Serum androgens and prostate cancer among 643 cases and 643 controls in the European Prospective Investigation into Cancer and Nutrition

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We examined the hypothesis that serum concentrations of circulating androgens and sex hormone binding globulin (SHBG) are associated with risk for prostate cancer in a case-control study nested in the European Prospective Investigation into Cancer and Nutrition (EPIC). Concentrations of androstenedione, testosterone, androstanediol glucuronide and SHBG were measured in serum samples for 643 prostate cancer cases and 643 matched control participants, and concentrations of free testosterone were calculated. Conditional logistic regression models were used to calculate odds ratios for risk of prostate cancer in relation to the serum concentration of each hormone. After adjustment for potential confounders, there was no significant association with overall risk for prostate cancer for serum total or free testosterone concentrations (highest *versus* the lowest thirds: OR, 1.02; 95% CI, 0.73–1.41 and OR, 1.07, 95% CI, 0.74–1.55, respectively) or for other androgens or SHBG. Subgroup analyses showed significant heterogeneity for androstenedione by cancer stage, with a significant inverse association of androstenedione concentration and risk for advanced prostate cancer. There were also weak positive associations between free testosterone concentration and risk for total prostate cancer among younger men and risk for high-grade disease. In summary, in this large nested case-control study, concentrations of circulating androgens or SHBG were not strongly associated with risk for total prostate cancer. However, our findings are compatible with a positive association of free testosterone with risk in younger men and possible heterogeneity in the association with androstenedione concentration by stage of disease; these findings warrant further investigation.

Key words: prospective; prostate cancer; serum; androgen; EPIC

Prostate cancer is the most common cancer in males in developed countries and the second most common cause of cancer mortality in men.[1] The etiology of the disease, however, remains

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poorly understood and the only established risk factors are age, family history of prostate cancer and ethnicity.[2] A prominent hypothesis suggests that steroid hormones, and in particular androgens, are involved in prostate cancer carcinogenesis.[3,4] Testosterone is the primary circulating androgen in men and is produced in the testes and to a much lesser extent in the adrenal cortex, from its precursor, androstenedione. Within the prostate itself, testosterone is converted by 5α-reductase type 2 to 5α-dihydrotestosterone (DHT), the most potent natural androgen. The bioavailability of circulating testosterone to tissues is largely determined by the concentration of sex hormone binding globulin (SHBG), which binds to testosterone in the blood thereby reducing the extent to which testosterone is free to cross cell membranes and enter target cells.

Androgens are important for the development and maintenance of the prostate gland through their influence on the proliferation and differentiation of the luminal epithelium, and data from *in vitro* and *in vivo* experiments strongly support their role in the development of prostate cancer.[3,4] For example, in animal models, the induction and growth of prostate tumors can be stimulated by the administration of testosterone.[5]

In humans, the evidence for a role of androgens in prostate cancer carcinogenesis is inconsistent. Androgen ablation is used as a treatment for advanced prostate cancer and the resultant dramatic lowering of circulating androgens can lead to marked regression of androgen sensitive tumours.[6,7] Furthermore, results from the Prostate Cancer Prevention Trial showed that use of finasteride, an inhibitor of 5α-reductase which results in reduced intraprostatic concentrations of DHT, caused a 24% reduction in the prevalence of prostate cancer over a 7-year period.[8] However, the evidence from epidemiological studies on the association of circulating androgen concentrations with the development of prostate cancer remains unclear. Findings from the 16 prospective epidemiological studies that have investigated prostate cancer risk in association with prediagnostic circulating concentrations of testosterone, free testosterone or SHBG have been inconclusive (reviewed in Refs. 3, 4 and 9), with most reporting either no association or only weak associations between serum measurements and risk for prostate cancer. Studies have also published on circulating concentrations of DHT and androstanediol glucuronide, a metabolite of DHT and a marker of 5α-reductase activity.[10] All 7 published studies found no association between prostate cancer risk and concentrations of DHT.[9,11,12] However, circulating DHT concentrations may not be the optimal marker of DHT levels within the prostate because circulating concentrations are also influenced by DHT produced in the testes (catalyzed by 5α-reductase type 2), and in the skin, hair follicles (Type 1) and liver (Type 1 and 2).[13] In contrast with the findings for DHT, studies of androstanediol glucuronide and risk for prostate cancer have been inconsistent; 5 early studies included in a meta-analysis found a higher mean concentration of androstanediol glucuronide among case patients compared to controls[9] but subsequent studies have found either no significant association[12,14,15] or a significant inverse association.[11]

In the present study, we examine the relationship between risk for prostate cancer and prediagnostic serum concentrations of androgens, androstanediol glucuronide and SHBG among 643 men with incident prostate cancer and 643 matched control participants participating in a large, European multi-centre cohort study (EPIC). We also evaluate these associations by stage of disease and other factors.

Material and methods

Study cohort

Between 1992 and 2000, ~500,000 individuals (150,000 were men), from 23 centers in 10 European countries were recruited into the European Prospective Investigation into Cancer and Nutrition (EPIC), a prospective study designed to investigate risk factors for different forms of cancer. The methods of recruitment and study design have been described in detail elsewhere.[16] In brief, participants completed an extensive questionnaire on dietary and nondietary data at recruitment, and about 400,000 individuals also provided a blood sample. The present study includes prostate cancer cases occurring after blood collection and individually matched male control participants from 7 of the 10 participating countries: Denmark, Germany, Greece, Italy, the Netherlands, Spain and the United Kingdom (UK). France and Norway were not included in the current study because these cohorts only included women. Sweden was not included but some data from Swedish cases have already been published elsewhere.[17]

A 30 mL blood sample was collected according to a standardized protocol. Filled syringes were kept at 5–10°C, protected from light, and transferred to a local laboratory for further processing and aliquoting, with the exception of participants recruited through the Oxford centre. Here, blood samples were collected throughout the United Kingdom and transported to a laboratory in Norfolk by mail at ambient temperature. Blood fractions (serum, plasma, red cells and buffy coat for DNA extraction) were aliquoted into 0.5 mL straws, which were then heat-sealed at both ends and stored in liquid nitrogen tanks at −196°C, except in Denmark where samples were stored in 1 mL tubes in nitrogen vapor at −150°C.

Participants gave written consent for the research, and approval for the study was obtained from the ethical review board of the International Agency for Research on Cancer and from all local institutions in regions where participants had been recruited for the EPIC study.

Follow-up for cancer incidence and vital status

Follow-up for diagnosis of prostate cancer is provided through record linkage with population-based cancer registries in 5 of the participating countries: Denmark, Italy, the Netherlands, Spain and the UK. In Germany and Greece, follow-up is active and is achieved through checks of insurance records and cancer and pathology registries as well as *via* self-reported questionnaires; self-reported incident cancers are verified through medical records. Data on vital status in most EPIC study centers were collected from mortality registries at the regional or national level, in combination with data collected by active follow-up (Greece). The 10th Revision of the International Statistical Classification of Diseases, Injuries and Causes of Death (ICD) was used, and cancer of the prostate as analyzed in the present study was defined as code C61. For each EPIC centre closure dates of the study period were defined as the latest dates of complete follow-up for both cancer incidence and vital status (dates varied between centers, from June 1999 to January 2003). Vital status is known for over 99% of men recruited. Current estimates are that within the EPIC cohort, 95.1% of men recruited are alive, 4.2% have died since recruitment, 0.26% have opted out of the study, 0.11% have moved and are untraceable and 0.35% have emigrated.

Selection of case patients and control participants

Case patients were men who developed prostate cancer after the date of blood collection and before the end of the study period, defined for each study centre by the latest date of follow-up. In total, the 7 sub-cohorts contributing to the present study included 127,811 men. After excluding men if they had previously been registered as having cancer at the time of blood collection (other than nonmelanoma skin cancer), had not provided a blood sample or had a missing date of blood collection, diagnosis or follow-up, 908 men had a diagnosis of prostate cancer by the end of each center's follow-up period. Men with prostate cancer who had been diagnosed with other tumors, except nonmelanoma skin cancer, were excluded from the current study, as were those with insufficient sera available at the time of assaying. Notably, only 88 of the 314 eligible case patients in Denmark still had blood samples

remaining. This analysis includes 643 case patients and 643 matched control participants, who are also included in a collaborative analysis of genetic and hormonal factors on prostate cancer risk.[18] These 643 case patients comprised 88 men recruited in Denmark, 187 in Germany, 9 in Greece, 60 in Italy, 25 in the Netherlands, 94 in Spain and 180 in the United Kingdom.

Data on the stage and grade of disease at diagnosis were collected from each centre, where possible. A total of 450 cases (70.0%) had information on TNM staging, or equivalent information; of these, 309 (48.1%) were classified as localized (TNM staging score of T1-2 and N0 and M0, or equivalent) and 141 (21.9%) were classified as advanced (T3-4 and/or N1-3 and/or M1, or equivalent). The Gleason score (or equivalent information) was available for 465 cases (72.3%); of these, 315 (49.0%) were classified as low-grade (Gleason score < 7 or equivalent, *i.e.* coded as moderately or well differentiated) and 150 (23.3%) were classified as high-grade (Gleason scores 7+ or equivalent, *i.e.* coded as poorly differentiated or undifferentiated). For 335 cases where information on both stage and grade was available, 76% of localized cases were also classified as low-grade and 57% of advanced cases were also classified as high-grade.

Each case patient was matched to a control subject selected at random among appropriate risk sets consisting of all male cohort members alive and free of cancer (except nonmelanoma cancer) at the time of diagnosis of the index case. An incidence density sampling protocol for control selection was used, such that controls could include participants who became a case later in time, while each control subject could also be sampled more than once. Matching criteria included: study centre, age at enrolment (± 6 months), time of day of blood collection (± 1 hr), follow-up time (as close as possible), time between blood draw and last consumption of food or drinks (<3, 3–6, >6 hr).

Laboratory assays

All hormone assays were performed by the laboratory of the Hormones and Cancer Team at the International Agency for Research on Cancer, Lyon, France. The laboratory personnel who conducted the assays were blinded to the case or control status of the participants providing the samples. Serum samples from each case-control set were assayed within the same batch, analyzed on the same day and with the same immunoassay kit. Three quality control serum samples, which were indistinguishable from the subject samples, were inserted into each assay batch.

Androstenedione and androstanediol glucuronide were measured by radio-immunoassay (RIA) with a double antibody system for the separation of free and bound antigen (Diagnostic Systems Laboratory, Webster, TX). Serum testosterone concentrations were measured by RIA (Immunotech, Marseilles, France). SHBG was measured by a solid phase "sandwich" immunoradiometric assay (Cis-Bio International, Gif-sur-Yvette, France). For some participants, particular hormone assay results were unavailable due to insufficient serum or to assay failure. Mean intra-batch coefficients of variation (CV) were estimated to be 3.5% (at 10.8 nmol/L) for androstenedione, 10.8% (at 13.9 nmol/L) for testosterone, 4.1% (at 10.2 nmol/L) for androstanediol glucuronide and 7.7% (at 33.3 nmol/L) for SHBG. Inter-batch CVs were 11.1% for androstenedione, 14.8% for testosterone, 9.9% for androstanediol glucuronide and 12.2% for SHBG. The lowest limits of detection, in terms of the lowest standard of the standard curve, were: 0.34 nmol/L for androstenedione, 0.31 nmol/L for testosterone, 1.71 nmol/L for androstanediol glucuronide and 5.2 nmol/L for SHBG. To identify possible outliers in the hormone data, we examined the distribution of each hormone using box and whisker plots and letter-value displays,[19] and identified values on the logarithmic scale beyond a cut-off equal to 3 times the interquartile range below or above the 25th or 75th percentile value, respectively. Three values for androstenedione were beyond the cut-off (2 very low values and 1 very high value) and values for testosterone and free testosterone from 1 man were below the cut-off: these individuals were excluded from the analyses of relevant hormones.

Indices of bioavailable testosterone were calculated by 2 alternative methods. In the first approach, serum concentrations of free testosterone, unbound to SHBG or albumin, were calculated from absolute concentrations of testosterone and SHBG using mass action equations, and assuming a constant serum albumin concentration of 43 g/L.[20,21] In the second approach, we simultaneously adjusted testosterone for SHBG, allowing for an independent effect of SHBG. Results were similar for both calculated free testosterone and testosterone adjusted for SHBG, and we present the data from analyses of free testosterone calculated from mass action equations.

The steroid hormone and SHBG concentrations measured among control participants in the current study were broadly similar to those reported among control participants in other prospective European studies of prostate cancer in men.[17,22,23]

Statistical analyses

Statistical analyses were performed with the Stata 9 statistical software package.[24] The hormone and SHBG concentrations were logarithmically transformed for statistical analyses to approximately normalize their frequency distributions. All tests of statistical significance were two-sided and p values below 0.05 were considered significant. We compared characteristics of cases and controls with the chi-squared test (for categorical variables) and the paired t-test (for continuous variables). The statistical significance of case-control differences in geometric mean hormone concentrations was evaluated by paired comparisons (t-tests) of case values *versus* the values in matched control participants in each case-control set. Pearson's partial correlation coefficients, adjusted for recruitment centre, assay batch and age at blood donation, were calculated to assess the correlations between the hormone and SHBG concentrations and continuous variables including age and anthropometric indices among controls. Analysis of covariance was used to examine whether geometric mean concentrations of serum hormones varied according to subject characteristics, including country, centre, body mass index (BMI), smoking, alcohol intake, physical activity, marital status, education, weight and height, with adjustment for recruitment centre, assay batch and age at blood donation where appropriate.

Conditional logistic regression models were used to calculate the odds ratios (ORs) for prostate cancer in relation to 3 categories of serum hormone concentrations, using cut points defined by the tertiles of hormone concentration among control participants for all centers combined. Likelihood ratio tests were used to assess heterogeneity, and tests for linear trend were conducted using continuous log-transformed values for each hormone and for SHBG. The effects of potential confounders (other than the matching criteria, controlled for by design) were examined by including additional variables in the logistic regression models. These variables were smoking (never, past, present), physical activity (inactive, moderately inactive, moderately active, active), alcohol intake (<8 g/day, 8–15 g/day, 16–39 g/day, ≥40 g/day), marital status (married/cohabiting or not married/cohabiting) and education (primary or none, secondary, degree level). We examined the importance of additional adjustment for BMI (in quintiles) in a separate multivariable model.

χ^2 tests were used to examine the heterogeneity of prostate cancer risk associated with a linear increase in androgen or SHBG level by stage and grade of the disease, and by country of recruitment, age (<60, 60+ years) and BMI (less than the median, equal to or greater than the median) at blood collection. The main analyses were repeated on a dataset restricted to case-control sets with diagnoses made 2 or more years after blood collection to reduce the possible impact of preclinical disease on hormone levels (N = case–control 473 pairs). For all subgroup analyses, we present results adjusted for potential confounders, including smoking, alcohol intake, marital status, physical activity and educational status.

Results

Characteristics of case patients and control participants

This study includes 643 case patients diagnosed with prostate cancer from recruitment until the end of follow-up, and 643 matched controls. Measurements were available for 626 matched case–control sets for androstenedione, 533 sets for testosterone, 490 sets for free testosterone, 636 sets for androstanediol glucuronide and 572 sets for SHBG. The baseline characteristics of case patients and control participants are shown in Table I. Participants had a median age of 61 years at blood collection (range = 43–76 years). Prostate cancer diagnosis followed blood collection by a median of 3.4 years (range = 0–9.1 years) and the median age at diagnosis was 65 years (range = 47–82 years). Case patients and control participants were similar with respect to weight, height, BMI, smoking, alcohol consumption, physical activity, marital status and educational attainment.

TABLE I – CHARACTERISTICS OF PROSTATE CANCER PATIENTS AND CONTROL PARTICIPANTS

Characteristic	Cases	Controls	*p* value[1]
Number	643	643	
Time to diagnosis (years)[2], *n* (%)			
<1 years	80 (12.4)	–	
1 years	90 (14.0)	–	
2 years	103 (16.0)	–	
3 years	114 (17.7)	–	
≥4 years	256 (19.9)	–	
Age (years), *n* (%)			
<50	17 (2.7)	17 (2.7)	
50–54	69 (10.7)	69 (10.7)	
55–59	186 (28.9)	184 (28.6)	
60–64	233 (36.2)	233 (36.2)	
65–70	67 (10.4)	69 (10.7)	
≥70	71 (11.0)	71 (11.0)	
Weight (kg), mean (SD)	79.4 (11.1)	80.5 (12.1)	0.07
Height (cm), mean (SD)	172.3 (6.8)	172.5 (7.0)	0.64
BMI (kg/m^2), mean (SD)	26.7 (3.5)	27.1 (3.6)	0.09
Smoking, *n* (%)[3]			
Never	201 (31.5)	171 (26.9)	
Former	291 (45.5)	289 (45.5)	
Current	147 (23.0)	175 (27.6)	0.09
Alcohol consumption, *n* (%)[3]			
<8 g/d	224 (34.9)	226 (35.3)	
8–15 g/d	123 (19.2)	137 (21.4)	
16–39 g/d	175 (27.3)	158 (24.6)	
≥40 g/d	119 (18.6)	120 (18.7)	0.65
Physical activity, *n* (%)[3]			
Inactive	128 (20.2)	107 (16.9)	
Moderately inactive	222 (35.0)	204 (32.3)	
Moderately active/active	284 (44.8)	321 (50.8)	0.09
Marital status, *n* (%)[3]			
Married or cohabiting	401 (87.7)	405 (89.2)	
Not married or cohabiting	56 (12.3)	49 (10.8)	0.49
Education, *n* (%)[3]			
Primary school or equivalent	234 (38.2)	236 (39.0)	
Secondary school	222 (36.2)	231 (38.2)	
University degree or equivalent	157 (25.6)	138 (22.8)	0.51
Stage, *n* (%)			
Localised	309 (48.1)	–	
Advanced	141 (21.9)	–	
Unknown	193 (30.0)	–	
Grade, *n* (%)			
Low grade[4]	315 (49.0)	–	
High grade[5]	150 (23.3)	–	
Unknown	178 (27.7)	–	

[1]*p* values relate to two-sided paired *t*-tests of equality of the means, or χ^2 tests of association, as appropriate.–[2]Time between blood collection and diagnosis among case patients.–[3]Denotes that the factor is unknown for some men–these men are excluded from the calculations of percentages and *p* values.–[4]Gleason score <7 or coded as well or moderately differentiated.–[5]Gleason score ≥7 or coded as poorly differentiated or undifferentiated.

Associations between hormones and other variables in control participants

Concentrations of hormones and SHBG did not vary significantly by recruitment centre, with the exception of androstenedione ($p < 0.01$), for which centre explained 3.5% of the variation in concentration between participants. Following adjustment for study centre and assay batch, there were significant inverse associations of age at blood collection with serum concentrations of free testosterone and androstanediol glucuronide, and a significant positive association with SHBG. Weight and BMI were positively associated with androstanediol glucuronide and inversely associated with testosterone, calculated free testosterone and SHBG ($p < 0.05$ for all, correlation coefficients are shown in Table II). Concentrations of androstenedione, testosterone and SHBG varied significantly by smoking status, with current smokers having higher concentrations of all 3 analytes than former or never smokers ($p < 0.01$ for all). Free testosterone concentrations were significantly higher among men not married or cohabiting than among married or cohabiting men ($p = 0.01$). There was no significant variation in any of the androgens or in SHBG by physical activity or educational status. These associations were not significantly different in case patients (data not shown).

Correlations between hormones in control participants

Pearson's partial correlation coefficients between steroid hormone levels in control participants, adjusted for recruitment centre, assay batch and age at blood donation, showed that all the androgens were positively correlated with each other (Table II), and that testosterone was positively correlated with SHBG ($r = 0.56$, $p < 0.001$). Testosterone was strongly positively correlated with calculated free testosterone ($r = 0.79$, $p < 0.001$). These correlations were similar in prostate cancer case patients (data not shown).

Circulating hormone levels in case patients and control participants

The geometric mean concentrations of androstenedione, testosterone, androstanediol glucuronide and SHBG were not significantly different between case patients and control participants, but mean free testosterone concentration was 4.2% higher in case patients than control participants ($p = 0.04$) (Table III).

The relative risks for prostate cancer by hormone levels from conditional logistic regression models adjusted for potential confounders are shown in Table IV. We observed no significant association between overall risk for prostate cancer and concentrations of androstenedione, testosterone, androstanediol glucuronide or SHBG, with or without adjustment for potential confounders, either individually or in combination. Mutual adjustment for hormones and SHBG (excluding free testosterone) also made little difference to the results. For prostate cancer risk in relation to free testosterone concentration, the test for linear trend using a continuous variable was weakly significant ($p_{trend} = 0.04$), although the analysis of risk in relation to thirds of free testosterone did not suggest a linear trend (OR of 0.89 (95% CI, 0.65–1.22) among men in the middle third, and 1.19 (95% CI, 0.84–1.69) among men in the highest *versus* the lowest third. This association became nonsignificant following adjustment for potential confounders including smoking, alcohol intake, marital status, physical activity and educational status (OR, 1.07, 95% CI, 0.74–1.55 for men in the highest *versus* the lowest third, $p_{trend} = 0.12$, Table IV). Additional adjustment for BMI made little difference to risk estimates and confidence intervals, and multivariable analyses are presented without adjustment for BMI.

Subgroup analysis by case characteristics

The associations between androgens, SHBG and risk for prostate cancer for localized and advanced disease are shown in Table IV. We found significant heterogeneity in the trend in risk with increasing concentrations of androstenedione between localized and advanced prostate cancer ($p_{\text{heterogeneity of trends}} = 0.01$),

TABLE II – PEARSON PARTIAL CORRELATION COEFFICIENTS[1] BETWEEN HORMONES[2], AGE, WEIGHT AND BODY MASS INDEX AMONG CONTROL PARTICIPANTS

	Androstenedione	Testosterone	Free testosterone	Androstanediol glucuronide	SHBG
Age	−0.06	0.01	−0.12#	−0.10*	0.15†
Weight	−0.02	−0.27†	−0.12#	0.15†	−0.29†
BMI	−0.01	−0.27†	−0.10*	0.18†	−0.32†
Testosterone	0.34†				
Free Testosterone	0.36†	0.79†			
Androstanediol glucuronide	0.27†	0.26†	0.31†		
SHBG	0.10*	0.56†	−0.05	0.04	

*$p < 0.05$.
#$p < 0.01$.
†$p < 0.001$.
[1]Adjusted for study centre and assay batch, and for age at blood collection where appropriate.–[2]Using log-transformed hormone data.

TABLE III – GEOMETRIC MEAN SERUM HORMONE CONCENTRATIONS (95% CONFIDENCE INTERVALS) AMONG PROSTATE CANCER CASE PATIENTS AND CONTROL PARTICIPANTS[1]

Hormone/binding protein	Number of cases/controls	Mean concentration (95% CI)		*p* for difference[2]
		Case patients	Control participants	
Androstenedione (nmol/l)	629/629	4.59 (4.47–4.72)	4.67 (4.54–4.80)	0.34
Testosterone (nmol/l)	533/533	15.8 (15.3–16.4)	15.6 (15.0–16.2)	0.50
Free testosterone (pmol/l)	490/490	282.1 (272.0–292.7)	269.4 (259.7–279.4)	0.04
Androstanediol glucuronide (nmol/l)	636/636	12.8 (12.2–13.4)	12.9 (12.3–13.5)	0.74
SHBG (nmol/l)	572/572	41.4 (40.0–42.9)	42.8 (41.3–44.3)	0.16

[1]Case patients and control participants were matched on recruitment centre, age at enrolment (±6 months), time of day of blood collection (±1 hr), follow-up time (as close as possible), time between blood draw and last consumption of food or drinks (<3, 3–6, >6 hr).–[2]Two-sided *p* values; paired *t*-test.

with increasing androstenedione associated with a reduction in risk for advanced disease (OR, 0.42, 95% CI, 0.19–0.91 in the highest *versus* the lowest third of the androstenedione distribution; $p_{trend} < 0.01$) whereas there was no significant association between androstenedione and risk of localized disease. There was no evidence of significant heterogeneity in the trends in risk between localized and advanced disease for testosterone, free testosterone, androstanediol glucuronide and SHBG (see Table IV), and no significant associations were seen between concentrations of these hormones or SHBG and risk for localized or advanced disease.

There was no significant heterogeneity between the trends for risk of low-grade or high-grade prostate cancer for any of the hormones or for SHBG. There were no significant associations between any hormone and low- or high-grade prostate cancer, although there was a weak positive association between free testosterone concentration and risk for high-grade cancer; the OR for the highest third *versus* the lowest third was 1.56 (95% CI, 0.67–3.64); $p_{trend} = 0.09$).

After excluding the first 2 years of follow-up (N = case-control 170 pairs excluded), results changed little, and we observed no significant associations between concentrations of any of the androgens or SHBG and risk for prostate cancer ($p > 0.05$ for all).

We next examined whether the associations of androgen and SHBG levels with risk for prostate cancer differed by age at blood collection (Table IV). There was no evidence of significant heterogeneity for any of the androgens or SHBG. When the associations between prostate cancer risk and hormone concentrations were examined separately by age at blood collection, we observed a significantly elevated risk with the highest third of free testosterone among men aged less than 60 years (OR, 1.46, 95% CI, 0.84–2.54; $p_{trend} = 0.02$), but no significant relationship among men who gave blood aged 60 years or above.

We also examined whether the associations of androgens and SHBG with prostate cancer risk differed by BMI (data not shown). There was no significant heterogeneity in the linear association of concentrations any of the androgens or SHBG with prostate cancer between men with a BMI less than the median (26.6 kg/m^2) and men with a BMI of equal to or greater than the median ($p_{heterogeneity\ of\ trends} > 0.05$ for all).

Discussion

We have examined the relationship between circulating androgens and risk for prostate cancer in a large prospective study among European men. Since the bioavailability of serum androgens to target tissues is largely determined by their binding to SHBG in the circulation, we also examined SHBG and calculated free testosterone concentrations in relation to risk for the disease. Our results suggest no strong associations between serum concentrations of androgens or SHBG and risk for total prostate cancer.

The findings from this study in general do not support the hypothesis that circulating androgens play a major role in the pathogenesis of prostate cancer and are in agreement with results from the majority of published prospective studies. A quantitative review[9] of the 8 prospective studies published up to 1999 showed no large differences in total testosterone (including a total of 817 cases and 2,107 controls) or non-SHBG bound testosterone (325 cases and 422 controls), with only one study within the meta-analysis[25] finding a significant positive association between SHBG-adjusted testosterone and risk for prostate cancer. A further 8 prospective studies of the relationship between circulating androgens and prostate cancer risk have since been published.[11,12,14,15,17,23,26,27] Results from 6 showed no significant association between risk for total prostate cancer and circulating total testosterone or free testosterone,[11,12,14,15,23,26] one reported a small but significant decrease in risk for increasing levels of total testosterone,[17] and another showed a significant positive linear relationship with free testosterone.[27] With respect to SHBG, the finding from the current study of no association between circulating concentrations and prostate cancer risk is consistent with results from the meta-analysis[9] and the 8 studies published subsequently.[11,12,14,15,17,23,26,27]

TABLE IV – ODDS RATIOS[1] (95% CONFIDENCE INTERVALS) FOR PROSTATE CANCER BY THIRDS OF SERUM ANDROGEN AND SHBG CONCENTRATIONS, OVERALL AND FOR SUBSETS DEFINED BY STAGE AND GRADE OF DISEASE AND BY AGE AT BLOOD COLLECTION

	No. cases/ controls	Third			p trend[2]	p Interaction
		1	2	3		
Total prostate cancer						
Androstenedione	629/629	1 (reference)	0.89 (0.68–1.17)	0.78 (0.57–1.07)	0.32	–
Testosterone	533/533	1 (reference)	0.87 (0.64–1.18)	1.02 (0.73–1.41)	0.70	–
Free testosterone	490/490	1 (reference)	0.83 (0.60–1.15)	1.07 (0.74–1.55)	0.12	–
Androstanediol glucuronide	636/636	1 (reference)	0.83 (0.63–1.09)	0.81 (0.60–1.08)	0.54	–
SHBG	572/572	1 (reference)	0.99 (0.74–1.34)	0.84 (0.60–1.16)	0.21	–
Localised prostate cancer						
Androstenedione	305/305	1 (reference)	1.06 (0.70–1.60)	0.96 (0.62–1.49)	0.42	–
Testosterone	256/256	1 (reference)	0.89 (0.56–1.42)	1.31 (0.82–2.12)	0.39	–
Free testosterone	232/232	1 (reference)	0.55 (0.33–0.92)	1.06 (0.58–1.92)	0.13	–
Androstanediol glucuronide	305/305	1 (reference)	0.88 (0.59–1.30)	1.02 (0.66–1.56)	0.73	–
SHBG	273/273	1 (reference)	0.79 (0.51–1.23)	0.73 (0.46–1.15)	0.48	–
Advanced prostate cancer						
Androstenedione	133/133	1 (reference)	0.90 (0.47–1.74)	0.42 (0.19–0.91)	0.005	0.01
Testosterone	112/112	1 (reference)	0.75 (0.38–1.49)	0.62 (0.26–1.47)	0.43	0.43
Free testosterone	98/98	1 (reference)	1.30 (0.59–2.87)	1.70 (0.70–4.13)	0.83	0.83
Androstanediol glucuronide	139/139	1 (reference)	0.85 (0.45–1.60)	0.64 (0.31–1.30)	0.74	0.74
SHBG	115/115	1 (reference)	1.29 (0.61–2.72)	1.03 (0.44–2.41)	0.33	0.33
Low grade prostate cancer						
Androstenedione	309/309	1 (reference)	0.79 (0.53–1.19)	0.65 (0.41–1.05)	0.61	–
Testosterone	257/257	1 (reference)	0.92 (0.58–1.46)	1.14 (0.70–1.87)	0.44	–
Free testosterone	238/238	1 (reference)	0.69 (0.43–1.12)	1.03 (0.60–1.78)	0.11	–
Androstanediol glucuronide	314/314	1 (reference)	0.93 (0.63–1.38)	0.82 (0.53–1.26)	0.96	–
SHBG	279/279	1 (reference)	1.11 (0.73–1.68)	0.83 (0.52–1.31)	0.60	–
High grade prostate cancer						
Androstenedione	145/145	1 (reference)	1.34 (0.68–2.61)	1.25 (0.63–2.63)	0.62	0.30
Testosterone	121/121	1 (reference)	1.28 (0.62–2.63)	1.42 (0.67–3.64)	0.31	0.40
Free testosterone	114/114	1 (reference)	1.53 (0.70–3.36)	1.56 (0.67–3.64)	0.09	0.30
Androstanediol glucuronide	149/149	1 (reference)	1.26 (0.66–2.43)	1.51 (0.78–2.91)	0.11	0.36
SHBG	135/135	1 (reference)	0.83 (0.37–1.87)	0.60 (0.27–1.36)	0.21	0.72
Age <60 years at blood collection						
Androstenedione	265/265	1 (reference)	1.01 (0.62–1.65)	0.90 (0.53–1.51)	0.39	–
Testosterone	225/225	1 (reference)	0.90 (0.53–1.51)	1.50 (0.89–2.51)	0.24	–
Free testosterone	211/211	1 (reference)	1.00 (0.59–1.72)	1.46 (0.84–2.54)	0.02	–
Androstanediol glucuronide	266/266	1 (reference)	0.84 (0.55–1.29)	0.92 (0.58–1.46)	0.76	–
SHBG	246/246	1 (reference)	1.37 (0.86–2.18)	0.87 (0.53–1.43)	0.27	–
Age ≥60 years at blood collection						
Androstenedione	361/361	1 (reference)	0.85 (0.60–1.20)	0.75 (0.50–1.12)	0.13	0.19
Testosterone	305/305	1 (reference)	0.85 (0.57–1.27)	0.74 (0.47–1.15)	0.57	0.20
Free testosterone	276/276	1 (reference)	0.70 (0.45–1.07)	0.80 (0.47–1.34)	0.80	0.06
Androstanediol glucuronide	366/366	1 (reference)	0.85 (0.59–1.23)	0.84 (0.56–1.24)	0.99	0.86
SHBG	323/323	1 (reference)	0.82 (0.54–1.25)	0.77 (0.48–1.23)	0.38	0.96

Tertile cutpoints are 4.1 and 5.5 nmol/l for androstenedione, 13.7 and 18.8 nmol/l for testosterone, 232.1 and 327.2 pmol/l for free testosterone, 10.4 and 16.9 nmol/l for androstanediol glucuronide and 36.3 and 51.6 nmol/l for SHBG.

[1]Case patients and control participants were matched on recruitment centre, age at enrolment (±6 months), time of day of blood collection (±1 hr), follow-up time (as close as possible), time between blood draw and last consumption of food or drinks (<3, 3–6, >6 hr). Adjustment was made for smoking (never, past, present), physical activity (inactive, moderately inactive, moderately active, active), alcohol intake (<8 g/day, 8–15 g/day, 16–39 g/day, ≥40 g/day), marital status (married or cohabiting) and education (primary or none, secondary, degree level).–[2]Test for trend using continuous log-transformed data.

Evidence from *in vitro* studies suggests that the intraprostatic conversion of testosterone to DHT by 5α-reductase type 2 might be the most important determinant of prostate cancer risk.[28] However, a potential limitation of the current study and all previous epidemiological studies is that it is not clear whether circulating hormone concentrations accurately reflect intraprostatic androgen levels. Our current understanding is that the serum concentration of androstanediol glucuronide, an end-metabolite of DHT, may provide the best serum marker of 5α-reductase activity,[29] although like serum DHT, it also partly reflects extraprostatic 5α-reductase type 1 activity. Furthermore, assays of circulating concentrations of androstanediol glucuronide, such as the radioimmunoassay used in the current study, usually only measure androstanediol 17-glucuronide, one of the two isomers of this metabolite. Since this is the predominant isomer in the circulation, representing more than 80% of total circulating androstanediol glucuronide,[30–32] it is likely that it is a useful proxy for total androstanediol glucuronide concentration in the circulation.

The results from the current study provide no evidence for an association between circulating concentrations of androstanediol glucuronide and risk for prostate cancer. This contrasts with the findings from a quantitative review of published data in 1999, which reported moderately elevated serum androstanediol glucuronide concentrations in 644 prostate cancer case patients relative to levels in 1,048 control participants.[9] However, our results are in agreement with 3 more recently published prospective studies, which also found no significant relationship,[12,14,15] although a fourth study[11] reported a significant but nonlinear inverse relationship. Taken together, these findings suggest that circulating concentrations of androstanediol glucuronide are not strongly associated with prostate cancer risk.

Prostate cancer stage and the grade of tumor differentiation, as 2 indicators of the aggressiveness of the disease, have been of interest in a number of previous epidemiological studies of circulating hormones and prostate cancer risk. This is in part due to increasing awareness that localized and/or low-grade tumors may have a different etiology compared to advanced and high-grade

tumors, and also because of changes in the characteristics of tumors being diagnosed, with growing numbers of early tumors being detected by prostate specific antigen (PSA) testing. Indeed, it has been suggested that some of the inconsistencies between findings from epidemiological studies of hormone concentrations in relation to the disease may be due to the changing tumor characteristics of cancers over time.[4] Testing for prostate cancer using the serum concentration of PSA has become more common during the follow-up period for this study. Data on PSA use in the EPIC cohort are not available, and therefore we were unable to distinguish between screen-detected and nonscreen-detected disease. Studies of annual rates of PSA testing in older middle aged men within the participating countries suggest rates of 6% in England and Wales, 7% in the Netherlands, 9% in Spain and 16% in Italy, compared to approximately 38% in US whites.[33–37]

In the current study, we found no evidence for a difference in the relationship between total or free testosterone, androstanediol glucuronide or SHBG and prostate cancer risk by stage of the disease, and these results are broadly consistent with the published literature.[12,14,22,25,38–40] The association of androstenedione concentration with risk differed significantly by stage: a significant inverse relationship with risk was observed for advanced disease only. This is a similar finding to recently published data on androstenedione and risk for aggressive (advanced and/or high-grade) prostate cancer in Australian men,[15] although the authors of this study also reported an inverse association between testosterone and aggressive disease. There is no obvious explanation for the apparent differences between the association of testosterone and androstenedione with risk for advanced disease in the current study. A similar relationship might have been expected given the close correlation between these hormones, with androstenedione being converted to testosterone by 17-β-hydroxysteroid dehydrogenase.[41]

We found no strong evidence that the association of serum androgens or SHBG with risk differed according the grade of disease in the current study. Although free testosterone concentration was positively associated with risk for high-grade disease, these findings were not statistically significant. However, we are aware that there is considerable measurement error in the determination of Gleason scores, with a high proportion of cases likely to be under-graded.[42,43] Indeed, in the present study, 43% of advanced cases were classified as low-grade, which is consistent with other data from Europe (Berrino F, Zigon G, Gatta G, personal communication) and most likely reflects some degree of under-grading, which may obscure any heterogeneity in the association by grade. Our findings with respect to prostate cancer grade are consistent with those from one other published study[27] but contrast with those from a second which suggested that elevated plasma total and free testosterone may increase the risk for low-grade prostate cancer,[12] and conversely, that there may be an inverse association between concentrations of these hormones and high-grade disease. Thus, overall findings for androgens in relation to prostate cancer grade in the published literature remain inconsistent.

We found no strong evidence for heterogeneity in the relationship of androgens or SHBG with prostate cancer risk by age at blood collection, although the results for free testosterone suggest a possible positive association with the risk for disease among younger men. These results for age are compatible with those from the majority of recent studies which have also reported no substantial difference in the relationship by age,[14,17,23] with the exception of the Health Professionals Follow-up Study which found a significant difference in the association between androstanediol glucuronide and risk by age.[12]

Because hormonal systems may be perturbed in obese men, we also assessed whether the relationship of sex hormones with prostate cancer differed by BMI at blood collection. We found no evidence that the association of sex hormones or SHBG is modified by body mass index at recruitment.

The conclusions that can be drawn from subgroup analyses in the current study, however, are limited by the small numbers in each group. Overall, this study had approximately 84% power at a statistical significance level of 0.05 to detect a relative risk for all prostate cancer of 1.5 among men in the highest third of the hormone distribution compared with the lowest third, but only more modest power to detect similar associations among subgroups defined by tumor subtype or subject characteristics: ~20–50% power in analyses of high-grade or advanced prostate cancer, or for analyses restricted to men with a normal body mass index, or young age at blood collection, and approximately 65% power in analyses of cases diagnosed at least 2 years following blood collection.

This study uses measurements of serum hormones and SHBG taken from single blood specimens provided by each participant and thus relies on the assumption that concentrations of hormones taken at one point in time are representative of long-term levels. While serum levels of testosterone and SHBG have been shown to be relatively stable over the medium term, with correlation coefficients of more than 0.8 for testosterone over a year,[44] and 0.61 for total testosterone, 0.58 for free testosterone and 0.71 for SHBG over 8 years,[45] little is known about the reliability of a single measurement as a marker of longer term levels in an individual.

A limitation of this study, as with most prospective studies of prostate cancer published to date, is that serum hormone concentrations are likely to reflect the hormonal milieu at a time when there were already early preclinical tumors rather than prior to the initiation of the tumorigenesis because the mean preclinical duration of prostate cancer has been estimated as being at least a decade.[46] In our study the mean time between blood sample donation and diagnosis in case patients was 3½ years. Thus, existing sub-clinical tumors at blood collection may have in some way influenced levels of circulating sex hormones. Exclusion of cases diagnosed in the first two-years of follow-up did not materially alter the main findings, but further examination of the role of androgens prior to the initiation of the disease remains to be determined with longer follow-up.

In conclusion, the findings from this large prospective study in European men show no strong association between circulating androgens or SHBG and risk for total prostate cancer. However, our findings are compatible with a positive association of free testosterone with risk in younger men, and with risk for high-grade disease, and also suggest that there may be variation in the association of androstenedione concentration with risk according to stage of disease. Further large or pooled analyses are needed to determine whether subgroup findings such as this are due to chance or represent true differences in prostate cancer etiology.

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