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ORIGINAL PAPER

Higher Dihydrotestosterone Is Associated with the Incidence of Lung Cancer in Older Men

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Abstract Advancing age is associated with increased cancer incidence, but the role of sex hormones as risk predictors for common cancers in older men remains uncertain. This study was performed to assess associations of testosterone (T), dihydrotestosterone (DHT) and estradiol (E2), with incident prostate, lung and colorectal cancer in community-dwelling older men. Plasma T, DHT and E2 were assayed using liquid chromatography-mass spectrometry between 2001 and 2004 in 3690 men. Cancer outcomes until 20 June 2013 were ascertained using data linkage. Analyses were performed using proportional hazards competing-risks models, and adjustments were made for potential confounding factors including smoking status. Results are expressed as subhazard ratios (SHR). There were 348, 107 and 137 cases of prostate, lung and colorectal cancers respectively during a median of 9.1-year follow-up. Mean T was comparable in current and non-smokers, whilst mean DHT was lower in ex- and current smokers compared to non-smokers. After adjusting for confounders including smoking, higher T or DHT was associated with an increased incidence of lung cancer (SHR = 1.30, 95% CI 1.06–1.60; $p = 0.012$ per 1 SD increase in T and SHR = 1.29, 95% CI 1.08–1.54; $p = 0.004$ for DHT). Sex hormones were not associated with prostate or colorectal cancer. In older men, higher T or DHT predict increased incidence of lung cancer over the next decade. Sex hormones are not associated with incident prostate or colorectal cancer. Further studies are warranted to determine if similar associations of sex hormones with lung cancer are present in other populations and to investigate potential underlying mechanisms.

Introduction

Sex hormone concentrations decline as men age [1]. Epidemiological studies in middle-aged and older men have predominantly focused on cardiovascular and mortality endpoints. These studies have demonstrated inverse or curvilinear

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associations of androgens with cardiovascular and mortality outcomes [2–4]. Cancer is a major cause of morbidity and mortality worldwide [5]. It has a steeply rising relationship with advancing age especially in men aged over 60 years [5]; however, associations of sex hormones with the incidence of cancer have not been well explored. The prostate is an androgen sensitive organ, and castration has been associated with improvement in advanced prostate cancer [6]; however, epidemiological studies have not shown a clear association of endogenous testosterone (T) and diagnosis of prostate cancer [7]. Previous meta-analyses have reported neutral associations of endogenous T with prostate cancer, but the majority of studies included in these meta-analyses are limited by the use of immunoassay for measurement of sex hormone levels [7, 8]. This method of sex hormone measurement is associated with non-specificity and assay dependent bias and is therefore less accurate compared to mass spectrometry (MS) [9, 10].

Mechanistic studies have also suggested a potential role for sex hormones to influence growth of other common cancers. Androgen receptor expression has been demonstrated on a variety of lung cancer cell lines, and a study using androgen receptor-expressing small cell lung cancer cell lines demonstrated marked growth stimulation with androgen exposure that was reversed by antiandrogens [11–13]. Androgen pathway manipulation was also associated with greater survival in male patients diagnosed with lung cancer [14]. A case-control study examining the androgen receptor genotype found a higher number of androgen receptor CAG repeats in men with colorectal cancer [15], and a genetic polymorphism connoting reduced androgen sensitivity in vivo [16]. This is further supported by a study demonstrating an inverse association of T and sex hormone binding globulin (SHBG) with the incidence of colorectal cancer in men [17]. Biological effects of T in men are also modulated by its 5α-reduction to dihydrotestosterone (DHT), a more potent androgen receptor ligand, and by its aromatisation to E2 [18–20]. Studies of androgen receptor-expressing small cell lung cancer cell lines have reported 5α-reductase activity in these cells, suggesting a potential for DHT effects on these cells [13]. Few studies have assessed the associations of T, DHT and E2, measured via liquid chromatography-tandem mass spectrometry (LC-MS/MS) with the incidence of cancer in men.

Given that sex hormones can modulate growth of specific cancer cell types [13], improved understanding of how sex hormones influence the incidence of common cancers may provide fresh therapeutic insights or novel biomarkers. This would also provide a more holistic view of how sex hormones may be associated with health in ageing men. Data from large epidemiological studies using MS-based steroid measurements would clarify the relationship between sex hormones and the incidence of common cancers in men. Therefore, our aim was to assess the association of sex hormones with the incidence of common cancers in older men. We tested the hypothesis that there would be parallel associations of T and DHT, but not E2 with the diagnosis of prostate and lung cancer, but not colorectal cancer.

Methods

Study Design and Population

This study is a longitudinal observational cohort study using the Health in Men Study (HIMS) cohort based in Perth, Western Australia. Methods used for recruitment of this cohort have previously been described [21]. In brief, between 1996 and 1999, 19,352 men were randomly selected from the electoral roll to participate in a randomised controlled trial of abdominal aortic aneurysm screening. A total of 12,203 (63.1%) men attended in 1996–1999 and completed a health questionnaire and a physical examination. This was termed wave 1 (W1) of data collection.

Baseline Assessment

Between 2001 and 2004, these 12,203 men were invited to participate in a second survey of which 4249 attended. These men completed a follow-up questionnaire and physical examination and had early morning fasting blood samples collected. This follow-up survey was termed wave 2 (W2) of data collection, and these men make up the inception cohort for the current study. The Human Research Ethics Committee of the University of Western Australia approved the study protocol, and all men provided written informed consent to participate in the study.

Baseline Assessment of Medical Comorbidities

Data from the health questionnaire and physical examination performed during W1 and W2, and information obtained from the Western Australian Data Linkage System (WADLS) were used to identify lifestyle habits, medication use and medical comorbidities at baseline.

The WADLS is a centralised linkage system which captures all private and public hospital admissions, death and cancer registry data in Western Australia [22]. The Western Australian Cancer Registry was established in 1981, and notification of cancer diagnoses including in situ and invasive neoplasms, non-melanoma skin cancers (excluding primary skin basal cell carcinoma and squamous cell carcinoma), is mandatory [23, 24]. Body mass index (BMI) was calculated using weight (kg) divided by height (m) squared. Diabetes mellitus was defined as men with a previous diagnosis of diabetes, men who reported the use of oral hypoglycaemics or insulin treatment or men who had fasting or random blood glucose levels of ≥7.0 mmol/l or ≥11.0 mmol/l, respectively.

Cancer diagnoses prior to W2 were identified using the *International Classification of Diseases*, 9th Revision (ICD-9) and 10th Revision (ICD-10) codes 140.x-209.x and C00-C97, respectively.

Follow-up Ascertainment of Incident Cancers

The WADLS was used to identify incident cancers between 2001 and 2004 and June 2013. Incident cancers were identified using the ICD-10 code C61 for prostate cancer, C33-34 for lung cancer and C18-C21 for colorectal cancer. Incident cancers included only primary malignant neoplasms and did not include in situ neoplasms, or neoplasms of uncertain or unknown behaviour. *International Classification of Diseases for Oncology* (ICD-O-3) morphological codes were used to identify lung cancer subtypes.

Laboratory Assays

Fasting morning blood samples were collected between 8.00 am and 10.30 am at W2, and plasma was stored at −80 °C until time of analysis. Plasma T, DHT and E2 were quantified using a single LC-MS/MS run without derivatisation using atmospheric pressure photoionization in positive mode for androgens and negative mode for estrogens, from 200-μl samples as previously reported [25, 26]. Precision profiles displayed coefficients of variation of <6% for T levels of >0.4 nmol/l, <13% for DHT levels of >0.7 nmol/l and <8% for E2 levels of >25 pmol/l. Measurements of luteinising hormone (LH) and SHBG were previously determined via chemiluminescent immunoassays on an Immulite 2000 analyzer (Diagnostic Products Corp.-Biomediq, Doncaster, Australia) with coefficients of variation of <7% for both hormones [27]. Calculations for cFT were performed using an empirical formula, which has been shown to provide close concordance to measured cFT levels compared to calculations based on equilibrium binding equations [28].

Statistical Analysis

Stata (version 13.1; StataCorp, Texas, USA) was used to perform statistical analysis. Differences between groups were assessed using *t* tests for continuous variables and Pearsons χ^2 test for categorical variables and are reported as means ± standard deviation (SD) and percentages, respectively. The associations of hormones and cancer incidence were assessed using proportional hazards competing-risks analysis as described by Fine and Gray [29]. We used this method of analysis as usual methods of survival analysis assuming that the event of interest (e.g. incident cancer) will eventually occur, regardless of whether a competing event (such as death) has occurred. The results of the longitudinal analyses are therefore expressed as a subhazard ratio (SHR) for every 1 SD increase in hormone level. Distributions of hormone variables were plotted as frequency histograms and visual inspection of hormonal data showed that T, DHT and E2 approximated the normal distribution whilst LH was skewed to the right. LH was therefore log transformed for the analyses. Men who had been diagnosed with the cancer of interest prior to baseline were excluded from the relevant prospective incidence analysis. Adjustments were made for age, BMI and smoking status (current, ex- or non-smoker), followed by vigorous physical activity, alcohol intake, diabetes mellitus, high density lipoprotein (HDL), triglycerides (TG) and previous diagnosis of cancer. Smoking is a strong risk factor for lung cancer; therefore, a priori adjustments for smoking exposure were included into the lung cancer models. These included smoking status, duration of smoking (years) and exposure (the greatest number of cigarettes smoked for ≥1 year). To assess for non-linear associations, restricted cubic splines with three knots were included in the fully adjusted model [30]. No significant non-linear associations were observed. A p value of <0.05 or a confidence interval (CI) which did not cross 1 was considered significant.

Results

Study Population

Of the 4249 men who provided an early morning blood sample, 18 were excluded due to missing data. Further exclusions included a history of orchidectomy (n = 56), androgen or antiandrogen therapy (n = 98) and a previous diagnosis of prostate cancer (n = 387). Men who had previously been diagnosed with colorectal cancer (n = 116) and lung cancer (n = 21) were further excluded from the colorectal cancer and lung cancer analysis, respectively. This left 3690 men whose data were analysed. Measurements of T, DHT and E2 were available for 3690, 3638 and 3673 men, respectively.

Baseline Characteristics

The median follow-up for prostate cancer was 9.1 years (range 0.06–11.6, interquartile range 7.4–10.1). For lung and colorectal cancer, median follow-up was 9.2 years (range 0.02–11.6, interquartile range 6.2–10.0) and 9.1 years (range 0.06–11.6, interquartile range 7.2–10.0), respectively. During this time, 348, 107, and 137 men developed prostate, lung and colorectal cancer, respectively. The baseline characteristics for this cohort are summarised in Table 1. Men who developed lung cancer were more likely to have a smoking history compared to men who did not, and men who developed colorectal cancer were more likely to be ex-smokers. Men with incident prostate cancer reported more vigorous physical activity at

Table 1 Baseline physical, biochemical and demographic characteristics of study participants at W2 (2001–2004) for the entire cohort, and in men who developed prostate, lung and colorectal cancer

	Entire sample	Prostate cancer		Lung cancer		Colorectal cancer	
		No	Yes	No	Yes	No	Yes
	n = 3690	n = 3342	n = 348	n = 3562	n = 107	n = 3436	n = 137
Variable	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Age (year)	77.0 ± 3.6	77.0 ± 3.6	76.8 ± 3.6	76.9 ± 3.6	77.5 ± 3.1	76.9 ± 3.6*	77.9 ± 3.8*
BMI (kg/m^2)	26.5 ± 3.6	26.5 ± 3.6	26.5 ± 3.5	26.5 ± 3.6	26.1 ± 3.6	26.5 ± 3.6	26.4 ± 3.6
Total T (nmol/l)	13.1 ± 4.9	13.1 ± 4.9	13.2 ± 4.4	13.1 ± 4.9	13.9 ± 5.4	13.1 ± 4.9	12.7 ± 4.4
cFT (pmol/l)	185 ± 55	185 ± 55	187 ± 50	185 ± 54	193 ± 61	185 ± 55	181 ± 50
DHT (nmol/l)	1.44 ± 0.7	1.43 ± 0.7	1.47 ± 0.7	1.43 ± 0.7	1.55 ± 0.8	1.44 ± 0.7	1.39 ± 0.7
E2 (pmol/l)	73.4 ± 29.1	73.3 ± 29.2	74.5 ± 28.0	73.4 ± 29.0	73.9 ± 30.7	73.3 ± 29.0	75.9 ± 32.5
LH (IU/l)	5.78 ± 5.27	5.84 ± 5.35*	5.19 ± 4.43*	5.76 ± 5.18	6.68 ± 5.13	5.75 ± 5.16	6.22 ± 5.48
SHBG (nmol/l)	42.4 ± 16.7	42.5 ± 16.9	42.0 ± 15.4	42.4 ± 16.8	44.4 ± 16.6	42.5 ± 16.9	42.9 ± 15.3
Smoking, *n* (%)							
Never	1226 (33.2)	1096 (32.8)	130 (37.4)	1222 (34.3)*	3 (2.8)*	1162 (33.8)*	34 (33.5)[a]
Ex	2259 (61.2)	2056 (61.5)	203 (58.3)	2155 (60.5)*	86 (80.4)*	2077 (60.5)*	100 (72.5)*
Current	204 (5.5)	189 (5.7)	15 (4.3)	184 (5.17)*	18 (16.8)*	196 (5.7)*	4 (2.9)*
Alcohol (drinks/week), *n* (%)							
Non-drinker	517 (14.7)	473 (14.9)	44 (13.4)	505 (14.9)	9 (8.7)	473 (14.8)	21 (16.0)
<15	2353 (67.0)	2130 (66.9)	221 (67.4)	2274 (67.1)	68 (66.0)	2188 (66.9)	87 (66.4)
15–28	486 (13.8)	437 (13.7)	49 (14.9)	462 (13.6)	20 (19.4)	451 (13.8)	16 (12.2)
>28	157 (4.5)	143 (4.5)	157 (4.5)	149 (4.4)	6 (5.8)	146 (4.5)	7 (5.3)
Vigorous physical activity, *n* (%)							
≥150 min/week	840 (22.8)	745 (22.3)*	95 (27.3)*	818 (23.0)	18 (17.0)	781 (22.8)	30 (21.7)
Diabetes mellitus	530 (14.4)	491 (14.7)	39 (11.2)	513 (14.4)	13 (12.2)	493 (14.4)	24 (17.4)

p values are for *t* tests or Pearson's χ^2 test as appropriate

*p value <0.05

baseline. Men who developed colorectal cancer were older compared to men who did not. Sex hormone concentrations were similar within each group; however, LH levels were lower in men with incident prostate cancer. Compared to non-smokers, mean T levels were lower in ex-smokers and non-significantly higher in current smokers (Table 2). Contrary to this, mean DHT levels were lower in both ex- and current smokers compared to non-smokers. In this cohort, older age was associated with lower T, DHT and E2 levels, and higher SHBG and LH levels.

No Associations of Sex Hormones and Incident Prostate Cancer

There were no associations of sex hormones with the incidence of prostate cancer (Table 3). The fully adjusted SHR for the incidence of prostate cancer with each SD increase in T was 1.00 (95% CI 0.90–1.12; p = 0.939). In a subcohort of men with T < 10 nmol/l, 70 men were diagnosed with prostate cancer during the follow-up period. T was not associated with incident prostate cancer in this subcohort of men after

Table 2 Differences in mean T and DHT concentrations at baseline according to smoking status

	Total T (nmol/l)			DHT (nmol/l)		
	Mean ± SE	Diff	p value	Mean ± SE	Diff	p value
Non-smoker	13.7 ± 0.14			1.53 ± 0.02		
Ex-smoker	12.7 ± 0.17	1.00	<0.001	1.38 ± 0.03	−0.15	<0.001
Current smoker	14.1 ± 0.36	0.45	0.219	1.41 ± 0.06	−0.12	0.031

Estimates represent the mean, standard error (SE) and differences (Diff) in hormone concentrations in ex-smokers and current smokers compared to non-smokers and their associated p values

Table 3 Competing-risks proportional hazards models for the association of hormone variables with the incidence of prostate cancer

	Model 1		Model 2	
	SHR (95%CI)	*p* value	SHR 95% (CI)	*p* value
T, nmol/l	1.01 (0.91, 1.12)	0.827	1.00 (0.90, 1.12)	0.939
cFT, pmol/l	1.04 (0.94, 1.15)	0.480	1.02 (0.92, 1.14)	0.701
DHT, nmol/l	1.05 (0.95, 1.15)	0.343	1.05 (0.95, 1.16)	0.308
E2, pmol/l	1.04 (0.94, 1.15)	0.460	1.04 (0.94, 1.16)	0.438
Log LH, IU/l	0.90 (0.81, 1.00)	0.051	0.91 (0.92, 1.02)	0.101
SHBG, nmol/l	0.96 (0.87, 1.07)	0.501	0.98 (0.88, 1.09)	0.698

Estimates represent subhazard ratios and 95% CIs for each SD increase in hormone level. Model 1 includes adjustments for age, BMI and smoking status. Model 2 includes adjustments for age, BMI, smoking status, physical activity, alcohol, diabetes, HDL, TG and history of prevalent cancer

adjusting for confounding factors (SHR = 0.74 for every 1 SD increase in T, 95% CI 0.44–1.23; $p = 0.241$) (data not shown). There were no associations of E2, LH or SHBG with the incidence of prostate cancer. Interactions of hormone variables with age were not significant.

Higher T or DHT Are Associated with Incident Lung Cancer

In the multivariate analysis, higher T, cFT and DHT were associated with an increased incidence of lung cancer. For every 1 SD (4.87 nmol/l) increment in total T, the adjusted risk of lung cancer increased by 30% (SHR = 1.30, 95% CI 1.06–1.60; $p = 0.012$). For every 1 SD (0.73 nmol/l) increment in DHT, the adjusted risk of lung cancer increased by 29% (SHR = 1.29, 95% CI 1.08–1.54; $p = 0.004$) (Table 4). E2, LH and SHBG were not associated with lung cancer. Adenocarcinomas (26.2%) and squamous cell carcinomas (19.6%) made up the largest proportion of lung cancer subtypes within this cohort; however, outcomes were insufficient to perform subtype-specific analyses.

Table 4 Competing-risks proportional hazards models for the association of hormone variables with the incidence of lung cancer

	Model 1		Model 2	
	SHR (95% CI)	*p* value	SHR (95% CI)	*p* value
T, nmol/l	1.24 (1.02, 1.50)	0.029	1.30 (1.06, 1.60)	0.012
cFT, pmol/l	1.24 (1.01, 1.52)	0.041	1.30 (1.04, 1.62)	0.022
DHT, nmol/l	1.24 (1.04, 1.49)	0.017	1.29 (1.08, 1.54)	0.004
E2, pmol/l	1.05 (0.86, 1.29)	0.619	1.07 (0.87, 1.30)	0.531
Log LH, IU/l	1.04 (0.83, 1.29)	0.753	1.04 (0.83, 1.29)	0.757
SHBG, nmol/l	1.08 (0.92, 1.27)	0.351	1.10 (0.93, 1.31)	0.276

Estimates represent subhazard ratios and 95% CIs for each SD increase in hormone level. Model 1 includes adjustments for age, BMI and smoking status. Model 2 includes adjustments for age, BMI, smoking status, physical activity, duration of smoking, smoking exposure (greatest number of cigarettes smoked for ≥1 year), alcohol, diabetes, HDL, TG and history of prevalent cancer

When men who developed lung cancer within the first 2 years of follow-up were excluded, T and DHT remained significantly associated with the incidence of lung cancer (SHR = 1.32, 95% CI 1.03–1.68; $p = 0.028$ for every 1 SD increase T and SHR = 1.30, 95% CI 1.06–1.59; $p = 0.012$ for DHT) (data not shown). However, stratification according to smoking status delineated clear differences between associations of T compared with DHT. The association of T and lung cancer incidence differed according to smoking status (p value for interaction = 0.015). In current smokers, higher T was associated with increased lung cancer incidence (SHR = 2.52, 95% CI 1.54–4.13; $p < 0.001$), but these associations were not significant in ex- or non-smokers (SHR = 1.16, 95% CI 0.91–1.48; $p = 0.236$ for ex-smokers and SHR = 1.10, 95% CI 0.60–2.00; $p = 0.758$ for non-smokers). When current smokers ($n = 202$) were excluded, the associations for T were not significant (SHR = 1.15, 95% CI 0.90–1.47; $p = 0.262$). By contrast, there was no significant interaction between DHT and smoking status, indicating a constant effect of DHT regardless of baseline smoking status (p value for interaction = 0.405). When current smokers were excluded, higher DHT was significantly associated with increased incidence of lung cancer (SHR = 1.26, 95% CI 1.03–1.54; $p = 0.026$) (data not shown).

Interactions were also tested to see if relationships differed according to age. There was a significant interaction between T and age ($p = 0.021$). The association of higher T and increased incidence of lung cancer was only present those older than 76 years. There were no significant interactions between other hormone variables and age (data not shown).

No Associations of Sex Hormones and Incident Colorectal Cancer

No associations of sex hormones, LH or SHBG with the incidence of colorectal cancer were observed in this cohort (supplementary Table 1). Interactions of hormone variables and age were not significant.

Discussion

In this cohort of community-dwelling older men, sex hormones were not associated with the incidence of prostate or colorectal cancer. On the contrary, higher T and DHT were independently associated with an increased incidence of lung cancer. T and DHT levels differed according to smoking status. In particular, DHT levels were lower in both ex- and current smokers compared to non-smokers. Therefore, results for DHT are unlikely to be due to residual confounding. In the

fully adjusted analyses, there were no associations of LH and SHBG with the incidence of prostate, lung or colorectal cancer.

In contrast to the earlier analysis from HIMS [31], we found no association of T with prostate cancer incidence. That initial observation may have been a chance finding as the current analysis utilised more accurate measurements of T with LC-MS/MS [32, 33], a more accurate cFT calculation, a longer duration of follow-up (3 additional years) and a larger number of incident prostate cancers (348 vs 297 cases). In addition, we were able to assess associations for DHT and E2, neither of which was associated with the incidence of prostate cancer. These results are similar to findings based on immunoassay measured sex hormone levels. For instance, a meta-analysis of 18 prospective studies found no differences of T in 3886 men who subsequently developed prostate cancer compared to 6438 controls who did not [8]. Similarly, no differences in immunoassay measured E2 were observed in 2186 men with prostate cancer compared to 3039 controls, and immunoassay measured DHT levels were similar in 1010 men with prostate cancer and 1445 controls [8]. However, both serum E2 and DHT measurements in men involve low circulating concentrations where direct immunoassay measurements are most inaccurate [10, 34].

Few cancer incidence studies have utilised MS for measurement of sex hormone levels. A case-cohort study of 275 men who developed prostate cancer and 1652 non-cases over an average follow-up of 4.7 years reported no associations of T and E2, measured via gas chromatography-mass spectrometry, with incident prostate cancer [35]. In the Copenhagen City Heart Study cohort, no associations of LC-MS/MS measured T and incident prostate cancer were observed in 4440 men followed for 22 years, 198 of whom developed prostate cancer [36]. Conversely, a cohort study consisting of 3255 men with 819 biopsy-detected prostate cancers reported a direct association of LC-MS/MS measured T with incident prostate cancer in men with $T < 10$ nmol/l, but not in the entire cohort or in men with $T \geq 10$ nmol/l, with no association seen for DHT [37]. We did not see any similar association of T with prostate cancer for men with $T < 10$ nmol/l in our analysis. A meta-analysis performed by Boyle et al. including 18 trials of immunoassay measured T and 2 trials of MS measured T also showed no associations of T with incident prostate cancer [7]. Overall, our results suggest neutral associations of T, DHT and E2 with incident prostate cancer in older men who are at the greatest risk.

Previous studies of immunoassay measured T have reported higher T levels in men who smoke [38]. In our previous analysis, higher T was associated with an increased incidence of lung cancer; however, residual confounding from smoking could not be excluded [31]. Few studies have reported associations of DHT or T measured via MS with smoking status. A higher prevalence of smoking was observed in men with the highest quartile of T in the Osteoporosis in Men (MrOS) Sweden cohort [39], whilst T and DHT levels were not significantly different according to smoking status in younger men [40]. In the current analysis, T was directly associated with lung cancer, and this association was driven by current smokers and was more apparent in those older than 76 years. Of note, our results indicate that for every 1 SD increase in DHT, the incidence of lung cancer is increased by ~30%. Mean DHT levels were lower in ex- and current smokers. Since smoking is a strong risk factor for lung cancer, lower DHT levels in men who are at highest risk due to smoking would influence associations towards the null. Thus, association of higher DHT with increased incidence of lung cancer is unlikely to be due to confounding from smoking status. Consistent with this observation, there was no interaction between DHT and smoking status with the outcome of incident lung cancer, and after excluding current smokers, higher DHT remained associated with increased incidence of lung cancer.

Our results are in contrast to findings from the Copenhagen City Heart Study cohort, whereby no associations of LC-MS/MS measured T and incident lung cancer were observed; however, that study included younger men and did not assess associations with DHT [36]. Our findings in vivo are supported by mechanistic studies demonstrating expression of genes involved in oxygen utilisation and cellular apoptosis when an adenocarcinoma non-small cell lung cancer (A549) cell line was exposed to androgens [11]. Other studies examining the A549 cell line have also shown cross talk between the androgen receptor and the epidermal growth factor receptor, with P38MAPK dependent activation of the mammalian target of rapamycin (mTOR) pathway, and stimulation of cellular proliferation by DHT [41]. It is therefore plausible that DHT may contribute to lung cancer growth.

Some epidemiological studies have suggested a role of sex hormones in the development of colorectal cancer. In a prospective study of 107, 859 men treated for prostate cancer, the incidence of subsequent colorectal cancer was higher in men who had undergone surgical or medical castration compared to men who did not receive androgen deprivation after excluding men who had received pelvic irradiation treatment for prostate cancer [42]. A prospective nested case-control study of 732 men and women with colorectal cancer and 1156 controls found an inverse association of higher T and SHBG with incident colorectal cancer in men but not women [17]. In women, hormone replacement therapy with conjugated equine estrogens and medroxyprogesterone acetate has been associated with a decreased risk of colorectal cancer [43]. Contrary to this, T was not associated with the incidence of colon cancer in either men or women in the Copenhagen City Heart Study cohort; however, DHT and E2 were not assessed [36]. Therefore, when accurately measured, sex hormones including T, DHT or E2 are not associated with the incidence of colorectal cancer.

We acknowledge several limitations associated with this study. This is an observational study; therefore, causality cannot be inferred. Our cohort consists almost entirely of older Caucasian males, limiting generalizability of our results to women or other ethnic backgrounds. Hormone data in this study were based on single measurements at baseline and serial measurements were not available. However, blood tests were taken in the early morning fasting period to minimise the effect of circadian variation on hormone levels, and sampling of blood T at a single time-point provides a reasonable estimate of hormone levels [44]. Information for other prognostic factors for cancer such as family history of cancer and occupational exposure were not available for this cohort; however, we comprehensively adjusted for available confounders to minimise residual confounding. Whilst the WADLS captures all cancer diagnoses in the state of Western Australia [22], routine measurement of prostate specific antigen (PSA) and prostate biopsy was not performed. Since prostate cancer has a long latency period, some may have been missed with identification of outcomes using the WADLS. Lastly, loss of follow-up could occur if men migrated out of Western Australia; however, few men in this age group migrate interstate or overseas and is estimated to occur in <1% of men [21].

Strengths of our study include a large, well characterised cohort with a narrow age range and a long period of follow-up. Compared to our previous analysis of the HIMS cohort using immunoassay measured T, we currently have a larger number of outcome events and more precise measures of T and its bioactive metabolites, DHT and E2, allowing more accurate characterisation of relationships within this cohort. We performed our analysis using a competing-risks approach and adjusted for potential confounding factors. Apart from limitations associated with prostate cancer diagnosis, ascertainment of outcome events using the WADLS allowed near complete capture of incident colorectal and lung cancer.

Conclusion

In conclusion, when measured accurately, T, DHT and E2 are not associated with the incidence of prostate and colorectal cancer in community-dwelling older men. Conversely, higher androgens were associated with an increased incidence of lung cancer. Higher plasma androgens, particularly DHT, represent a potential biomarker for incidence of lung cancer in older men. This result requires validation in other cohorts and additional studies are required to establish if DHT is a biomarker or even a contributory factor for lung cancer, as there are potential implications when considering the benefits vs risks of testosterone treatment of older men. Further studies would be needed to explore whether androgen deprivation might have any effects on lung cancers refractory to conventional therapy.

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Conflict of Interest The authors declare that they have no conflict of interest.

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