

## Serum testosterone, sex hormone-binding globulin and sex-specific risk of incident type 2 diabetes in a retrospective primary care cohort

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**1 Serum testosterone, sex hormone-binding globulin and sex-specific risk of incident type 2 diabetes**  
**2 in a retrospective primary care cohort**

**3 Running title:** Serum testosterone and incident diabetes

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31 **SUMMARY**

32 **Objective:** Previous studies suggest that androgens have a sexually dimorphic impact on metabolic  
33 dysfunction. However, the sex-specific link between circulating androgens and risk of type 2 diabetes  
34 mellitus (T2DM) has not been examined in a large scale, longitudinal cohort, a task we undertook in this  
35 study.

36 **Design:** A retrospective cohort study in a UK primary care database.

37 **Patients:** We included men and women with available serum testosterone and sex hormone-binding  
38 globulin (SHBG) results.

39 **Measurements:** We categorized serum concentrations according to clinically relevant cut-off points and  
40 calculated crude and adjusted T2DM Incidence Rate Ratios (IRRs and aIRRs).

41 **Results:** Serum testosterone concentrations were available in 70,541 men and 81,889 women; serum  
42 SHBG was available in 15,907 men and 42,034 women. In comparison to a reference cohort with serum  
43 testosterone  $\geq 20$ nmol/l, men with lower serum testosterone had a significantly increased risk of T2DM,  
44 with the highest risk in those with serum testosterone  $< 7$ nmol/l (aIRR 2.71, 95% CI 2.34-3.14,  $p < 0.001$ ).  
45 In women, the risk of T2DM started to increase significantly when serum testosterone concentrations  
46 exceeded 1.5nmol/l, with the highest risk in women with serum testosterone  $\geq 3.5$ nmol/l (aIRR 1.98, 95%  
47 CI 1.55-2.52,  $p < 0.001$ ). These observations were verified in a continuous rather than categorized analysis.  
48 The risk of T2DM increased in men and women with serum SHBG  $< 40$ nmol/L and  $< 50$ nmol/L,  
49 respectively.

50 **Conclusions/Interpretation:** In this longitudinal study, we found sexually dimorphic associations  
51 between serum testosterone and risk of incident T2DM. Androgen deficiency and excess should be  
52 considered important risk factors for diabetes in men and women, respectively.

53 **Key words:** Testosterone, androgens, hypogonadism, sex hormone-binding globulin, diabetes, metabolic  
54 diseases, population health

55 **INTRODUCTION**

56 Sex differences are critical in the epidemiology and pathophysiology of metabolic disease, with  
57 an increased incidence of type 2 diabetes mellitus (T2DM) and cardiovascular disease in men <sup>1</sup>. Sex  
58 hormones such as androgens may mediate these differences, but the association between androgens and  
59 metabolic dysfunction is complex and sex-specific <sup>2</sup>. Androgen excess has recently been identified as an  
60 independent risk factor for non-alcoholic fatty liver disease (NAFLD) in women <sup>3</sup>, and promotes lipid  
61 accumulation in female adipose tissue as well as systemic lipotoxicity <sup>4</sup>. Female-to-male gender  
62 reassignment patients undergoing androgen therapy develop dyslipidemia and abnormal body  
63 composition <sup>5,6</sup>. Mirroring this, the adverse metabolic phenotype of male androgen deficiency bears a  
64 striking similarity to that of female androgen excess; lower testosterone levels in men are associated with  
65 impaired glucose homeostasis, hepatic steatosis and coronary artery disease <sup>7-9</sup>. A number of meta-  
66 analyses support a sex-specific relationship between androgens and the risk of metabolic dysfunction, and  
67 suggest that low circulating sex hormone-binding globulin (SHBG) concentrations may be metabolically  
68 harmful in both sexes <sup>9,10</sup>.

69 Delineating an independent role for androgens in the pathogenesis of T2DM is confounded by  
70 changes in body composition, body mass index and lean mass observed in disorders of androgen excess  
71 and deficiency <sup>11</sup>. Against the background of a global epidemic of T2DM <sup>12</sup>, there is an urgent health need  
72 to understand the sexually dimorphic role played by androgens in the pathogenesis of hyperglycemia. The  
73 shared constellation of risk factors observed in women with androgen excess and men with androgen  
74 deficiency suggests that circulating androgen concentrations common to both disorders may be  
75 metabolically disadvantageous <sup>2</sup>. To our knowledge, however, no large longitudinal studies have  
76 specifically examined the association between circulating androgen exposure *per se* and risk of T2DM in  
77 a sex-specific context.

78 The aim of this study was to investigate the independent sex-specific association between serum  
79 testosterone concentrations and the risk of hyperglycaemia in men and women by undertaking a  
80 retrospective cohort study in a large and diverse UK population base.

81 **MATERIALS AND METHODS**

82 **Database**

83 A large primary care database in the UK with contribution from over 700 general practices (14  
84 million patients) was utilized for this study. Data from practices that use VISION Electronic Medical  
85 Record (EMR) are gathered, anonymized and released for research purpose <sup>13</sup>. The resulting database,  
86 The Health Improvement Network (THIN) database holds data on demographic characteristics, clinical  
87 diagnosis, physical measurement, laboratory results and prescriptions. The THIN database is broadly  
88 representative of the UK population structure <sup>14</sup> and has been utilized for numerous epidemiological  
89 studies, including studies on T2DM <sup>15,16</sup> and sex hormones <sup>3,17,18</sup>.

90 **Testosterone and Sex Hormone Binding Globulin (SHBG) measurements**

91 Men or women over the age of 16 who had a measurement of the serum concentration of  
92 testosterone or SHBG between 1<sup>st</sup> of Jan 2000 and 15<sup>th</sup> of May 2016 were eligible to take part in the  
93 study. Common clinical indications for these measurements include suspected polycystic ovary syndrome  
94 (PCOS) in women, infertility investigations in both sexes and erectile dysfunction in men <sup>19,20</sup>. Where  
95 multiple measurements were available in one individual, the first measurement was utilized. Patients with  
96 the outcome of interest (T2DM) preceding the index date were excluded from the study.

97 **Exposure categories**

98 To explore non-linear relationships, establish gradient increase and assess risk within the normal  
99 range, measurements were categorized by applying clinically relevant cut-off points for serum  
100 concentrations (nmol/L)<sup>3</sup>. For women, testosterone was grouped as <1.0nmol/L (reference group), 1.0-  
101 1.49, 1.5- 1.99, 2.0-2.49, 2.5-2.99, 3.0-3.49 and >3.5 nmol/L. For men, the groups were as follows: <7, 7-  
102 9.9, 10.0-14.9, 15-19.9, >20.0nmol/L (reference group) nmol/L. For both sexes, SHBG was categorized  
103 as >60.0nmol/L (reference group), 50.0-59.9, 40.0-49.9, 30.0-39.9, 20.0-29.9 and < 20 nmol/L. Exposures  
104 were also treated as continuous variables and risk of T2DM assessed.

105 **Follow-up period**

106 The date of measurement of testosterone or SHBG served as the index date. Each participant was  
107 followed-up from the index date until the exit date. Exit date was defined as the earliest of the following  
108 dates: outcome (diagnosis of T2DM), study end, death or the date they left the general practice or the  
109 general practice stopped contributing to the database.

110 **Outcome and covariates**

111 A clinical diagnosis of T2DM by the general practitioner was the outcome of interest. In the UK,  
112 the Quality Outcome Framework (QOF) in general practices ensures high quality data on important  
113 comorbidities such as cardiovascular disease, hypertension and T2DM <sup>21</sup>. Within the database, diagnostic  
114 codes for T2DM were identified based on Read codes, a hierarchical coding system to record signs,  
115 symptoms, procedures and diagnosis in primary care <sup>3</sup>. Covariates that are independent predictors of  
116 T2DM other than the exposure of interest were selected on the basis of biological plausibility and  
117 previous literature <sup>22</sup>. These included age, body mass index (BMI), Townsend deprivation score and  
118 smoking status.

119 **Statistical analysis**

120 Baseline data of each category in the serum testosterone and SHBG cohorts was reported  
121 separately for men and women as mean (standard deviation) or median (interquartile range [IQR]) as  
122 appropriate for continuous variables and as proportions for categorical variables. Crude Incidence Rate  
123 Ratio (IRR) and adjusted Incidence Rate Ratio (aIRR) were calculated by applying Poisson regression  
124 offsetting for the person years of follow-up. Covariates adjusted for in the model were age, BMI,  
125 Townsend quintiles and smoking status. In women, an additional model included polycystic ovary  
126 syndrome (PCOS) as a covariate to explore if the risk of T2DM in women was independent of a diagnosis  
127 of PCOS. In an additional sensitivity analysis, when adjusting for PCOS. we accepted the presence of  
128 hirsutism and anovulation as indicative of PCOS given that the diagnosis is underreported in primary  
129 care.

130           Where missing data existed (BMI, Townsend or smoking), we created a separate category so that  
131 all available data is utilized in the analysis. BMI was categorized as per WHO recommendation into  
132  $<25.0\text{kg/m}^2$ ,  $25\text{-}29.0\text{kg/m}^2$  and  $>30\text{kg/m}^2$ . All analyses were performed in STATA 14.0.

### 133 **Subgroup analysis**

134           In women, we performed stratified analysis by age ( $<50$  years and 50 years and above) to explore  
135 if the association was similar before and after the average age of menopause. A similar age-stratified  
136 analysis was also carried out in men. In addition to this, in those patients with simultaneous  
137 measurements of testosterone and SHBG, a free androgen index (FAI) was calculated  $[(\text{Tx}100)/\text{SHBG}]$ ,  
138 and risk of T2DM calculated to control for the confounding effect of low SHBG levels.

### 139 **Ethical Approval**

140           This study used routinely collected, anonymised primary care data. Patients were not involved in  
141 the study, and therefore no consent was required. Research using THIN data was approved by the NHS  
142 South-East Multicentre Research Ethics Committee in 2003, with the condition that studies undergo  
143 independent scientific review<sup>23</sup>. Approval for this analysis was obtained from the Scientific Review  
144 Committee for the use of THIN data in January 2018 (SRC reference number 17THIN106).



145 **RESULTS**

146 *Characteristics of the cohorts with serum testosterone and SHBG measurements*

147 A total of 152,430 participants in the cohort with available serum testosterone measurement  
148 results (testosterone cohort; 70,541 men and 81,889 women) and a total of 57,942 participants (15,907  
149 men and 42,035 women) in the SHBG cohort, both derived from the THIN database, met the inclusion  
150 criteria and were included in the current study. Median follow-up in the testosterone cohort was 3.3  
151 years (IQR:1.5-6.1) in men and 3.2 (IQR:1.3-6.2) years in women. In the SHBG cohort, median follow-  
152 up was 2.8 (1.3-4.9) years in men and 2.8 (1.2-5.4) in women. The mean age for men was 51.6 (SD  
153 14.8) years in the testosterone cohort and 51.7 (SD 16.0) years in the SHBG cohort. For women, mean  
154 age was 33.2 (SD 10.9) years in the testosterone cohort and 32.1 (SD 10.6) years in in the SHBG  
155 cohort. In total, 40,464 (57.4%) men in the testosterone cohort and 9,795 (61.6%) men in the SHBG  
156 cohort were overweight or obese (BMI  $\geq 25\text{kg/m}^2$ ). Among women, 36,640 (44.7 %) were obese or  
157 overweight in the testosterone cohort and 19,270 (45.8%) in the SHBG cohort. Approximately 21% of  
158 men and 22% of women were smokers across both testosterone and SHBG cohorts (**Table 1**). A diagnosis  
159 of PCOS was only documented in 6.3% (N=5,136) and 7.9% (N=3,303) of the female testosterone and  
160 SHBG cohorts, respectively. However, clinical features suggestive of PCOS, anovulation and clinical  
161 evidence of hirsutism, were documented in 25.8% and 11.2% of the female testosterone cohort,  
162 respectively, and in 26.9% and 12.1% of the female SHBG cohort, respectively.

163 Biochemical evidence of male androgen deficiency (serum testosterone  $< 7\text{nmol/L}$ ) was observed  
164 in 5,862 men (8.3%). Biochemical evidence of female androgen excess (serum testosterone  $> 2\text{nmol/L}$ )  
165 was observed in 20,565 women (25.1%); of those, 2,481 women (3.0%) had severe androgen excess  
166 (serum testosterone  $\geq 3.5\text{ nmol/L}$ ). Serum SHBG concentrations  $< 20\text{nmol/L}$  were observed in 2,517  
167 (15.8%) men and 3,733 (8.9 %) women (**Suppl. Tables 1-4**).

168 *Association between sex hormones and T2DM risk in men*

169 Among 70,541 men with serum testosterone measurements, 3,156 developed T2DM during the  
170 follow-up period. As expected, increasing age, overweight/obesity, smoking and higher social deprivation  
171 conferred an increased risk for T2DM (**Suppl. Tables 5 and 6**).

172 After adjusting for age, BMI, Townsend index and smoking status, aIRR for T2DM in men  
173 increased with decreasing categories of serum testosterone concentrations, most notably a 271% higher  
174 risk of developing T2DM in those with testosterone levels <7nmol/L, compared to the reference category  
175 of  $\geq 20$ nmol/L (aIRR 2.71, 95% CI 2.34-3.14,  $p < 0.001$ , **Table 2**). However, the risk of T2DM increased  
176 even within the normal male testosterone range (15-19.99 nmol/L, aIRR 1.29, 95% CI 1.13-1.47,  
177  $p < 0.001$ ; 10-14.99 nmol/L, aIRR 1.90, 95% CI 1.68-2.15,  $p < 0.001$ , **Table 2 & Figure 1A+B**).

178 In the SHBG cohort, among 15,907 men studied, there were 708 cases of incident T2DM during  
179 the follow-up period. After adjusting for age, BMI, Townsend index and smoking status, the risk of  
180 T2DM increased in men with SHBG levels <40nmol/L; aIRR of incident T2DM increased across  
181 categories of decreasing SHBG concentrations as compared to the reference category ( $\geq 60$ nmol/L) and  
182 the risk was more than 5-fold higher in the group with SHBG <20nmol/L (aIRR 5.74, 95% CI 3.72-8.87,  
183 **Table 2 & Figure 1C+D**).

#### 184 *Association between sex hormones and T2D risk in women*

185 Among 81,889 women with serum testosterone measurements, 1,282 developed T2DM during  
186 the follow-up period. After adjusting for age, BMI, Townsend index and smoking status, T2DM aIRR  
187 tended to be higher with increasing serum testosterone levels. The risk increased significantly for serum  
188 testosterone levels >1.5 nmol/L, as compared to reference category (<1nmol/L), and continued to increase  
189 across each category of serum testosterone concentrations thereafter, with a two-fold increase in risk  
190 observed in women with serum testosterone  $\geq 3.5$  nmol/L (aIRR 1.98, 95% CI 1.55-2.52,  $p < 0.001$ , **Table**  
191 **2 & Figure 2A+B**). Further adjustment for a diagnosis of PCOS or clinical features of suspected PCOS  
192 (hirsutism or anovulation) did not substantially change results (aIRR in subgroup of women with

193 testosterone levels  $>3.5$  nmol/L = 1.89, 95% CI 1.48-2.42,  $p<0.001$  and 1.76, 95% CI 1.38-2.25,  $p<0.001$   
194 respectively, **Suppl. Table 7**).

195 In the SHBG cohort, among 42,034 women studied, there were 597 cases of incident T2DM  
196 during the follow-up period. The risk of incident T2DM increased with each category of decreasing  
197 SHBG concentration. Women with serum SHBG concentrations  $<20$ nmol/L had a 9-fold higher risk of  
198 developing T2DM compared to the reference category of  $\geq 60$ nmol/L (aIRR 9.23, 95% CI 6.61-12.88,  
199  $p<0.001$ ), after adjustment for age, BMI, Townsend index and smoking status (**Table 2 & Figure 2C+D**).  
200 Additional adjustment for a diagnosis of PCOS and clinical features of suspected PCOS did not alter the  
201 risk of T2DM (aIRR 9.13, 95% CI 6.53-12.75,  $p<0.001$  and aIRR 8.88, 95% CI 6.36-12.42,  $p<0.001$   
202 respectively, **Suppl. Table 8**).

### 203 *Analysis of sex hormones as a continuous variable*

204 In men, for every nmol/L decrease in testosterone, the risk of T2DM increased by 5% (aIRR 1.05,  
205 95% CI 1.04-1.06,  $p<0.001$ ). In women, for every nmol/L increase in testosterone, the risk of T2DM  
206 increased by 10% (aIRR 1.10, 95% CI 1.06-1.14,  $p<0.001$ ). In the analysis of SHBG, for every nmol/L  
207 decrease in SHBG the risk of T2DM increased by 3% in both men and women (aIRR 1.03, 95% CI 1.03-  
208 1.04,  $p<0.001$ , in both sexes).

### 209 *Free androgen index and risk of T2DM*

210 Only 40% women ( $n=34,578$ ) and 16% of men ( $n=12,178$ ) had undergone a simultaneous  
211 measurement of serum SHBG and testosterone. Using these to calculate the free androgen index (FAI),  
212 we found that FAI was positively associated with risk of T2DM in women (aIRR 1.03, 95% CI 1.02-1.04,  
213  $p<0.001$ ), but not in men (aIRR 1.00, 95% CI 0.997-1.004,  $p=0.789$ ).

### 214 *Subgroup analyses*

215 Subgroup analysis stratified by age ( $<50$  and  $\geq 50$  years) did not alter the observed associations. In  
216 both age groups, a gradient increase in risk of T2DM was observed with increasing testosterone  
217 concentrations in women and decreasing testosterone concentrations in men (**Suppl. Fig 1 and Suppl.**

218 **Tables 9-12).** Increased aRRs for T2DM were noted with lower concentrations of SHBG in both age  
219 groups in men and women (**Suppl. Fig 2 and Suppl. Tables 13-16).**

## 220 **DISCUSSION**

221 In this large retrospective cohort study, we have demonstrated that androgens confer an  
222 independent sex-specific effect on the risk of incident T2DM. To our knowledge, this is the largest study,  
223 and the first longitudinal analysis, to address the impact of serum testosterone on risk of development of  
224 T2DM in both men and women. In the female cohort, aRRs for T2DM increased significantly once  
225 serum testosterone concentrations increased above 1.5nmol/L; even those with circulating testosterone  
226 levels between 1.5 and 1.99nmol/L, conventionally considered within the normal physiological range for  
227 women, already had a 23% increased risk of T2DM compared to the reference group. Perhaps even more  
228 surprisingly, once male serum testosterone concentrations dropped below 20nmol/L, the risk of T2DM  
229 began to increase; men with circulating concentrations between 15 and 19.99nmol/L, i.e. within the  
230 normal physiological male range, had a 28% increased risk of T2DM over the study period. Reduced  
231 SHBG concentrations in both sexes, but particularly in women, also potently increased the risk of T2DM.  
232 This finding is in agreement with observations from some previous studies, which demonstrated a  
233 stronger inverse association between SHBG levels and risk of T2DM in women compared to men<sup>10,24</sup>.  
234 This inverse relationship with T2DM appears to be particularly strong in postmenopausal women<sup>25</sup>. A  
235 2011 meta-analysis, however, found that higher SHBG levels were equally associated with a reduced risk  
236 of metabolic syndrome in both sexes<sup>26</sup>.

237 A systematic review and meta-analysis, which included a total of 3825 men and 4795 women in  
238 36 cross-sectional studies, as well as 368 cases from 7 prospective study populations, previously  
239 demonstrated that increased serum testosterone was associated with a 60% higher risk of T2DM in  
240 women; higher testosterone levels in men reduced the risk of T2DM by 42%<sup>10</sup>. Goodman-Gruen *et al*  
241 also observed sex differences in the association between serum androgens and glucose tolerance status in  
242 an older community cohort of 775 men and 633 women above the age of 55<sup>27</sup>. Men with impaired fasting

243 glucose, impaired glucose tolerance and T2DM had significantly lower levels of serum testosterone,  
244 while women with T2DM had significantly higher levels of bioavailable testosterone, independent of total  
245 body fat, fat distribution, physical activity and smoking. However, our study is the only longitudinal  
246 retrospective analysis to comprehensively evaluate these associations.

247 A number of key insights into the role of androgen excess in the development of metabolic  
248 dysfunction is provided by studies in women with polycystic ovary syndrome (PCOS), a disorder  
249 affecting up to 10% of the female population and primarily defined by the presence of hyperandrogenism  
250 and ovulatory dysfunction<sup>28</sup>. We have recently demonstrated that lean women with PCOS have an almost  
251 two-fold increased risk of NAFLD, a hepatic manifestation of metabolic dysfunction, and that androgen  
252 excess is an independent mediator of this increased risk<sup>3</sup>. Androgen-mediated adipose tissue lipotoxicity  
253 may contribute to this increase in NAFLD risk<sup>4,29</sup>. PCOS women are at significantly increased risk of  
254 impaired glucose tolerance and T2DM at a young age, irrespective of body weight<sup>30</sup>. A recent large  
255 Danish population register study concluded that the risk of T2DM was four-fold higher for women with  
256 PCOS, and diagnosed 4 years earlier, compared to women in the background population<sup>31</sup>.

257 Male androgen deficiency occurs as a consequence of primary testicular pathology,  
258 hypothalamic-pituitary disorders, obesity or as part of the ageing process in older men<sup>32,33</sup>. Additionally,  
259 iatrogenic hypogonadism due to androgen deprivation therapy is observed in men with prostate cancer<sup>34</sup>.  
260 Whilst the relationship between obesity and hypogonadism in men is complex and bidirectional<sup>35</sup>, data  
261 from male cohorts treated with short term androgen deprivation therapy show that hypogonadism directly  
262 induces metabolically deleterious changes in body composition, with increases in weight and in  
263 percentage fat body mass<sup>36</sup>. However, studies of androgen deprivation therapy, which result in significant  
264 hypogonadism, are not an ideal model to compare to the relatively modest reductions in testosterone  
265 observed in community-dwelling older men. The results of our study are particularly surprising, given  
266 that an increased risk of T2DM was apparent at circulating testosterone concentrations considered  
267 physiologically normal, but below the reference group of 20nmol/L, independent of age, obesity and other  
268 potential confounding factors. However, our results do not imply endorsement of testosterone

269 pharmacotherapy to restore circulating testosterone levels above 20nmol/L in otherwise healthy men.  
270 Studies investigating a potential beneficial impact of androgen therapy on metabolic outcomes in men  
271 with testosterone concentrations in the low or low-normal range have shown at best conflicting results. A  
272 recent double-blind placebo-controlled trial of testosterone treatment in 788 older men showed no impact  
273 on serum glucose or HbA1C<sup>37</sup>; another study showed no change in insulin sensitivity after 36 months of  
274 treatment in 308 community-dwelling men<sup>38</sup>. The 2018 Endocrine Society Clinical Practice Guideline on  
275 testosterone therapy in men with hypogonadism no longer recommend screening men with T2DM for low  
276 serum testosterone, and advise against using testosterone therapy to improve glycaemic control<sup>39</sup>.

277         Low circulating SHBG has been consistently identified as a surrogate marker for T2DM in both  
278 sexes in a number of smaller studies and meta-analyses<sup>10,40,41</sup>, and our study supports these observations.  
279 In a meta-analysis of 13 population-based studies with 1,912 incident cases of T2DM, low SHBG was  
280 associated with increased risk of T2DM in women, irrespective of menopausal status<sup>40</sup>. SHBG levels are  
281 typically higher in women, and our data confirm that reduced circulating concentrations are associated  
282 with a higher risk of T2DM than that observed in men. SHBG is a critical mediator of the association  
283 between sex steroids and metabolic dysfunction. The majority of circulating testosterone is bound to  
284 SHBG, such that only the unbound or 'free' fraction is capable of exerting effects in target tissues<sup>42</sup>.  
285 Therefore, reduced SHBG levels in women are a surrogate marker of increased circulating active  
286 androgens. Insulin is a potent regulator of hepatic SHBG output, which is suppressed in the context of  
287 hyperinsulinemia, leading to reduced SHBG, and therefore increased free androgens, in insulin resistant  
288 states such as PCOS in women<sup>43</sup>. It is unlikely, however, that SHBG independently plays a causal role in  
289 the pathophysiology of metabolic diseases such as T2DM. Low SHBG and testosterone levels in men are  
290 likely to be mediated by obesity in a population already at increased risk<sup>44</sup>. We found that FAI in men did  
291 not have a negative linear association with T2DM risk, indicating that low SHBG rather than testosterone  
292 is the predominantly associated with metabolic risk in men. This supports the observations of Bhasin<sup>45</sup>,  
293 but conflicts with those of Haring *et al*, who found that declining testosterone rather than SHBG levels  
294 were the main driver of metabolic syndrome in a large German cohort<sup>46</sup>. It is important to note that FAI

295 must be interpreted with caution in both men and women, and is particularly inaccurate in women when  
296 the SHBG concentration falls below 30nmol/l <sup>47</sup>.

297         This study has a number of important limitations, not least its retrospective nature. Detailed  
298 clinical phenotyping in studies of this type are not possible. There are also no detailed data available on  
299 laboratory assays used to measure serum testosterone. This is not of particular concern in men, as  
300 physiologically higher testosterone concentrations do not represent a challenge for quantification by either  
301 radioimmunoassay (RIA) or tandem mass spectrometry techniques. In women, however, where low  
302 circulating concentrations pose significant analytical and quantification difficulties for standard RIAs, the  
303 consensus is that today measurements should be performed by liquid chromatography-tandem mass  
304 spectrometry to improve quantification and avoid cross reactivity <sup>48</sup>. Furthermore, we have no information  
305 on the time of day blood sampling for serum testosterone took place; in men, Endocrine Society  
306 guidelines emphasize that morning samples are crucial to accurately diagnose hypogonadism <sup>49</sup>. Lastly,  
307 we must assume that testosterone data were obtained from men and women with a clinical indication for  
308 serum measurement; this introduces a potential bias by indication. However, we believe that these  
309 limitations are ameliorated by the large patient numbers and the clear and potent gradient towards sex-  
310 specific T2DM risk in the study population.

311         In conclusion, in the largest retrospective longitudinal study of its kind, we have demonstrated  
312 evidence of a sexually dimorphic role for androgens in mediating the risk of T2DM. Reduced SHBG  
313 levels in both sexes, but particularly in women, significantly increase the risk of T2DM. These data  
314 further define androgens as a novel metabolic risk factor in men and women, but potential mechanisms  
315 underpinning these observations remain to be clarified. We suggest that women with androgen excess and  
316 men with androgen deficiency should be systematically screened for T2DM. Future studies will be  
317 required to show if reducing androgens in women, and increasing androgens in men, will improve overall  
318 metabolic health and risk of progression to overt T2DM.

319

320

321 **AUTHOR CONTRIBUTIONS**

322 MWOR, WA and KN conceptualized the manuscript. MG, BK, AS, KAT and KN designed the  
323 methodology. MG, KM, AS and KN performed data cleaning and analysis. MWOR, MG, BK, AS, WA,  
324 KNM and KN wrote the manuscript. MWOR, BK, AS, TM, WH, KAT, KNM, AAT OHF, KN and WA  
325 reviewed and edited the final manuscript. WA and KN were responsible for overall supervision. All  
326 authors contributed to the interpretation of the data and approved the final manuscript for submission.



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**Table 1:** Baseline characteristics of the testosterone and SHBG cohorts stratified by sex

| Characteristics                           | Men                |                 | Women              |                 |
|---|--------------------|-----------------|--------------------|-----------------|
|   | Serum testosterone | Serum SHBG      | Serum testosterone | Serum SHBG      |
| <b>Population</b>                         |                    |                 |                    |                 |
| n (%)                                     | 70,541 (46.28)     | 15,907 (27.45)  | 81,889 (53.72)     | 42,034 (72.55)  |
| <b>Age (years)</b>                        |                    |                 |                    |                 |
| mean (SD)                                 | 51.6 (14.8)        | 51.7 (16.0)     | 33.2 (10.9)        | 32.1 (10.6)     |
| <b>Townsend index</b> n (%)               |                    |                 |                    |                 |
| 1 (least deprived)                        | 20,017 (28.38)     | 3,997 (25.13)   | 18,470 (22.55)     | 8,753 (20.82)   |
| 2   | 15,481 (21.95)     | 3,427 (21.54)   | 15,688 (19.16)     | 7,688 (18.29)   |
| 3   | 13,687 (19.40)     | 3,033 (19.07)   | 17,043 (20.81)     | 8,681 (20.65)   |
| 4   | 10,997 (15.59)     | 2,565 (16.12)   | 15,295 (18.68)     | 8,155 (19.40)   |
| 5 (most deprived)                         | 7,374 (10.45)      | 2,186 (13.74)   | 10,269 (12.54)     | 5,955 (14.17)   |
| Missing or implausible data               | 2,985 (4.23)       | 699 (4.39)      | 5,124 (6.26)       | 2,802 (6.67)    |
| <b>BMI (kg/m<sup>2</sup>) categorised</b> |                    |                 |                    |                 |
| n (%)                                     |                    |                 |                    |                 |
| <25                                       | 19,195 (27.21)     | 3,995 (25.11)   | 32,519 (39.71)     | 15,975 (38.00)  |
| 25-30                                     | 25,962 (36.80)     | 5,817 (36.57)   | 16,849 (20.58)     | 8,445 (20.09)   |
| >30                                       | 14,502 (20.56)     | 3,978 (25.01)   | 19,791 (24.17)     | 10,825 (25.75)  |
| Missing or implausible data               | 10,882 (15.43)     | 2,117 (13.31)   | 12,730 (15.55)     | 6,789 (16.15)   |
| <b>Smoking status</b>                     |                    |                 |                    |                 |
| n (%)                                     |                    |                 |                    |                 |
| Non-smokers                               | 53,311 (75.57)     | 12,264 (77.10)  | 61,288 (74.84)     | 31,557 (75.07)  |
| Smokers                                   | 15,325 (21.72)     | 3,377 (21.23)   | 18,020 (22.01)     | 9,312 (22.15)   |
| Missing or implausible data               | 1,905 (2.70)       | 266 (1.67)      | 2,581 (3.15)       | 1,165 (2.77)    |
| <b>Confounding conditions</b>             |                    |                 |                    |                 |
| PCOS                                      |                    |                 | 5,136 (6.27)       | 3,303 (7.86)    |
| Anovulation                               |                    |                 | 21,148 (25.83)     | 11,288 (26.85)  |
| Hirsutism                                 |                    |                 | 9,133 (11.15)      | 5,064 (12.05)   |
| <b>Follow-up in years</b>                 |                    |                 |                    |                 |
| median (IQR)                              | 3.3 (1.5 – 6.1)    | 2.8 (1.3 – 4.9) | 3.2 (1.3 – 6.2)    | 2.8 (1.2 – 5.4) |

**Table 2.** Risk of incident T2DM according to the category of serum testosterone and SHBG at baseline

|   | IRR (95% CI); p-value           |                               |                               |                               |
|---|---------------------------------|-------------------------------|-------------------------------|-------------------------------|
|   | Adjusted <sup>1</sup>           | Adjusted <sup>2</sup>         | Adjusted <sup>3</sup>         | Adjusted <sup>4</sup>         |
| <b>MEN</b>  |                                 |                               |                               |                               |
| <b>Serum testosterone concentration categories (nmol/L)</b> |                                 |                               |                               |                               |
| < 7   | 3.82 (3.31-4.41);<br>p<0.001    | 2.60 (2.25-3.00);<br>p<0.001  | 2.71 (2.34-3.14);<br>p<0.001  |                               |
| 7 - 9.99  | 3.70 (3.24-4.22);<br>p<0.001    | 2.46 (2.15-2.81);<br>p<0.001  | 2.57 (2.24-2.94);<br>p<0.001  |                               |
| 10 - 14.99  | 2.40 (2.13-2.71);<br>p<0.001    | 1.83 (1.62-2.06);<br>p<0.001  | 1.90 (1.68-2.15);<br>p<0.001  |                               |
| 15 - 19.99  | 1.45 (1.27-1.66);<br>p<0.001    | 1.25 (1.09-1.43);<br>p=0.001  | 1.29 (1.13-1.47);<br>p<0.001  |                               |
| ≥ 20  | <i>Ref</i>                      | <i>Ref</i>                    | <i>Ref</i>                    |                               |
| <b>Serum SHBG concentration categories (nmol/L)</b>         |                                 |                               |                               |                               |
| <20   | 8.23 (5.37-12.63);<br>p<0.001   | 5.00 (3.24-7.71);<br>p<0.001  | 5.74 (3.72-8.87);<br>p<0.001  |                               |
| 20 - 29.99  | 4.30 (2.83-6.53);<br>p<0.001    | 2.92 (1.91-4.44);<br>p<0.001  | 3.20 (2.09-4.87);<br>p<0.001  |                               |
| 30 - 39.99  | 3.33 (2.19-5.08);<br>p<0.001    | 2.45 (1.60-3.74);<br>p<0.001  | 2.61 (1.71-3.99);<br>p<0.001  |                               |
| 40 - 49.99  | 1.56 (0.98-2.50);<br>p=0.063    | 1.28 (0.80-2.06);<br>p=0.298  | 1.36 (0.85-2.17);<br>p=0.207  |                               |
| 50 - 59.99  | 1.07 (0.61-1.87);<br>p=0.825    | 0.88 (0.50-1.54);<br>p=0.654  | 0.91 (0.52-1.60);<br>p=0.748  |                               |
| ≥60   | <i>Ref</i>                      | <i>Ref</i>                    | <i>Ref</i>                    |                               |
| <b>WOMEN</b>  |                                 |                               |                               |                               |
| <b>Serum testosterone concentration categories (nmol/L)</b> |                                 |                               |                               |                               |
| < 1   | <i>Ref</i>                      | <i>Ref</i>                    | <i>Ref</i>                    | <i>Ref</i>                    |
| 1.0 - 1.49  | 1.21 (1.02-1.43);<br>p=0.030    | 1.12 (0.95-1.33);<br>p=0.184  | 1.12 (0.94-1.32);<br>p=0.204  | 1.11 (0.94-1.32);<br>p=0.213  |
| 1.5 - 1.99  | 1.45 (1.23-1.70);<br>p<0.001    | 1.26 (1.07-1.48);<br>p=0.005  | 1.23 (1.05-1.45);<br>p=0.011  | 1.23 (1.04-1.44);<br>p=0.013  |
| 2.0 - 2.49  | 1.70 (1.42-2.04);<br>p<0.001    | 1.34 (1.12-1.61);<br>p=0.002  | 1.30 (1.08-1.56);<br>p=0.005  | 1.28 (1.07-1.54);<br>p=0.008  |
| 2.5 - 2.99  | 2.07 (1.67-2.58);<br>p<0.001    | 1.59 (1.27-1.97);<br>p<0.001  | 1.53 (1.23-1.90);<br>p<0.001  | 1.50 (1.20-1.87);<br>p<0.001  |
| 3.0 - 3.49  | 2.51 (1.90-3.32);<br>p<0.001    | 1.74 (1.31-2.30);<br>p<0.001  | 1.68 (1.27-2.23);<br>p<0.001  | 1.62 (1.22-2.15);<br>p=0.001  |
| ≥ 3.5   | 3.00 (2.36-3.82);<br>p<0.001    | 2.09 (1.64-2.67);<br>p<0.001  | 1.98 (1.55-2.52);<br>p<0.001  | 1.89 (1.48-2.42);<br>P<0.001  |
| <b>Serum SHBG concentration categories (nmol/L)</b>         |                                 |                               |                               |                               |
| <20   | 19.76 (14.36-27.21);<br>p<0.001 | 8.96 (6.42-12.50);<br>p<0.001 | 9.23 (6.61-12.88);<br>p<0.001 | 9.13 (6.53-12.75);<br>p<0.001 |
| 20 - 29.99  | 8.66 (6.29-11.93);<br>p<0.001   | 4.45 (3.20-6.19);<br>p<0.001  | 4.48 (3.22-6.24);<br>p<0.001  | 4.44 (3.19-6.18);<br>p<0.001  |
| 30 - 39.99  | 4.66 (3.31-6.57);<br>p<0.001    | 2.69 (1.90-3.82);<br>p<0.001  | 2.70 (1.91-3.84);<br>p<0.001  | 2.69 (1.90-3.82);<br>p<0.001  |
| 40 - 49.99  | 2.99 (2.04-4.38);<br>p<0.001    | 2.05 (1.40-3.02);<br>p<0.001  | 2.08 (1.41-3.05);<br>p<0.001  | 2.07 (1.41-3.05);<br>p<0.001  |
| 50 - 59.99  | 1.64 (1.02-2.64);<br>p=0.043    | 1.29 (0.80-2.08);<br>p=0.295  | 1.29 (0.80-2.07);<br>p=0.304  | 1.29 (0.80-2.08);<br>p=0.301  |
| ≥60   | <i>Ref</i>                      | <i>Ref</i>                    | <i>Ref</i>                    | <i>Ref</i>                    |

**O'Reilly MW *et al.***

<sup>1</sup> *Adjusted for age,*

<sup>2</sup> *Adjusted for age, BMI,*

<sup>3</sup> *Adjusted for age, BMI, Townsend index, smoking status*

<sup>4</sup> *Adjusted for age, BMI, Townsend index, smoking status, PCOS*

*Abbreviations: IRR, incidence rate ratio; SHBG, sex hormone-binding globulin; T2DM, type 2 diabetes mellitus*

**FIGURE LEGENDS**

**Figure 1:** Risk of incident type 2 diabetes (T2DM) according to serum testosterone and sex hormone-binding globulin (SHBG) concentration categories in men. (A) Adjusted Incidence Rate Ratios (aIRRs) for diabetes in 70,541 men with serum testosterone measurements. (B) Distribution of 70,541 men across each quintile of serum testosterone concentration. (C) aIRRs for serum SHBG concentrations for incident diabetes in 15,907 men. (D) Distribution of 15,907 men across each category of serum SHBG concentration.

**Figure 2:** Risk of incident type 2 diabetes (T2DM) according to serum testosterone and sex hormone-binding globulin (SHBG) concentrations in women. (A) Adjusted Incidence Rate Ratios (aIRRs) for incident diabetes in 81,889 women with serum testosterone measurements. (B) Distribution of 81,889 women across each category of serum testosterone concentration. (C) aIRRs for serum SHBG concentrations for incident diabetes in 42,034 women with serum SHBG measurements. (D) Distribution of 42,034 women across each category of serum SHBG concentration.