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CLINICAL STUDY

# In men older than 70 years, total testosterone remains stable while free testosterone declines with age. The Health in Men Study

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## Abstract

*Objective*: An age-related decline in serum total and free testosterone concentration may contribute to ill health in men, but limited data are available for men >70 years of age. We sought to determine the distribution and associations of reduced testosterone concentrations in older men.
*Design*: The Health in Men Study is a community-representative prospective cohort investigation of 4263 men aged ≥70 years. Cross-sectional hormone data from 3645 men were analysed.
*Methods*: Early morning sera were assayed for total testosterone, sex hormone binding globulin (SHBG) and LH. Free testosterone was calculated using the Vermeulen method.
*Results*: Mean (±S.D.) serum total testosterone was 15.4±5.6 nmol/l (444±162 ng/dl), SHBG 42.4±16.7 nmol/l and free testosterone 278±96 pmol/l (8.01±2.78 ng/dl). Total testosterone correlated with SHBG (Spearman's $r=0.6$, $P<0.0001$). LH and SHBG increased with age ($r=0.2$, $P<0.0001$ for both). Instead of declining, total testosterone increased marginally ($r=0.04$, $P=0.007$) whilst free testosterone declined with age ($r=-0.1$, $P<0.0001$). Free testosterone was inversely correlated with LH ($r=-0.1$, $P<0.0001$). In multivariate analyses, increasing age, body mass index (BMI) and LH were associated with lower free testosterone.
*Conclusions*: In men aged 70–89 years, modulation of androgen action may occur via an age-related increase in SHBG and reduction in free testosterone without a decline in total testosterone concentration. Increasing age, BMI and LH are independently associated with lower free testosterone. Further investigation would be required to assess the clinical consequences of low serum free testosterone, particularly in older men in whom total testosterone may be preserved.

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## Introduction

In middle-aged men, total and free serum testosterone concentrations fall by 0.8 and 2% per year respectively contributing to an increasing prevalence of reduced circulating androgens with advancing age (1–4). Androgen deficiency, defined by the presence of suggestive symptoms and either low total testosterone (<6.94 nmol/l, <200 ng/dl) or low free testosterone (<309 pmol/l, <8.91 ng/dl in the presence of intermediate total testosterone) affects 6% of men aged 40–69 years, increasing to 12.3% after 8.8 years follow-up (5). In older men with low concentration of testosterone, testosterone replacement therapy has been reported to increase physical performance and lean body mass, and to offer some beneficial effects on sexual function, mood and bone mineral density (6–10). Reduced serum free testosterone concentrations have been associated with impaired cognitive function and increased likelihood of Alzheimer's disease in longitudinal studies (11, 12), and more recently with both increased insulin resistance and risk of metabolic syndrome as a marker of cardiovascular risk (13–16).

There is increasing interest in the possible beneficial effects of exogenous testosterone supplementation on cognitive, physical and metabolic functions in older men (17). However, this is a controversial area with overviews of controlled trials of testosterone therapy in men indicating only modest improvements in body composition and limited effects on general and sexual health (17–19). Thus, there is an ongoing debate over the utility of exogenous testosterone therapy in men who are not clearly hypogonadal and also over the exact thresholds at which testosterone replacement should be considered (17–22). A total testosterone <10.4 nmol/l (<300 ng/dl) or free testosterone <170 pmol/l



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(<5 ng/dl) has been suggested as indicative of androgen deficiency in men, allowing for variation in local reference laboratory ranges (21). In contrast, thresholds of 11 nmol/l of total testosterone and 225 pmol/l of free testosterone have been suggested for the diagnosis of partial androgen deficiency in the ageing male (23). However, unless age-adjusted reference ranges can be incorporated into the clinical guidelines, a single cut-off may overestimate the number of men aged >70 years with 'low' testosterone concentrations (2, 17, 24).

Total testosterone concentrations were lower in men aged 80 years and above compared with those aged 75–79 years in a South Australian study of 195 older men (25). A major study examining testosterone and oestradiol concentrations in 2623 men aged >65 years found that free testosterone declines with increasing age, body mass index (BMI) and poorer self-reported health status with a lesser effect of age on total testosterone (26). The key question remains whether serum total and free testosterone concentrations continue to decline in a linear fashion at the upper range of age, or whether a plateau is established allowing men aged 70–85+ years to maintain relatively preserved circulating androgens. To address these issues we analysed serum total and free testosterone concentrations in community-dwelling men aged 70–89 years, aiming to determine whether or not total and free serum testosterone concentrations continue to decline with age above 70 years. Secondary aims were to clarify the appropriateness of age-adjusted reference ranges for prevalence of reduced total and free testosterone concentrations, to identify factors associated with low serum free testosterone and examine the utility of luteinising hormone (LH) as a marker of primary gonadal failure in older men.

## Participants and methods

### *Study population*

The Health in Men Study (HIMS) is a prospective follow-up investigation of participants in a trial of screening for abdominal aortic aneurysms (27). Briefly, between April 1996 and January 1999, community-dwelling men resident in Perth, Western Australia, aged 65–83, were randomly selected from the electoral roll and invited to attend a screening clinic, enrolment to vote being compulsory for Australian citizens. On arrival at the clinic, the trial was explained to each participant and written consent was obtained. A risk factor questionnaire covering the aspects of medical history and lifestyle relevant to cardiovascular disease was then completed. In this questionnaire, participants were also asked about their daily consumption of alcohol in a usual week (measured in standard drinks of 10 g alcohol). Of 17 432 eligible men, 12 203 (70%) attended initial screening. Between October 2001 and August 2004, surviving men were invited to participate in a follow-up study, which included another health questionnaire and a single early morning blood sample drawn for isolation of DNA and biochemical analysis. Out of ~9000 surviving men, 4263 attended follow-up representing 35% of the men initially screened and 47% of surviving men potentially able to participate. Height (in centimetres), weight (in kilograms), girth at hips and waist (in centimetres) and blood pressure were measured using standard procedures. The Human Research Ethics Committee of the University of Western Australia approved the study protocol.

### *Laboratory methods*

Blood samples were collected between 0800 and 1030 h. Serum was prepared immediately following phlebotomy and stored at −80 °C until assayed. Biochemical and hormone assays were performed in the Biochemistry Department, PathWest, Royal Perth Hospital, Western Australia. Serum total testosterone, sex hormone binding globulin (SHBG) and LH were determined by chemiluminescent immunoassays on an Immulite 2000 analyser (Diagnostic Products Corp.-Biomediq, Doncaster, Australia). Between-day imprecision (coefficient of variation) for testosterone: 11.2% at 7.2 nmol/l and 8.9% at 18 nmol/l, for SHBG: 6.7% at 5.2 nmol/l and 6.2% at 81 nmol/l, and for LH: 6.4% at 2.3 IU/l and 5.8% at 19 IU/l. The working range of the testosterone assay was 0.7–55 nmol/l; the sensitivities of the SHBG and LH assays were 2 nmol/l and 0.1 IU/l, respectively. The established reference intervals for these assays are total testosterone 8–35 nmol/l, SHBG 10–70 nmol/l and LH 1–8 IU/l. Free testosterone, specifically the portion not bound to either SHBG or albumin, was calculated from total testosterone and SHBG using the Vermeulen method (28). Total testosterone in nmol/l can be multiplied by 28.8 to convert the units into ng/dl.

### *Statistical analysis*

We used the statistical package SPSS, version 11.5 (SPSS Inc., Chicago, IL, USA), to analyse the data. Initial descriptive analyses showed that the serum concentrations of all hormones were skewed to the right. As logarithmic transformations did not improve normality (as judged by the Kolmogorov–Smirnov and Shapiro–Wilks statistics), non-parametric tests were used throughout. We used Spearman's rank-order correlation to test the strength of the association between variables, such as testosterone and LH. Kruskal–Wallis tests were used to determine differences between groups and post-hoc comparisons were made with the Mann–Whitney *U* test. The Jonckheere–Terpstra test was used to assess trends across age groups (e.g. decline in total testosterone concentration across the different age groups). Binary logistic regression was used to explore

predictors of a low free testosterone, with covariates fitted in a forward stepwise manner in the multivariate analysis. A few extreme outliers were present in all hormone data, but their presence did not affect the behaviour of the data and, for this reason, they were not excluded. $\alpha$ was set at 5%.

## Results

### *Characteristics of participants*

Available sera were assayed to provide hormone data for 4165 men. After exclusion of men receiving hormonal therapy ($n=113$ including orchidectomy, long-acting gonadotrophin-releasing hormone agonists, finasteride, androgen blockade with cyproterone acetate, bicalutamide, flutamide or nilutamide), men receiving any form of testosterone supplementation ($n=26$) and those with prostate cancer ($n=381$), we included the results for 3645 men in the analysis. Baseline characteristics of these participants are shown in Table 1. Of these 3645 men, 84.5% were married, 14.1% had completed primary school education only, 63.5% had completed part or whole of high school education and 22.0% possessed a tertiary qualification. One third (35.2%) had never smoked and 94.5% were not smoking. Most of them (82.5%) consumed 0–14 standard drinks per week. High blood pressure (systolic $\geq 140$ mmHg or diastolic $\geq 90$) was present in 61.2%.

### *Population distributions of total and free testosterone, SHBG and LH*

The mean ($\pm$S.D.) serum total testosterone of participants was $15.4 \pm 5.6$ nmol/l ($444 \pm 162$ ng/dl; Fig. 1A), whereas mean calculated free testosterone was $278 \pm 96$ pmol/l ($8.01 \pm 2.78$ ng/dl; Fig. 1B). Mean SHBG was $42.4 \pm 16.7$ nmol/l and LH $5.8 \pm 5.3$ IU/l (Fig. 1C and D). Of the whole cohort, 497 men (13.6%) had a total testosterone concentration of $<10$ nmol/l (288 ng/dl). Total testosterone was strongly correlated with SHBG (Spearman's $r=0.6$, $P<0.0001$; Fig. 2A). Total

**Table 1** Physical characteristics of the study population, and distribution of serum total and free testosterone, sex hormone binding globulin (SHBG) and luteinising hormone (LH) across age groups.

| | **Whole cohort** ($n=3645$) | **70–74 years** ($n=1383$) | **75–79 years** ($n=1555$) | **80–84 years** ($n=584$) | **85+ years** ($n=123$) |
|---|---|---|---|---|---|
| Age (years) | | | | | |
| Mean | 76.97 | 73.58 | 77.36 | 82.02 | 86.20 |
| S.D. | 3.60 | 0.94 | 1.42 | 1.35 | 0.78 |
| BMI (kg/m$^2$) | | | | | |
| Mean | 26.48 | 26.80 | 26.45 | 25.98 | 25.63 |
| S.D. | 3.60 | 3.63 | 3.59 | 3.64 | 2.74 |
| BMI $\geq 30$ kg/m$^2$ (%) | 14.7 | 16.6 | 14.0 | 13.7 | 6.5 |
| Total testosterone (nmol/l) | | | | | |
| Mean | 15.43 | 15.15 | 15.64 | 15.58 | 15.21 |
| S.D. | 5.61 | 5.44 | 5.71 | 5.86 | 4.99 |
| Median | 14.80 | 14.60 | 14.90 | 14.80 | 15.00 |
| 75th centile | 18.40 | 18.00 | 18.60 | 18.60 | 18.60 |
| 25th centile | 11.70 | 11.50 | 11.90 | 11.70 | 11.50 |
| 5th centile | 7.89 | 7.82 | 8.04 | 7.15 | 6.70 |
| 2.5th centile | 6.43 | 6.52 | 6.64 | 5.23 | 6.17 |
| Free testosterone (pmol/l) | | | | | |
| Mean | 278 | 284 | 281 | 262 | 250 |
| S.D. | 96 | 97 | 96 | 97 | 84 |
| Median | 270 | 277 | 272 | 255 | 246 |
| 75th centile | 326 | 329 | 330 | 303 | 312 |
| 25th centile | 222 | 229 | 223 | 212 | 193 |
| 5th centile | 152 | 164 | 155 | 123 | 133 |
| 2.5th centile | 122 | 139 | 125 | 81 | 83 |
| LH (IU/l) | | | | | |
| Mean | 5.80 | 4.79 | 6.04 | 7.16 | 7.66 |
| S.D. | 5.32 | 3.39 | 5.70 | 6.82 | 7.34 |
| Median | 4.34 | 3.92 | 4.44 | 5.14 | 5.22 |
| 75th centile | 6.55 | 5.78 | 6.76 | 8.16 | 8.93 |
| 25th centile | 3.02 | 2.74 | 3.15 | 3.36 | 3.36 |
| SHBG (nmol/l) | | | | | |
| Mean | 42.42 | 39.41 | 42.64 | 47.58 | 48.89 |
| S.D. | 16.72 | 14.49 | 16.38 | 20.40 | 17.20 |
| Median | 39.60 | 37.15 | 40.20 | 43.85 | 45.20 |
| 75th centile | 50.33 | 46.80 | 50.55 | 55.08 | 57.40 |
| 25th centile | 31.40 | 29.70 | 31.50 | 35.40 | 37.20 |

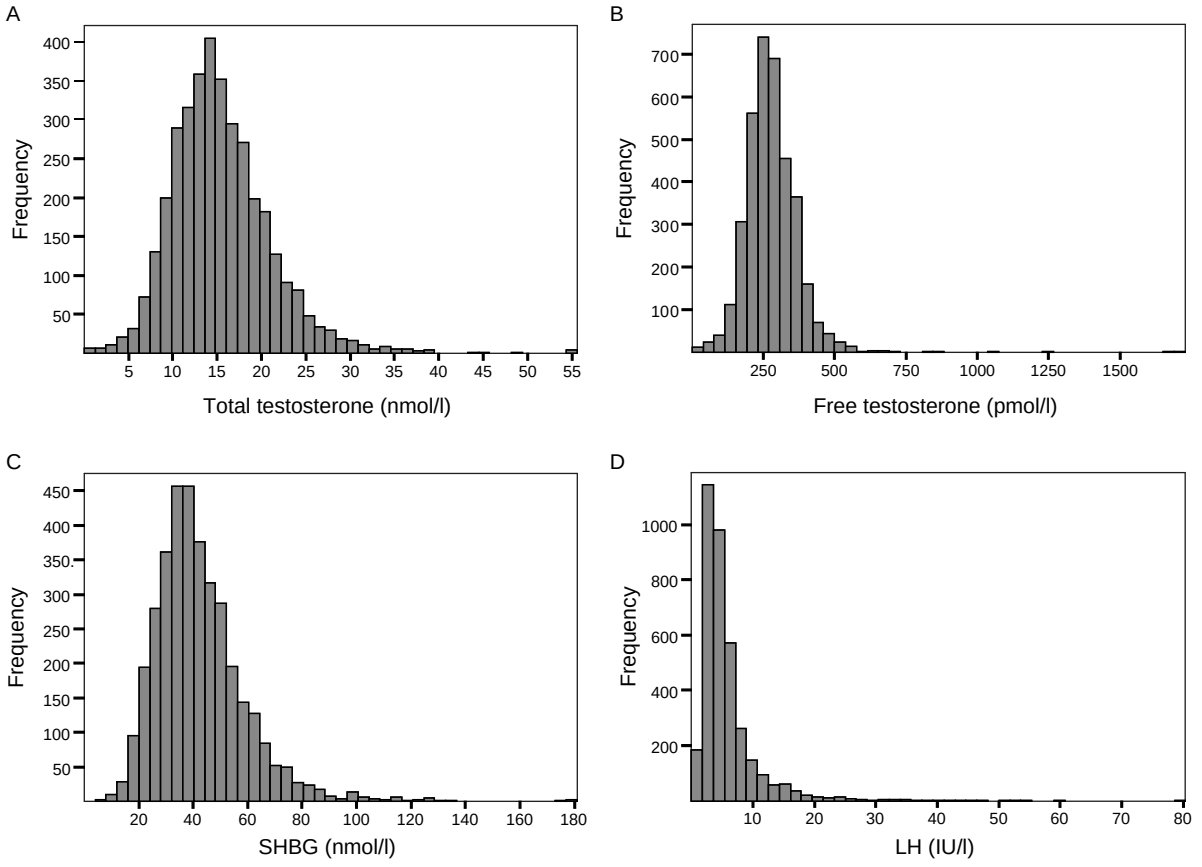


**Figure 1** Frequency distributions of total testosterone (nmol/l, A), free testosterone (pmol/l, B), SHBG (nmol/l, C) and LH (IU/l, D) for 3645 men aged 70–89 years.

testosterone was not correlated with LH ($r=0.01$, $P=0.500$; Fig. 2B), but free testosterone showed a weak inverse correlation with LH ($r=-0.1$, $P<0.0001$; Fig. 2C).

In order to estimate the utility of a single LH measurement in distinguishing primary gonadal failure from downregulation of the hypothalamo–pituitary–gonadal axis, men were classified as having hypergonadotrophic hypogonadism if total testosterone was $<8$ nmol/l ($<230$ ng/dl) and LH $>12$ IU/l ($>1.5$ times upper limit of normal range), Leydig cell impairment if total testosterone was 8–15 nmol/l (230–432 ng/dl) with LH $>12$ IU/l, and hypogonadotrophic hypogonadism if total testosterone was $<8$ nmol/l with LH $\leq 12$ IU/l (29). Using these criteria, there were only 56 men (1.5%) with hypergonadotrophic hypogonadism, 127 (3.5%) with Leydig cell impairment and 134 (3.7%) with hypogonadotrophic hypogonadism.

### *Relationship between age and total and free testosterone, SHBG and LH*

There was a weak positive association between higher total testosterone concentration and increasing age (Spearman's $r=0.04$, $P=0.007$; Fig. 3A). In contrast, free testosterone fell with increasing age ($r=-0.1$, $P<0.0001$; Fig. 3B). SHBG increased with age ($r=0.02$, $P<0.0001$; Fig. 3C), as did LH ($r=0.2$, $P<0.0001$; Fig. 3D). Means ($\pm$S.E.M.) for total testosterone in the age groups 70–74, 75–79, 80–84 and 85+ years were $15.1\pm0.15$ nmol/l, $15.6\pm0.14$ nmol/l, $15.6\pm0.24$ nmol/l and $15.2\pm0.45$ nmol/l (Fig. 4A). This difference was not significant ($\chi^2=5.471$, $P=0.140$, Kruskal–Wallis test; Jonckheere–Terpstra test for trend $P=0.053$). Of men with total testosterone $<8$ nmol/l, 74 (representing 5.4% of the men in that age category) were 70–74 years old, 75 (4.8%) were 75–79 years, 33 (5.7%) were 80–84 years and 8 (6.5%) were 85+ years.

Calculated free testosterone fell sequentially across age groups from $284\pm2.6$ pmol/l to $281\pm2.4$ pmol/l, $262\pm4$ pmol/l and $250\pm7.6$ pmol/l respectively (Fig. 4B). This difference was significant ($\chi^2=40.374$, $P<0.0001$, Kruskal–Wallis test; Jonckheere–Terpstra test for trend $P<0.0001$), although there was no significant difference between men aged 70–74 and 75–79 ($P=0.261$, Mann–Whitney $U$ test), and men aged 80–84 and 85+ years ($P=0.271$,

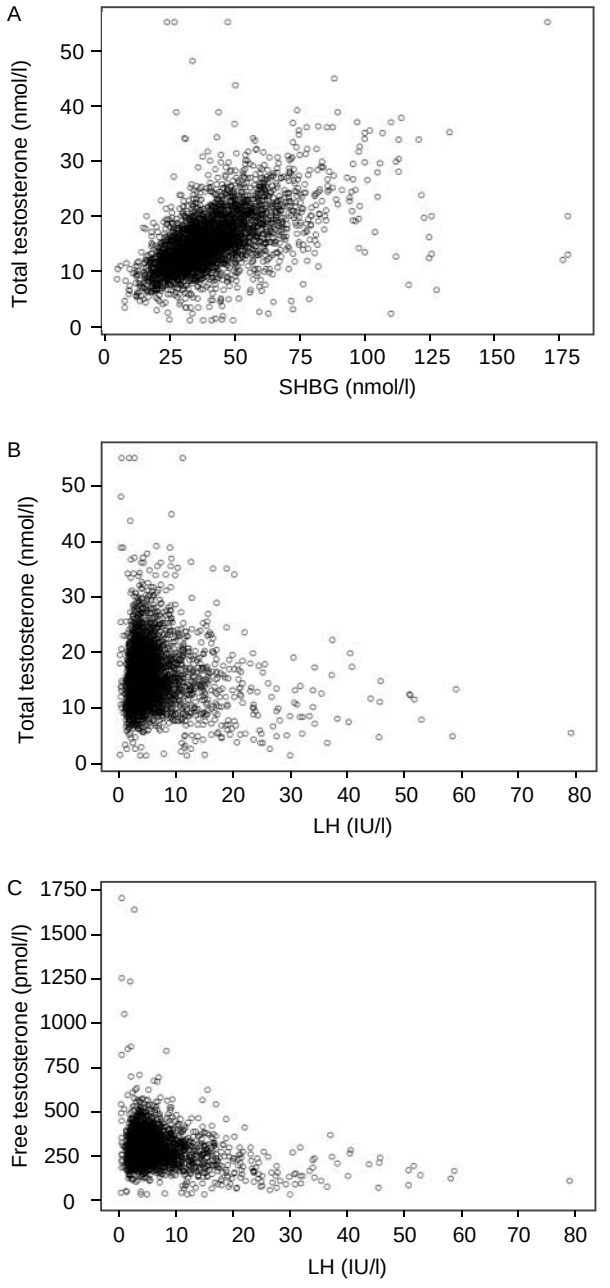


**Figure 2** Correlations between serum total testosterone with SHBG and LH, and free testosterone with LH. Total testosterone (nmol/l) correlates with SHBG (nmol/l; Spearman's $r=0.6$, $P<0.0001$) (A). Total testosterone was not correlated with LH (IU/l) ($r=0.01$, $P=0.5$) (B), but free testosterone (pmol/l) was inversely correlated with LH ($r=-0.1$, $P<0.0001$) (C).

Mann–Whitney *U* test). Differences between all other age groups were significant ($P<0.001$, Mann–Whitney *U* test). Of men with free testosterone < 210 pmol/l, 239 were 70–74 years old, representing 17.3% of men in that age category, 300 (19.3%) were 75–79 years, 143 (24.5%) were 80–84 years and 41 (33.3%) were 85+ years.

SHBG increased with increasing age from 39.4 ± 0.4 nmol/l to 42.6 ± 0.4 nmol/l, 47.6 ± 0.8 nmol/l and 48.9 ± 1.6 nmol/l respectively (Fig. 4C). The difference between age groups was significant ($\chi^2=115.917$, $P<0.0001$, Kruskal–Wallis test and Jonckheere–Terpstra test for trend $P<0.0001$), although there was no significant difference between men aged 80–84 and 85+ ($P=0.155$, Mann–Whitney *U* test). Significant differences existed between all other groups ($P<0.0001$, Mann–Whitney *U* test).

LH increased across age groups from 4.8 ± 0.1 IU/l in the group aged 70–74 years to 6.0 ± 0.1 IU/l, 7.2 ± 0.3 IU/l and 7.7 ± 0.7 IU/l in the older age groups (Fig. 4D). The difference between groups was significant ($\chi^2=100.286$, $P<0.0001$, Kruskal–Wallis test and Jonckheere–Terpstra test for trend $P<0.0001$). Again, there was no significant difference between men aged 80–84 and 85+ years ($P=0.525$, Mann–Whitney *U* test). Differences between all other groups were significant ($P\leq0.01$, Mann–Whitney *U* test).

The mean, S.D., median, 75th and 25th centiles for serum total testosterone, free testosterone, SHBG and LH are presented in Table 1, with the 5th and 2.5th centiles for total and free testosterone concentration.

### *Univariate and multivariate analyses*

Binary logistic regression analysis was performed after defining low free testosterone as the lowest 20% of values (Table 2A). This threshold was selected to allow the lowest quintile of free testosterone values to be compared with the remaining values and corresponds to a calculated free testosterone concentration of 210 pmol/l, which is between the concentrations of 170 and 225 pmol/l previously reported as indicative of androgen deficiency (21, 23). In univariate analyses, increasing age, BMI and LH were associated with low free testosterone concentration, whereas marital status, education level, smoking status and usual alcohol consumption were not. There was a negative correlation between SHBG and BMI (Spearman's $r=-0.3$, $P<0.0001$; data not shown). In multivariate analysis with variables fitted in a forward stepwise manner, independent predictors of low free testosterone were increasing age, BMI and LH (Table 2B). Thus, a man in the oldest age group of 85+ years had a greater than twofold increased risk of having a free testosterone concentration in the lowest quintile of values compared with a man aged 70–74 years. A man with BMI $\geq 30$ kg/m$^2$ had an 80–90% greater risk of having a free testosterone in the lowest quintile of values compared with a man with BMI $<30$ kg/m$^2$. An incremental increase in LH of 1 IU/l resulted in a 10% increase in the risk of low free testosterone.

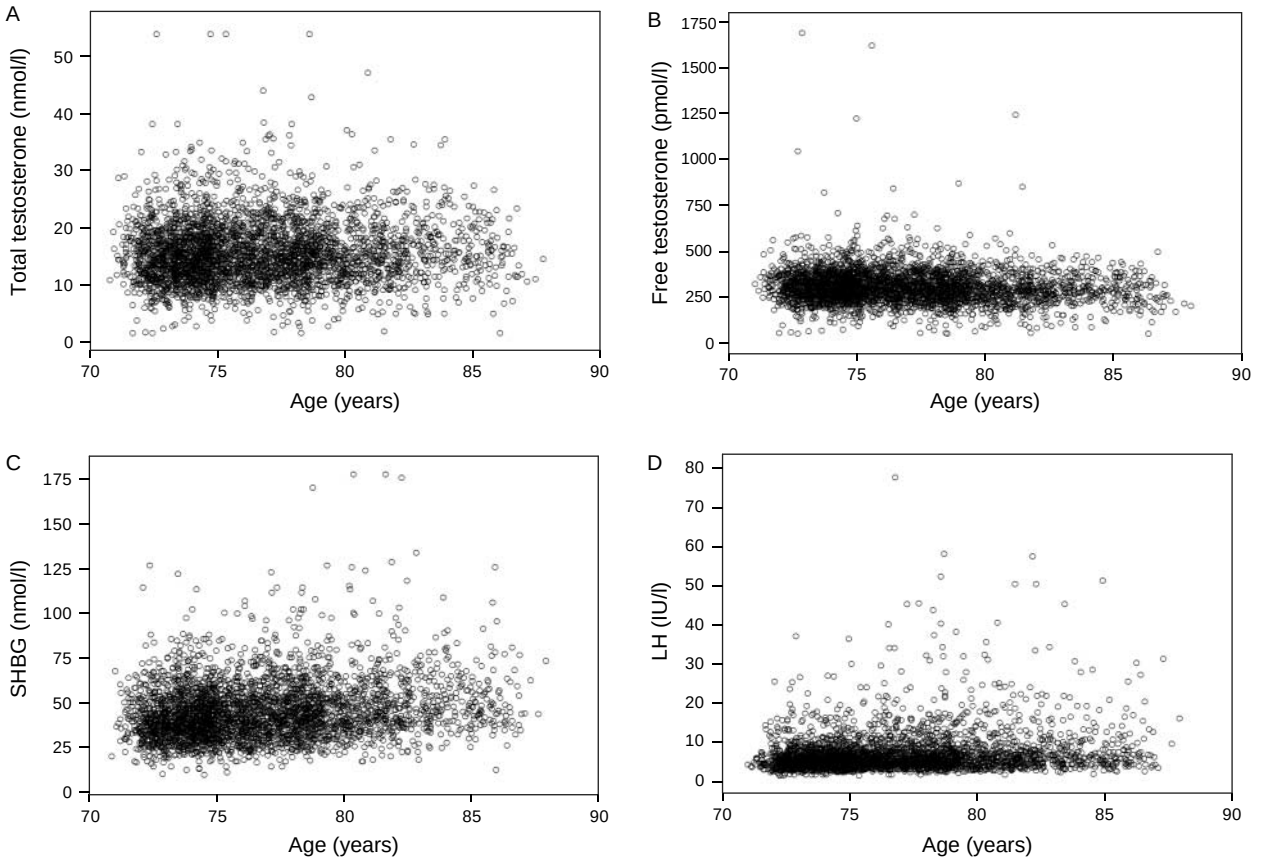


**Figure 3** Association of hormone levels with age in older men. There was a trend for total testosterone (nmol/l) to increase with age (Spearman's $r=0.04$, $P=0.007$) (A). Free testosterone (pmol/l) fell with increasing age ($r=-0.1$, $P<0.0001$) (B). SHBG (nmol/l) increased with age ($r=0.02$, $P<0.0001$) (C), as did LH (IU/l) ($r=0.02$, $P<0.0001$) (D).

## Discussion

Our results indicate that in men aged 70–89 years, total testosterone does not fall with advancing age, while free testosterone does. These findings differ from those of previous studies of male cohorts spanning from middle to old age, typically 40–80 years (1–5, 25, 30, 31), and also from a previous report of lower total testosterone in men age >80 years (25). However, the mean serum total testosterone of our cohort is similar to the concentration of $14.7\pm5.6$ nmol/l ($423\pm162$ ng/dl) reported in a recent survey of men older than 65 years (26). In that study, total testosterone concentration was also correlated with SHBG and tended to fall only slightly with increasing age, whereas free testosterone showed a clearer decline in older age groups. In our study, total testosterone remained stable with age, while the magnitude of the cross-sectional decline in free testosterone with age was of the order of 0.8% per annum when compared with an increase in SHBG of approximately 1.6% per annum.

One explanation for the observed decline in free but not total testosterone concentration in our cohort is that across ages 70–85+ years, increased SHBG, which increases the binding of testosterone, reduces the proportion of free testosterone (14, 17), with this effect being particularly prominent in men aged 80+ years. In men older than 61 years, LH has been shown to increase longitudinally with age (30). This is consistent with the positive correlation between LH and age in our cross-sectional analysis. The fact that LH is inversely correlated with free testosterone suggests that the magnitude of the decline in free testosterone with increasing age is biologically relevant, and leads to increased LH secretion. But the decline in free testosterone with age would also be consistent with inadequate or impaired compensatory responses at both pituitary and testicular levels (32, 33), with our data suggesting that only a relatively low proportion of older men have primary gonadal failure.

Ageing and androgen deficiency share numerous signs and symptoms, and the impact of reduced circulating androgens on the ageing process is an area of growing research interest (17, 18, 34). In men spanning ages 50–89 years, low bioavailability of testosterone is associated with loss of weight and reduced physical activity (35, 36). In addition, there is evidence that graded doses of i.m. testosterone increase

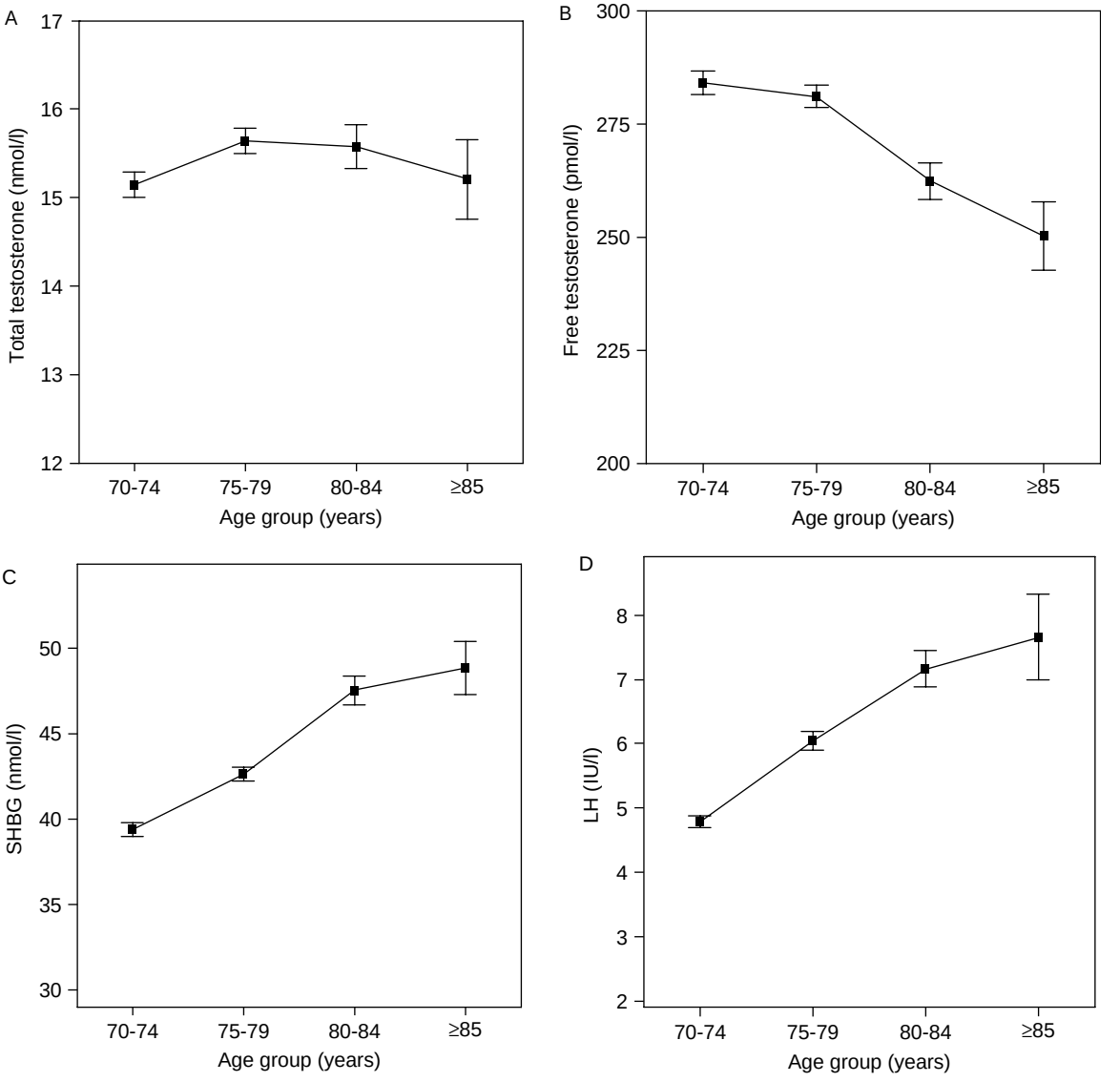


**Figure 4** Changes in total testosterone, free testosterone, SHBG and LH with age. Data are mean (±S.E.M.) for total testosterone (nmol/l), free testosterone (pmol/l), SHBG (nmol/l) and LH (IU/l) in groups of men aged 70–74, 75–79, 80–84 and 85+ years. Error bars show mean ±1S.E.M.

fat-free mass, reduce fat mass and improve the muscle strength of healthy men aged 60–75 years without evidence of testosterone deficiency (37). However, that study employed relatively high doses of testosterone, which limits its applicability to the context of community populations. Oral androgen supplementation may also exert some effect to increase muscle mass and decrease body fat (38). However, contraindications and potential adverse effects of exogenous testosterone are well recognised (18–21, 37). Thus, the case for androgen supplementation in older men is stronger in the presence of documented androgen deficiency, which relies on appropriate threshold concentrations for total and free testosterone (17, 18, 21).

Given that total testosterone concentrations did not decline in a linear fashion with age in men ≥70 years, a threshold of 6.4 nmol/l 3(185 ng/dl) for total testosterone would identify the lowest 2.5% of levels among men in our cohort and would be only slightly below the threshold suggested by Araujo *et al.* for identifying androgen deficiency in middle-aged to older men (5). In contrast, free testosterone concentrations showed a continued age-related decline. Further research is needed to clarify the clinical relevance of lower free testosterone concentrations in older men, and the potential utility of age-adjusted reference ranges based on the 2.5th or 5th centiles in our study. It is important to remember that clinical guidelines recommend that if

**Table 2** Predictors of lower serum free testosterone in older men. Univariate and multivariate analyses were performed to identify factors associated with serum free testosterone concentration in the lowest 20%. High blood pressure was defined as systolic ≥140 mmHg or diastolic ≥90, (and both readings less than the very high category). Very high blood pressure was defined as systolic ≥160 or diastolic ≥95. Odds ratios are the increased (>1.0) or decreased (<1.0) likelihood of a man in that category possessing a free testosterone level in the lowest quintile of values compared with a man in the reference category.

| | Odds ratio | 95% CI |
|---|---|---|
| (A) | | |
| Age group | | |
| 70–74 | 1.00 | – |
| 75–79 | 1.14 | 0.95–1.38 |
| 80–84 | 1.54 | 1.22–1.94 |
| 85+ | 2.46 | 1.65–3.65 |
| Marital status | | |
| Never married | 1.00 | – |
| Now married | 0.84 | 0.54–1.30 |
| Separated | 0.88 | 0.41–1.90 |
| Divorced | 0.78 | 0.43–1.41 |
| Widowed | 0.67 | 0.38–1.18 |
| De facto | 0.30 | 0.05–1.02 |
| Education | | |
| No school | 1.00 | – |
| Primary school | 1.30 | 0.28–6.03 |
| Incomplete high school education | 1.22 | 0.27–5.59 |
| Completed high school | 1.25 | 0.27–5.75 |
| Tertiary qualification | 1.28 | 0.28–5.90 |
| Ever smoked | 1.12 | 0.95–1.34 |
| Smoking frequency | | |
| Not at all | 1.00 | – |
| Not every day | 0.86 | 0.57–1.29 |
| Every day | 0.64 | 0.25–1.65 |
| Alcohol use (standard drinks/week) | | |
| 0–14 | 1.00 | – |
| 15–28 | 0.94 | 0.74–1.20 |
| 29+ | 1.11 | 0.75–1.64 |
| Blood pressure | | |
| Normal or low | 1.00 | – |
| High | 0.95 | 0.79–1.14 |
| Very high | 0.92 | 0.74–1.16 |
| BMI≥30 (kg/m$^2$) | 1.78 | 1.45–2.20 |
| LH (IU/l) | 1.10 | 1.08–1.12 |
| (B) | | |
| Age group | | |
| 70–74 | 1.00 | – |
| 75–79 | 1.02 | 0.84–1.24 |
| 80–84 | 1.24 | 0.97–1.59 |
| 85+ | 2.09 | 1.37–3.17 |
| BMI≥30 (kg/m$^2$) | 1.91 | 1.54–2.37 |
| LH (IU/l) | 1.10 | 1.08–1.12 |

A – all covariates fitted separately. B – all covariates fitted in a forward, stepwise manner.

an initial measurement of testosterone concentration is low, at least one repeat sample is obtained for confirmation before accepting a diagnosis of testosterone deficiency (17, 21, 29).

The results of a previous study showed that men aged 40 years or above who had a total testosterone <8.7 nmol/l (<250 ng/dl) or low free testosterone on two or more measurements were almost twice as likely to die over a mean follow-up period of 4.3 years compared with men with normal levels (39). While our study is based on a single measurement of testosterone, the proportion of men who met this threshold was 7.8% ($n=284$). Longitudinal follow-up of our cohort is required to determine the actual mortality risk of low testosterone concentration in men beyond the age of 70 years. Potential confounding effects, including concomitant illness and cardiovascular risk factors, would need to be considered when analysing the relationship between hormone levels and mortality.

In this population, age, BMI and LH concentrations were independently associated with low free testosterone. An association between increased BMI and reduced total and free testosterone and SHBG was also observed in the Tromso study (40). In addition, the Massachusetts Male Aging Study recently reported that overweight men (BMI ≥25 kg/m$^2$) have lower total testosterone and SHBG (41). Our results extend these findings from men aged 40–74 years into the ages of 70–89, with increasing BMI associated with lower free testosterone despite the negative correlation of BMI with SHBG. Therefore, increasing weight (BMI) is associated with both lower free testosterone and reduced SHBG, when, in isolation, a lower SHBG would usually be accompanied by a higher free testosterone.

Strengths of this study are the focus on men ≥70 years, the availability of detailed medical and drug histories allowing exclusion of men with prostate cancer and those receiving hormonal therapy, and the large sample size. Our analysis of 3645 men is larger than the study of Orwoll *et al.* with 2623 men ≥65 years from six centres (26), previously regarded as the largest cohort of older men in which sex steroids were available. We collected morning blood samples from each participant to minimise potential confounding effects of circadian variation in testosterone concentrations.

Limitations of this study include the use of a single blood sample. Greater precision could have been achieved by taking more than one sample, but this was not within the scope of the study and sampling at a single time-point offers a reasonable estimate of testosterone levels (42). There are accepted limitations in the use and interpretation of automated testosterone assays (43–48). In our study, total testosterone was assayed using an automated immunoassay possessing acceptable accuracy for men, although it would be inappropriate for women (46, 47). Data derived from a specific immunoassay of total testosterone should not be extrapolated to other populations without considering the potential of different testosterone assays to give varying results (48). Also, free testosterone was derived using the Vermeulen method (28), which may not provide an exact estimate of circulating free testosterone (49). However, these methods have been used extensively for large-scale studies where mass-spectrometry measurement of total testosterone and direct assay of free testosterone using equilibrium dialysis would be impractical (5, 11, 14, 24, 26). Another relevant

consideration is the selection of the 4263 study participants from an original cohort of 12 203 men surveyed several years previously. A sustained effort was made to recruit as many of the originally surveyed men into the follow-up study as possible, but it could be that a 'healthy survivor' effect is present in our cohort, with a censoring effect on men with lower testosterone levels due to general ill health (50). It is conceivable that older men with lower total testosterone concentrations declined to participate in the study, or that stable total testosterone concentration, higher SHBG or lower free testosterone might be factors associated with greater longevity. Thus, our findings could be regarded as indicative of testosterone distribution in a relatively healthier cohort of community-dwelling older men, rather than representative of the population as a whole.

In summary, in men aged 70–89 years, serum free testosterone decreases with increasing age, although the concentration of total testosterone remains relatively stable. SHBG and LH increase with age, with an inverse correlation between LH and free testosterone. Further, investigation of the clinical consequences of low serum free testosterone, particularly in older men in whom total testosterone may be preserved, is required to guide the diagnosis of androgen deficiency in men over 70 years of age and to establish if hormone replacement has a role to play in these men.

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## Note

Whilst this article was in production, another 7 men were found to have had a diagnosis of prostate cancer, orchidectomy or a disorder of the genital organs. Exclusion of these men left 3638 men for analysis of testosterone, SHBG and LH levels. This did not change the result of the statistical analyses.

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