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ORIGINAL PAPER

Lower Circulating Androgens Are Associated with Overall Cancer Risk and Prostate Cancer Risk in Men Aged 25–84 Years from the Busselton Health Study

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Abstract

Androgens, notably testosterone (T), have been implicated in development of several common cancers and prostate cancer; however, precise mechanisms remain unclear. This study assessed prospective associations of serum T, dihydrotestosterone (DHT) and estradiol (E2) with overall cancer (excluding skin cancer), prostate, colorectal and lung cancer risk in 1574 community-dwelling men aged 25–84 years. Sex hormones were assayed using mass spectrometry and men were followed for 20 years with outcomes ascertained using data linkage. Over 20 years, there were 289, 116, 48 and 22 men who developed any cancer, prostate cancer, colorectal cancer and lung cancer, respectively. Androgens in the lowest quartile were associated with an increased overall cancer risk (HR = 1.36, 95% CI 1.05–1.76, $p = 0.020$ for T; and HR = 1.30, 95% CI 1.00–1.69, $p = 0.049$ for DHT comparing the lowest vs other quartiles). T in the lowest quartile was associated with an increased risk of prostate cancer (HR = 1.53, 95% CI 1.02–2.29, $p = 0.038$ comparing the lowest vs other quartiles). The association between androgens and overall cancer risk remained similar after excluding prostate cancer outcomes; however, results were not significant. There were no associations of T, DHT or E2 with colorectal or lung cancer risk; however, LH in the highest quartile was associated with an increased risk of lung cancer (HR = 4.55, 95% CI 1.70–12.19, $p = 0.003$ for the highest vs other quartiles). Whether T is a biomarker of poor health in men with any cancer or prostate cancer requires further confirmation as does the nature and mechanism of the association of a high LH with future lung cancer.

Keywords Testosterone · Dihydrotestosterone · Estradiol · Cancer incidence

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Introduction

Cancer carries a high burden of disease worldwide with prostate, colorectal and lung cancers being the most common cancers in men [1]. Androgens are required for prostate development and growth, and androgen deprivation therapy is standard treatment for advanced prostate cancer [2]. However, the role of androgens in the development of prostate cancer is unclear and a meta-analysis found no prospective associations of circulating testosterone (T) with subsequent risk of incident prostate cancer [3]. Recently, sex hormones have also been implicated in the development of lung cancer, with higher circulating androgens reported to be associated with incidence of lung cancer in older men [4, 5]. A case-control study demonstrated a higher number of androgen receptor (AR) cytosine-adenine-guanine (CAG) repeats (signifying decreased AR transcriptional activity) in men with colorectal cancer compared to healthy controls as well as with a poorer 5-year overall survival

[6]. However, prospective cohort studies have not associated androgens with risk of colorectal cancer [4, 5, 7].

Previous epidemiological studies of sex hormones and cancer risk in men have predominantly utilised immunoassays for sex hormone measurement. This mono-analyte method of sex hormone measurement is relatively non-specific through cross-reactivity with other steroids as well as assay-dependent bias compared to the more specific, multi-analyte reference method of liquid chromatography-tandem mass spectrometry (LC-MS/MS) [8, 9]. Moreover, the biological effects of T are modulated by its conversion to the more potent androgen dihydrotestosterone (DHT) through 5α-reductase activity, and to estradiol (E2) by aromatase [10, 11]. Few epidemiological studies have utilised LC-MS/MS for sex hormone measurement which measures serum T, DHT and E2 in a single run unlike steroid immunoassays. In a study of 3255 middle-aged men followed for 4 years, LC-MS/MS measured T was associated with prostate cancer risk in men with T < 10 nmol/L but not in men with higher T levels [12]. No associations of DHT and prostate cancer risk were observed in that study, and outcomes for other common cancers or associations with E2 were not available [12]. On the contrary, no associations of LC-MS/MS measured serum T, DHT or E2 were found with prostate, colorectal or lung cancer in older men [5].

Testosterone treatment is commonly used in clinical practice even for men without pathologic hypogonadism, which remains the sole approved indication. Hence, epidemiological studies using LC-MS/MS measured sex hormones are therefore required to clarify the associations of T and DHT with cancers. The aim of the current study was therefore to assess if LC-MS/MS measured serum T, DHT and E2 are associated prospectively with the incidence of any cancer, prostate cancer, colorectal cancer and lung cancer in a cohort of men of a wide age range followed for 20 years.

Methods

Study Population and Participants

The population of Busselton, a coastal area in the southwest of Western Australia, has been regularly surveyed since the Busselton Health Study was established in 1966 [13]. Greater than 90% of this population consists of individuals with Anglo-Celtic ancestry. In 1994/1995, a follow-up health survey of survivors from previous surveys was conducted as previously described [14]. A total of 2143 men participated in the survey and provided a blood sample.

Baseline Measurements and Cancer Outcomes

Participants in the 1994/1995 survey completed a comprehensive health and lifestyle questionnaire, underwent various measurements and tests and provided a blood sample [14]. Information on marital status, occupation, smoking, alcohol intake, minutes of moderate and vigorous leisure time physical activity per usual week, diabetes and use of medications was obtained by questionnaire. Leisure time physical activity was calculated as (minutes/week of moderate activities) + 2 × (minutes/week of vigorous activity) and categorised as (0–149, 150+ min/week) where 150 min/week is the recommended level of physical activity sufficient for health benefits [15]. Alcohol consumption was categorised as light, moderate and heavy if intake was < 140 g/week, 140–420 g/week and > 420 g/week, respectively. Anthropometric measures were obtained using standardised protocols by trained assessors, which included measurements of weight and height. Body mass index was defined as weight (kg) divided by height (m) squared.

Blood samples were obtained from the participants after an overnight fast at the time of survey. Serum was separated and stored at − 70 °C. Serum T, DHT and E2 were quantified within a single LC-MS/MS run without derivatisation using atmospheric pressure photo-ionisation for positive mode for androgens and negative mode for oestrogens, from 200 μL samples as previously described [16]. Between-run imprecision for T was 8.6% at a concentration of 5.3 nmol/L and 7.9% at 26.9 nmol/L. For DHT, it was 11.3% at a concentration of 1.3 nmol/L and 9.1% at 5.3 nmol/L, and for E2, it was 14.5% at a concentration of 73 pmol/L and 9.9% at 279 pmol/L. Luteinising hormone (LH) was assayed using a two-step noncompetitive chemiluminometric immunoassay (Abbott Architect, Abbott Diagnostics, North Ryde, NSW, Australia) with between-run imprecision of 5.6% at 4.8 IU/L. Sex hormone-binding globulin (SHBG) was assayed using a solid-phase, two-site enzyme immunometric assay with chemiluminescent substrate (Immulite 2000xPi; Siemens Healthcare, Bayswater, Vic., Australia) with between-run imprecision of 3.4% at 39.4 nmol/L.

The Human Research Ethics Committee of the Department of Health of Western Australia (project number 2011/60) gave permission to access the cancer and death records of the survey participants for the period between the 1st of January 1980 to the 30th of June 2014 using record linkage to cancer registrations and deaths [17]. International Classification of Diseases, 9th revision (ICD-9) codes were used up to 30th June 1999, and International Classification of Diseases, 10th revision (ICD-10) codes for subsequent events. History of cancer at baseline in 1994/1995 cohort was based on any cancer registration during the 15 years before the survey (i.e. 1980 to 1994/1995). Cancer outcome events during the 20-year follow-up from survey attendance in 1994/1995 to 30th June 2014 were from cancer and death records during this period. Four outcome events were analysed: time to first

fatal or non-fatal cancer (excluding skin cancer), prostate cancer, colorectal cancer and lung cancer. ICD-10 codes used to identify any cancer were ICD-10 C00-C42 and C45-C97. Prostate, colorectal and lung cancers were identified using ICD-10 C61, ICD-10 C18-C21 and ICD-10 C33-C34 respectively.

Statistical Analyses

Statistical analyses were performed using SAS® 9.4. The associations between hormone levels (T, DHT, E2, LH, SHBG) and cancer outcomes were examined using Cox proportional hazards regression modelling. Risk factors were examined as a continuous variable and in quartiles to assess for non-linear associations. Based on these results, quartile 1 (Q1) was also compared against quartiles 2–4 (Q2–Q4) of each hormone variable with overall and prostate cancer outcomes. The estimated hazard ratios with 95% confidence interval (CI) and p value are reported for each risk factor in relation to each of the four cancer outcomes after adjustment for potential confounders (age, marital status, occupation, smoking, alcohol consumption, leisure time physical activity (LTPA) 150+ min/week, BMI and diabetes). A p value of < 0.05 or a CI that did not cross 1.0 was considered statistically significant.

Results

There was a total of 2143 men who participated in the 1994/1995 Busselton Health Survey. After excluding men who were aged outside the range of 25–84 years at baseline (177), those with a history of cancer at baseline (79), those taking androgens or anti-androgens or who had undergone orchidectomy (20) and those with missing data on key variables (255), there remained a total of 1612 participants. To avoid the potential of reverse causation, participants who died or were diagnosed with cancer within the first 2 years of follow-up were also excluded (38) leaving a total of 1574 men for analyses.

Baseline Characteristics

Baseline characteristics of the cohort are presented in Table 1. The average age was 51 years, 16% were smokers, 39% were moderate/heavy alcohol drinkers, 48% met the recommended physical activity requirement of 150 min/week, mean BMI was 26.7 kg/m^2 and 5% had diabetes.

During the 20-year follow-up period, a total of 289 men (18.4%) developed any cancer, 116 (7.4%) developed prostate cancer, 48 (3.0%) developed colorectal cancer and 22 (1.4%) developed lung cancer (Table 1).

Table 1 Characteristics of the cohort and number of cancer events. Data are shown as mean (SD), percent or number (%) of cancer outcomes

Characteristic	($n = 1574$)
Age (years)	51.1 (14.7)
Marital status	
Married/living with partner	85.3
Other	14.7
Occupation	
Managers/administrators	25.2
Professionals	15.1
Tradespersons	13.0
Clerks/sales persons	7.8
Plant operators/labourers	10.7
Indept. means/pensioners	24.6
Unknown	3.7
Smoking status	
Never	41.9
Former	42.0
Current	16.1
Alcohol consumption	
None	4.0
Ex	6.2
Light	48.5
Moderate/heavy	39.1
Unknown	2.3
Leisure time physical activity	
< 150 min/week	51.8
≥ 150 min/week	48.2
BMI (kg/m^2)	26.7 (3.4)
Diabetes	5.4
Testosterone (nmol/L)	13.5 (4.8)
Dihydrotestosterone (nmol/L)	1.72 (0.72)
Estradiol (pmol/L)*	59.1 (29.6)
Luteinising hormone (IU/L)	3.89 (2.90)
Sex hormone binding globulin (nmol/L)	29.0 (12.4)
Cancer outcomes	
Any cancer	289 (18.4)
Prostate cancer	116 (7.4)
Colorectal cancer	48 (3.0)
Lung cancer	22 (1.4)

*$n = 1491$

There was no correlation between T and LH in this population of men ($r = 0.024$, $p = 0.342$). Compared to the men who were excluded due to a diagnosis of cancer within the first 2 years of follow-up, men included in the study were younger (mean age 51.1 vs 69.3 years), more likely to be pensioners, be former smokers and have a history of diabetes, and had higher androgen concentrations as well as lower E2 and LH concentrations (Supplementary Table 1).

Associations of Hormone Variables with Any (Non-skin) Cancer

The risk of any cancer showed associations with T and DHT but not with E2, LH or SHBG. After adjusting for potential confounders, including age, marital status, occupation, smoking, alcohol consumption, LTPA, BMI and diabetes, the risk of any cancer did not exhibit a decreasing trend with increasing T (trend $p = 0.223$) but was highest for people in the lowest quartile (Q1) of T and was significantly higher for people in Q1 than for people in Q2 ($p = 0.044$) and Q3 ($p = 0.037$) (Tables 2 and 3). When Q2–Q4 were combined into a single group, the adjusted risk of any cancer was 36% higher for those in Q1 versus Q2–Q4 for T (HR = 1.36, 95% CI 1.05–1.76, $p = 0.020$) (Table 3). Similarly, the adjusted risk of any (non-skin) cancer did not exhibit a decreasing trend with increasing DHT (trend $p = 0.105$) but was highest for people in the lowest quartile of DHT and was significantly higher for people in Q1 than for people in Q4 ($p = 0.049$) (Tables 2 and 3). When Q2–Q4 were combined into a single group, the adjusted risk of any (non-skin) cancer was 30% higher for those in Q1 versus Q2–Q4 for DHT (HR = 1.30, 95% CI 1.00–1.69, $p = 0.049$) (Table 3). These results were similar when LH was added to the fully adjusted model (HR = 1.37, 95% CI 1.06–1.77, $p = 0.017$ comparing the lowest versus other quartiles of T, and HR = 1.31, 95% CI 1.01–1.71, $p = 0.042$ comparing the lowest versus other quartiles of DHT). After excluding prostate cancer from overall cancer outcomes, the direction of association did not change; however, results were no longer significant (HR = 1.26, 95% CI 0.90–1.76, $p = 0.185$ comparing the lowest versus other quartiles of T, and HR = 1.39, 95% CI 0.99–1.94, $p = 0.053$ comparing the lowest versus other quartiles of DHT).

Associations of Hormone Variables with Prostate Cancer

The adjusted risk of prostate cancer exhibited a decreasing trend with increasing T (trend $p = 0.047$) (Table 2). When Q2–Q4 were combined into a single group, the adjusted risk of prostate cancer was 53% higher for those in Q1 versus Q2–Q4 for T (HR = 1.53, 95% CI 1.02–2.29, $p = 0.038$) (Table 3). There was no clear relationship with DHT, E2, LH or SHBG. The associations of T and prostate cancer were independent of LH concentrations (HR = 1.54, 95% CI 1.03–2.31, $p = 0.034$ comparing the lowest versus other quartiles of T after including LH into the fully adjusted model).

Associations of Hormone Variables with Other Cancers

There was no apparent relationship between any hormone variable and risk of colorectal cancer (Table 2). Whilst the estimated adjusted risk of lung cancer decreased with increasing T and across the quartile groups, this did not reach statistical significance (trend $p = 0.105$) possibly due to lack of statistical power through there being only 22 cases of lung cancer. There was no evidence of associations with DHT, E2 and SHBG. There was a trend for higher LH to be associated with increased risk of lung cancer ($p = 0.126$) (Table 2). For LH, there was a considerably higher estimated risk of lung cancer in people in the highest quartile (Q4) for LH (Supplementary Table 2). When Q1–Q3 were combined into a single group, the adjusted risk of lung cancer was 4.55 times higher for those in Q4 versus Q1–Q3 (HR = 4.55, 95% CI 1.70–12.19, $p = 0.003$).

Discussion

In this cohort of men aged 25–84 years, serum T and DHT, measured by LC-MS/MS, in the lowest quartile were associated with a 30% increased risk of overall cancer and a 50% increased risk of prostate cancer. LH in the highest quartile was associated with higher lung cancer risk. There were no associations of sex hormone variables with colorectal cancer in this cohort of men.

In our study, T and DHT in the lowest quartile were associated with an increased overall cancer risk. The direction of

Table 2 Adjusted hazard ratios (95% confidence interval) and p values for hormone levels in relation to cancer outcomes

Hormone	Any cancer	Prostate cancer	Colorectal cancer	Lung cancer
T (nmol/L)	0.92 (0.81, 1.05) $p = 0.223$	0.81 (0.66, 1.00) $p = 0.047$	1.04 (0.76, 1.42) $p = 0.801$	0.65 (0.39, 1.09) $p = 0.105$
DHT (nmol/L)	0.90 (0.79, 1.02) $p = 0.105$	0.84 (0.69, 1.03) $p = 0.093$	0.87 (0.63, 1.20) $p = 0.396$	0.74 (0.46, 1.21) $p = 0.230$
E2 (pmol/L)	0.97 (0.87, 1.10) $p = 0.654$	0.85 (0.70, 1.04) $p = 0.109$	1.12 (0.85, 1.46) $p = 0.428$	1.05 (0.70, 1.57) $p = 0.817$
LH (nmol/L)	0.95 (0.85, 1.07) $p = 0.416$	0.89 (0.72, 1.10) $p = 0.284$	0.71 (0.46, 1.11) $p = 0.135$	1.15 (0.96, 1.39) $p = 0.126$
SHBG (IU/L)	0.95 (0.83, 1.09) $p = 0.485$	0.93 (0.75, 1.15) $p = 0.503$	1.11 (0.81, 1.53) $p = 0.511$	0.76 (0.45, 1.29) $p = 0.313$

p value is from trend test and hazard ratio is for a one SD change in hormone level; SD = 4.8 nmol/L for T, 0.72 nmol/L for DHT, 29.6 pmol/L for E2, 2.90 IU/L for LH and 12.4 nmol/L for SHBG

Adjustments were made for age, marital status, occupation, smoking, alcohol consumption, leisure time physical activity, BMI and diabetes

Table 3 Adjusted hazard ratios (95% confidence interval) and *p* values for quartiles and Q1 versus Q2–Q4 of T, DHT and E2 in relation to overall and prostate cancer outcomes

Hormone		Any cancer	Prostate cancer
T	Q1 (T < 10.17 nmol/L)	1.00 (reference level)	1.00 (reference level)
	Q2 (10.17 ≤ T < 12.95)	0.72 (0.53, 0.99)	0.62 (0.37, 1.03)
	Q3 (12.95 ≤ T < 16.49)	0.71 (0.51, 0.98)	0.75 (0.46, 1.23)
	Q4 (T ≥ 16.49)	0.81 (0.57, 1.14)	0.58 (0.33, 1.01)
T	Q1 (T < 10.1)	1.36 (1.05, 1.76)*	1.53 (1.02, 2.29)*
	Q2–Q4 (T ≥ 10.17)	1.00 (reference level)	1.00 (reference level)
DHT	Q1 (DHT < 1.235 nmol/L)	1.00 (reference level)	1.00 (reference level)
	Q2 (1.235 ≤ DHT < 1.672)	0.85 (0.62, 1.16)	1.11 (0.68, 1.81)
	Q3 (1.672 ≤ DHT < 2.106)	0.74 (0.53, 1.03)	0.68 (0.40, 1.18)
	Q4 (DHT ≥ 2.106)	0.71 (0.50, 1.00)	0.76 (0.44, 1.31)
DHT	Q1 (DHT < 1.235)	1.30 (1.00, 1.69)*	1.17 (0.76, 1.80)
	Q2–Q4 (DHT ≥ 1.235)	1.00 (reference level)	1.00 (reference level)
E2	Q1 (E2 < 37.9 pmol/L)	1.00 (reference level)	1.00 (reference level)
	Q2 (37.9 ≤ E2 < 55.1)	0.97 (0.69, 1.36)	0.82 (0.49, 1.37)
	Q3 (55.1 ≤ E2 < 75.7)	0.93 (0.66, 1.31)	1.06 (0.65, 1.75)
	Q4 (E2 ≥ 75.7)	0.91 (0.65, 1.27)	0.60 (0.34, 1.04)
E2	Q1 (E2 < 37.9)	1.07 (0.81, 1.41)	1.23 (0.82, 1.86)
	Q2–Q4 (E2 ≥ 37.9)	1.00 (reference level)	1.00 (reference level)

*$p < 0.05$

Adjustments were made for age, marital status, occupation, smoking, alcohol consumption, leisure time physical activity, BMI and diabetes

association did not change but was not significant after excluding prostate cancers from overall cancer outcomes, likely due to reduced power. These results differ from a study of 4453 men and 4318 women aged 20–94 years who were followed for 30 years whereby 1140 cancers occurred [7]. In that study, there were no associations of T measured via immunoassay with the incidence of any cancer in either men or women [7]. Data for DHT, E2, LH and SHBG were not available in that study [7]. Several studies examining associations of sex hormones and cause-specific mortality have been performed. An association of lower T with cancer-specific mortality has been observed in the European Prospective Investigation Into Cancer in Norfolk (EPIC-Norfolk) study [18] and in the Study of Health in Pomerania (SHIP) study [19]. However, no associations of T and cancer-specific mortality were observed in the Massachusetts Male Aging Study (MMAS) [20] and the Rancho-Bernardo Study [21]. Of note, in the Concord Health and Aging in Men Project (CHAMP) study which included LC-MS/MS sex hormones measured longitudinally over a 5-year period, T, DHT and E2 in the lowest quartile were associated with higher cancer mortality [22]. Progressive declines in serum T, DHT and E2 over the baseline, 2-year and 5-year follow-up were also associated with higher cancer mortality in that study [22]. A higher proportion of men with hypogonadism have been reported in patients with cancer spanning a wide age range [23]. Lower total T in cancer patients has been associated with increased symptom burden, and lower T levels have also been reported in patients with cancer with cachexia versus matched cancer patients without cachexia [23, 24]. In a large cross-sectional study of 1563 men aged 25 years and older, those who reported a history of cancer had lower T compared to those who did not have a previous diagnosis of cancer [25]. It is therefore plausible that low T in this setting may be a consequence of (i.e. biomarker for) poor health rather than a risk factor for or cause of cancer. Measures were undertaken to minimise reverse causality in this study, including the exclusion of men who experienced an outcome within the first 2 years of follow-up as well as men who had a previous history of cancer. However, our results may be reflective of residual confounding that could not be accounted for.

We found an inverse association of T and prostate cancer in this cohort of men, with ~ 50% increase in risk of prostate cancer in men with T < 10.17 nmol/L. This is in contrary to findings from other studies of LC-MS/MS measured T and prostate cancer risk. In a case-cohort study using the Osteoporotic Fractures in Men (MrOS) population which consisted of 275 cases (14.3%) of prostate cancer and 1652 non-cases in men aged ≥ 65 years, T and E2 measured via gas chromatography were not associated with prostate cancer [26]. In that study, self-reported prostate cancer cases were ascertained over approximately 5 years and confirmed via medical record screening [26]. Similarly, LC-MS/MS-measured T, DHT and E2 were not associated with prostate cancer risk in the Health In Men Study (HIMS) which consisted of older men followed for 9 years whereby 348 cases (9.4%) of

prostate cancers occurred and were identified through the same data linkage system as our current study [5]. Compared to MrOS and HIMS, our population consisted of a larger proportion of younger and middle-aged men. In another study of men aged 50–75 years who were at high risk of prostate cancer and underwent routine prostate biopsies at 2 and 4 years of follow-up, a direct association of LC-MS/MS-measured T and prostate cancer risk was reported in those with T < 10 nmol/L but not in men with higher T levels, and DHT was not associated with prostate cancer in these men [12]. In that study, a higher proportion of men were diagnosed with prostate cancer compared to our population (25.2% vs 7.4%) which mostly consisted of Gleason score 6 tumours [12]. It is plausible that detection of higher grade tumours is more frequent in an unscreened population such as ours; however, information regarding tumour stage and grade was not available in our population for direct comparison. Of note, a meta-analysis of 18 prospective studies reported no associations of T, DHT or E2 with prostate cancer risk; however, the majority of studies included in this meta-analysis utilised immunoassay for measurement of sex hormone concentrations [3]. Several observational studies have suggested an association of low T and prostate cancer. Morgentaler et al. found an increased prevalence of prostate cancer diagnosis in men with low T [27], and Hoffman et al. found a greater percentage of positive prostate biopsies in men with low compared to normal free T but not total T levels [28]. Prostate cancer patients with low total T have also been observed to have higher Gleason scores compared to those with normal T levels [29]. In men with prostate cancer, low total T was also shown to be a predictor of higher Gleason scores [30], positive lymph node involvement [30] and extraprostatic disease [31]. Improvement of T and LH levels has also been demonstrated in prostate cancer patients after treatment of prostate cancer with prostatectomy [32, 33]. This is in keeping with our findings of an increased incidence of prostate cancer in men with low T. Our results therefore most likely reflect suppression of the gonadal axis due to prostate disease; however, further studies examining cohorts of a wide age range, and studies with data regarding grade or stage of prostate cancer are required to examine the effect of age and extent of disease on the association of androgens and prostate cancer risk.

Androgens have been implicated in colorectal cancer growth and development; however, any specific role in the origins of colorectal cancer is unclear. In a case-control study, patients with colorectal cancer were found to have longer CAG repeats in the androgen receptor gene (associated with decreased transcriptional activity of the androgen receptor) compared to healthy controls [6]. In our study, sex hormones were not associated with colorectal cancer development. These findings are consistent with results from the HIMS and Copenhagen City Heart Study cohorts whereby no associations of T, DHT or E2 were found with colorectal cancer risk [5, 7]. Overall, our results do not support a role for sex hormones in the development or growth of colorectal cancer in men.

In this study, there were no significant associations of T, DHT or E2 with the incidence of lung cancer in men. This is in contrary to findings from the HIMS cohort which included 107 cases of lung cancer whereby higher DHT was associated with increased incidence of lung cancer [5]. Compared to HIMS, the Busselton Health Survey consisted of a larger proportion of younger men with fewer men being diagnosed with lung cancer (22 cases). Lack of associations seen in this study is therefore likely due to lack of power from a small number of outcome events. We found an association of LH in the highest quartile with increased lung cancer risk which was not observed in the HIMS cohort [5]. The presence of LH receptors on several small and non-small cell lung cancer (SCLC and NSCLC) cell lines has been reported, with in vitro studies suggesting proliferative effects of LH on the HTB183 NSCLC cell line as well as the CRL2062 and CRL5853 SCLC cell lines [34]. In the same study, LH receptor mRNA was also detected in four of eight human NSCLC samples [34]. However, given the small number of lung cancer cases in our study, these results should be interpreted with caution.

We acknowledge several limitations associated with this study. Given the observational nature of this data, the direction of causality cannot be inferred. Whilst baseline measures of sex hormones were available, we did not have longitudinal measurements of sex hormone data for these men. The men in this study were predominantly Caucasian; therefore, results cannot be generalised to other ethnicities or to women. There were a limited number of outcome events for colorectal and lung cancer; therefore, weak to moderate associations may have been missed. Prostate cancer has a long latency period, and we did not perform routine prostate biopsies or prostate-specific antigen (PSA) screening in these men; however, record linkage captures all cancer diagnoses in Western Australia [17]. Therefore, clinically relevant prostate cancer cases are identified through this method. Strengths of this study include a long period of follow-up with a cohort spanning a wide age range and followed for two decades. Sex hormones were measured via LC-MS/MS and we were able to assess associations of DHT and E2 with cancer outcomes in these men. We used record linkage to capture outcome events in this study, allowing near complete capture of all clinically relevant cancers.

In conclusion, in this population of community-dwelling men spanning a wide age range and followed for two decades, lower T and DHT were associated with an increased incidence of any (non-skin) cancer, whilst lower T was associated with an increased incidence of prostate cancer. No associations of androgens with lung cancer were observed; however, higher LH was associated with an increased risk of lung cancer. There were no associations of sex hormones with colorectal

cancer incidence. Associations of low T and increased overall cancer risk may be reflective of poorer health status in these men. Further studies using LC-MS/MS-measured sex hormones are required to clarify the relationship between T and prostate cancer risk in men. Ongoing epidemiologic and mechanistic studies are required to validate findings of higher LH and lung cancer risk.

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