RESEARCH

SHBG levels in primary infertile men: a critical interpretation in clinical practice

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Abstract

Objective: We aimed to test the association between age, BMI and sex-hormone–binding globulin (SHBG) in a homogenous cohort of white-European men presenting for primary couple's infertility.
Design: Retrospective study.
Methods: Data from 1547 infertile men were analysed. Health-significant comorbidities were scored with the Charlson comorbidity index (CCI). Fasting serum hormones were measured in every patient. Age was considered according to quartile groups (<33, 33-41, >41 years) and BMI as normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2) and obesity (>30 kg/m^2). Descriptive statistics and linear regression analysis tested the associations between age, BMI and SHBG.
Results: Median SHBG levels increased across quartiles of age and decreased along with BMI increases (all $P < 0.001$). For each year increase in age, SHBG increased 0.32 nmol/L; conversely, for each unit increase in BMI, SHBG decreased by 1.1 nmol/L (all $P < 0.001$). SHBG levels decline with increasing BMI was greater than SHBG progressive increase with age. Overall, BMI explained 3.0 times more of the variability in SHBG than did ageing. At multivariate linear model, age and BMI were the most significant factors influencing SHBG concentration (all $P < 0.001$), after accounting for CCI, albumin levels and smoking status.
Conclusions: We found a wide distribution of SHBG concentrations across age and BMI values in primary infertile men. The association between BMI and lowered SHBG levels seems to be greater than the association of ageing with increased SHBG.

Key Words
- infertility
- testosterone
- hypogonadism
- sex hormone-binding globulin
- obesity

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Introduction

In postpuberal males the testes contribute to more than 95% of total testosterone (tT) in serum, where it equilibrates between protein-bound (98%) and free hormone (1–2%) fractions (1). Circulating testosterone is bound either to low-affinity proteins (primarily albumin but also transcortin and orosomucoid) or to the high-affinity glycoprotein sex-hormone-binding globulin (SHBG) (2). These binding proteins have potential clinical relevance since they influence both tissue bioavailability and metabolic clearance rate of testosterone, while regulating the amount of free testosterone (fT) available for eventual biological actions in the tissues (1, 2). The tight SHBG-testosterone binding renders testosterone fraction biologically inactive, as testosterone is unable

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to diffuse through cell membranes when bound to SHBG, while contributing to the major proportion of tT measured, that is, the standard test to identify men with testosterone deficiency (2, 3, 4). Therefore, variations in SHBG concentration may have a considerable impact on tT deficiency diagnosis in clinical practice (1, 2).

Despite most of current scientific guidelines suggest measuring fT only in patients with borderline tT and if there is concern that the patient may have altered SHBG concentrations (4, 5), the importance of calculated fT (cFT) and SHBG over tT alone in assessing symptoms of androgen deficiency in men with sexual dysfunctions has been recently outlined (3, 6). Similarly, Ring *et al.* investigated the clinical utility of testing SHBG/cFT values in infertile men (7).

Several factors are known to influence serum SHBG levels such as ageing, obesity, diabetes mellitus (DM), thyroid diseases and cirrhosis (8, 9, 10). Of relevance, SHBG was found to linearly increase with ageing while decreasing with increasing BMI values (8, 9, 11). Additionally, SHBG *per se* has also been shown to be an independent determinant of DM, metabolic syndrome (MetS), cardiovascular diseases (CVD) and overall mortality risk (12, 13, 14). Overall, all these latter conditions have been observed to be highly prevalent in men with male factor infertility (MFI) (15, 16, 17, 18).

As a whole, there is limited information regarding the variability of serum SHBG distributions in clinical populations of men in whom SHBG concentrations may impact medical decision-making, such as throughout the work-up of men with MFI.

These observations prompted us to retrospectively investigate the distribution of serum SHBG in a homogeneous cohort of white-European men presenting for primary couple's infertility in the real-life setting, with a specific focused analysis dedicated to the relationship between SHBG levels, age and BMI.

Materials and methods

In this retrospective study, we analysed data from 1547 consecutive white-European men assessed at a single academic centre for couple's primary infertility between September 2014 and August 2019. Patients were enrolled if they were ≥18 and ≤60 years old and had MFI only. MFI was defined after a comprehensive diagnostic evaluation of all the female partners carried out by expert infertility-trained gynaecologists. According to the World Health Organization (WHO) criteria, infertility was defined as not conceiving a pregnancy after at least 12 months of unprotected intercourses regardless of whether or not a pregnancy ultimately occurs (19). Primary infertility was defined when a couple was never able to conceive (19).

Patients were assessed with a thorough self-reported medical history including age and comorbidities. The Charlson comorbidity index (CCI) was applied to score health-significant comorbidities, coded using the International Classification of Diseases, 9th revision (20). Likewise, BMI was calculated for each patient and categorized according to the NIH definitions of 'normal' (below 24.9 kg/m^2), 'overweight' (from 25 to 29.9) and 'obese' (30+) (21). Testes volume was assessed using a Prader orchidometer (22) by a single expert uro-andrologist, calculating the mean value between the two sides. Smoking habit was investigated according to the pack-year history and then categorized in two groups as follows: no smokers (never and former smokers) or current smokers (23).

Varicocele was clinically assessed in every patient and further confirmed by ultrasound examination (24).

Venous blood samples were drawn from each patient between 07:00 h and 11:00 h after an overnight fast (1). Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured using a heterogeneous competitive magnetic separation assay (Bayer Immuno 1 System, Bayer Corp.). Inhibin B (InhB) was measured by an ELISA (Beckman Coulter AMH Gen II ELISA). Total testosterone was measured via a direct chemiluminescence immunoassay (ADVIA Centaur; Siemens Medical Solutions Diagnostics) and SHBG levels were measured via a solid-phase chemiluminescent immunometric assay on Immulite 2000 (Medical Systems SpA, Genoa, Italy). Serum albumin and SHBG values were measured and utilized to determine cFT using the validated Vermeulen formula (25). Chromosomal analysis and genetic testing were performed in every patient (karyotype analysis and tests for Y-chromosome microdeletions and cystic fibrosis mutations) (26). The same laboratory was used for the analysis of all parameters for all patients.

Patients underwent at least two consecutive semen analyses, both showing below standard values for normal semen parameters according to the WHO criteria (27). For the specific purposes of this analysis, we considered semen volume, sperm concentration, progressive sperm motility and normal morphology. Sperm DNA fragmentation index (SDF) was measured by sperm chromatin structure assay (SCSA) in every patient (28).

Men with genetic abnormalities (any type) were excluded from final analysis; likewise, patients who had

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testicular or pituitary surgery and/or previous vasectomy and men who were on pharmacological agents (any) that could affect tT values (i.e. clomiphene citrate) at the time of investigation were removed from the final analysis. No cases of liver cirrhosis or chronic use of steroids that could have an influence on SHBG values have been found.

Data collection followed the principles outlined in the Declaration of Helsinki. All patients signed an informed consent agreeing to share their own anonymous information for future studies. The study was approved by the IRCCS San Raffaele Hospital Ethical Committee (Prot. 2014 – Pazienti Ambulatoriali).

Statistical methods

Distribution of data was tested with the Shapiro–Wilk test. Data are presented as medians (interquartile range; IQR) or frequencies (proportions). A 95% CI was estimated for the association of categorical parameters. First, demographics characteristics, hormonal values and semen parameters were compared among patients as segregated according to quartiles of age (namely, <33 years, 33–40 years, and ≥41 years) with the Kruskal–Wallis test and the Chi-square test. Similarly, descriptive statistic was applied to the whole cohort as segregated according to BMI categories (21). To explore the effects of age and BMI on SHBG, men were divided into four groups (9): (1) nonobese younger, (BMI <30 and age <37); (2) obese younger (BMI ≥30 and age <37); (3) nonobese older (BMI <30 and age ≥37); and (4) obese older men (BMI ≥30 and age ≥37). SHBG among groups was compared with Kruskal–Wallis test with multiple comparisons. Subsequently, we graphically explored the relationship between SHBG with age and BMI using the locally weighted scatterplot smoothing (LOWESS) method to account for possible non-linear relationships (29). Finally, univariable (UVA) and multivariable (MVA) linear regression analyses tested the associations between clinical variables (e.g. age, BMI, CCI, albumin and smoking habits) and SHBG values. Statistical analyses were performed using SPSS v.26 (IBM Corp.) and Stata 14.0 (StataCorp, College Station, TX, USA). All tests were two sided, and statistical significance level was determined at $P<0.05$.

Results

Table 1 lists descriptive statistics of the entire cohort of patients as segregated according to quartiles of age at first assessment. Patients' BMI and CCI increased among quartiles of age (all $P\leq0.03$). Total testosterone ($P=0.03$) and cFT ($P<0.001$) decreased with age; on the contrary, SHBG increased across quartiles of age ($P<0.001$). Table 2 lists descriptive statistics of the entire cohort of patients as segregated according to BMI groups. Accordingly, median tT, cFT and SHBG values decreased across BMI groups ($P<0.001$) (Table 2). In terms of semen parameters, both sperm concentration and sperm motility decreased across age and BMI groups (all $P\leq0.04$); conversely, SDF increased with age and BMI increases (Tables 1 and 2).

Figure 1 shows SHBG distribution among patients subcategorized in groups by age and BMI. As depicted, SHBG values changed across the four groups ($P<0.001$), with lowest SHBG value being observed in obese-younger men (19.9 (15-23); $P<0.01$ vs all groups), while the highest value being observed in nonobese-older men (33 (25-43); $P<0.01$ vs all groups) (Fig. 1).

Figure 2 shows the LOWESS curves depicting the association between SHBG with age and BMI. In this context, a negative linear correlation was found between SHBG and BMI ($P<0.001$); conversely, SHBG linearly increases with age ($P<0.001$). SHBG was positively correlated with tT ($P<0.001$) but not with cFT ($P=0.08$) (data not shown).

At linear regression analysis, SHBG was found to decrease by 1.1 nmol/L (95% CI: −1.2, −0.8) for each unit increase in BMI; conversely, SHBG increased 0.32 (95% CI: 0.2, 0.4) nmol/L for each year increase in age ($P<0.001$ for both effects) (Table 3). The progressive decline in SHBG with increasing BMI was greater than the progressive increase in SHBG with age. Overall, BMI explained 3.0 times more of the variability in SHBG than did ageing ($r^2=0.06$ for BMI and $r^2=0.02$ for age). At multivariate linear model, age and BMI emerged as the most significant factors influencing SHBG concentration (all $P<0.001$), after accounting for CCI, albumin levels and smoking status (Table 3).

Discussion

Testosterone plays a pivotal role in overall men's health (1), therefore the identification and management of hypogonadal individuals is crucial in clinical practice. This is particularly relevant in patients at higher risk of hypogonadism and when the restoration of physiological testosterone levels might have important clinical and prognostic rebounds, such as in infertile men and in those with sexual dysfunction (30, 31).

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Table 1 Descriptive statistics of the whole cohort segregated according to quartiles of age (No. = 1547).

	Overall	Age <33 years	Age 33–40 years	Age = 41 years	
	n = 1547 (100%)	*n* = 287 (18.5%)	*n* = 841 (54.3%)	*n* = 419 (27.2%)	*P*-value[a]
Age (years)	37.0 (33–41) (18–60)	31.0 (29–32) (18–32)	37.0 (35–38) (33–40)	44.0 (42–47) (41–60)	
BMI (kg/m^2)	25.1 (23.3–27.2) (18.5–44.8)	24.7 (22.8–27.5) (18.5–39.2)	25.1 (23.3–27.2) (18.5–44.8)	25.3 (23.6–27.1)[b] (18.5–41.4)	0.03
CCI (score)	0.0 (0–0)	0.0 (0–0)	0.0 (0–0)	0.0 (0–0)[b,c]	<0.001
Mean (S.D.)	0.1 (0.5) (0.0–8.0)	0.06 (0.3) (0.0–3.0)	0.08 (0.4) (0.0–8.0)	0.2 (0.7) (0.0–8.0)	
CCI = 1 (No. (%))	110 (7.1)	12 (4.2)	46 (5.5)	52 (12.4)	<0.001
Type 2 DM (No. (%))	35 (2.3)	3 (1.0)	12 (1.4)	20 (4.7)	<0.01
Length of infertility (month)	21 (15–36) (12.0–228.0)	18.0 (12–24) (12.0–168.0)	23.0 (12–32) (12.0–162.0)	24.0 (12–36) (12.0–228.0)	<0.001
Testis volume (average Prader value)	15.0 (12–20) (2.0–25.0)	15.0 (11–20) (2.0–25.0)	15.0 (12–20) (2.0–25.0)	15.0 (12–20) (3.0–25.0)	0.1
Smoking status (No. (%))					0.03
Never smoked/former smokers	1100 (71.1)	186 (64.7)	605 (71.9)	309 (73.6)	
Active smokers	447 (28.9)	101 (35.2)	236 (28.1)	110 (26.3)	
FSH (mUI/mL)	5.5 (3.3–11.0) (0.1–99.1)	5.2 (3.2–11.7) (1.1–74.0)	5.2 (3.2–11.0) (0.1–99.1)	5.7 (3.4–10.9) (0.1–64.7)	0.14
LH (mUI/mL)	4.2 (2.9–5.9) (0.1–77.0)	4.5 (3.3–6.3) (1.1–35.3)	4.1 (2.8–5.9) (0.1–77.0)	4.2 (2.7–5.8) (0.1–27.0)	0.3
tT (ng/mL)	4.6 (3.4–5.7) (0.2–26.4)	4.6 (3.5–5.9) (0.5–15.1)	4.5 (3.4–5.7) (0.2–26.4)	4.2 (3.2–5.6)[b] (0.2–23.5)	0.03
cFT (pg/mL)	90.9 (70.4–118.0) (1.1–940.0)	98.1 (76.7–130.0) (1.1–667.0)	91.5 (70.3–117.0)[b] (2.8–688.0)	85.1 (65.1–112.0)[b,c] (9.1–940.0)	<0.001
SHBG (nmol/L)	31.0 (23–41) (2.4–104.0)	27.3 (21–37) (7.0–79.0)	32.0 (23–40)[b] (2.4–104.0)	34.0 (25–44)[b,c] (6.0–103)	<0.001
InhB (pg/mL)	105.6 (44.2–166.3) (0.5–538.0)	102.9 (43.8–168.9) (0.5–303.0)	108.4 (47.5–163.6) (0.5–538.0)	100.5 (38.7–166.6) (0.6–521.9)	0.6
Albumin (pg/mL)	46.7 (44.7–48.7) (17.9–61.3)	47.3 (45.0–48.9) (17.9–57.1)	46.8 (44.9–48.6) (23.3–60.2)	46.1 (44.1–48.2)[b,c] (22.6–61.3)	<0.001
Semen volume (mL)	3.0 (2.0–4.0) (1.0–16.0)	3.0 (2.0–4.0) (1.0–9.0)	3.0 (2.0–4.0) (1.0–16.0)	3.0 (2.0–4.0)[b] (1.0–9.0)	0.04
Sperm concentration (×106/mL)	13.8 (3.2–37.0) (0.1–455.0)	14.0 (3.6–34) (0.1–455.0)	13.0 (3.0–39.1) (0.1–198.4)	11.0 (2.9–40.0)[b] (0.1–305.0)	0.03
Progressive motility (%)	20.0 (8.0–36.0) (0.0–84.0)	24.0 (6.0–40.0) (0.0–82.0)	21.0 (8–35) (0.0–78.0)	16.0 (8.0–34.0)[b] (0.0–84.0)	0.02
Normal morphology (%)	3.0 (1.0–10.0) (0.0–100.0)	2.0 (1.0–10.5) (0.0–80.0)	2.0 (1.0–10.0) (0.0–100.0)	2.0 (1.0–11.0) (0.0–91.0)	0.4
SDF (SCSA) (%)	35.5 (21.9–52.9) (0.4–99.9)	23.5 (15.9–44.1) (0.3–97.7)	35.0 (21.9–50.6) (1.4–99.8)	40.8 (25.9–58.0)[b,c] (0.5–93.3)	0.01

Data presented as median (IQR) (range).
[a]*P* value according to the Kruskal–Wallis test, as indicated; [b]$P < 0.05$ vs Age < 33 years group; [c]$P < 0.05$ vs 33–40 years group.
CCI, Charlson comorbidity index; DM, diabetes mellitus; SDF, sperm DNA fragmentation index; tT, total testosterone.

Current guidelines support the combination of clinical symptoms/signs and low tT values for the diagnosis of male hypogonadism, but they all highlight that tT could provide misleading information in all those conditions known to alter SHBG levels (4, 5). Previous studies have investigated the importance of SHBG testing throughout the evaluation of men's gonadal status. In this context, Rastelli *et al.* analysed data from 2622 men presenting for sexual dysfunction at a single centre and found that higher SHBG, regardless of circulating tT, was associated with either subjective or objective androgen deficiency features (3). Thereof, they stressed the clinical importance of SHBG testing in conjunction with tT in order to evaluate men with sexual dysfunction and the suspicion of suffering from hypogonadal status. Similarly, Ring *et al.* determined the utility of adding SHBG to standard tT testing for the diagnosis of male hypogonadism in 168 infertile men (7). Authors found that using tT levels alone would potentially misclassify 20–53% of men as eugonadal while actually being hypogonadal.

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Table 2 Descriptive statistics of the whole cohort segregated according to BMI categories (No. = 1547).

	BMI 18.5–24.9	BMI 25–29.9	BMI ≥30	
	n = 737 (47.6%)	*n* = 670 (43.3%)	*n* = 140 (9.0%)	***P*-value[a]**
Age (years)	36.0 (33–41) (18–60)	38.0 (34–41) (18–60)	37.0 (34–43) (23–60)	<0.002
CCI (score)	0.0 (0–0)	0.0 (0–0)	0.0 (0–0)	0.2
Mean (S.D.)	0.09 (0.4) (0.0–8.0)	0.1 (0.5) (0.0–8.0)	0.1 (0.4) (0.0–4.0)	
CCI ≥ 1 (No. (%))	43 (5.9)	42 (6.3)	12 (8.5)	0.2
Type 2 DM (No. (%))	14 (1.9)	14 (2.1)	2 (1.4)	0.14
Length of infertility (month)	18 (12–24) (12.0–180.0)	24.0 (12–36) (12.0–228.0)	24.0 (15–38) (12.0–204.0)	<0.001
Testis volume	15.0 (12–20) (2.0–25.0)	15.0 (12–20) (2.0–25.0)	18.0 (11–23) (2.0–25.0)	0.7
Smoking status (No. (%))				0.1
No smokers/Former smokers	541 (73.4) 460 (68.6)	95 (67.9)		
Active smokers	196 (26.6) 210 (31.4)	45 (32.1)		
FSH (mUI/mL)	5.2 (3.2–10.2) (0.6–99.1)	5.7 (3.4–11.0) (0.9–74.0)	7.1 (3.9–12.7)[b] (0.1–38.2)	0.02
LH (mUI/mL)	4.1 (2.9–5.9) (0.1–35.3)	4.1 (2.9–5.9) (0.6–36.3)	4.3 (2.7–6.3) (0.8–77.0)	0.7
tT (ng/mL)	4.9 (3.8–6.1) (0.2–23.5)	4.3 (3.2–5.5)[b] (0.1–17.0)	3.3 (2.7–4.6)[b,c] (1.1–22.5)	<0.001
cFT (pg/mL)	94.8 (74.4–123.0) (2.8–667.0)	89.4 (70.0–115.0)[b] (1.1–940.0)	74.7 (59.8–102.0)[b,c] (10.1–688.0)	<0.001
SHBG (nmol/L)	35.0 (27–44) (2.4–104.0)	29.0 (22–38)[b] (7.5–95.0)	22.5 (18–31)[b,c] (6.0–104.0)	<0.001
InhB (pg/mL)	118.4 (53.7–175.3) (0.5–465.8)	99.9 (40.0–163.4)[b] (0.9–538.0)	70.0 (22.8–118.6)[b,c] (0.5–307.6)	<0.001
Albumin (pg/mL)	47.1 (45.1–48.9) (27.1–60.2)	46.6 (44.8–48.5)[b] (17.9–61.3)	45.1 (43.4–47.5)[b,c] (26.1–52.1)	<0.001
Semen volume (mL)	3.0 (2.0–4.0) (1.0–9.0)	3.0 (2.0–4.0) (1.0–16.0)	3.0 (2.0–4.0) (1.0–8.0)	0.1
Sperm concentration ($\times 10^6$/mL)	18.3 (3.8–36.6) (0.1–159.0)	17.0 (4.6–40.3) (0.1–455.0)	10.6 (2.6–33)[b] (0.1–305.9)	0.01
Progressive motility (%)	25.0 (10.0–39.5) (0.0–84.0)	24.0 (10–36) (0.0–72.0)	16.0 (5.0–31.0)[b] (0.0–82.0)	0.01
Normal morphology (%)	3.0 (1.0–10.7) (0.0–94.0)	2.5 (0.0–10.0) (0.0–100.0)	6.0 (1.0–14.0) (0.0–85.0)	0.2
SDF (%)	33.1 (20.8–49.7) (1.4–97.7)	38.0 (23.6–52.8) (0.3–96.4)	38.5 (17.7–59.7)[b] (10.0–99.8)	0.04

Data presented as median (IQR) (range).
[a]*P* value according to the Kruskal–Wallis test, as indicated; [b]$P < 0.05$ vs BMI 18.5–24.9 group; [c]$P < 0.05$ vs 25–29.9 group.
CCI, Charlson comorbidity index; DM, diabetes mellitus; SDF, sperm DNA fragmentation index; tT, total testosterone.

Conversely, 20% of patients were diagnosed with hypogonadism but were actually eugonadal when using SHBG for cFT evaluation (7). Therefore, in addition of standard serum tT levels, SHBG should be considered as an important tool during the diagnostic work-up of infertile men for a more accurate definition of the androgen milieu. Moreover, since the main peripheral organ that produces SHBG is the liver, which is considered the central metabolic organ, SHBG levels assessment is usefull in a more comprehensive understanding of the relevance of certain metabolic diseases eventually resulting in changed SHBG serum levels (32, 33).

It is well known that several conditions may alter serum SHBG concentrations (8, 9, 10, 11), with a subsequent impact on the actual interpretation of circulating tT values. However, there is limited information regarding the variability of serum SHBG distributions in infertile patients. Of relevance, infertile men *per se* do represent a critical subset of subjects, since they have been demonstrated to recapitulate many characteristics and disorders of the aging men at a significantly younger age, and in a relatively short time frame.

The findings of this real-life study showed that SHBG values significantly increase across ageing but decline

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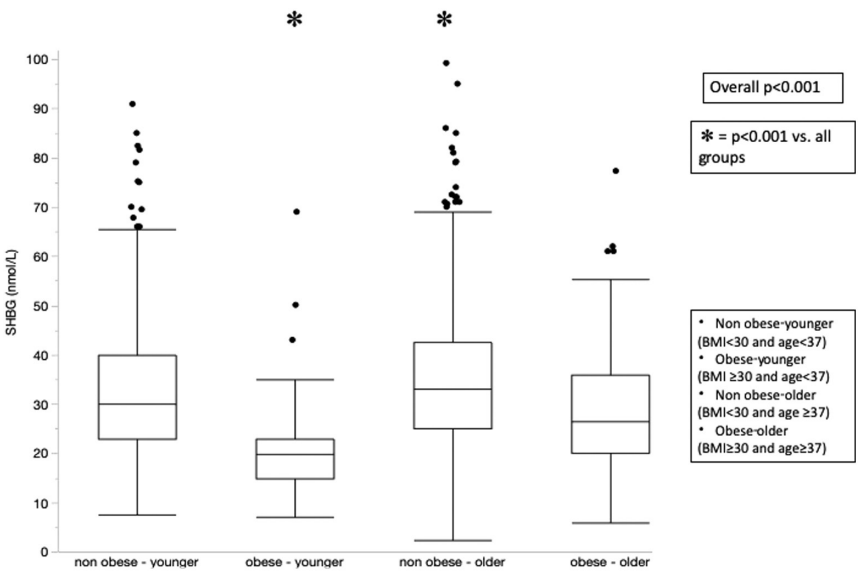

Figure 1
Serum SHBG distribution among patients subcategorized in groups by age and BMI.

according to increasing BMI categories. Of clinical relevance, this latter negative association observed between increasing BMI categories and decreased circulating SHBG levels, in our homogenous large cohort of white-European primary infertile men, was even more relevant than the well-known association observed with the ageing process (8, 9, 11).

Previous Authors have reported SHBG variability in men from the general population and in those with sexual dysfunctions. In the CARDIA Male hormone study, data from 474 black and 695 white men, aged 24–31 years, have been analysed to investigate aging-related changes in SHBG, tT and bioavailable testosterone according to changes in BMI (34). The authors found that SHBG significantly increased along with age for men, whose BMI decreased. Moreover, there were progressively smaller increases in SHBG for men whose BMI was stable or whose BMI increased modestly. The relationship between age and SHBG was lost among men whose BMI increased most (34). A remarkably wide distribution of SHBG concentrations was reported in a study with 1000 men presenting for sexual symptoms (8). The authors found a nearly 20-fold difference in values over the range of SHBG results, with younger men having lower mean SHBG concentrations than older ones. Likewise, Cooper *et al*. analysed the impact of age and BMI on SHBG in 3671 men who underwent laboratory testing for testosterone deficiency (9). As expected, their results showed that SHBG was negatively correlated with BMI but positively associated with age. In particular, the association between obesity and lowered SHBG was greater than the association of ageing with increased SHBG (9). Our results confirm those previous findings, with the further observation that BMI eventually impacts on SHBG distribution at a larger extent than age on the same parameter (namely, 3.0 times more). Overall, the recognition of this large variability of SHBG values in infertile men should be considered in the interpretation of androgen status in clinical practice, particularly in overweight and obese men, in which SHBG testing appears of utmost clinical importance. The exact mechanism underlying this association has not been clarified yet but reported hypothesis are the suppression of hepatic SHBG synthesis by elevated concentrations of insulin and an obesity-induced increase in oestrogen levels that may contributes in determining negative feedback at the pituitary level (35, 36, 37, 38). Similarly, the association of increasing age with increasing

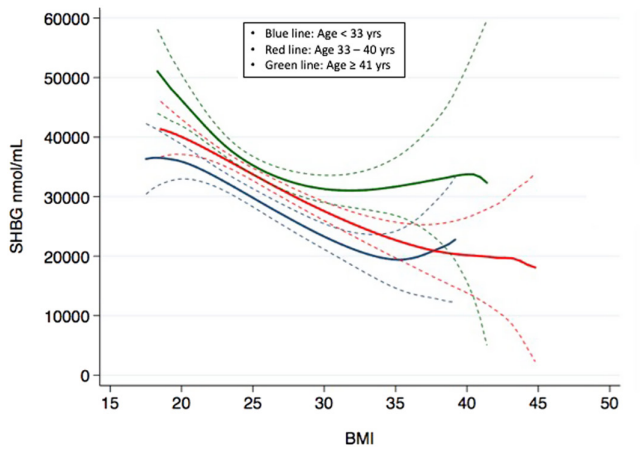

Figure 2
LOWESS curves depicting the relationship between serum SHBG, age and BMI.

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Table 3 Linear regression models predicting SHBG values in the whole cohort.

	UVA model			MVA model		
	Beta	*P* value	95% CI	Beta	*P* value	95% CI
Age	0.32	<0.001	0.21–0.43	0.37	<0.001	0.25–0.49
BMI	−1.07	<0.001	−1.24–−0.82	−1.21	<0.001	−1.43–−0.97
CCI	1.29	0.06	−0.09–2.68	0.78	0.28	−0.66–2.13
Albumin	0.02	0.83	−0.15–0.18	−0.08	0.33	−0.24–0.08
Smoking status Yes vs No	0.1	0.96	−1.54–1.63	0.58	0.48	−1.07–2.25

MVA, multivariate model; UVA, univariate model.

SHBG has been demonstrated in prior studies (8, 9, 39, 40). Data from the European Male Aging Study (EMAS) demonstrated a steady rise in SHBG with increasing age among men aged 40–70 years (40). However, the reason for this increase is unknown. Our study confirms a linear relationship between age and SHBG along with a negative correlation between age and BMI even in younger men, overall in theory at a lower risk of comorbid diseases than those of greater age, if they were not infertile and, therefore, with an epidemiologically recognized risk of a lower health status than the fertile counterpart (15, 16, 17, 18).

The clinical strength of our study is several fold. This study is innovative because it is the first to provide detailed SHBG values for a large population of men specifically presenting for primary couple's infertility. Second strength is that we have comprehensively investigated a relatively large homogenous cohort of patients with a thorough clinical evaluation and an accurate assessment of possible factors that may alter SHBG values, such as recreational habits and health comorbidities (8). Third strength is SHBG measurement by a single laboratory, thus providing homogenous results in a specific cohort of white-European men presenting for primary couple's infertility. This latter aspect would further provide our results with a strong characterization in the real-life setting.

Our study is not devoid of limitations. First, although these analyses have taken into consideration a homogenous cohort of white-European men, they report the findings of a single center-based retrospective study, thus raising the possibility of selection biases; thereof, larger studies across different centers and cohorts are needed to validate our findings. Second, our study did not include a control group of normal fertile men. Third, we did not use gas chromatography–mass spectrometry, which is considered the gold standard for measuring circulating tT levels; in contrast, to reflect common practice of a clinical biochemistry laboratory, we elected to measure circulating tT using commercially available analytic methods. Furthermore, serum tT levels were measured using a commercial assay distributed by a company that has modified the assay's normal range throughout the time interval over which data have been extracted, thus leading to a potential bias and a consequent misinterpretation of normal ranges of the hormonal milieu. Lastly, fT values were calculated based on the validated Vermeulen formula, rather than the gold standards of equilibrium dialysis (41), which may have introduced some further bias.

Conclusions

The findings of this retrospective study revealed a remarkably wide distribution of SHBG concentrations across age and BMI in primary infertile men. The association between increasing BMI values and lowered SHBG concentrations emerged to be greater than the association of ageing with increased SHBG. These results outline the clinical importance of a probable significant variability in terms of SHBG concentrations in the real-life diagnostic and therapeutic work-up of men presenting for couple's infertility, along with the concomitant suspicion of a relevant hypogonadism. Further large cohort studies are needed to corroborate our results, even in different ethnicity settings.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
L B and A S designed the study, collected data, performed statistical analyses and wrote the manuscript. P C designed the study and collected

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data. W C, E P, L C, F B, D O, E V, N S, G F, M P, and C A collected data. W C, E P, L C, F B, D O, E V, N S, G F, M P, C A, E M, F M, and A S collected data, interpreted results, and revised the manuscript critically. L B, P C, W C, E P, L C, F B, D O, E V, N S, G F, M P, C A, E M, F M, and A S interpreted results and revised the manuscript critically. All authors and co-authors approved the final version of the manuscript to be published.

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