 2016]. These findings are important because T2DM is a classifying component of the MetS [Alberti et al. 2009]. We have further investigated associations between SHBG and other variables associated with mortality in men with T2DM testosterone.

Previous data [Tint et al. 2016] showing associations between SHBG and age as well as mortality have been replicated in our cohort of men with T2DM who had not received testosterone treatment. We found a clear and significant association between SHBG level and age and Figure 1 shows that stratifying T2DM men by SHBG quartiles results in subgroups with increasing mean ages. As expected, we found associations between SHBG and relevant variables; significant negative associations were found with HbA1c, BMI and TG while the association with HDL-c was positive. However, no association was observed between SHBG and BP. We recognise that the observed values of these variables may be altered by treatment. Further, a negative correlation between SHBG and statin use was observed, a finding compatible with reports that statin use is associated with increased risk of T2DM [Abbas et al. 2012, Sattar et al. 2010]. The interaction of SHBG with TG appears interesting and worthy of further study. Importantly, TG levels may largely reflect untreated values as this variable is not included in primary care diabetes treatment guidelines with neither fibrate or nicotinic treatment being recommended (<https://www.nice.org.uk/guidance/cg181/chapter/1-Recommendations#lipid-modification-therapy-for-the-primary-and-secondary-prevention-of-cvd-2> – accessed on 04/04/2018). This may explain why TG was the only factor, other than age, SHBG and TT that was associated with all-cause mortality. HbA1c, BP, TC targets are included in primary care guidelines (<http://www.nhsemployers.org/-/media/Employers/Documents/Primary-care-contracts/QOF/2014-15/2014-15-General-Medical-Services-contract---Quality-and-Outcomes-Framework.pdf?la=en&hash=69CF3870EB19476ECDFF3A5CBAA35F08C34E00DC> – accessed on 04/04/2018) and this may account for the lack of association with mortality. It is clear that increasing HDL-c using therapy has largely failed to reduce mortality [Barter et al. 2007, AIM-HIGH investigators et al. 2011].

Previous studies show both age and SHBG are independently and significantly associated with mortality though a more detailed analysis of these associations was not included [Tint et al. 2016]. Accordingly, we stratified the men by median age (66 years) and median SHBG concentration (35nmol/l) and examined the association between combinations of these variables and mortality. We stratified SHBG by median value because unlike TT [Hackett et al. 2016b, 2017b] there is little data regarding associations between the serum level of the protein and pathology. Our data (Table 5, Figure 2a) show the percentage of men who died was greater in those aged >66 years and was highest in men aged >66 years with SHBG >35nmol/l. Interestingly, SHBG level was not associated with mortality in men aged ≤66 years. We similarly examined the association between combinations of age (stratified by median, 66 years) and TT (stratified by 12 nmol/l, the definition of HG). Table 5 and Figure 2b show TT <12 nmol/l was significantly associated with increased mortality in both men aged less than and, greater than 66 years. Thus, TT demonstrates a different pattern of association with age and mortality to SHBG. Though SHBG and TT demonstrate different age-related associations with mortality, they are closely related and, our results may reflect an independent effect of SHBG or the influence of free testosterone. Our analysis showed that if cFT was substituted for TT, it as well as SHBG and age remained significantly associated with mortality (Table 4 footnote). We recognised that using cFT may not represent physiological reality and a study based on measured values of free testosterone would be useful.

SHBG is a predictor of mortality by a mechanism that is unclear. Serum levels of the peptide are regulated by sex hormones and influenced by other hormonal and non-hormonal factors including drugs such as statins [Rosner et al. 2010, Tint et al. 2016]. Indeed, the wide range of serum values in reference groups (eg. 10-57 nmol/l) and our study (7.9-185.5 nmol/l) seem to us to argue against this protein having a simple, direct causative effect on mortality. Thus, we interpret the analysis of associations between mortality and dichotomised combinations of SHBG and TT (Table 6) as showing the influence of the peptide is mediated by its ability to bind the hormone thereby reducing its free concentration. Thus, the reference category (SHBG>35 nmol/l, TT<12 nmol/l) describes the combination likely to result in the lowest levels of free hormone and the one most positively associated with mortality.

We believe this study has identified interesting associations between SHBG, age and mortality in diabetic men not receiving testosterone but has limitations. The numbers studied did not allow analysis of larger combinations of variables and detailed information on changes to therapy during the study was not available. Thus, the impact of therapy on serum levels of SHBG and other variables could not be assessed. We did not have a complete data set on all patients and information on smoking was absent. Further, having levels of measured, rather than cFT, would have allowed proper assessment of the role of the active moiety. Information on statin treatment was obtained at the end of follow-up. However, as the Quality Outcomes Framework has been used in English primary care since 2004 with statin treatment in T2DM incentivised, we expect many patients on statins at final visit/death were treated prior to study start [Livingston et al. 2015].

Conclusions

Our data support the work of Tint et al [2016] showing that SHBG is associated with mortality in men with T2DM. We extended these findings showing for the first time, that this association is mediated by age; it was only found in men aged >66 years. The mechanism for the association of the peptide with mortality and the influence of age is unclear though as suggested by Svartberg et al [2014] and our analysis, may involve the mediation of free testosterone levels by SHBG. The importance of SHBG in gonadal health is indicated by Rastrelli et al 2017. They reported that higher levels of SHBG are independently of TT, associated with markers of androgen deficiency.

**Disclosures**

Professor Geoffrey I Hackett has received honoraria for acting as a speaker for Bayer plc who provided the grant. Professor Sudarshan Ramachandran has received educational grants to attend meetings and honoraria for serving as a speaker for Besins Health Care Ltd. Professor Geoffrey I Hackett has spoken at various national and international meetings on testosterone and PDE5I treatments in men and sits on the committee of the European Society for Sexual Medicine. Professor Farid Saad works for Bayer AG, but had no role in study design or data analysis.

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Author contributions

Professors Geoffrey I Hackett and Sudarshan Ramachandran had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Professor Geoffrey I Hackett.

Analysis and interpretation of data: Professor Geoffrey I Hackett, Professor Sudarshan Ramachandran and Professor Richard C Strange.

Drafting of the manuscript: Professor Geoffrey I Hackett, Professor Richard C Strange, Professor Anthony A Fryer, Professor Farid Saad and Professor Sudarshan Ramachandran.

Critical revision of the manuscript for important intellectual content: Professor Geoffrey I Hackett, Professor Sudarshan Ramachandran, Professor Anthony A Fryer, Professor Farid Saad and Professor Richard C Strange.

Statistical analysis: Professor Geoffrey I Hackett, Professor Richard C Strange and Professor Sudarshan Ramachandran.

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# Corona et al

# Corona et al

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Legends

**Table 1.** Characteristics of 364 men with T2DM and after stratification by mortality.

**Table 2.** Individual linear regression models describing associations between SHBG (dependent variable) and relevant factors.

**Table 3.** Cox regression analysis describing associations between mortality (dependent variable) and factors associated with SHBG. All factors were included in a single model comprising 309 T2DM men with complete data on the variables listed.

**Table 4a.** A single Cox regression analysis used to describe associations, in 309 T2DM men with complete data, between survival and, age, SHBG, TT, TC and TG as independent variables.

**Table 4b.** A single Cox regression analysis used to describe associations between mortality (dependent variable) and age, SHBG and TT (independent variables) in 362 T2DM men with data.

**Table 5.** Mortality data and Cox regression analysis in T2DM men stratified by median age (66 years) and a. median value for SHBG (35 nmol/l) or b. TT (12 nmol/l). The analyses included SHBG and TT (when not included as stratified pair wise combination with age) as continuous variables. The Kaplan-Meier plots graphically (Figures 2a and 2b) display the survival of the pairwise categories.

**Table 6**. Cox regression analysis was used to determine associations between mortality and combinations of SHBG (≤ and >35 nmol/l) and TT (< and ≥12 nmol/l) in T2DM men aged > 66 years.

**Figure 1.**The mortality rate plotted against stratified SHBG quartiles. The mean age of each SHBG category together with mean follow-up are shown.

Figure 2a: The Kaplan-Meier plot graphically displays the survival of combinations of age at initial visit and SHBG, both categorised by median values).

Figure 2b: The Kaplan-Meier plot graphically displays the survival of combinations of age at initial visit (categorised by median value) and TT (categorised by 12nmol/l, used in the classification of HG).

**Figure 3:** The Kaplan-Meier plot graphically displays the survival of combinations of SHBG (categorised by median value) and TT (categorised by 12nmol/l, used in the classification of HG) at initial visit in men > 66 years.