# Associations Between Circulating Reproductive Hormones and SHBG and Prevalent and Incident Metabolic Syndrome in Community-Dwelling Older Men: The Concord Health and Ageing in Men Project

Benjumin Hsu, Robert G. Cumming, Vasi Naganathan, Fiona M. Blyth, David G. Le Couteur, Markus J. Seibel, Louise M. Waite, and David J. Handelsman

School of Public Health (B.H., R.G.C.), University of Sydney, Sydney 2006, New South Wales, Australia; Centre of Education and Research on Ageing (B.H., R.G.C., V.N., F.M.B., D.G.L.C., L.M.W.) and ANZAC Research Institute (R.G.C., D.G.L.C., M.J.S., D.J.H.), University of Sydney and Concord Hospital, Sydney 2139, New South Wales, Australia

**Context:** The causal relationship between metabolic syndrome and reproductive hormones is unclear.

**Objective:** This study sought to examine the cross-sectional, longitudinal, and predictive associations between reproductive hormones and SHBG and metabolic syndrome in older men.

**Design, Setting, and Participants:** Men ages 70 years and older from the Concord Health and Ageing in Men Project study (n = 1705) were assessed at baseline and 2-year follow-up. At baseline, T, dihydrotestosterone (DHT), estradiol, and estrone were measured by liquid chromatography-tandem mass spectrometry, and SHBG, LH, and FSH by immunoassay. Metabolic syndrome was defined using the P National Cholesterol Education Program (NCEP) Adult Treatment Panel III criteria.

**Results:** In cross-sectional data, significant associations between each of T, SHBG, DHT, and calculated free testosterone (cFT) with the metabolic syndrome remained significant after multivariate adjustment. In longitudinal analyses, however, only lower SHBG was significantly associated with incident metabolic syndrome over the 2-year follow-up (*P* for linear trend = .04).

**Conclusions:** Although low serum T, DHT, SHBG, and cFT were associated cross-sectionally with metabolic syndrome among community-dwelling older men, over a 2-year follow-up period only SHBG remained significant after multivariate adjustment. This suggests that lowered circulating androgens (T and DHT) may be biomarkers rather than causally related to incident metabolic syndrome. **(*J Clin Endocrinol Metab* 99: E2686–E2691, 2014)**



Low levels of T and SHBG are associated with metabolic syndrome and its risk factors, including hypertension, obesity, insulin resistance, and dyslipidemia (1–3); however, the relationship is less clear in older men and the T measurements used immunoassays rather than mass spectrometry (4). It is important to determine whether observed associations between T, SHBG, and other reproductive hormones with metabolic syndrome represents a causal relationship, or rather biomarkers of risk.

An unproven belief in the "andropause" hypothesis that modest decreases in circulating T cause somatic features of aging, rather than representing a nonspecific adaptive hypothalamic response to chronic disease, has led to a dramatic increase in T prescribing in Australia and

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received May 24, 2014. Accepted September 24, 2014.
First Published Online September 26, 2014

Abbreviations: BMI, body mass index; cFT, calculated free testosterone; CHAMP, Concord Health and Ageing in Men Project; CI, confidence interval; DHT, dihydrotestosterone; E1, estrone; E2, estradiol; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MATeS, Men in Australia Telephone Survey.

elsewhere (5). This has been accompanied by an excess of cardiovascular events reported in a randomized, placebo-controlled trial as well as some but not all observational cohort studies (6–8).

Our study aimed to examine the relationships between circulating reproductive hormones and SHBG with the metabolic syndrome in older men both cross-sectionally and longitudinally over a 2-year follow-up period.

## Materials and Methods

### Study subjects

The Concord Health and Ageing in Men Project (CHAMP) is a longitudinal, observational study of aging conducted among Australian men (9). Baseline measurements were collected between 2005 and 2007 and 2-year follow-up between 2007 and 2009 (9).

### Reproductive hormone measurement

Serum from an early morning fasting blood sample was stored at −80°C until assay. Serum T, DHT, estradiol (E2), and estrone (E1) were measured by a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (10). Serum LH, FSH, and SHBG were measured by automated immunoassays with coefficients of variation of 1.0–2.0% (11). Calculated free testosterone (cFT) levels were computed using a validated empirical (12).

### Outcome measurement

Metabolic syndrome was defined using the National Cholesterol Education Program (NCEP) Adult Treatment Panel III criteria, which involves the presence of at least three or more of waist circumference $>$ 102 cm, fasting glucose $\geq$ 5.6 mmol/L and/or on diabetes treatment, triglycerides $\geq$ 1.7 mmol/L, high-density lipoprotein cholesterol $<$ 1.03 mmol/L, and systolic blood pressure $\geq$ 130 mm Hg and/or diastolic blood pressure $\geq$ 85 mm Hg and/or on antihypertensive treatment (3).

### Statistical analysis

Of the 1705 men who completed the baseline assessments, 1299 men were included in cross-sectional analyses, after excluding men on either androgen or antiandrogen treatments (n = 20), or missing data for one or more risk factors (n = 386) and/or reproductive hormones (n = 25). Most missing data were for baseline fasting glucose (n = 369) due to technical laboratory failures. For the longitudinal analyses, loss to follow-up was for death (n = 99) and declining to attend (n = 222).

Cross-sectional and longitudinal associations between prevalent and incident metabolic syndrome and baseline hormone quartiles were assessed by logistic regression. Of the 978 men with longitudinal data, 400 with metabolic syndrome at baseline were excluded from the longitudinal analyses. The model building for all analyses included relevant covariates notably age, body mass index (BMI), and smoking status.

All categorical (quartile) analyses were verified by fitting the reproductive hormones and SHBG as continuous variables. Models were fitted using SPSS software version 20 (IBM) and SAS software 9.3 (SAS Institute).

### Ethics approval

The CHAMP study was approved by the Concord Hospital Human Research Ethics Committee, and participants provided written informed consent.

## Results

The 1299 men included in the baseline cross-sectional analyses (age, 76.7 ± 5.4 y; range, 70–97 y; BMI, 28 ± 4.1 kg/m$^2$; Table 1) were predominantly Caucasian (49% Australians, 20% Italians, 4% Greek, 4% British, 3% Chinese) with 37% (n = 481) having metabolic syndrome.

Of 578 men in the longitudinal analyses (age, 76.4 ± 5.1 y; BMI, 27 ± 3.4 kg/m$^2$; Table 1) 106 (18%) developed incident metabolic syndrome over the 2-year follow-up period.

There were no differences in age, BMI, T, or SHBG, and all the individual risk factors of metabolic syndrome in men excluded from analyses due to missing fasting glucose (n = 369) compared with the men included (data not shown).

In cross-sectional unadjusted analyses, lower quartiles of androgens (T and DHT) and SHBG but not E2 and E1 were associated with presence of metabolic syndrome (Table 2) and remained associated after multivariate-adjustment. Significant linear trends ($P < .001$) were observed across T, DHT, SHBG and cFT quartiles, with men having lower levels more likely to have metabolic syndrome. The association with E1 (but not E2) was significant after multivariate adjustment despite not being significant in univariate model.

In the longitudinal analyses, T, DHT, SHBG, and cFT were associated with incident metabolic syndrome in unadjusted models (Table 2) but only SHBG remained a statistically significant predictor of new metabolic syndrome in multivariate-adjusted models (significant linear trend, $P < .04$) whereby men with lower circulating SHBG levels more likely to develop metabolic syndrome (Table 2). Subanalyses adjusting for self-reported hypothyroidism, hyperthyroidism, liver disease, and renal diseases in further multivariate models did not

**Table 1.** Baseline Characteristics of Subjects in the Cross-Sectional Analytic Sample and in the Longitudinal Analytic Sample

| Characteristic | Cross-Sectional Analytic Sample (n = 1299) | Longitudinal Analytic Sample (n = 578) |
|---|---|---|
| Age, y | 76.7 (5.4) | 76.4 (5.1) |
| BMI, kg/m$^2$ | 27.9 (4.1) | 26.5 (3.4) |
| <25 | 313 (24%) | 197 (34%) |
| 25–<30 | 623 (48%) | 305 (53%) |
| ≥30 | 363 (28%) | 75 (13%) |
| Smoking status | | |
| Non-smoker | 475 (37%) | 237 (41%) |
| Ex-smoker | 748 (58%) | 313 (54%) |
| Current smoker | 76 (6%) | 28 (5%) |
| T, ng/ml | 4.2 (1.9) | 4.6 (1.9) |
| DHT, ng/ml | 0.4 (0.3) | 0.4 (0.3) |
| SHBG, nmol/L | 49.5 (21.0) | 51.8 (20.0) |
| E2, pg/ml | 24.8 (9.1) | 24.8 (8.6) |
| E1, pg/ml | 40.0 (16.0) | 40.4 (15.1) |
| LH, IU/L | 9.4 (8.1) | 9.1 (8.3) |
| FSH, IU/L | 14.4 (14.5) | 13.9 (14.4) |
| cFT, pmol/L | 59.5 (22.9) | 63.1 (21.9) |
| No. of metabolic syndrome criteria | | |
| 0 | 64 (5%) | 26 (5%) |
| 1 | 346 (27%) | 237 (41%) |
| 2 | 408 (31%) | 209 (36%) |
| 3 | 288 (22%) | 83 (14%) |
| 4 | 149 (12%) | 19 (3%) |
| 5 | 44 (3%) | 4 (1%) |
| Incident metabolic syndrome | | 106 (18%) |

Data are presented as either Mean (SD) or N (%).

modify the findings for hormones or SHBG (data not shown).

Forty-five percent of men were on hypolipidemic medication, mostly statins; another subanalysis stratified by either treated with statins or free of statins revealed similar findings between the two groups and similar findings as the main analyses (data not shown).

The findings using hormone levels as continuous (linear) variables confirmed the findings using quartiles. For each 1-SD reduction in hormones and SHBG level, men had adjusted odds ratios for prevalent metabolic syndrome at baseline of 1.10 (95% confidence interval [CI], 1.04–1.16) for T, 1.12 (95% CI, 1.07–1.18) for DHT, and 1.16 (95% CI, 1.10–1.28) for SHBG. In the longitudinal analyses, for each 1-SD reduction in SHBG there was an odds ratio of 1.09 (95% CI, 1.00–1.18) for development of metabolic syndrome over the 2-year follow-up period.

The interaction term between SHBG and BMI in the overall analyses was $P = 0.02$, suggesting possible effect modification. However, in the subgroup analyses stratified by BMI (<25, 25–30, and >30 kg/m$^2$), there were no differences in the associations between SHBG and incident metabolic syndrome in any group (data not shown).

## Discussion

To our knowledge, this study provides the first comprehensive examination of cross-sectional and longitudinal associations between all the major bioactive reproductive hormones and metabolic syndrome in older men based on LC-MS steroid analyses. In cross-sectional analyses, men with low levels of androgens (T and DHT) and SHBG, but not estrogens (E2, E1) had more than 2-fold higher odds of exhibiting metabolic syndrome. In longitudinal analyses, however, only lower circulating levels of SHBG were predictive of incident metabolic syndrome over a 2-year follow-up period. This suggests that androgens (T and DHT) may be biomarkers of risk and/or reflect the underlying changes in SHBG but in any case not likely to predict or explain incident metabolic syndrome in older men.

Our CHAMP data revealed longitudinal findings consistent with the Framingham Heart Study in which SHBG, but not T, was associated with incident metabolic syndrome (1). In contrast with CHAMP and Framingham, other prospective studies have all found longitudinal associations between T and metabolic syndrome (2). The discrepancies between studies may be due to different steroid assays, with most using direct

**Table 2.** Unadjusted, Age-Adjusted, and Multivariate-Adjusted Odds Ratios for Cross-Sectional and Longitudinal Associations Between Reproductive Hormones Quartiles and Metabolic Syndrome

| | Cross-Sectional (n = 1299) | | | Longitudinal (n = 578) | | |
|---|---|---|---|---|---|---|
| **Hormone Quartile** | **Unadjusted** | **Age Adjusted** | **Multivariate Adjusted[a]** | **Unadjusted** | **Age Adjusted** | **Multivariate Adjusted[a]** |
| T | | | | | | |
| Highest | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Third | 1.80 (1.26–2.57) | 1.81 (1.27–2.58) | 1.67 (1.13–2.47) | 1.15 (0.64–2.07) | 1.20 (0.66–2.17) | 1.20 (0.64–2.23) |
| Second | 2.86 (2.02–4.03) | 2.86 (2.02–4.05) | 2.01 (1.37–2.95) | 1.24 (0.68–2.26) | 1.21 (0.66–2.22) | 0.91 (0.48–1.74) |
| Lowest | 3.81 (2.69–5.40)[b] | 4.03 (2.83–5.73)[b] | 2.30 (1.55–3.41)[b] | 2.06 (1.13–3.75)[b] | 2.16 (1.18–3.97)[b] | 1.32 (0.68–2.56) |
| DHT | | | | | | |
| Highest | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Third | 1.53 (1.06–2.19) | 1.53 (1.06–2.20) | 1.23 (0.83–1.82) | 1.07 (0.59–1.93) | 1.06 (0.58–1.92) | 1.07 (0.57–1.98) |
| Second | 3.21 (2.27–4.55) | 3.15 (2.22–4.47) | 2.29 (1.56–3.35) | 1.40 (0.77–2.54) | 1.30 (0.71–2.38) | 1.16 (0.61–2.20) |
| Lowest | 4.29 (3.03–6.08)[b] | 4.47 (3.15–6.34)[b] | 2.37 (1.61–3.49)[b] | 2.28 (1.26–4.12)[b] | 2.31 (1.27–4.21)[b] | 1.55 (0.81–2.97) |
| SHBG | | | | | | |
| Highest | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Third | 1.69 (1.17–2.44) | 1.66 (1.15–2.41) | 1.32 (0.86–1.99) | 1.31 (0.67–2.57) | 1.26 (0.64–2.48) | 1.10 (0.54–2.24) |
| Second | 2.66 (1.87–3.79) | 2.57 (1.79–3.69) | 1.70 (1.14–2.54) | 2.48 (1.33–4.60) | 2.12 (1.13–4.01) | 1.76 (0.91–3.44) |
| Lowest | 5.01 (3.53–7.09)[b] | 4.81 (3.36–6.89)[b] | 3.10 (2.09–4.59)[b] | 2.98 (1.58–5.62)[b] | 2.47 (1.28–4.77)[b] | 1.89 (0.96–3.74)[‡] |
| E2 | | | | | | |
| Highest | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Third | 0.96 (0.70–1.33) | 0.95 (0.69–1.32) | 1.13 (0.78–1.63) | 0.52 (0.28–0.97) | 0.50 (0.26–0.93) | 0.49 (0.25–0.96) |
| Second | 0.85 (0.62–1.17) | 0.84 (0.61–1.16) | 1.07 (0.74–1.55) | 0.91 (0.52–1.60) | 0.86 (0.49–1.51) | 0.99 (0.54–1.83) |
| Lowest | 1.10 (0.80–1.52) | 1.11 (0.80–1.53) | 1.35 (0.93–1.97) | 0.65 (0.36–1.19) | 0.61 (0.33–1.12) | 0.67 (0.35–1.28) |
| E1 | | | | | | |
| Highest | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Third | 0.96 (0.70–1.33) | 0.97 (0.70–1.34) | 1.22 (0.84–1.77) | 0.74 (0.39–1.39) | 0.74 (0.39–1.40) | 0.87 (0.43–1.73) |
| Second | 1.02 (0.74–1.41) | 1.01 (0.73–1.39) | 1.34 (0.92–1.93) | 1.20 (0.68–2.12) | 1.11 (0.62–1.98) | 1.51 (0.80–2.84) |
| Lowest | 1.13 (0.82–1.55) | 1.15 (0.83–1.58) | 1.50 (1.03–2.18) | 1.17 (0.64–2.13) | 1.13 (0.62–2.07) | 1.49 (0.77–2.90) |
| LH | | | | | | |
| Highest | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Third | 1.20 (0.87–1.64) | 1.08 (0.78–1.49) | 1.09 (0.76–1.57) | 1.45 (0.76–2.75) | 1.27 (0.66–2.43) | 1.48 (0.75–2.95) |
| Second | 0.99 (0.72–1.37) | 0.86 (0.62–1.20) | 0.84 (0.58–1.23) | 1.17 (0.62–2.22) | 0.94 (0.49–1.81) | 0.84 (0.41–1.69) |
| Lowest | 0.84 (0.60–1.16) | 0.72 (0.52–1.01) | 0.67 (0.46–0.99) | 1.49 (0.80–2.78) | 1.16 (0.61–2.21) | 1.07 (0.54–2.12) |
| FSH | | | | | | |
| Highest | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Third | 1.05 (0.76–1.44) | 0.99 (0.72–1.36) | 0.98 (0.68–1.42) | 1.59 (0.84–3.03) | 1.45 (0.75–2.77) | 1.28 (0.64–2.56) |
| Second | 1.16 (0.84–1.59) | 1.05 (0.75–1.45) | 1.10 (0.76–1.60) | 1.30 (0.66–2.56) | 1.12 (0.56–2.22) | 1.14 (0.55–2.35) |
| Lowest | 0.84 (0.61–1.17) | 0.75 (0.54–1.05) | 0.74 (0.51–1.09) | 1.59 (0.84–3.02) | 1.33 (0.69–2.56) | 1.33 (0.67–2.65) |
| cFT | | | | | | |
| Highest | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Third | 2.01 (1.42–2.84) | 2.00 (1.41–2.83) | 1.73 (1.18–2.53) | 1.28 (0.71–2.28) | 1.30 (0.72–2.33) | 1.26 (0.68–2.33) |
| Second | 2.32 (1.66–3.24) | 2.36 (1.68–3.30) | 1.61 (1.11–2.34) | 1.11 (0.62–2.02) | 1.10 (0.60–2.00) | 0.81 (0.43–1.53) |
| Lowest | 3.16 (2.22–4.37)[b] | 3.55 (2.51–5.02)[b] | 1.90 (1.29–2.80)[b] | 1.95 (1.08–3.54) | 2.27 (1.24–4.18) | 1.33 (0.69–2.59) |

Prevalent metabolic syndrome was defined as having three or more risk factors.

Incident metabolic syndrome was defined as free of metabolic syndrome at baseline and having three or more risk factors at follow-up.

[a] Model was adjusted for age, BMI and smoking status.

[b] Linear trend, $P < .05$.

sex steroid immunoassays, which have poorer accuracy, especially at low circulating T levels such as those prevailing among older men (13). Other studies also used different measures and definitions for metabolic syndrome such as a higher boundary cutoff for fasting glucose of > 6.1 mmol/L, using nonfasting glucose levels, or have used antidiabetes medication to define hyperglycemia (2). The men in previous longitudinal studies were also much younger than in CHAMP, with a mean age of approximately 50 years. Nevertheless, our study has a shorter follow-up period (2-years) which may underestimate an association reported in previous studies with longer follow-up.

Interestingly, our study is the first to report a strong association, albeit cross-sectional, between DHT and metabolic syndrome. Although DHT a pure (nonaromatizable) androgen is mainly produced by the conversion of T if may also be produced by the alternative (backdoor) pathway that bypasses T, under some pathophysiological circumstances thereby varying independently of T (14).

Further longitudinal studies are warranted to investigate the role of DHT in male aging.

A previous study reported the associations between metabolic syndrome and SHBG were strongly modified by BMI, which was not confirmed in our analyses (2). The role of SHBG and/or aging in mediating metabolic syndrome of older men remains incompletely understood (15). Although obesity has prominent and reversible effects in lowering circulating SHBG (16), recent genetic studies also suggest that SHBG may mediate apparently independent effects on risk of diabetes and cardiovascular disease (17–18). Nevertheless, these effects may be mediated via modulation of sex steroid action, given that all bioactive sex steroids are bound strongly to circulating SHBG (19). Thus, there remain doubts as to the causal relevance and/or mechanism of action of SHBG in mediating metabolic syndrome.

The lack of statistically significant longitudinal associations with T in men regardless of BMI status suggests that androgen levels in older men may be biomarkers of metabolic syndrome rather than causal factors. This supports the possibility that low T may be caused by metabolic syndrome, not the other way around. Hence, the present findings provide no support for the "andropause" hypothesis that proposes that T treatment may be beneficial for older men.

A major strength of our study is that we were able to compare cross-sectional and longitudinal findings, which enabled us to investigate the possible causal direction. Another strength is that we used LC-MS/MS, the current gold standard for steroid assays, together with comprehensive, multianalyte steroid profiling rather than the single-analyte focus of steroid immunoassays, especially becasue androgen status is mediated by two bioactive androgens, not just T (20). CHAMP includes a large and representative group of older Australian men, as demonstrated by similar sociodemographic and health characteristics in the nationally representative Men in Australia Telephone Survey (MATeS) study (21).

Limitations of our study include the short follow-up period and the 20% loss to follow-up, mostly due to inevitable high mortality rate of an older male population. Another limitation was the lost data for fasting glucose, which was due to laboratory error but, as it was unrelated to any characteristics of the men, represents a form of data missing at random, making it unlikely to cause bias (22). Diurnal variation and seasonal variation in hormone concentrations are unlikely to have influenced our results becasue blood was sampled consistently in the morning and seasonal variations in men are minimal (23). In our multivariate model analyses, we have adjusted for BMI, but not insulin resistance, which may be a potential modifiable risk factor. Another limitation is that in using fasting blood glucose to define metabolic syndrome, diabetes, or impaired glucose tolerance could not be defined by using an oral glucose tolerance test and/or HbA1C.

Our findings add to evidence that low circulating levels of T, DHT, and SHBG are strongly associated cross-sectionally with metabolic syndrome in older men but that only SHBG was longitudinally associated with incident metabolic syndrome over 2-year follow-up period. Our longitudinal study suggests that androgens, T, and DHT may be biomarkers of metabolic syndrome and not causally related to onset of metabolic syndrome over time.

## Acknowledgments

We thank Helen Creasey and the late Philip Sambrook for their contribution in the study concept and design.

Address all correspondence and requests for reprints to: David J. Handelsman, ANZAC Research Institute, Sydney 2139, New South Wales, Australia. E-mail: djh@anzac.edu.au.

R.G.C., D.J.H., M.J.S., L.M.W., V.N., D.G.L.C., and F.M.B. contributed to the formulation of the study concept, design, methods, subject recruitment and data collection.

B.H. performed the analyses and wrote the manuscript; R.G.C. and D.J.H. wrote portions of the manuscript; V.N., F.M.B., D.G.L.C., M.J.S., and L.M.W. reviewed the manuscript and contributed to discussion.

This work was supported by the NHMRC Project Grant (No. 301916), Sydney Medical School Foundation and Ageing and Alzheimer's Institute. B.H. is funded by the Sydney Medical School Foundation.

Disclosure Summary: B.H., F.M.B., V.N., D.G.L.C., M.J.S., L.M.W., and D.J.H. have nothing to declare. R.G.C. received an honorarium from Eli Lilly Australia for an education event.

## References

1. **Bhasin S, Jasjua GK, Pencina M, et al.** Sex hormone-binding globulin, but not testosterone, is associated prospectively and independently with incident metabolic syndrome in men: The framingham heart study. *Diabetes Care*. 2011;34:2464–2470.
2. **Brand JS, Rovers MM, Yeap BB, et al.** Testosterone, sex hormone-binding globulin and the metabolic syndrome in men: An individual participant data meta-analysis of observational studies. *PLoS One*. 2014 Jul;9:e10040914.
3. **Grundy SM, Brewer HB Jr., Cleeman JI, Smith SC Jr., Lenfant C; American Heart Association, National Heart, Lung, and Blood Institute.** Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation.* 2004; 109:433–438.
4. **Handelsman DJ, Wartofsky L.** Requirement for mass spectrometry sex steroid assays in the Journal of Clinical Endocrinology and Metabolism. *J Clin Endocrinol Metab*. 2013 Oct;98:3971–3973.
5. **Handelsman DJ.** Pharmacoepidemiology of testosterone prescribing in Australia, 1992–2010. *Med J Aust*. 2012;196:642–645.

6. **Basaria S, Coviello AD, Travison TG, et al.** Adverse events associated with testosterone administration. *N Engl J Med*. 2010;363:109–122.
7. **Finkle WD, Greenland S, Ridgeway GK, et al.** Increased risk of non-fatal myocardial infarction following testosterone therapy prescription in men. *PLoS One*. 2014 Jan;9:e8580529.
8. **Vigen R, O'Donnell CI, Barón AE, et al.** Association of testosterone therapy with mortality, myocardial infarction, and stroke in men with low testosterone levels. *JAMA*. 2013 Nov;310:1829–1366.
9. **Cumming RG, Handelsman D, Seibel MJ, et al.** Cohort Profile: The Concord Health and Ageing in Men Project (CHAMP). *Int J Epidemiol*. 2009;38:374–378.
10. **Harwood DT, Handelsman DJ.** Development and validation of a sensitive liquid chromatography-tandem mass spectrometry assay to simultaneously measure androgens and estrogens in serum without derivatization. *Clin Chim Acta*. 2009;409:78–84.
11. **Ly LP, Sartorius G, Hull L, et al.** Accuracy of calculated free testosterone formulae in men. *Clin Endocrinol (Oxf)*. 2010;73:382–388.
12. **Sartorius G, Ly LP, Sikaris K, McLachlan R, Handelsman DJ.** Predictive accuracy and sources of variability in calculated free testosterone estimates. *Ann Clin Biochem*. 2009;46:137–143.
13. **Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H.** Position statement: Utility, limitations, and pitfalls in measuring testosterone: An Endocrine Society position statement. *J Clin Endocrinol Metab*. 2007;92:405–413.
14. **Auchus RJ.** The backdoor pathway to dihydrotestosterone. *Trends Endocrinol Metab*. 2004;15:432–438.
15. **Hammond GL, Wu TS, Simard M.** Evolving utility of sex hormone-binding globulin measurements in clinical medicine. *Curr Opin Endocrinol Diabetes Obes*. 2012;19:183–189.
16. **Corona G, Rastrelli G, Monami M, et al.** Body weight loss reverts obesity-associated hypogonadotropic hypogonadism: A systematic review and meta-analysis. *Eur J Endocrinol*. 2013;168:829–843.
17. **Ding EL, Song Y, Manson JE, et al.** Sex hormone-binding globulin and risk of type 2 diabetes in women and men. *N Engl J Med*. 2009;361:1152–1163.
18. **Perry JR, Weedon MN, Langenberg C, et al.** Genetic evidence that raised sex hormone binding globulin (SHBG) levels reduce the risk of type 2 diabetes. *Hum Mol Genet*. 2010;19:535–544.
19. **Travison TG, Zhuang WV, Lunetta KL, et al.** The heritability of circulating testosterone, oestradiol, oestrone and sex hormone binding globulin concentrations in men: The Framingham Heart Study. *Clin Endocrinol*. 2014;80:277–282.
20. **Handelsman DJ.** Mechanisms of action of testosterone–unraveling a Gordian knot. *N Engl J Med*. 2013 Sep;369:1058–1912.
21. **Holden CA, McLachlan RI, Pitts M, et al.** Men in Australia Telephone Survey (MATeS): A national survey of the reproductive health and concerns of middle-aged and older Australian men. *Lancet*. 2005;366:218–224.
22. **Little R, Rubin D.**2002. Statistical analysis with missing data. 2nd ed. Hoboken: John Wiley, and Sons;12–19, 47, 119.
23. **Brambilla DJ, Matsumoto AM, Araujo AB, McKinlay JB.** The effect of diurnal variation on clinical measurement of serum testosterone and other sex hormone levels in men. *J Clin Endocrinol Metab*. 2009;94:907–913.