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Article type : 8 Requested Commentary

Biochemical Evaluation of Male Androgen Status: Beyond Total Testosterone

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Word count 1,138; Figure: 1.

Key words: testosterone, SHBG, hypogonadism

Disclosure Summary: Mathis Grossmann has received research funding from Bayer Pharma, Novartis, Weight Watchers, Lilly and speaker's honoraria from Besins Healthcare.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/CEN.13552](https://doi.org/10.1111/CEN.13552)

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Male hypogonadism is a clinical diagnosis confirmed by consistent biochemical findings. Clinical guidelines agree that the initial diagnostic test to confirm clinically suspected androgen deficiency should be a fasting morning total testosterone concentration by a reliable assay. They also agree that a diagnosis of androgen deficiency should only be made in men with consistent symptoms and signs, and unequivocally and repeatedly low serum testosterone concentrations¹⁻³.

However, what should be done when the total testosterone is near the lower limit of the reference range, but not clearly low? Most guidelines suggest that obtaining a free testosterone concentration either by equilibrium dialysis or by calculation with a validated algorithm should be the next step^{1,2}. Because serum sex hormone binding globulin (SHBG) concentrations affect total testosterone concentrations and their interpretation (Figure), a free testosterone measurement is also recommended if a condition associated with alterations in circulating SHBG is suspected^{1,2}. In practice, however, equilibrium dialysis is usually not available, and some formulae used to calculate free testosterone have not been fully validated^{4,5}. Moreover, while recent progress has been made towards standardisation of reference intervals for total testosterone⁶, normal ranges for free testosterone remain currently less well defined.

Recently, Australian guidelines have proposed that, rather than relying on free testosterone measured by suboptimal methodology, at high and low ends of the reference interval, serum total testosterone should primarily be interpreted in relation to SHBG concentrations³.

However, there has been relatively little evidence that SHBG provides information in the assessment of male androgens status additional to that of total testosterone, a gap that is addressed by Rastrelli et al. in this issue of Clinical Endocrinology⁷.

Previous epidemiological studies trying to identify the best biochemical measure of androgen status have compared total to free⁸ or bioavailable⁹ testosterone. These studies reported that free and bioavailable testosterone are better predictors of clinical androgen

status than total testosterone with respect to symptoms⁸, as well as with respect to end-organ deficits compatible with androgen deficiency such as low muscle strength, bone density or haemoglobin^{8,9}. In these studies, free and bioavailable testosterone were calculated rather than measured directly. Given that the formulae used to calculate free and bioavailable testosterone incorporate SHBG, the differential associations of calculated vs total testosterone with androgen deficiency-like features might reflect possible, unaccounted for roles of SHBG. There is evidence that SHBG may not be just a passive carrier for sex steroids, and epidemiological studies suggest that SHBG may predict health outcomes such as diabetes¹⁰ or mortality¹¹ independently of circulating testosterone.

Rastrelli et al.⁷ report results of a cross-sectional study among a cohort of 2,622 middle-aged men referred to a single academic centre in Italy for sexual dysfunction. Most men had normal total testosterone concentrations (mean 15.3 nmol/L), suggesting that sexual dysfunction was not due to androgen deficiency in a substantial proportion. This study tested the hypothesis that circulating SHBG concentrations are associated with markers of androgen status independently of total testosterone. Rastrelli et al.⁷ report the interesting and novel observation that in this large cohort, higher SHBG concentrations were independently associated with both subjective as well as objective markers of androgen deficiency. Higher SHBG was associated with a higher frequency of androgen deficiency-like symptoms by self-reported questionnaire, and with lower frequency of sexual intercourse, independent of confounders such as age or body mass index. Higher SHBG was also associated with lower prostate specific antigen and haemoglobin, which are non-specific markers of androgen action. These associations remained significant after adjustment for total testosterone.

The authors interpret their findings to represent evidence that high SHBG restrains the biological activity of testosterone, hence supporting the free hormone hypothesis, that posits that only free testosterone or bioavailable testosterone (loosely bound to albumin) but not tightly SHBG-bound testosterone is biologically active. In support of this, Rastrelli et al.⁷ report that among men with borderline total testosterone concentrations (defined as 8-12 nmol/L), only calculated free testosterone (potentially reflecting both testosterone synthesis and tissue availability), but not SHBG (reflecting testosterone tissue availability

only) was more closely associated with markers of androgen deficiency than total testosterone. Studies measuring serum hormone concentrations cannot conclusively prove the free hormone hypothesis that remains controversial^{4,5}. Serum hormone concentrations, even if quantitated by accurate assay technology, oversimplify biology as the concentrations of testosterone and its metabolites (estradiol and dihydrotestosterone) in target tissues, which depend not only on tissue uptake but also on local metabolism, may differ from serum concentrations.

Unfortunately, luteinizing hormone (LH) concentrations were not reported in this study. Compensatory increases in LH concentrations in response to increased SHBG concentrations would provide further supportive evidence for genuine androgen deficiency in these men. Overall, Rastrelli's⁷ findings show that, in their population, high SHBG concentrations are inversely associated with markers of androgen action, independently of testosterone. If there is a causal relationship between SHBG concentrations and androgen effects, it remains to be determined whether the effect of SHBG is mediated indirectly by decreasing free testosterone concentrations or by a direct effect of SHBG on tissues^{10,11}.

An important limitation is that, by design, observational studies cannot infer causality. Androgen deficiency-like symptoms and peripheral markers of androgen status are relatively non-specific. It remains possible that, in some men, comorbid burden and obesity could cause both androgen deficiency-like symptoms as well as reductions in serum testosterone, via illness-associated hypothalamic suppression¹². Cause and consequence can only be determined in clinical trials. Therefore, observational studies cannot clarify who benefits from testosterone treatment, but are useful to select participant characteristics for enrolment in clinical trials.

So, how do we incorporate these findings into current clinical practice? In most men with organic hypogonadism due to defined pituitary and testicular disease, total testosterone concentrations will be unequivocally low. Neither SHBG nor free testosterone will add diagnostic information and their measurement is not necessary¹³. These men instead need careful evaluation for an underlying cause, including measurements of gonadotrophin concentrations to distinguish primary from secondary hypogonadism.

127
128 Middle aged and older men, especially when obese and suffering from comorbidities,
129 commonly present with nonspecific symptoms and modestly reduced testosterone
130 concentrations relative to reference ranges based on healthy young men. In this context,
131 additional biochemical testing might be useful, for example, to exclude hypogonadism when
132 low total testosterone is due to low SHBG because of obesity or diabetes (Figure). In
133 contrast, there is no evidence that biochemical testing beyond total testosterone is useful
134 for identifying men that will benefit testosterone treatment. The T trials, which provide the
135 best evidence to date regarding the benefits of testosterone treatment in older men,
136 recruited participants based on total testosterone concentrations¹⁴.

137
138 Finally, weight loss and optimizing comorbidities may, in addition to providing general
139 health benefits, increase endogenous serum testosterone concentrations, and improve
140 androgen deficiency-like symptoms in older men¹². Therefore, lifestyle measures and
141 meticulous care of comorbidities should be a cornerstone focus in the management of
142 age-related hypogonadism, irrespective of whether testosterone treatment is considered or
143 not.

155 **Figure Legend**

156 Figure: Changes in SHBG are associated with parallel changes in total testosterone, limiting
157 its diagnostic utility.

This figure illustrates schematically how, because of the relationship of total testosterone concentrations with SHBG concentrations, reliance on total testosterone may be misleading in situations where SHBG concentrations are altered. On the one hand, SHBG is reduced by obesity, insulin resistance and exposure to exogenous androgens; in this situation, total testosterone below the reference interval does not confirm a diagnosis of androgen deficiency, especially if circulating serum gonadotrophin concentrations are not elevated. Reliance on total testosterone may lead to an overdiagnosis of hypogonadism.

On the other hand, SHBG can be markedly increased with liver disease, hyperthyroidism or antiepileptic therapies. In this situation, hypogonadism may be present despite a total testosterone in the reference range, especially if gonadotrophins are elevated.

Note that albumin bound (bioavailable) testosterone has been omitted for clarity. For illustrative purposes, the relative proportions of total to free testosterone are not to scale; only 1-2% of circulating total testosterone is non-protein bound (i.e. free).

Note that increases in total testosterone concentrations in response to increased SHBG occur only in men with an intact hypothalamic-pituitary-testicular (HPT) axis. Hypogonadal men with HPT axis pathology are incapable of increasing testosterone production in response to increasing SHBG concentrations.

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Title:

Biochemical evaluation of male androgen status: Beyond total testosterone

Date:

2018-04-01

Citation:

Grossmann, M. (2018). Biochemical evaluation of male androgen status: Beyond total testosterone. CLINICAL ENDOCRINOLOGY, 88 (4), pp.538-540. <https://doi.org/10.1111/cen.13552>.

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