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## Effects of testosterone and sex hormone binding globulin on lung function in males and females: a multivariable Mendelian Randomisation study

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8 **The effects of testosterone and SHBG on lung function in males and females: A multivariable MR**  
9 **study**

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12

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28 **Abstract**

29 **Background:** Observational studies suggest that testosterone (TT) and sex hormone-binding globulin  
30 (SHBG) may have beneficial effects on lung function, but these findings might be spurious due to  
31 confounding and reverse causation. We addressed these limitations by using multivariable  
32 Mendelian randomisation (MVMR) to investigate the independent causal effects of TT and SHBG on  
33 lung function.

34 **Methods:** We first identified genetic instruments by performing genome-wide association analyses of  
35 TT and SHBG in the large UK Biobank, separately in males and females. We then assessed the  
36 independent effects of TT and SHBG on FEV<sub>1</sub>, FVC, and FEV<sub>1</sub>/FVC using one-sample MVMR. We  
37 addressed pleiotropy, which could bias MVMR, using several methods that account for it. We  
38 performed subgroup MVMR analyses by obesity, physical activity and menopausal status, and  
39 assessed associations between TT and SHBG with lung function decline. Finally, we compared the  
40 MVMR results with those of observational analyses in UK Biobank.

41 **Findings:** In the MVMR analyses there was evidence of pleiotropy, but results were consistent when  
42 accounting for it. We found a strong beneficial effect of TT on FVC and FEV<sub>1</sub> in both males and  
43 females, but a moderate detrimental effect of SHBG on FEV<sub>1</sub> and FEV<sub>1</sub>/FVC in males only. Subgroup  
44 analyses suggested stronger effects of TT among obese and older males. The observational analyses,  
45 in line with previous studies, agreed with MRMV for TT, but not for SHBG.

46 **Interpretation:** These findings suggest that testosterone improves lung function in males and  
47 females, while SHBG has an opposite independent effect in males.

48

49 **Word count abstract:** 248 words

50

51 **Keywords:** Testosterone; sex hormone-binding globulin; lung function; multivariable Mendelian  
52 randomisation; GWAS.

53 **Summary box**

54 What is already known on this topic

55 A few epidemiologic studies have shown that higher testosterone levels are associated with better  
56 lung function in men and experimental studies have highlighted possible protective effects of  
57 testosterone in the lungs. Our recent observational analysis of the UK Biobank study also showed a  
58 significant beneficial association of sex hormone-binding globulin (SHBG) levels with forced vital  
59 capacity (FVC) and forced expiratory volume in one second (FEV<sub>1</sub>), both in males and females.  
60 However, whether testosterone and SHBG have a causal effect on lung function in both sexes  
61 remains unknown, as associations from observational studies may arise from confounding or reverse  
62 causation.

63 What this study adds

64 By using multivariable Mendelian Randomisation, which addresses the limitations of observational  
65 studies, our study in the large UK Biobank provides strong evidence that higher testosterone levels  
66 lead to higher FEV<sub>1</sub> and FVC levels in both men and women from the general population. Conversely,  
67 SHBG has a negative effect on FEV<sub>1</sub> and FEV<sub>1</sub>/FVC in men.

68 How this study might affect research, practice or policy

69 Our findings stress the need for further research on testosterone supplementation in people at risk  
70 of accelerated lung function decline and suggest the need for active screening for lung function  
71 impairment in people with low testosterone levels.

72 **Introduction**

73 Sex hormones influence lung physiology and respiratory disease. Clinical studies have reported low  
74 levels of testosterone in male patients with chronic obstructive pulmonary disease (COPD).[1] This  
75 was initially thought to reflect endocrine dysfunctions due to the disease or to the long-term use of  
76 corticosteroids in COPD patients.[1–3] However, recent laboratory based research has provided  
77 stronger evidence for an overall beneficial effect of androgens on respiratory health.[4–8] In line with  
78 this hypothesis, epidemiological studies have reported positive associations of lung function with  
79 levels of testosterone, testosterone precursors (dehydroepiandrosterone (DHEA)), testosterone  
80 metabolites (DHT), and androgen receptor expression in the airway epithelium in men [2,9,10], with  
81 some positive associations also reported in women.[11–15] One study also reported higher forced  
82 vital capacity (FVC) and forced expiratory volume in one second (FEV<sub>1</sub>) in men with higher levels of  
83 sex hormone-binding globulin (SHBG), which binds androgens and oestrogens with high affinity  
84 regulating their bioavailability to tissues and target cells.[9]

85 In agreement with previous cross-sectional studies, our recent observational analysis of the UK  
86 Biobank study (UKB) showed a positive association of total testosterone (TT) with FEV<sub>1</sub> and FVC in  
87 men.[13] This was not observed in women, but a longitudinal analysis showed that women with  
88 higher TT levels at baseline had reduced decline in FEV<sub>1</sub> and FVC over the follow-up. In this study we  
89 also reported a significant positive association of SHBG levels with FEV<sub>1</sub> and FVC, both in men and  
90 women, with participants with the greatest increase in SHBG over 4 years of follow-up also having  
91 higher FVC at the end of follow-up. Although the underlying mechanisms are unclear, there is  
92 increasing evidence that SHBG might have biological effects that are independent of those explained  
93 by its binding to sex steroids.[16–19] SHBG could itself function as a hormone or as a signal  
94 transduction factor. A recent Mendelian Randomisation (MR) study reported a positive causal effect  
95 of SHBG on asthma, supporting the hypothesis of beneficial effects of SHBG in the lungs.[20]

96 Overall, these findings, mostly based on cross-sectional studies, suggest a protective effect of both TT  
97 and SHBG on lung function. However, they might be spurious and arise from confounding by factors  
98 such as smoking, physical activity and diet, or from reverse causation, whereby it is lung function  
99 affecting hormone levels rather than vice-versa (e.g., hormones influenced by hypoxemia[1–3]). The  
100 MR approach addresses these limitations by using genetic instruments as proxies for the risk factor  
101 of interest. MR is thereby not affected by traditional confounding or reverse causation and thus  
102 provides stronger evidence of a possible causal effect and its direction. MR has been shown to not  
103 always confirm observational evidence (see for example [21,22]), and it therefore represents an  
104 important complementary approach to observational studies when experimental studies (randomized  
105 controlled trials - RCTs) are not feasible. The validity of MR relies on some assumptions, the most  
106 problematic being the absence of pleiotropy, where a genetic instrument affects the outcome

107 through additional pathways independent from the risk factor of interest.[23] While pleiotropy can  
108 bias MR findings, methods have been developed to both detect and control for it.[24]

109 Here we used one-sample MR to investigate the presence of a causal effect of TT and SHBG on FEV<sub>1</sub>,  
110 FVC and FEV<sub>1</sub>/FVC, separately in males and females, using data from the large UKB. As levels of SHBG  
111 and TT are partly determined by the same genes, we used multivariable MR (MVMR), where TT and  
112 SHBG are modelled together to estimate their effects independent of each other. First, genetic  
113 instruments for the MVMR were selected by performing a genome-wide association (GWA) analysis  
114 of TT and SHBG, stratified by sex in UKB. Then, several MVMR methods were used to identify and  
115 address pleiotropy. As secondary analyses, we performed subgroup analyses to investigate possible  
116 effect modification by obesity, physical activity, and menopausal status in females and age above 50  
117 years in males. We also assessed the effect of TT and SHBG on lung function decline using 8-year  
118 follow-up data on a subset of the UKB study population. Finally, we compared the results of our main  
119 MVMR analyses with observational estimates of the association of TT and SHBG with lung function  
120 obtained in UKB.

## 121 **Material and methods**

122 We first performed a GWA analysis to identify SNPs associated with TT and SHBG to be used as  
123 genetic instruments. We then performed a one-sample MVMR to investigate the effect of TT and  
124 SHBG on lung function. We applied different MVMR methods to test the robustness of the findings.  
125 Using MVMR, we also investigated possible effect modification in population subgroups, and we  
126 assessed the effects of TT and SHBG on lung function decline. Finally, for comparison, we performed  
127 an observational analysis of the association of TT and SHBG with lung function. All analyses were  
128 performed using UKB data and were stratified by sex. A figure describing this workflow (Figure S1)  
129 and further details on the methods are provided in the Supplementary Methods.

### 130 UK Biobank data

131 We included subjects from the UKB, which is a large population-based study in the United Kingdom  
132 [25]. Details on the study design of UKB [25] and genotyping/imputation methods [26] have been  
133 published elsewhere. UKB has received ethics approval from the National Health Service National  
134 Research Ethics Service (Ref 11/NW/0382) and all participants provided written consent.

135 Within UKB, we created 3 datasets (Figure 1), which were subsequently stratified by sex: 1)  
136 “discovery hormone dataset” (2/3 of sample: N=323,144), used in the GWA analyses of TT and SHBG;  
137 2) “replication hormone dataset” (1/3 of sample: N=161,572), used for the replication of GWA  
138 findings of TT and SHBG and for the MVMR analyses; 3) “lung function dataset” (N=341,826), used  
139 for the MVMR analyses and for the observational analyses. The “lung function dataset” used to  
140 obtain the lung function effect estimates included only subjects with previously defined good-quality  
141 spirometry data (see Supplementary Methods). In the MVMR analyses, the overlap between the  
142 hormone replication dataset and the lung function datasets was about 70%.

143 For lung function we considered three measures: FEV<sub>1</sub> (mL), FVC (mL), and FEV<sub>1</sub>/FVC (%). The lung  
144 function estimates were based only on subjects with spirometry data that passed the quality control  
145 criteria developed by Prof. Tobin and coll. in the UK BiLEVE study[27], who created UKB variables for  
146 “best” measures. As lung function “best measure” were derived for white participants, and therefore  
147 only these were included in the lung function dataset, while the hormone datasets also included a  
148 very small proportion of participants from other ethnicities (5.4%).

### 149 GWA analysis for genetic instrument selection

150 To identify genetic instruments for TT and SHBG, we performed GWA analyses stratified by sex in the  
151 “discovery hormone dataset” on (natural) log-transformed TT and SHBG values. We used linear  
152 mixed-models implemented in BOLT-LMM[28], which account for relatedness and fine-scale  
153 population structure, adjusted for age, genotyping batch/array, and centre. We selected  
154 independent single nucleotide polymorphism (SNPs; linkage disequilibrium (LD),  $r^2 < 0.05$ ) with p-

155 value  $<5 \times 10^{-8}$  and tested them for replication in the “*replication* hormone dataset”. We considered  
156 SNPs as replicated if the direction of their effect was the same as in the discovery dataset, and their  
157 Bonferroni-corrected one-side p-value was statistically significant.

158 In total, we replicated 450 SNPs (Supplementary Table S1 and S2 and Manhattan plots in  
159 Supplementary Figures S2 and S3). For TT, we replicated 92 SNPs in males and 63 in females, which  
160 explained 8.1% and 3.6% of the variance in TT levels, respectively. For SHBG, we replicated 213 SNPs  
161 in males and 153 in females, which explained 20.5% and 13.0% of SHBG variance.

## 162 MVMR analyses

163 We performed one-sample MVMR analyses, with estimates of the causal effects of TT and SHBG on  
164 lung function derived from the SNP-hormone (G-X) and the SNP-lung function (G-Y) association  
165 estimates, using different MVMR methods (see Supplementary Methods for details and background  
166 information).[24,29]

167 As G-X estimates, we used the effect estimates of our GWA replication analysis and removed SNPs  
168 that were in LD ( $r^2 > 0.1$ ) between the SNP lists of TT and SHBG. In total, 178 independent SNPs were  
169 included in the male MVMR analyses, and 174 in the female analyses. To obtain the G-Y estimates,  
170 again stratified by sex, we estimated the effect of each SNP on FEV<sub>1</sub>, FVC and FEV<sub>1</sub>/FVC in UKB using  
171 BOLT-LMM and adjusting for age, age<sup>2</sup>, height, genotyping batch/array, and centre. G-X and G-Y  
172 estimates are reported in Supplementary Table S2.

173 The validity of MR heavily relies on the absence of “horizontal” pleiotropy, which occurs when a  
174 genetic instrument affects the outcome through pathways that are independent from the risk factor  
175 of interest.[23] We therefore applied six MVMR methods, of which two assume no pleiotropy (two-  
176 stage least squares (2SLS) and fixed-effect inverse-variance weighted (FE-IVW) meta-analysis), while  
177 the others account for pleiotropy but make different assumptions about it: random-effects IVW (RE-  
178 IVW) meta-analysis, robust regression, weighted median estimation, and MR-Egger regression (see  
179 Supplementary Methods for details and references).

180 Possible pleiotropy was detected, and reported in the Supplementary Tables (Q test, Sargan test, and  
181 MR-Egger intercept p-value). To assess the validity of our genetic instruments, we took two  
182 approaches to identify and remove potential pleiotropic SNPs which were subsequently excluded in  
183 sensitivity analyses; 1) a statistical approach using MVMR-PRESSO[30] to detect outliers, and 2) using  
184 PhenoScanner ([www.phenoscanter.medschl.cam.ac.uk](http://www.phenoscanter.medschl.cam.ac.uk)) to identify SNPs associated with potential  
185 sources of pleiotropy identified a priori: smoking, weight-related traits, diabetes, insulin, leptin and  
186 adiponectin (n= 36 SNPs). In addition, to better understand the relevance of potential pleiotropic  
187 pathways, we further investigated MVMR-PRESSO outliers by searching for association of these SNPs

188 with any other traits in previous GWA studies ( $p < 5 \times 10^{-8}$ ) using PhenoScanner; and used Leave-One-  
189 Out RE-IVW analysis to identify the most influential pleiotropic SNPs.

#### 190 MVMR Secondary analyses

191 We performed three subgroup analyses to investigate effect modification by potential modifiers: 1)  
192 obesity, as several studies have reported interactions between sex-hormones and obesity on lung  
193 function and asthma[22,31]); 2) moderate physical activity, as randomised controlled trials in COPD  
194 patients have suggested that testosterone supplementation might be more effective when combined  
195 with exercise training[3,13,32], and our previous observational analysis[13] highlighted a stronger  
196 positive effect of testosterone levels in physically active men; 3) menopausal status in females and  
197 age 50 years in males, as the effects of the SHBG and testosterone might vary with age and age-  
198 related changes in hormonal milieu.

199 We also performed MVRM to assess the effect of TT and SHBG on lung function decline using a  
200 subset of the UKB study population with available longitudinal data. We included 6,812 males and  
201 7,470 females with a mean follow-up time of 8.3 years. We obtained the G-Y estimates stratified by  
202 sex by estimating the effect of each SNP on yearly decline of FEV<sub>1</sub>, FVC and FEV<sub>1</sub>/FVC in UKB using the  
203 same model as in the main analysis (Supplementary Methods). Not all the 450 SNPs passed QC and  
204 therefore less SNPs were included in the MVMR analyses (see Supplementary Table S2 for the G-Y  
205 estimates of the included SNPs).

#### 206 Observational analyses

207 For comparison, we also performed cross-sectional observational analyses in UKB of the association  
208 of log-transformed TT and SHBG values with the three lung function outcomes, separately by sex,  
209 adjusted for the same covariates as in our previous study in UKB.[13] This analysis is different as both  
210 TT and SHBG were included in the same multiple linear regression models in order to estimate their  
211 independent association with lung function.[13] Moreover, our previous analysis was performed on a  
212 much smaller samples size (n=10,581) as it was based on longitudinal data only.

#### 213 Results presentation

214 To help interpretation of the results and figures (based on log-transformed hormone values), we  
215 calculated the average regression coefficient (beta) across MVMR methods and multiplied it by  
216  $\ln(1.1)$  to display the effect of a 10% increase in SHBG or TT on lung function. The effect estimates  
217 reported in the Supplementary Tables represent the effect on lung function of a 1-unit increase in  
218 log-transformed SHBG and TT.

## 219 **Results**

220 The characteristics of the participants included in the GWA and MVMR analyses are reported in Table  
221 1. Results of the GWA for genetic instrument selection and derivation of G-X and G-Y estimates for  
222 MVMR analysis are described in the methods and Supplementary Methods.

### 223 MVMR – Testosterone

224 In males, the MVMR analysis did not show consistent evidence across the various MVMR methods of  
225 an effect of TT on any lung function outcome but there was strong evidence of pleiotropy (Figure 2,  
226 Supplementary Table S3). After removing 13 SNPs identified by MVMR-PRESSO as pleiotropic  
227 (outliers), we found consistent evidence for a large beneficial effect of higher TT being associated  
228 with higher FVC and FEV<sub>1</sub> across all methods, although there was evidence of residual pleiotropy. A  
229 10% increase in TT was associated with 15.5mL higher FVC and 17.0mL higher FEV<sub>1</sub>, and therefore a  
230 small beneficial effect on FEV<sub>1</sub>/FVC (Figure 3 and Supplementary Table S4A). The Leave-One-Out  
231 analysis carried-out to investigate the effect of the 13 pleiotropic SNPs, identified two SNPs  
232 (rs56196860 in *FKBP4* and rs62062271 in *MAPT*) as the most influential pleiotropic SNPs that were  
233 driving the results towards the null (Supplementary Figures S4 and S5). Investigation of these SNPs in  
234 PhenoScanner showed that rs56196860 was not associated with any other trait at genome-wide  
235 significance level ( $p < 5 \times 10^{-8}$ ), but rs62062271 was associated with several human blood-cell traits in  
236 the GWA study by Astle *et al.* (Supplementary Table S5).[33] Of note, by lowering the significance  
237 level to  $p < 0.05$  in PhenoScanner, revealed that 9 out of the 13 pleiotropic SNPs were associated with  
238 blood-cell traits, including rs56196860 which was associated with platelet related outcomes.

239 In females, we found a beneficial effect of TT on FVC and FEV<sub>1</sub> (Figure 2, Supplementary Table S3),  
240 with 4.0mL higher FVC and 3.8mL higher FEV<sub>1</sub>. There was strong evidence of pleiotropy, but the  
241 results remained similar after removing 10 SNPs identified as outliers (Figure 3, Supplementary Table  
242 S4A).

### 243 MVMR – SHBG

244 In males, the MVMR analyses showed a detrimental effect of SHBG on FEV<sub>1</sub>/FVC (Figure 2,  
245 Supplementary Table S3), with about 0.07% lower FEV<sub>1</sub>/FVC. A detrimental effect on FEV<sub>1</sub> (-10.5mL)  
246 was also found, but only with the robust and weighted median MVMR methods. None of the MVMR  
247 methods showed an effect of SHBG on FVC. Again, there was strong evidence of pleiotropy, and the  
248 analyses removing 13 outlier SNPs showed a detrimental effect for SHBG on both FEV<sub>1</sub>/FVC and FEV<sub>1</sub>  
249 across all MVMR methods (Figure 3, Supplementary Table S4A). Of note, it is interesting that the  
250 detrimental effect of SHBG was accompanied by a beneficial effect of TT on both FEV<sub>1</sub> and FEV<sub>1</sub>/FVC  
251 (Figure 3).

252 In females, we did not find consistent evidence of an effect of SHBG on FVC or FEV<sub>1</sub>/FVC (Figure 2,  
253 Supplementary Table S3), while there was evidence of a small detrimental effect on FEV<sub>1</sub> (-2.0mL).  
254 However, when repeating the analysis excluding 10 outlier SNPs (which reduced but did not  
255 eliminate the heterogeneity), no significant effect was found in any of the MVMR analyses (Figure 3,  
256 Supplementary Table S4A).

257 For the analyses of both TT and SHBG and in both sexes, when we removed potential sources of  
258 pleiotropy identified *a priori* (36 SNPs), the results did not change (Supplementary Table S4B).

#### 259 MVMR – Secondary analyses

260 In males, the subgroup analyses showed a stronger effect of TT on FVC and FEV<sub>1</sub> in obese males  
261 (Figure 4A-B, Supplementary Table S6-A), although a statistically significant interaction with obesity  
262 was only found for one MVMR method (2SLS; FVC: +32.7mL in obese vs +3.8mL in non-obese,  
263 p=0.010). Furthermore, the beneficial effect of TT and detrimental effect of SHBG on FEV<sub>1</sub>/FVC (TT:  
264 +0.12%; SHBG: -0.11%) were restricted to males older than 50, with no effect found in younger males  
265 (Figure 4C, Supplementary Table S6-D). We found no evidence of modifying effects of obesity,  
266 moderate physical activity, or menopause in females (Supplementary Table S6).

267 In the MVMR analyses assessing the effect on lung function decline, we did not find a significant  
268 effect of TT or SHBG on the decline of any of the three lung function outcomes (Supplementary Table  
269 S7).

#### 270 Observational analyses

271 In the observational analyses, a 10% increase in TT was independently associated with higher FVC  
272 (+10.4mL) and FEV<sub>1</sub> (+9.3mL) in males (Figure 2, Supplementary Table S8). Beneficial statistically  
273 significant associations, although of small magnitude, were also observed between TT and FEV<sub>1</sub> in  
274 females (+0.87mL) and between TT and FEV<sub>1</sub>/FVC in both males (+0.04%) and females (+0.03%). The  
275 same analysis showed beneficial association of SHBG with higher FVC in both males (+6.6mL) and  
276 females (+6.0mL). SHBG was also beneficially, but less strongly, associated with FEV<sub>1</sub>, and thus  
277 detrimentally associated with FEV<sub>1</sub>/FVC.

278 **Discussion**

279 Our multivariable Mendelian Randomisation (MVMR) showed a beneficial effect of testosterone on  
280 FVC and FEV<sub>1</sub> in males, with a more moderate beneficial effect in females, independent from the  
281 effect of SHBG. While this provides causal support for previous observational evidence, our findings  
282 for SHBG contrast with the observational evidence in showing a detrimental effect of SHBG on  
283 FEV<sub>1</sub> and FEV<sub>1</sub>/FVC in males, with no effect of SHBG in females.

284 The MVMR finding of a beneficial effect of testosterone on FVC in males is consistent with  
285 observational findings from previous studies and from our own analysis.[2,9,10,13] While in the  
286 initial analysis there was no consistent evidence of an effect across MVMR methods, this appeared to  
287 be driven by pleiotropy, where genetic instruments affect the outcome through alternative  
288 independent pathways (phenotypes). Moreover, it suggested “negative” pleiotropy, where the effect  
289 of a genetic instruments on the alternative phenotype is in the opposite direction compared to its  
290 effect on testosterone. After removing pleiotropic SNPs identified by MVMR-PRESSO, we found a  
291 strong beneficial effect of TT and a weaker detrimental effect of SHBG on FVC in males. This suggests  
292 that a few SNPs associated with higher levels of testosterone are also associated with other factors  
293 that negatively impact lung function. Further investigation of the identified pleiotropic SNPs suggests  
294 that pleiotropy might occur through the effect of these SNPs on blood-cell subtypes, and indeed  
295 several blood-cell types have been previously associated with lung function and its decline.[34,35]  
296 Heterogeneity remained, although reduced, when we removed outlier SNPs, thus suggesting the  
297 presence of some unresolved pleiotropy. However, when we removed *a priori* considered potential  
298 sources of pleiotropy the results did not change, suggesting the presence of other unknown sources  
299 of pleiotropy. Moreover, the consistency of the findings across the different MVMR methods  
300 accounting for pleiotropy provides reassurance of the robustness of our results.

301 The MVMR analysis also suggests a moderate beneficial effect of testosterone on FVC in females.  
302 Significant beneficial effects of testosterone were found in all female analyses, including the  
303 subgroup analyses.[34] Few studies have investigated the associations between circulating  
304 testosterone and lung function in females. Recent cross-sectional analyses showed no significant, or  
305 modest detrimental, associations between free testosterone levels and FVC in women.[10,13]  
306 However, the longitudinal analysis over an 8-year follow-up by Lenoir *et al.*[13] showed a slower  
307 decline in FVC in females with higher levels of total and free testosterone at baseline. This is  
308 consistent with another study showing higher FVC in women with higher levels of DHEAs, a major  
309 precursor of testosterone in women.[12]

310 In addition to its action on lung development and maturation early in life, testosterone might  
311 increase FEV<sub>1</sub> through direct bronchodilator effects, and FVC through direct effects on respiratory or  
312 skeletal muscle strength, or it could affect both lung function measures by reducing inflammatory

313 responses and subsequent airway remodelling.[3,4,7,8,14] In our cross-sectional MVMR analyses, we  
314 found that TT had some beneficial effect on FVC, whereas we found no significant effect of TT in the  
315 longitudinal analysis with FVC decline. A first possible explanation might be a beneficial effect of TT in  
316 attaining higher maximum peak FVC during lung growth. However, at least in females, the slower FVC  
317 decline observed by Lenoir et al.[13] in females with higher testosterone might rather point towards  
318 a protective effect of testosterone in sustaining lung capacity in adulthood, rather than a beneficial  
319 effect during lung growth in early life. An alternative hypothesis might be a positive short-term effect  
320 of testosterone. Such a “short-term” effect was also proposed by Lenoir et al.[13] as an alternative  
321 explanation for the positive cross-sectional association between TT and FVC but lack of association  
322 with FVC decline in men. In the ECRHS, Pesce et al.[12] found that low level of the major precursor of  
323 testosterone in women (DHEA-S) was associated with FVC but not FVC decline and suggested that  
324 this might point towards a short-term, maybe reversible, effect of DHEA-S deficiency on pulmonary  
325 vital capacity. Similarly, a short-term positive effect of testosterone (possibly through broncho-  
326 relaxing properties, or anabolic effects on muscle strength) might explain why we observe strong  
327 associations in the cross-sectional analysis, but not in the longitudinal analysis. We further cannot  
328 exclude a small effect of TT on lung function decline that we could not observe after a short follow-  
329 up. Longitudinal studies, with lung function trajectories from childhood to adulthood would be useful  
330 to investigate whether the effect of TT is mostly on lung growth, and whether this takes place  
331 similarly in males and females.

332 Testosterone deficiency has been frequently reported in patients with COPD.[1,3,32] However, to  
333 date, small clinical trials of male patients with COPD failed to show any protective effect of  
334 testosterone supplementation on lung function, although it was associated with an increase in lean  
335 body mass and a reduced risk of respiratory hospitalizations.[3,32,36] Moreover, a recent phase 2A  
336 trial in men and postmenopausal women with COPD showed that a selective androgen receptor  
337 modulator (GSK2881078) increased leg strength, at least in men, and lean body mass in both men  
338 and women, but no changes in lung function were observed.[37] As suggested by Mohan et al.[9], it  
339 is possible that testosterone might have a beneficial effect in preserving lung function through  
340 mechanisms independent from those driving the lower testosterone levels in patients with COPD. It  
341 is also possible that the duration of the intervention trials was too short to observe a protective  
342 effect on lung function measures. In an additional analysis [data not shown], we did assess the  
343 association of TT and SHBG with airflow obstruction ( $FEV_1/FVC < \text{lower limit of normal}$ ) and did not  
344 find a clear association in either males or females, in line with the null association observed for the  
345  $FEV_1/FVC$  ratio.

346 There is increasing interest in a possible role of SHBG in several diseases.[16–20,38] SHBG binds to  
347 circulating sex hormones; in particular, 40%-65% of testosterone is bound to SHBG, which reduces

348 the concentration of unbound (“free”) testosterone.[39] In our observational analysis, which was  
349 adjusted for testosterone, SHBG showed positive associations with FVC in males and females,  
350 consistent with previous observational studies.[9,13] In contrast, our MVMR analysis showed  
351 significant independent detrimental effects of SHBG on FVC, FEV<sub>1</sub> and FEV<sub>1</sub>/FVC in males after  
352 removing pleiotropic SNPs. This detrimental effect of SHBG was always accompanied by a beneficial  
353 effect of TT. Therefore, one might speculate that SHBG has a detrimental effect on lung function  
354 through its binding to testosterone.[36]

355 In females, we found no evidence for a detrimental effect of SHBG on lung function. Of note,  
356 oestradiol also binds to SHBG, and in contrast to androgens, oestrogens may also have pro-  
357 inflammatory properties and might detrimentally impact lung function.[4,7,40] Therefore, the lack of  
358 effect of SHBG on lung function in females might be partly explained by SHBG binding, not only to  
359 testosterone, but also to oestradiol in females, and the balance between the two may be driving the  
360 final effect.

361 The “free hormone” hypothesis assumes that only unbound testosterone can bind to androgen  
362 receptors and thus exert its effect, and measurement of free testosterone has been recommended  
363 to establish clinical diagnoses, particularly in men with altered SHBG levels and in women[41].  
364 However, more recent evidence suggests that testosterone binding to SHBG is important for reaching  
365 and becoming active in target tissues[41], and that SHBG might even increase sex hormones signal  
366 transduction.[42] In this study we used MVMR with the aim to investigate the independent effects of  
367 SHBG and TT on lung function (i.e. whether for a given value of TT, individuals with higher SHBG have  
368 higher lung function, and vice-versa), and hence we did not consider any calculated index to reflect  
369 the proportion of TT that is not bound to SHBG.

370 The large sample size allowed us to investigate possible interactions based on *a priori* hypotheses.  
371 We observed that the effects of TT and SHBG on FVC in men were more marked in obese than in  
372 non-obese individuals, although the effects were in the same direction in the two groups and there  
373 was no consistent evidence of interaction. We found no evidence of interaction with physical activity  
374 and menopausal status in women, while we found that the small negative effect of SHBG on  
375 FEV<sub>1</sub>/FVC was limited to older men.

376 There are some limitations in our study. Like many previous observational studies on hormones, TT  
377 was not measured using a standardized LC-MS/MS assay, which is the gold-standard method.  
378 Regarding our findings for FEV<sub>1</sub>/FVC, a marker of obstruction, only pre-bronchodilator lung function  
379 measurements were available in UKB, and this does not allow us to differentiate between reversible  
380 airflow obstruction (as in asthma) and fixed obstruction (as in COPD). However, our findings mostly  
381 provided evidence of an effect of TT on FVC, while there was little evidence for an effect on  
382 FEV<sub>1</sub>/FVC. As we only used UK Biobank data, which predominantly includes white participants, and  
12

383 good spirometry data was limited to participants of Caucasian ancestry, our findings should be  
384 generalised with caution. Finally, we did not find an effect on lung function decline in our secondary  
385 MVMR analyses in contrast to our previous observational study[13]. However, this could be due to  
386 the greatly reduced number of UKB participants in the longitudinal analysis (N=14,282) compared  
387 to the main MVMR analyses (N=341,826) and the relatively short follow-up time (average 8 years),  
388 and there could be a small effect that we would be underpowered to detect. Future availability of  
389 longitudinal lung function data from large samples and over a long period of time will allow further  
390 MR investigations to provide conclusive evidence on the effects of sex hormones on lung function  
391 decline.

## 392 **Conclusion**

393 Our findings show that higher genetically-determined testosterone levels are associated with higher  
394 FVC and FEV<sub>1</sub> in both males and females. In contrast, higher genetically-determined SHBG levels are  
395 associated with lower FEV<sub>1</sub> and FEV<sub>1</sub>/FVC in males. These findings suggest a beneficial causal effect of  
396 testosterone and an adverse causal effect of SHBG on adult lung function. Our findings stress the  
397 need for further research to investigate whether testosterone therapy might be used to slow down  
398 lung function impairment among selected groups of people at risk of accelerated lung function  
399 decline and in older age. They also suggest the need for active screening for lung function  
400 impairment in people with low-testosterone levels.

401 **Statements**

402 Author contributions: DAvdP participated in the study design, analysis, and interpretation of the  
403 data, and drafting of the manuscript, tables and figures. CM, BL and DAvdP participated in  
404 determining the study design and interpretation of the data. CM, BL, AL, SD, JFP, FGR and DJ were  
405 involved in data collection and interpretation of the data. SOS, BL, CM, DJ, SD and FGR obtained  
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415 Competing Interest: DJ reports to have obtained a grant from the European Union. All other authors  
416 declare that there is no conflict of interest.

417 Ethics approval: UK Biobank has received ethics approval from the National Health Service National  
418 Research Ethics Service (Ref 11/NW/0382).

419 Data sharing: All data relevant to perform the multivariable Mendelian randomisation analyses are  
420 included as supplementary information (G-X and G-Y in Supplementary Table S2). Individual  
421 participant data may be obtained from a third party and are not publicly available. Please consult the  
422 data access policy of the UK Biobank at <https://www.ukbiobank.ac.uk/>.

423 **References**

- 424 1 Atlantis E, Fahey P, Cochrane B, et al. Endogenous testosterone level and testosterone  
425 supplementation therapy in chronic obstructive pulmonary disease (COPD): a systematic  
426 review and meta-analysis. *BMJ Open* 2013;3. doi:10.1136/bmjopen-2013-003127
- 427 2 Svartberg J, Schirmer H, Medbø A, et al. Reduced pulmonary function is associated with  
428 lower levels of endogenous total and free testosterone. The Tromsø study. *Eur J Epidemiol*  
429 2007;22:107–12. doi:10.1007/s10654-006-9095-9
- 430 3 Baillargeon J, Urban RJ, Zhang W, et al. Testosterone replacement therapy and  
431 hospitalization rates in men with COPD. *Chron Respir Dis* 2019;16:1479972318793004.  
432 doi:10.1177/1479972318793004
- 433 4 Kalidhindi RSR, Ambhore NS, Balraj P, et al. Androgen receptor activation alleviates airway  
434 hyperresponsiveness, inflammation, and remodeling in a murine model of asthma. *Am J*  
435 *Physiol Lung Cell Mol Physiol* 2021;320:L803–18. doi:10.1152/ajplung.00441.2020
- 436 5 Fuseini H, Yung JA, Cephus JY, et al. Testosterone Decreases House Dust Mite-Induced Type 2  
437 and IL-17A-Mediated Airway Inflammation. *J Immunol* 2018;201:1843–54.  
438 doi:10.4049/jimmunol.1800293
- 439 6 Sathish V, Martin YN, Prakash YS. Sex steroid signaling: Implications for lung diseases.  
440 *Pharmacol Ther* 2015;150:94–108. doi:10.1016/j.pharmthera.2015.01.007
- 441 7 Wang X, Huang L, Jiang S, et al. Testosterone attenuates pulmonary epithelial inflammation  
442 in male rats of COPD model through preventing NRF1-derived NF-κB signaling. *J Mol Cell Biol*  
443 2021;13:128–40. doi:10.1093/jmcb/mjaa079
- 444 8 Aono K, Matsumoto J, Nakagawa S, et al. Testosterone deficiency promotes the development  
445 of pulmonary emphysema in orchietomized mice exposed to elastase. *Biochem Biophys Res*  
446 *Commun* 2021;558:94–101. doi:10.1016/j.bbrc.2021.04.051
- 447 9 Mohan SS, Knuiman MW, Divitini ML, et al. Higher serum testosterone and  
448 dihydrotestosterone, but not oestradiol, are independently associated with favourable  
449 indices of lung function in community-dwelling men. *Clin Endocrinol (Oxf)* 2015;83:268–76.  
450 doi:10.1111/cen.12738
- 451 10 Han Y-Y, Yan Q, Yang G, et al. Serum free testosterone and asthma, asthma hospitalisations  
452 and lung function in British adults. *Thorax* 2020;75:849–54. doi:10.1136/thoraxjnl-2020-  
453 214875
- 454 11 DeBoer MD, Phillips BR, Mauger DT, et al. Effects of endogenous sex hormones on lung  
455 function and symptom control in adolescents with asthma. *BMC Pulm Med* 2018;18:58.  
456 doi:10.1186/s12890-018-0612-x
- 457 12 Pesce G, Triebner K, van der Plaats DA, et al. Low serum DHEA-S is associated with impaired  
458 lung function in women. *EClinicalMedicine* 2020;23:100389.  
459 doi:10.1016/j.eclinm.2020.100389
- 460 13 Lenoir A, Fuertes E, Gómez-Real F, et al. Lung function changes over 8 years and testosterone  
461 markers in both sexes: UK Biobank. *ERJ Open Res* 2020;6. doi:10.1183/23120541.00070-2020
- 462 14 Zein JG, McManus JM, Sharifi N, et al. Benefits of Airway Androgen Receptor Expression in  
463 Human Asthma. *Am J Respir Crit Care Med* Published Online First: March 2021.  
464 doi:10.1164/rccm.202009-3720OC
- 465 15 Han Y-Y, Forno E, Witchel SF, et al. Testosterone-to-estradiol ratio and lung function in a  
466 prospective study of Puerto Rican youth. *Ann Allergy Asthma Immunol* 2021;127:236-242.e1.  
467 doi:10.1016/j.anai.2021.04.013

- 468 16 Arathimos R, Millard LAC, Bell JA, et al. Impact of sex hormone-binding globulin on the  
469 human phenome. *Hum Mol Genet* 2020;29:1824–32. doi:10.1093/hmg/ddz269
- 470 17 Simó R, Sáez-López C, Barbosa-Desongles A, et al. Novel insights in SHBG regulation and  
471 clinical implications. *Trends Endocrinol Metab* 2015;26:376–83.  
472 doi:10.1016/j.tem.2015.05.001
- 473 18 Dimou NL, Papadimitriou N, Gill D, et al. Sex hormone binding globulin and risk of breast  
474 cancer: a Mendelian randomization study. *Int J Epidemiol* 2019;48:807–16.  
475 doi:10.1093/ije/dyz107
- 476 19 Zhao J v, Schooling CM. Sex-specific Associations of Sex Hormone Binding Globulin with CKD  
477 and Kidney Function: A Univariable and Multivariable Mendelian Randomization Study in the  
478 UK Biobank. *J Am Soc Nephrol* 2021;32:686–94. doi:10.1681/ASN.2020050659
- 479 20 Arathimos R, Granell R, Haycock P, et al. Genetic and observational evidence supports a  
480 causal role of sex hormones on the development of asthma. *Thorax* 2019;74:633–42.  
481 doi:10.1136/thoraxjnl-2018-212207
- 482 21 Markozannes G, Kannelopoulou A, Dimopoulou O, et al. Systematic review of Mendelian  
483 randomization studies on risk of cancer. *BMC Med* 2022;20:41. doi:10.1186/s12916-022-  
484 02246-y
- 485 22 van der Plaats DA, Pereira M, Pesce G, et al. Age at menopause and lung function: a  
486 Mendelian randomisation study. *Eur Respir J* 2019;54. doi:10.1183/13993003.02421-2018
- 487 23 Davies NM, Holmes M v., Davey Smith G. Reading Mendelian randomisation studies: A guide,  
488 glossary, and checklist for clinicians. *BMJ (Online)* 2018;362:k601. doi:10.1136/bmj.k601
- 489 24 Sheehan NA, Didelez V, Burton PR, et al. Mendelian randomisation and causal inference in  
490 observational epidemiology. *PLoS Med* 2008;5:1205–10. doi:10.1371/journal.pmed.0050177
- 491 25 Sudlow C, Gallacher J, Allen N, et al. UK Biobank: An Open Access Resource for Identifying the  
492 Causes of a Wide Range of Complex Diseases of Middle and Old Age. *PLoS Med*  
493 2015;12:e1001779. doi:10.1371/journal.pmed.1001779
- 494 26 Bycroft C, Freeman C, Petkova D, et al. The UK Biobank resource with deep phenotyping and  
495 genomic data. *Nature* 2018;562:203–9. doi:10.1038/s41586-018-0579-z
- 496 27 Wain L V, Shrine N, Miller S, et al. Novel insights into the genetics of smoking behaviour, lung  
497 function, and chronic obstructive pulmonary disease (UK BiLEVE): a genetic association study  
498 in UK Biobank. *Lancet Respir Med* 2015;3:769–81. doi:10.1016/S2213-2600(15)00283-0
- 499 28 Loh P-R, Tucker G, Bulik-Sullivan BK, et al. Efficient Bayesian mixed-model analysis increases  
500 association power in large cohorts. *Nat Genet* 2015;47:284–90. doi:10.1038/ng.3190
- 501 29 Grant AJ, Burgess S. Pleiotropy robust methods for multivariable Mendelian randomization.  
502 *Stat Med* 2021;40:5813–30. doi:https://doi.org/10.1002/sim.9156
- 503 30 Verbanck M, Chen CY, Neale B, et al. Detection of widespread horizontal pleiotropy in causal  
504 relationships inferred from Mendelian randomization between complex traits and diseases.  
505 *Nat Genet* 2018;50:693–698. doi:10.1038/s41588-018-0099-7
- 506 31 Han Y-Y, Forno E, Celedón JC. Sex Steroid Hormones and Asthma in a Nationwide Study of  
507 U.S. Adults. *Am J Respir Crit Care Med* 2020;201:158–66. doi:10.1164/rccm.201905-0996OC
- 508 32 Pan L, Wang M, Xie X, et al. Effects of anabolic steroids on chronic obstructive pulmonary  
509 disease: a meta-analysis of randomised controlled trials. *PLoS One* 2014;9:e84855.  
510 doi:10.1371/journal.pone.0084855
- 511 33 Astle WJ, Elding H, Jiang T, et al. The Allelic Landscape of Human Blood Cell Trait Variation  
512 and Links to Common Complex Disease. *Cell* 2016;167:1415-1429.e19.  
513 doi:10.1016/j.cell.2016.10.042

- 514 34 Tan WC, Bourbeau J, Nadeau G, et al. High eosinophil counts predict decline in FEV(1): results  
515 from the CanCOLD study. *Eur Respir J* 2021;57. doi:10.1183/13993003.00838-2020
- 516 35 Wu X, Wang C, Li H, et al. Circulating white blood cells and lung function impairment: the  
517 observational studies and Mendelian randomization analysis. *Ann Med* 2021;53:1118–28.  
518 doi:10.1080/07853890.2021.1948603
- 519 36 Svartberg J, Aasebø U, Hjalmsen A, et al. Testosterone treatment improves body  
520 composition and sexual function in men with COPD, in a 6-month randomized controlled  
521 trial. *Respir Med* 2004;98:906–13. doi:10.1016/j.rmed.2004.02.015
- 522 37 Mohan D, Rossiter H, Watz H, et al. Selective androgen receptor modulation for muscle  
523 weakness in chronic obstructive pulmonary disease: a randomised control trial. *Thorax*  
524 2023;78:258–66. doi:10.1136/thorax-2021-218360
- 525 38 Day FR, Hinds DA, Tung JY, et al. Causal mechanisms and balancing selection inferred from  
526 genetic associations with polycystic ovary syndrome. *Nat Commun* 2015;6:8464.  
527 doi:10.1038/ncomms9464
- 528 39 de Ronde W, van der Schouw YT, Pierik FH, et al. Serum levels of sex hormone-binding  
529 globulin (SHBG) are not associated with lower levels of non-SHBG-bound testosterone in  
530 male newborns and healthy adult men. *Clin Endocrinol (Oxf)* 2005;62:498–503.  
531 doi:10.1111/j.1365-2265.2005.02252.x
- 532 40 Card JW, Zeldin DC. Hormonal influences on lung function and response to environmental  
533 agents: lessons from animal models of respiratory disease. *Proc Am Thorac Soc* 2009;6:588–  
534 95. doi:10.1513/pats.200904-020RM
- 535 41 Keevil BG, Adaway J. Assessment of free testosterone concentration. *J Steroid Biochem Mol*  
536 *Biol* 2019;190:207–11. doi:10.1016/j.jsbmb.2019.04.008
- 537 42 Li H, Pham T, McWhinney BC, et al. Sex Hormone Binding Globulin Modifies Testosterone  
538 Action and Metabolism in Prostate Cancer Cells. *Int J Endocrinol* 2016;2016:6437585.  
539 doi:10.1155/2016/6437585

540 **Figure legends**

541

542 **Figure 1:** Flow diagram of sample selection from UK Biobank (UKB) for the one-sample multivariable  
543 Mendelian Randomisation (MVMR) analyses. We first undertook genome-wide association analyses  
544 (GWA), separately by sex, in the “*hormone discovery dataset*” to identify genetic instruments (SNPs)  
545 for Total Testosterone (TT) and Sex Hormone Binding Globulin (SHBG), with replication in the  
546 “*hormone replication dataset*”. The MVMR estimates of the effects of TT and SHBG on lung function  
547 were then derived in UKB, separately by sex, from the SNP-hormone (G-X; “*hormone replication*  
548 *dataset*”) and the SNP-lung function (G-Y; “*lung function dataset*”) estimates. The “lung function  
549 dataset” included only UKB subjects with previously defined good-quality spirometry data.

550

551 **Figure 2:** Results of the MVMR analysis of the effect of SHBG and testosterone on A) FVC, B) FEV<sub>1</sub>,  
552 and C) FEV<sub>1</sub>/FVC separately in males and females. Results expressed as the effect of a 10% increase in  
553 SHBG or TT on lung function.

554 FE-IVW = Fixed effect Inverse variance weighted meta-analysis; RE-IVW = Random effect Inverse  
555 variance weighted meta-analysis; 2SLS = Two-Stage least squares regression.

556

557 **Figure 3:** Results of the MVMR analysis after excluding pleiotropic SNPs identified by MVMR-PRESSO  
558 (outliers), for the effect of SHBG and TT on: A) FVC, B) FEV<sub>1</sub>, and C) FEV<sub>1</sub>/FVC, separately in males and  
559 females. Results expressed as the effect of a 10% increase in SHBG or TT on lung function.

560

561 **Figure 4:** Subgroup analysis showing significant interaction by obesity (A-B) and age (C) in males.  
562 Outlier SNPs and “weak” SNPs (F-statistic  $\leq 10$ ) were excluded. Results expressed as the effect of a  
563 10% increase in SHBG or TT on lung function.

564 \* Nominal significant interaction (p-values for interaction  $< 0.05$ ).

## Tables

**Table 1.** Population characteristics per dataset.

	Discovery hormone dataset		Replication hormone dataset		Lung function dataset	
	<i>Males</i>	<i>Females</i>	<i>Males</i>	<i>Females</i>	<i>Males</i>	<i>Females</i>
<b>N</b>	140,941	163,661	70,321	82,033	158,693	183,133
<b>Age (years), median [min-max]</b>	58 [39-72]	57 [39-70]	58 [37-73]	58 [40-71]	58 [39-72]	57 [39-71]
<b>&gt;50 years, N (%)</b>	104,389 (74%)	119,869 (73%)	51,978 (74%)	60,245 (73%)	117,735 (74%)	135,419 (74%)
<b>Height (cm), mean (SE)</b>	176 (0.02)	162 (0.02)	176 (0.03)	162 (0.02)	176 (0.02)	163 (0.01)
<b>BMI (kg/m<sup>2</sup>), mean (SE)</b>	27.8 (0.01)	27.1 (0.01)	27.8 (0.02)	27.1 (0.02)	27.8 (0.01)	27.0 (0.01)
<b>Smoking status</b>						
<b>Never-smoker, N (%)</b>	69,047 (49%)	97,637 (60%)	34,598 (49%)	48,877 (60%)	77,469 (49%)	106,280 (58%)
<b>Ex-smoker, N (%)</b>	54,148 (38%)	51,304 (31%)	26,898 (38%)	25,598 (31%)	62,432 (39%)	60,883 (33%)
<b>Current-smoker, N (%)</b>	17,529 (12%)	14,461 (9%)	8,699 (12%)	7,428 (9%)	18,755 (12%)	15,913 (9%)
<b>Pack years (years), mean (SE)</b>	26.1 (0.09)	20.1 (0.07)	26.2 (0.13)	20.4 (0.11)	25.6 (0.09)	19.9 (0.07)
<b>Menopause, N (%)</b>	-	98,791 (60%)	-	49,884 (61%)	-	111,601 (61%)
<b>Moderate physical activity, N (%)*</b>	67,420 (50%)	75,356 (49%)	33,546 (50%)	37,614 (49%)	76,836 (50%)	85,322 (49%)
<b>Hormone measurements</b>						
<b>N Testosterone (%)</b>	140,216 (99%)	139,591 (85%)	69,933 (99%)	69,916 (85%)	-	-
<b>Testosterone (nmol/L), median (IQR)</b>	11.62 (4.71)	1.01 (0.65)	11.61 (4.70)	1.01 (0.66)	-	-
<b>N SHBG (%)</b>	129,700 (92%)	150,365 (92%)	64,804 (92%)	75,325 (92%)	-	-
<b>SHBG (nmol/L), median (IQR)</b>	36.96 (20.25)	56.46 (37.40)	36.93 (20.21)	56.25 (37.03)	-	-
<b>Lung function outcomes</b>						
<b>FEV<sub>1</sub> (L), mean (SE)</b>	-	-	-	-	3.35 (0.002)	2.43 (0.001)
<b>FVC (L), mean (SE)</b>	-	-	-	-	4.46 (0.002)	3.20 (0.001)
<b>FEV<sub>1</sub>/FVC (%), mean (SE)</b>	-	-	-	-	74.91 (0.02)	75.96 (0.01)
<b>Airflow obstruction**, N (%)</b>	-	-	-	-	15,341 (10%)	14,976 (8%)

SHBG: sex hormone binding globulin.

\* Reported 4 or more days doing 10 minutes or more of moderate physical activities like carrying light loads, cycling at normal pace (Does not include walking).

*\*\* Airflow obstruction =  $FEV_1/FVC < \text{Lower limit of normal (LLN)}$  based on GLI-2012.*