

## Associations between Testosterone Levels and Incident Prostate, Lung, and Colorectal Cancer. A Population-Based Study

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### Abstract

**Background:** The relationship between testosterone and cancer is relatively unexplored. We sought to examine whether testosterone and related hormones are associated with incident prostate, lung, and colorectal cancer.

**Methods:** This was a population-based cohort study. Demographic and clinical predictors of cancer, and testosterone, sex hormone-binding globulin (SHBG), and luteinizing hormone (LH) were measured between 2001 and 2004 in 3,635 community-dwelling men aged 70 to 88 years (mean 77 years). Cancer notifications were obtained via electronic record linkage until December 31, 2010.

**Results:** During a mean follow-up period of  $6.7 \pm 1.8$  years, there were 297, 104, and 82 cases of prostate, colorectal, and lung cancer. In adjusted competing risks proportional hazards models, each one SD increase in free testosterone was associated with a 9% increase in prostate cancer risk (95% confidence interval [CI], 1.00–1.18), but other hormones were not significantly associated. No significant associations were observed between hormonal parameters and colorectal cancer. Higher total testosterone was associated with lung cancer. Compared with the mean of 15 nmol/L, men with levels of 20 nmol/L were 1.38 times more likely to be cases (95% CI, 1.21–1.57), whereas those with levels of 30 nmol/L were 3.62 times more likely to be cases (95% CI, 2.53–5.18). Higher free testosterone was also associated with lung cancer, though SHBG and LH were not. Associations were maintained after exclusion of current smokers.

**Conclusions:** Higher free testosterone was associated with incident prostate cancer. Higher testosterone levels may also be associated with lung cancer.

**Impact:** Further studies should investigate whether these risks apply to men receiving testosterone therapy. *Cancer Epidemiol Biomarkers Prev*; 21(8); 1319–29. ©2012 AACR.

### Background

Cancer is a leading cause of death and burden of disease (1). Lifestyle factors, such as smoking, poor diet, and physical inactivity account for a significant proportion of cases, but sex hormones are thought to have a role in the aetiology of some cancers.

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The prostate is an androgen-dependent organ; adequate levels of testosterone and its potent  $5\alpha$ -reductase (5AR) derived metabolite, dihydrotestosterone (DHT), are required for normal development, growth, and function (2). Development is inhibited in 46, XY individuals with androgen insensitivity syndromes or 5AR deficiency, whereas androgen deprivation therapy leads to atrophy of the prostate and other male reproductive tissues (3, 4). Males who undergo orchidectomy in adolescence or early adulthood rarely develop prostate cancer, whereas testosterone therapy can hasten progression of the disease (2). Conversely, androgen deprivation therapy is an effective palliative treatment and remains the gold standard (5).

However, the relationship between endogenous testosterone and incident prostate cancer remains controversial. Some prospective studies have reported that higher testosterone levels are associated with increased risk (6, 7), although the majority have not found a statistically significant association (8, 9). However, many of the latter studies are underpowered and have failed to consider the problem of competing risks. The question of whether

testosterone levels are related to prostate cancer risk is important, due to growing interest in testosterone therapy, and the relatively high prevalence of the disease. Latent disease is found in 70% of men aged over 80 years, and it has been suggested that all men with testosterone above castrate levels would develop at least low-grade prostate cancer if they lived long enough (10). The risk/benefit ratio of therapy is therefore yet to be established.

Studies of hormone replacement therapy in women suggest both deleterious and beneficial effects. The Women's Health Initiative trials reported an increase in lung cancer mortality and invasive breast cancer in women receiving conjugated equine estrogens (CEE) and medroxyprogesterone acetate, although colorectal cancer incidence was reduced (11, 12). However, the relevance of these findings to men, if any, is unknown. We also recently reported an association between endogenous testosterone levels and lung cancer mortality in this cohort, although we lacked sufficient cases to conduct multivariate analyses after exclusion of smokers (13). Testosterone stimulates proliferation of small-cell lung cancer cell lines (14), but smoking can also increase testosterone (15). Whether this association would be maintained in analyses of incident lung cancer, rather than mortality alone (providing sufficient cases to allow exclusion of current smokers), is unclear.

We therefore conducted a competing risks analysis to explore associations between endogenous sex hormones and incident cancer in a cohort of men aged 70 to 88 years at baseline. We limited our endpoints to prostate, lung, and colorectal cancer, as these are the most common cancers in men (excluding skin), which might also be plausibly influenced by sex hormones. We hypothesized that men with high testosterone would be more likely to be diagnosed with prostate cancer, but that testosterone levels would be unrelated to diagnosis of lung or colorectal cancer.

## Materials and Methods

### Study population

Participants were drawn from the population-based Health in Men Study, which is described in detail elsewhere (16). Participants were resident in Perth, Western Australia, and were mostly Caucasian. Subjects were randomly selected from the electoral roll (enrolment to vote being compulsory in Australia). In wave 1 (W1) between 1996 and 1999, 12,203 men aged 65 years and older participated. During 2001 to 2004 (wave 2, W2), 4,249 participated and provided early morning blood samples. Questionnaires assessing a variety of risk factors and demographic items were completed at both time points. The Human Research Ethics Committee of the University of Western Australia provided ethical approval for the study, and all men gave written informed consent to participate.

### Laboratory methods

Blood samples were collected at W2 between 0800 and 1030 hours. Biochemical assays were carried out at Royal Perth and Fremantle hospitals, as previously reported (17). Serum total testosterone, sex hormone-binding globulin (SHBG), and luteinizing hormone (LH) were determined by chemiluminescent immunoassays on an Immulite 2000 analyzer (Diagnostic Products Corp. Biomediq). Between-day imprecision for total testosterone was 11.2% at 7.2 nmol/L and 8.9% at 18 nmol/L; for SHBG it was 6.7% at 5.2 nmol/L and 6.2% at 81 nmol/L; and for LH it was 6.4% at 2.3 IU/L and 5.8% at 19 IU/L. Working ranges for these assays are 0.7 to 55 nmol/L for testosterone, 2 to 180 nmol/L for SHBG, and 0.1 to 200 IU/L for LH, whereas normal male ranges are 8 to 35 nmol/L for testosterone, 10 to 70 nmol/L for SHBG, and 1 to 8 IU/L for LH. Free testosterone, the fraction not bound to SHBG or albumin, was estimated with Vermeulen's method (18). The total testosterone assay accounts for the majority of variance (>80%) in the free testosterone estimate; detailed analyses of its predictive accuracy have been published elsewhere (19, 20). Serum glucose, high- and low-density lipoprotein, total cholesterol, and triglycerides were assayed using a Roche Hitachi 917 analyzer (Roche Diagnostic GmbH).

### Other measurements

Height (in centimeters), weight (in kilograms), waist and hip circumference (in centimeters), and blood pressure were measured at W1 and W2. Questionnaire data (W1 and W2) and biochemistry (W2) were used to flag dyslipidaemia and diabetes. Questionnaire and clinical data (W1 and W2) were used to identify hypertension. Men were asked about tobacco use at both time points and alcohol use at W1.

### Cancer diagnoses

We obtained previous and incident cancer diagnoses via the Western Australian Data Linkage System (WADLS). WADLS provides electronic linkage to the state's population health collections and includes records from the mortality, hospital, and cancer registries (21). The latter was established in 1981. Notification of cancer and other neoplasms is mandatory in Western Australia, including all *in situ* neoplasms, all malignant neoplasms of the skin (other than primary squamous or basal cell carcinomas), and all neoplasms of the central nervous system, whether malignant or benign (22). Cancer diagnoses are coded with the International Classification of Diseases for Oncology (ICDO-3) system. For our analyses, we considered topography codes C18, C19, C20, and C21 to indicate colorectal cancer; C33 and C34 to indicate lung cancer; and C61.9 to indicate prostate cancer. Incident cases comprised only primary invasive malignancies detected before December 31, 2010. We ignored metastatic sites, neoplasms in which primary or metastatic status was uncertain, and neoplasms of unknown behavior. *In situ* carcinomas were not included.

### Statistical analysis

We used Stata 11.2 to analyze the data (StataCorp, 2011). Of 4,249 men providing sera, testosterone and LH were successfully assayed in 4,165 and SHBG in 4,162. From these, we excluded orchidectomized men, those with prostate cancer, and those receiving antiandrogens, 5AR inhibitors, GnRH analogs, or testosterone therapy, leaving 3,636 men (including 3 without SHBG). One man subsequently withdrew consent, leaving 3,635 participants for analysis. No men reported taking aromatase inhibitors. We explored each cancer of interest separately. To minimize the possibility of reverse causality, we excluded men who had previously been diagnosed with the outcome of interest. We excluded 23 men with a previous diagnosis of lung cancer from the incident lung cancer analysis, and 115 men with previous colorectal cancer from the colorectal cancer models. To assess associations between baseline factors (such as smoking or testosterone level) and each cancer of interest, we used the Mann-Whitney *U* test and Pearson  $\chi^2$  test for continuous and categorical data, respectively. We used Spearman rank-order correlation to investigate associations between individual hormonal parameters. To explore associations between hormonal parameters and time to diagnosis of cancer, we used competing risks models, as described by Fine and Gray (23). We chose the competing risks approach because alternatives such as the Cox model have an important limitation: they assume the outcome of interest will eventually occur. That is, individuals who die before developing cancer are treated as if they were right censored and could later be diagnosed, which is impossible. To explore whether associations between hormonal parameters and cancer were curvilinear, we initially entered hormones into the models as restricted cubic splines. Associations with colorectal and lung cancer appeared curvilinear and were modeled with this approach. We report sub-hazard ratios (sub-HR) at specific points along the spline that may be of interest to the reader; they do not represent division of the data at the quintiles, but rather show how the hazard function changes across the data. In contrast, associations with prostate cancer were linear, or near linear and were subsequently modeled as Z-scores. We modeled the relationship between testosterone and prostate cancer as a linear effect because measures such as the Bayesian information criterion indicated this to be the most parsimonious model. Sub-HRs reflect the effect of a 1 *SD* increase in hormone level in those models. Adjustments were made for age, waist to hip ratio (WHR), smoking status (never/ex-/current smoker), smoking exposure (greatest amount of tobacco smoked for as long as 1 year, and years of smoking), diabetes, and previous diagnosis of cancer (other than the cancer of interest) before blood sampling. We also examined the effect of adjusting for body mass index rather than WHR; this did not alter the results. Additional models in which hormonal parameters were analyzed as categorical variables split at the quintiles are presented as Supplementary Material. All tests were two-

sided, and *P* values less than 0.05 were considered significant.

### Results

Mean follow-up duration was  $6.7 \pm 1.8$  years (range 0.1–9.2 years), comprising 24,398, 23,625, and 24,256 person-years, for the prostate, colorectal, and lung cancer analyses. During this time, 297 men were diagnosed with prostate cancer, 104 with colorectal cancer, and 82 with lung cancer. There were 886 competing risk events (death from any cause) in men included in prostate cancer analyses, 867 in colorectal cancer analyses, and 870 in lung cancer models. Total testosterone was significantly correlated with SHBG ( $\rho = 0.61$ ;  $P < 0.001$ ), but not LH ( $\rho = 0.01$ ;  $P = 0.441$ ). There was a significant weak negative correlation between free testosterone and LH ( $\rho = -0.11$ ;  $P < 0.001$ ). Detailed descriptive statistics of the hormonal data are published elsewhere (17).

#### Baseline characteristics of men by cancer diagnosis

Men diagnosed with prostate cancer during follow-up had significantly higher baseline free testosterone than those who were not (Table 1). Prostate cancer cases were also less likely to have diabetes. No significant differences were observed in other hormones. With regard to colorectal cancer, sex hormones did not differ significantly, though higher LH levels in cases approached significance. Men diagnosed with lung cancer had significantly higher total testosterone at baseline, although higher free testosterone and LH levels approached significance. Men with lung cancer were more likely to be current or previous smokers, had smoked for longer, and were heavier smokers.

#### Associations between hormone levels and incident cancer

Relationships between total and free testosterone levels, and prostate cancer were approximately linear, with higher levels associated with increased risk (Fig. 1). There was no clear association between testosterone and colorectal cancer. Higher total and free testosterone was strongly associated with lung cancer.

#### Prostate cancer

In univariate competing risks models, each 1 *SD* increase in free testosterone was associated with a 10% increase in the risk of prostate cancer (Table 2). After adjustment for age, WHR, smoking status and exposure, diabetes, alcohol use, and previous diagnosis of cancer (other than prostate), free testosterone continued to be associated with incident prostate cancer (sub-HR, 1.09; 95% confidence interval [CI], 1.00–1.18). Total testosterone, SHBG, and LH were not significantly associated with prostate cancer in either univariate or multivariate models. A significant protective association was observed for diabetes in the free testosterone model (sub-HR, 0.68; 95%

**Table 1.** Baseline (2001–2004) demographic, biochemical, and clinical characteristics of the entire sample, and of men with, and without incident prostate, colorectal, and lung cancer

| Variable                                  | Entire sample<br>(n = 3,635)<br>Mean ± SD | Prostate cancer                |                               | P value | Colorectal cancer              |                               | P value | Lung cancer                    |                              | P value |
|---|---|--------------------------------|-------------------------------|---------|--------------------------------|-------------------------------|---------|--------------------------------|------------------------------|---------|
|   |   | No<br>(n = 3,338)<br>Mean ± SD | Yes<br>(n = 297)<br>Mean ± SD |         | No<br>(n = 3,416)<br>Mean ± SD | Yes<br>(n = 104)<br>Mean ± SD |         | No<br>(n = 3,529)<br>Mean ± SD | Yes<br>(n = 82)<br>Mean ± SD |         |
| Age (y)                                   | 77.0 ± 3.6                                | 77.0 ± 3.6                     | 76.7 ± 3.6                    | 0.148   | 76.9 ± 3.6                     | 78.7 ± 3.8                    | <0.001  | 76.9 ± 3.6                     | 77.9 ± 3.0                   | 0.001   |
| WHR                                       | 0.97 ± 0.1                                | 0.97 ± 0.1                     | 0.97 ± 0.1                    | 0.182   | 0.97 ± 0.1                     | 0.97 ± 0.1                    | 0.903   | 0.97 ± 0.1                     | 0.97 ± 0.1                   | 0.694   |
| Total testosterone<br>(nmol/L)            | 15.4 ± 5.6                                | 15.4 ± 5.6                     | 15.8 ± 5.3                    | 0.103   | 15.5 ± 5.6                     | 15.1 ± 4.7                    | 0.688   | 15.4 ± 5.5                     | 17.7 ± 8.5                   | 0.026   |
| Free testosterone<br>(pmol/L)             | 278 ± 96                                  | 277 ± 96                       | 290 ± 96                      | 0.043   | 278 ± 97                       | 268 ± 69                      | 0.344   | 278 ± 94                       | 314 ± 165                    | 0.089   |
| SHBG (nmol/L)                             | 42.4 ± 16.7                               | 42.5 ± 16.9                    | 41.6 ± 14.9                   | 0.690   | 42.5 ± 16.9                    | 42.8 ± 15.7                   | 0.567   | 42.4 ± 16.7                    | 44.8 ± 17.6                  | 0.345   |
| LH (IU/L)                                 | 5.8 ± 5.3                                 | 5.8 ± 5.3                      | 5.2 ± 4.5                     | 0.178   | 5.8 ± 5.1                      | 6.5 ± 6.0                     | 0.054   | 5.8 ± 5.2                      | 6.5 ± 7.4                    | 0.091   |
| Years smoked (y)                          | 33.0 ± 16.0                               | 33.1 ± 16.0                    | 31.5 ± 16.1                   | 0.136   | 33.2 ± 15.9                    | 30.6 ± 15.2                   | 0.207   | 32.5 ± 15.9                    | 43.4 ± 15.2                  | <0.001  |
| Greatest tobacco<br>use (cigarettes/week) | 178 ± 143                                 | 177 ± 143                      | 186 ± 152                     | 0.770   | 178 ± 144                      | 172 ± 130                     | 0.982   | 176 ± 141                      | 208 ± 140                    | 0.004   |
|   | n (%)                                     | n (%)                          | n (%)                         |         | n (%)                          | n (%)                         |         | n (%)                          | n (%)                        |         |
| Hypertension                              | 2,777 (76.4)                              | 2,563 (76.8)                   | 214 (72.1)                    | 0.066   | 2,593 (75.9)                   | 85 (81.7)                     | 0.170   | 2,693 (76.3)                   | 67 (81.7)                    | 0.255   |
| Dyslipidaemia                             | 2,746 (75.5)                              | 2,520 (75.5)                   | 226 (76.1)                    | 0.818   | 2,579 (75.5)                   | 74 (71.2)                     | 0.311   | 2,675 (75.8)                   | 56 (68.3)                    | 0.117   |
| Diabetes mellitus                         | 573 (15.8)                                | 540 (16.2)                     | 33 (11.1)                     | 0.022   | 538 (15.8)                     | 22 (21.2)                     | 0.138   | 555 (15.7)                     | 14 (17.1)                    | 0.741   |
| Smoking status                            |   |                                |                               | 0.320   |                                |                               | 0.022   |                                |                              | <0.001  |
| Never smoked                              | 1,215 (33.4)                              | 1,104 (33.1)                   | 111 (37.4)                    |         | 1,160 (34.0)                   | 26 (25.0)                     |         | 1,212 (34.4)                   | 2 (2.5)                      |         |
| Exsmoker                                  | 2,220 (61.1)                              | 2,049 (61.4)                   | 171 (57.6)                    |         | 2,062 (60.3)                   | 76 (73.1)                     |         | 2,136 (60.5)                   | 63 (76.8)                    |         |
| Current smoker                            | 200 (5.5)                                 | 185 (5.5)                      | 15 (5.0)                      |         | 194 (5.7)                      | 2 (1.9)                       |         | 181 (5.1)                      | 17 (20.7)                    |         |
| Alcohol consumption<br>(drinks/week)      |   |                                |                               | 0.697   |                                |                               | 0.916   |                                |                              | 0.222   |
| Nondrinker                                | 509 (14.0)                                | 472 (14.1)                     | 37 (12.5)                     |         | 478 (14.0)                     | 17 (16.3)                     |         | 499 (14.1)                     | 7 (8.5)                      |         |
| <15 drinks                                | 2,488 (68.4)                              | 2,286 (68.5)                   | 202 (68.0)                    |         | 2,338 (68.5)                   | 70 (67.3)                     |         | 2,419 (68.6)                   | 55 (67.1)                    |         |
| 15–28 drinks                              | 482 (13.3)                                | 437 (13.1)                     | 45 (15.1)                     |         | 452 (13.2)                     | 13 (12.5)                     |         | 462 (13.1)                     | 16 (19.5)                    |         |
| ≥29 drinks                                | 156 (4.3)                                 | 143 (4.3)                      | 13 (4.4)                      |         | 148 (4.3)                      | 4 (3.9)                       |         | 149 (4.2)                      | 4 (4.9)                      |         |

NOTE: All parameters measured at W2 (2001–2004), except alcohol use, which was measured at W1 (1996–1999). Years smoked and greatest tobacco use shown for current and exsmokers only. *P* values are for Mann–Whitney *U* test or Pearson  $\chi^2$  test, as appropriate.

CI, 0.47–0.98), and all other multivariate models (data not shown). An association was also apparent for free testosterone when modeled as a categorical variable, but this did not reach statistical significance (Supplementary Table S1). Cancer grade was unavailable for 88.9% of cases.

To assess the possibility of reverse causality, and also the effect of possible undiagnosed cases in the sample (which would bias estimates toward the null), we excluded cases diagnosed within 2 years of blood sampling ( $n = 98$ ). Free testosterone continued to be significantly associated with incident prostate cancer, whereas an association between higher LH and lower prostate cancer risk approached significance in multivariate analyses (Table 3).

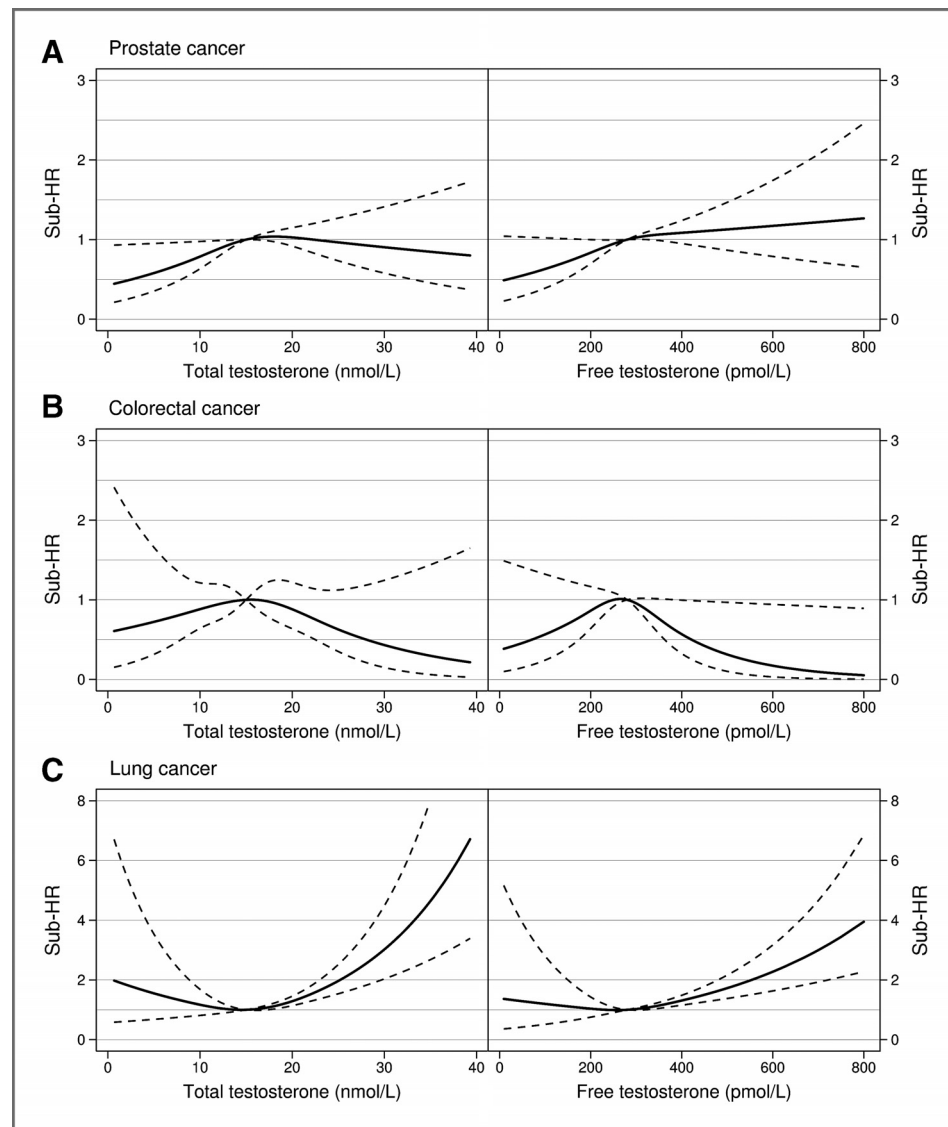
### Colorectal cancer

In both univariate and multivariate models, total and free testosterone, SHBG, and LH were not significantly associated with colorectal cancer, though CIs were large owing to the small number of cases (Table 4). Results were similar when hormones were modeled as categorical variables (Supplementary Table S2).

### Lung cancer

Higher total and free testosterone was associated with lung cancer in both univariate and adjusted models (Table 5). Results were similar when hormones were modeled as categorical variables (Supplementary Table S3). In multivariate models, men with total testosterone levels of 20 nmol/L and 30 nmol/L, were 1.38 times and 3.62 times more likely to be diagnosed than men with the mean of 15 nmol/L. Men with a free testosterone level of 400 pmol/L were 1.41 times more likely to be diagnosed than those with a level of 280 pmol/L. However, men with lung cancer were more likely to smoke, which can increase testosterone levels (15).

Among cases, current smokers had significantly higher mean total testosterone than exsmokers (23.7 vs. 16.1 nmol/L;  $P = 0.005$ ). Levels were also higher than those of never smokers (14.4 nmol/L), though there were insufficient numbers to test this statistically. Similarly, in the entire cohort, total testosterone was higher in current smokers than exsmokers (17.0 vs. 15.0 nmol/L;  $P < 0.001$ ), and never smokers (15.9 nmol/L;  $P = 0.007$ ). However, current smokers who were lung cancer cases



**Figure 1.** Univariate competing risks proportional hazards models exploring associations between total and free testosterone levels, and incident cancer of the prostate (A), colon and rectum (B), and lung (C). Sex hormones entered into the models as restricted cubic splines. Reference values for sub-HRs are 15 nmol/L for total testosterone, and 280 pmol/L for free testosterone. Dashed lines denote 95% CIs.

( $n = 17$ ) had higher total testosterone (23.7 vs. 16.3 nmol/L;  $P = 0.002$ ) than current smokers in the rest of the cohort ( $n = 181$ ). Current smokers who were lung cancer cases had smoked for longer than the rest of the cohort (60.6 vs. 56.8 years;  $P = 0.024$ ), but a difference in quantity of tobacco smoked (current smokers among lung cancer cases had smoked more) was not significant (196.6 vs. 175.0 cigarettes/week;  $P = 0.392$ ).

Testosterone did not differ significantly (16.1 vs. 15.0 nmol/L;  $P = 0.281$ ) between exsmokers in the lung cancer cases ( $n = 63$ ) and exsmokers in the rest of the cohort ( $n = 2,136$ ). Of cases who were exsmokers, smoking cessation occurred  $21.3 \pm 14.2$  years (range 1–58 years) before baseline. The average time to diagnosis for all cases was  $3.9 \pm 2.1$  years (range 0.4–8.0 years) after baseline.

To address the possibility of residual confounding, we repeated our analyses after excluding current smokers ( $n = 198$ ). After applying exclusion criteria, total and free

testosterone continued to be associated with lung cancer in both univariate and multivariate analyses (Table 6). These associations were maintained after additional exclusion of cases diagnosed within 2 years of blood sampling (Supplementary Table S4).

## Discussion

In this study of older men, higher baseline free testosterone levels were associated with incident prostate cancer, whereas higher total and free testosterone levels were associated with incident lung cancer. Testosterone was not significantly associated with colorectal cancer.

Our findings are consistent with those of Gann et al. (7). Their prospective, nested case-control analysis comprised 222 prostate cancer cases and 390 controls with a mean age of 62 years. In a multivariate model, adjusted for age, smoking status, estradiol, dihydrotestosterone, androstanediol glucuronide, and SHBG, men with total

**Table 2.** Competing risks proportional hazards models exploring associations between hormone levels and incident prostate cancer

| Variable                  | Univariate |           |         | Multivariate |           |         |
|---------------------------|------------|-----------|---------|--------------|-----------|---------|
|                           | Sub-HR     | 95% CI    | P value | Sub-HR       | 95% CI    | P value |
| <b>Total testosterone</b> | 1.07       | 0.97–1.18 | 0.151   | 1.05         | 0.94–1.17 | 0.407   |
| <b>Free testosterone</b>  | 1.10       | 1.01–1.19 | 0.023   | 1.09         | 1.00–1.18 | 0.047   |
| <b>SHBG</b>               | 0.98       | 0.87–1.09 | 0.658   | 0.94         | 0.83–1.06 | 0.277   |
| <b>LH</b>                 | 0.89       | 0.75–1.05 | 0.169   | 0.90         | 0.76–1.06 | 0.190   |

NOTE: Sub-HRs indicate the effect of 1 SD increase in hormone level. Multivariate models adjusted for age, WHR, smoking status (never/ex-/current smoker), smoking exposure (maximum quantity of tobacco smoked and years of smoking), diabetes, alcohol use, and previous diagnosis of cancer (other than prostate) before blood sampling. *P* values are for Wald test.

testosterone above the highest quartile were 2.6 times more likely to be cases than those below the lowest quartile. Similar results were observed in a prospective study of 794 middle-aged men comprising 114 cases. Higher free, but not total testosterone was significantly associated with increased risk (6). Additionally, population differences in testosterone level have recently been associated with population disparities in prostate cancer incidence (24).

However, conflicting results were observed in case-cohort analyses of the Melbourne Collaborative Cohort Study, which involved 17,049 men aged 27 to 75 years, comprising 524 cases and a subcohort of 1,859 (25). Total testosterone, estradiol, SHBG, and adrenal androgens were not associated with overall prostate cancer incidence, but the risk of aggressive disease was approximately halved with a doubling of testosterone or androstenedione level. Higher DHEA sulfate (DHEA-S) was also associated with reduced risk of aggressive cancer.

Despite widespread perception of a relationship between testosterone and prostate cancer risk, the majority of epidemiological studies are inconclusive. A recent pooled analysis of 18 prospective studies comprising 3,886 cases and 6,438 controls found no association between total testosterone and incident prostate cancer

(8). A weak, nonsignificant association was observed for free testosterone levels above the highest quintile (relative risk [RR], 1.11; 95% CI, 0.96–1.27), whereas similar weak, nonsignificant associations were observed for DHEA-S and androstenediol glucuronide. However, a significant inverse association was observed for higher levels of SHBG (RR, 0.86; 95% CI, 0.75–0.98). The majority of testosterone is bound to either SHBG or albumin; less than 2% circulates unbound or "free." This free portion is hypothesized to be most biologically active (26). Thus, higher levels of SHBG are likely associated with reduced availability of testosterone in target tissues. Although associations were weak, the pooled analysis is suggestive of a role for testosterone in prostate cancer.

Despite inconclusive studies, a role for endogenous testosterone in the development of prostate cancer remains plausible (the "androgen hypothesis"). Androgens are necessary for the normal growth and function of the prostate (2), whereas prolonged testosterone administration induces malignancy in rodents (27, 28). Conversely, androgen deprivation therapy rapidly halts tumor progression (5). However, the androgen hypothesis is challenged by some puzzling observations. Prostate cancer incidence rises precisely as androgen levels fall, whereas Morgentaler et al. found an unusually large

**Table 3.** Competing risks proportional hazards models exploring associations between hormone levels and incident prostate cancer after exclusion of cases diagnosed within 2 years of blood sampling

| Variable                  | Univariate |           |         | Multivariate |           |         |
|---------------------------|------------|-----------|---------|--------------|-----------|---------|
|                           | Sub-HR     | 95% CI    | P value | Sub-HR       | 95% CI    | P value |
| <b>Total testosterone</b> | 1.11       | 0.99–1.24 | 0.055   | 1.10         | 0.97–1.25 | 0.140   |
| <b>Free testosterone</b>  | 1.14       | 1.05–1.24 | 0.003   | 1.13         | 1.03–1.24 | 0.013   |
| <b>SHBG</b>               | 0.97       | 0.85–1.10 | 0.630   | 0.97         | 0.84–1.11 | 0.615   |
| <b>LH</b>                 | 0.81       | 0.69–0.96 | 0.016   | 0.86         | 0.73–1.01 | 0.063   |

NOTE: Sub-HRs indicate the effect of 1 SD increase in hormone level. Multivariate models adjusted for age, WHR, smoking status (never/ex-/current smoker), smoking exposure (maximum quantity of tobacco smoked and years of smoking), diabetes, alcohol use, and previous diagnosis of cancer (other than prostate) before blood sampling. *P* values are for Wald test.

**Table 4.** Competing risks proportional hazards models exploring associations between hormone levels and incident colorectal cancer

| Variable                           | Univariate |           |         | Multivariate |           |         |
|------------------------------------|------------|-----------|---------|--------------|-----------|---------|
|                                    | Sub-HR     | 95% CI    | P value | Sub-HR       | 95% CI    | P value |
| <b>Total testosterone (nmol/L)</b> |            |           | 0.428   |              |           | 0.437   |
| 5                                  | 0.72       | 0.32–1.66 |         | 0.66         | 0.29–1.51 |         |
| 10                                 | 0.88       | 0.64–1.21 |         | 0.85         | 0.61–1.17 |         |
| 15                                 | 1          |           |         | 1            |           |         |
| 20                                 | 0.88       | 0.64–1.22 |         | 0.90         | 0.65–1.26 |         |
| 30                                 | 0.43       | 0.15–1.24 |         | 0.44         | 0.14–1.36 |         |
| <b>Free testosterone (pmol/L)</b>  |            |           | 0.107   |              |           | 0.078   |
| 100                                | 0.57       | 0.24–1.32 |         | 0.44         | 0.18–1.05 |         |
| 200                                | 0.86       | 0.64–1.17 |         | 0.78         | 0.58–1.07 |         |
| 280                                | 1          |           |         | 1            |           |         |
| 300                                | 0.96       | 0.90–1.02 |         | 0.97         | 0.91–1.03 |         |
| 400                                | 0.57       | 0.32–1.00 |         | 0.59         | 0.33–1.05 |         |
| <b>SHBG (nmol/L)</b>               |            |           | 0.892   |              |           | 0.841   |
| 20                                 | 1.11       | 0.51–2.39 |         | 1.19         | 0.55–2.60 |         |
| 30                                 | 0.99       | 0.79–1.23 |         | 1.03         | 0.82–1.31 |         |
| 40                                 | 1          |           |         | 1            |           |         |
| 50                                 | 1.06       | 0.77–1.46 |         | 1.02         | 0.73–1.43 |         |
| 60                                 | 1.04       | 0.68–1.60 |         | 0.98         | 0.62–1.56 |         |
| <b>LH (IU/L)</b>                   |            |           | 0.258   |              |           | 0.513   |
| 0.5                                | 0.61       | 0.32–1.17 |         | 0.69         | 0.36–1.33 |         |
| 2                                  | 0.73       | 0.48–1.10 |         | 0.79         | 0.53–1.20 |         |
| 5                                  | 1          |           |         | 1            |           |         |
| 10                                 | 1.14       | 0.95–1.37 |         | 1.09         | 0.90–1.30 |         |
| 15                                 | 1.13       | 0.79–1.64 |         | 1.07         | 0.74–1.53 |         |

NOTE: Sex hormones entered into the models as restricted cubic splines. Multivariate models adjusted for age, WHR, smoking status (never/ex-/current smoker), smoking exposure (maximum quantity of tobacco smoked and years of smoking), diabetes, alcohol use, and previous diagnosis of cancer (other than colorectal) before blood sampling. *P* values are for Wald test.

prevalence of prostate cancer in a sample of hypogonadal men (29). Others have reported high rates of androgen deficiency in newly diagnosed, untreated men with prostate cancer (30). However, gonadotropins were also low in the latter study, suggesting tumor-mediated disruption of the hypothalamic-pituitary-gonadal axis. Gonadotropins and testosterone have been shown to rise following radical prostatectomy, supporting this hypothesis (31). Additionally, elevated clearance of testosterone has been observed in men with prostate cancer (32), further suggesting reverse causality. The long latency of the disease also suggests the likelihood of undiagnosed cases in previous studies, producing inconclusive or spurious associations.

Nevertheless, the rising incidence in older age when testosterone levels are lowest is more difficult to explain. Given testosterone's regulatory role in prostatic cell differentiation, some have argued that adequate testosterone is necessary to prevent the pathological changes that occur in the initial stages of the disease (33, 34). The Prostate Cancer Prevention Trial initially appeared to lend support to this concept. Although overall prostate cancer inci-

dence was reduced in men randomized to the 5AR inhibitor finasteride, the treatment group was more likely to be diagnosed with aggressive disease (35). However, subsequent analyses suggest this finding is an artefact (36, 37). Prostate cancer typically progresses slowly, and it is more likely that the long latency of the disease accounts for its clinical emergence in later life. Although 85% of diagnoses are made in those aged more than 65 years (38), autopsy studies have found latent disease as early as the fourth decade of life (39).

A role for testosterone in other cancers in men is less certain, and surprisingly unexplored. The EPIC-Norfolk study found an association between low testosterone and mortality from any cancer, but this was not maintained in full multivariate models (40). The authors did not explore cause-specific cancer mortality. In our study, we also explored the association between testosterone and colorectal cancer but did not find a statistically significant association. However, a protective effect is biologically plausible; the antiandrogen cyproterone acetate enhances carcinogenesis in a rat model, whereas testosterone has suppressive effects (41). Human colorectal cancer

**Table 5.** Competing risks proportional hazards models exploring associations between hormone levels and incident lung cancer

| Variable                           | Univariate |           |         | Multivariate |           |         |
|------------------------------------|------------|-----------|---------|--------------|-----------|---------|
|                                    | Sub-HR     | 95% CI    | P value | Sub-HR       | 95% CI    | P value |
| <b>Total testosterone (nmol/L)</b> |            |           | <0.001  |              |           | <0.001  |
| 5                                  | 1.55       | 0.68–3.53 |         | 1.24         | 0.54–2.85 |         |
| 10                                 | 1.17       | 0.81–1.69 |         | 1.05         | 0.72–1.53 |         |
| 15                                 | 1          |           |         | 1            |           |         |
| 20                                 | 1.29       | 1.14–1.45 |         | 1.38         | 1.21–1.57 |         |
| 30                                 | 3.02       | 2.03–4.49 |         | 3.62         | 2.53–5.18 |         |
| <b>Free testosterone (pmol/L)</b>  |            |           | <0.001  |              |           | <0.001  |
| 100                                | 1.20       | 0.51–2.80 |         | 0.95         | 0.40–2.25 |         |
| 200                                | 1.04       | 0.75–1.43 |         | 0.94         | 0.68–1.31 |         |
| 280                                | 1          |           |         | 1            |           |         |
| 300                                | 1.03       | 0.99–1.06 |         | 1.04         | 1.00–1.09 |         |
| 400                                | 1.31       | 1.15–1.49 |         | 1.41         | 1.23–1.63 |         |
| <b>SHBG (nmol/L)</b>               |            |           | 0.310   |              |           | 0.416   |
| 20                                 | 1.03       | 0.60–1.78 |         | 1.02         | 0.57–1.83 |         |
| 30                                 | 1.00       | 0.79–1.28 |         | 1.00         | 0.77–1.29 |         |
| 40                                 | 1          |           |         | 1            |           |         |
| 50                                 | 1.05       | 0.94–1.18 |         | 1.05         | 0.93–1.19 |         |
| 60                                 | 1.14       | 0.94–1.38 |         | 1.13         | 0.92–1.40 |         |
| <b>LH (IU/L)</b>                   |            |           | 0.525   |              |           | 0.879   |
| 0.5                                | 0.66       | 0.26–1.70 |         | 0.78         | 0.31–2.01 |         |
| 2                                  | 0.77       | 0.42–1.39 |         | 0.86         | 0.48–1.55 |         |
| 5                                  | 1          |           |         | 1            |           |         |
| 10                                 | 1.14       | 0.90–1.44 |         | 1.04         | 0.83–1.31 |         |
| 15                                 | 1.17       | 0.75–1.83 |         | 1.01         | 0.65–1.57 |         |

NOTE: Sex hormones entered into the models as restricted cubic splines. Multivariate models adjusted for age, WHR, smoking status (never/ex-/current smoker), smoking exposure (maximum quantity of tobacco smoked and years of smoking), diabetes, alcohol use, and previous diagnosis of cancer (other than lung) before blood sampling. *P* values are for Wald test.

suppressor genes show evidence of regulation by testosterone (42), whereas treatment with CEE and medroxyprogesterone acetate, which has androgenic properties, reduced colorectal cancer incidence in women (11). Other, sufficiently powered, long-running observational studies are required to investigate this possibility.

A relationship between testosterone and lung cancer is also plausible. Sex differences exist in normal lung development, probably mediated by sex hormones (43, 44). The androgen receptor (AR) is present in normal and malignant lung tissue, and testosterone alters expression of genes regulating metabolism and apoptosis in malignant cells (45). Small-cell lung cancer cell lines are stimulated by testosterone, an effect blocked by AR antagonists (14). However, smoking can increase testosterone (15), suggesting the possibility of confounding. Nevertheless, the association between testosterone and lung cancer was maintained after excluding current smokers, and exsmokers retained in analyses ceased smoking an average of 21 years before baseline. Reverse causality cannot be dismissed, as cultured lung cancer cells have been shown to secrete hormones, including estradiol and LH (46). Alter-

natively, cancer-related antibodies may interfere with immunoassay measurement of testosterone (47). These seem the most plausible explanations. However, the possibility that testosterone might stimulate growth of lung cancer is troubling and requires further investigation.

Several limitations must be acknowledged in this observational study. We followed participants via record linkage, rather than recalling them for testing, such as digital rectal examination, prostate-specific antigen (PSA), or biopsy. Lung and colorectal cancers are unlikely to remain undiagnosed, and record linkage is probably adequate for detection. However, given the long latency of prostate cancer, false negatives in our data are highly likely, although the effect of this would be to introduce bias in favor of the null hypothesis. We could not explore whether associations differed by cancer grade, as this information was absent for the majority of cases, and we did not ask about family history of cancer. We were also unable to explore the effects of increased PSA testing, which may result in increased registration of low-grade cancers that would have otherwise gone undetected. PSA testing is subsidized by the Australian public health system and



**Table 6.** Competing risks proportional hazards models exploring associations between testosterone levels and incident lung cancer in men who have never smoked or are exsmokers

| Variable                           | Univariate |           |         | Multivariate |            |         |
|------------------------------------|------------|-----------|---------|--------------|------------|---------|
|                                    | Sub-HR     | 95% CI    | P value | Sub-HR       | 95% CI     | P value |
| <b>Total testosterone (nmol/L)</b> |            |           | <0.016  |              |            | 0.006   |
| 5                                  | 1.80       | 0.74–4.37 |         | 1.36         | 0.55–3.39  |         |
| 10                                 | 1.27       | 0.86–1.88 |         | 1.10         | 0.74–1.65  |         |
| 15                                 | 1          |           |         | 1            |            |         |
| 20                                 | 1.15       | 0.99–1.33 |         | 1.30         | 1.07–1.57  |         |
| 30                                 | 2.10       | 1.24–3.53 |         | 2.96         | 1.52, 5.76 |         |
| <b>Free testosterone (pmol/L)</b>  |            |           | 0.001   |              |            | 0.002   |
| 100                                | 1.66       | 0.70–3.91 |         | 1.27         | 0.53–3.07  |         |
| 200                                | 1.18       | 0.85–1.63 |         | 1.06         | 0.76–1.48  |         |
| 280                                | 1          |           |         | 1            |            |         |
| 300                                | 1.01       | 0.97–1.05 |         | 1.03         | 0.98–1.07  |         |
| 400                                | 1.22       | 1.06–1.40 |         | 1.33         | 1.10–1.60  |         |

NOTE: Sex hormones entered into the models as restricted cubic splines. Multivariate models adjusted for age, WHR, smoking status (never/exsmoker), smoking exposure (maximum quantity of tobacco smoked and years of smoking), diabetes, alcohol use, and previous diagnosis of cancer (other than lung) before blood sampling. *P* values are for Wald test.

may be used more frequently than in other regions; one study reported that 43% of Western Australian men aged 40 to 80 years were tested (48). Additionally, age at baseline did not differ between men with and without prostate cancer in our study, suggesting PSA testing may be common. However, evidence suggests urological practice is conservative in Western Australia and that prostate cancer may be underdiagnosed. In a recent study of 5,145 men undergoing an initial biopsy for the disease between 1998 and 2004, (approximately 85% of all biopsies), high-grade cancer (Gleason sum  $\geq 7$ ) was detected in 69% of cases (49). Thus, the threshold for biopsy seems to be high, and the spectrum of disease in our sample may be shifted upward relative to studies from other regions with a high prevalence of PSA testing. Limitations also include the single blood sample, lack of other hormone data, such as DHT, and estimation of free testosterone. However, a single measurement of testosterone is considered adequate in the context of large sample sizes (50), and we lacked the resources to directly measure free testosterone and assay other hormones. Strengths of our study include the large, population-based sample, adjustment for competing risks, focus on a narrow age range, and near-complete capture of endpoints via electronic record linkage (at least with regard to lung and colorectal cancer). In particular, our large sample size may explain why we found statistically significant associations between free testosterone and prostate cancer, when other studies have not.

In summary, our results suggest that higher free testosterone is associated with incident prostate cancer. The association between free testosterone and prostate cancer was weak, and did not remain statistically significant when free testosterone was modeled as a categorical variable. An association remained apparent, however,

and it should be remembered that loss of information and statistical power are among the problems that occur when continuous exposures are categorized (51, 52). Testosterone may also be associated with lung cancer, though this most likely reflects reverse causality or confounding, and requires validation in other studies. Endocrine Society guidelines for testosterone prescribing recommend against treating men with prostate cancer, and suggest ongoing monitoring of prostate health as appropriate for the patient (53). Our findings support this paradigm. Given continued uncertainty over the risks of testosterone therapy, there is a need for long-term trials to establish the risk/benefit ratio of treatment. To date, the majority of trials have been too small to adequately explore the risk of cancer. Consideration should be given to increasing the power of future trials to enable the detection of any carcinogenic signal.

#### Disclosure of Potential Conflicts of Interest

The authors have no conflicts of interest to declare.

#### Authors' Contributions

**Conception and design:** Z. Hyde, L. Flicker, G.J. Hankey, B.B. Yeap  
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