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Endogenous sex steroid hormones and risk of liver cancer among US men: Results from the Liver Cancer Pooling Project

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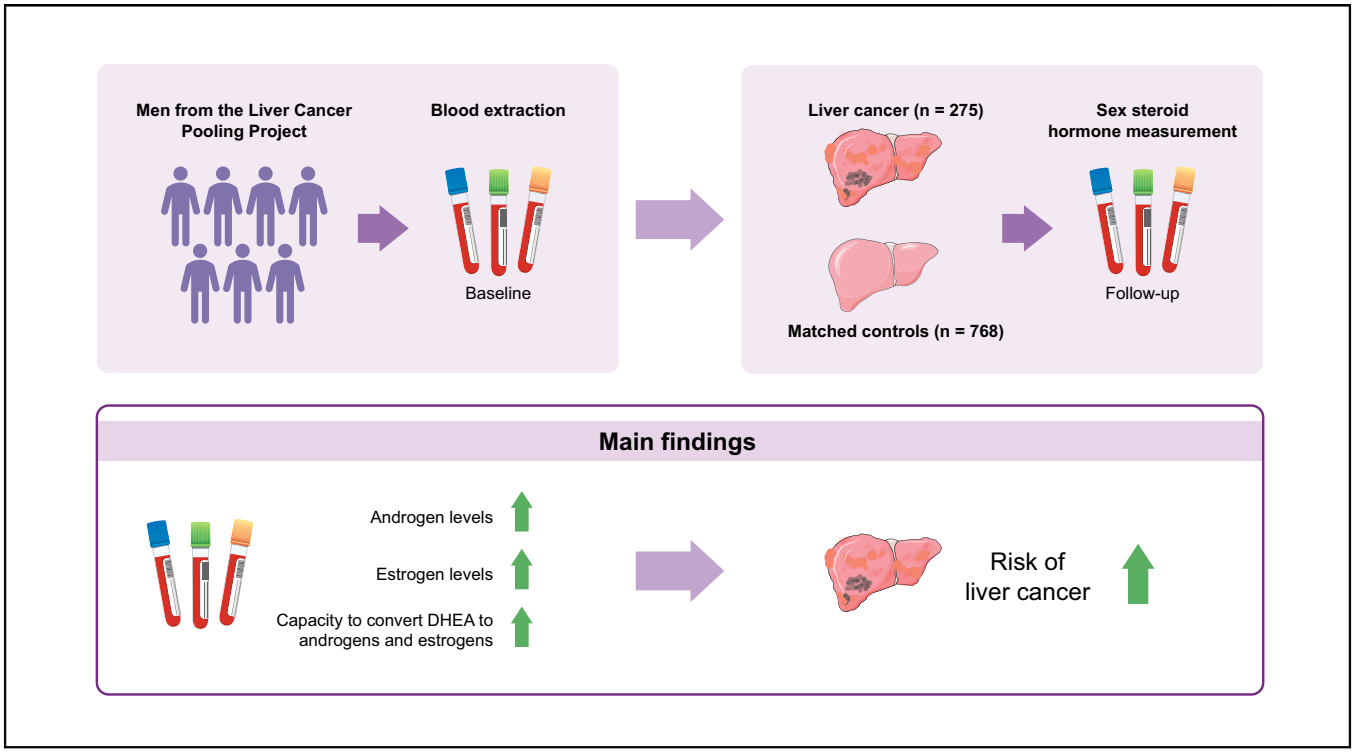
Graphical abstract



Highlights

- Higher concentrations of androgen were associated with increased risk of liver cancer.
- Higher concentrations of oestrogen were also associated with increased risk of liver cancer.
- A greater capacity to convert DHEA to androgens and oestrogens could be associated with increased risk of liver cancer among men.

Impact and implications

This study does not fully support the current hormone hypothesis as both androgen and oestrogen levels were associated with increased risk of liver cancer among men. The study also found that higher DHEA levels were associated with lower risk, thus suggesting the hypothesis that greater capacity to convert DHEA could be associated with increased liver cancer risk among men.

https://doi.org/10.1016/j.jhepr.2023.100742

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Endogenous sex steroid hormones and risk of liver cancer among US men: Results from the Liver Cancer Pooling Project

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JHEP Reports **2023.** https://doi.org/10.1016/j.jhepr.2023.100742

Background & Aims: Incidence rates of liver cancer in most populations are two to three times higher among men than women. The higher rates among men have led to the suggestion that androgens are related to increased risk whereas oestrogens are related to decreased risk. This hypothesis was investigated in the present study via a nested case-control analysis of pre-diagnostic sex steroid hormone levels among men in five US cohorts.

Methods: Concentrations of sex steroid hormones and sex hormone-binding globulin were quantitated using gas chromatography–mass spectrometry and a competitive electrochemiluminescence immunoassay, respectively. Multivariable conditional logistic regression was used to calculate odds ratios (ORs) and 95% CIs for associations between hormones and liver cancer among 275 men who subsequently developed liver cancer and 768 comparison men.

Results: Higher concentrations of total testosterone (OR per one-unit increase in $\log_2$ = 1.77, 95% CI = 1.38–2.29), dihydrotestosterone (OR = 1.76, 95% CI = 1.21–2.57), oestrone (OR = 1.74, 95% CI = 1.08-2.79), total oestradiol (OR = 1.58, 95% CI=1.22–20.05), and sex hormone-binding globulin (OR = 1.63, 95% CI = 1.27–2.11) were associated with increased risk. Higher concentrations of dehydroepiandrosterone (DHEA), however, were associated with a 53% decreased risk (OR = 0.47, 95% CI = 0.33–0.68).

Conclusions: Higher concentrations of both androgens (testosterone, dihydrotestosterone) and their aromatised oestrogenic metabolites (oestrone, oestradiol) were observed among men who subsequently developed liver cancer compared with men who did not. As DHEA is an adrenal precursor of both androgens and oestrogens, these results may suggest that a lower capacity to convert DHEA to androgens, and their subsequent conversion to oestrogens, confers a lower risk of liver cancer, whereas a greater capacity to convert DHEA confers a greater risk.

Impact and implications: This study does not fully support the current hormone hypothesis as both androgen and oestrogen levels were associated with increased risk of liver cancer among men. The study also found that higher DHEA levels were associated with lower risk, thus suggesting the hypothesis that greater capacity to convert DHEA could be associated with increased liver cancer risk among men.

Keywords: Androgen; Oestrogen; Sex steroid hormone; Liver cancer; Male.
Received 31 October 2022; received in revised form 28 February 2023; accepted 3 March 2023; available online 22 March 2023

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Introduction

Liver cancer is the sixth most commonly diagnosed cancer worldwide, with an estimated 906,000 new cases and 830,000 deaths occurring in 2020.[1] Both incidence and mortality rates of liver cancer are two to three times higher among men than women in most regions. The major histological types of liver cancer are hepatocellular carcinoma (HCC), comprising 75–85% of cases, and intrahepatic cholangiocarcinoma (ICC), comprising 10–15% of cases.[1]

Major risk factors for liver cancer include chronic infection with HBV or HCV, consumption of aflatoxin-contaminated foods, heavy alcohol intake, smoking, and the constellation of metabolic disorders including metabolic syndrome, obesity, type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD).[1,2] Although differences in lifestyle risk factors, such as alcohol consumption and cigarette smoking, may partly explain the male predominance,[1] it has been hypothesised that sex steroid hormones play a role in liver cancer aetiology, with androgens being related to increased risk and oestrogens being related to decreased risk.[3] Experimental animal studies using castration and hormone dosing strategies have reported that androgens accelerated hepatocarcinogenesis whereas oestrogens attenuated tumour formation.[4–6] However, epidemiology studies have reached conflicting conclusions, perhaps because of limited sample sizes, differences in the dominant risk factors in various populations, and differences in study designs.[7–11] These studies have mainly examined testosterone and oestradiol, whereas few studies have investigated the role of other sex steroid hormones or sex hormone-binding globulin (SHBG), which regulates the bioavailability of circulating sex steroid hormones.[12]

Previously, our team examined the associations between sex steroid hormones and risk of liver cancer among postmenopausal women in the Liver Cancer Pooling Project (LCPP).[13] We found that, among US women, higher level of 4-androstenedione (4-dione) was associated with lower risk, and SHBG with higher risk. To better understand the relationship between sex steroid hormones and liver cancer, we conducted a nested case-control study among men in the LCPP.

Materials and methods

Study population

The LCPP consists of US-based cohort studies who are members of the National Cancer Institute (NCI) Cohort Consortium. Of the 14 LCPP cohorts, four were included in the current analysis: the Cancer Prevention Study-II Nutrition Cohort (CPS-II),[14] the Health Professionals Follow-Up Study (HPFS),[15,16] the Physicians' Health Study (PHS),[17] and the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO).[18] In addition, the Multiphasic Health Checkup cohort (MHC), a cohort of Kaiser Permanente Northern California health plan members, was included[19] (Table S1). All studies received Institutional Review Board and data sharing approvals from their host institutions. Informed consent was obtained from all participants. Before contributing, the NCI de-identified participant-level data and serum or plasma samples.

Liver cancer was defined via use of the International Classification of Diseases, 10th version topography code C22.[20] Where available, ICD-O-3 morphology codes were used to ascertain histology status (HCC: 8170–8175; ICC: 8032–8033, 8041, 8050, 8070–8071, 8140–8141, 8160, 8260, 8480, 8481, 8490, and 8560).[21] All men were cancer-free at baseline. Men who subsequently developed liver cancer (*i.e.* case men) were matched, using incidence density matching, to control men. The matching criteria included parent cohort, age at baseline, race/ethnicity, and date of baseline blood draw (within a fixed 3-month period). A total of 275 cases and 768 controls were included in the analysis with 27 participants from CPS-II, 36 from HPFS, 120 from PHS, 352 from the PLCO, and 508 from MHC.

Laboratory methods

Serum/plasma samples from the cases and controls were assayed for concentrations of testosterone and oestradiol.[22–24] Assays were performed at the Pharmacogenomics Laboratory of Université Laval (Quebec, Canada) and were quantitated using gas chromatography–mass spectrometry (GC–MS/MS). A total of 90 blinded quality control (QC) samples were included in the batching scheme. Within-batch coefficients of variation (CVs) were 5.7% for testosterone and 10.7% for oestradiol (Table S2). SHBG was measured in the majority of samples, however, 111 of 120 of the PHS samples did not have adequate volume to measure SHBG. The nine PHS samples that did have adequate volume were included in all analyses. A competitive electrochemiluminescence immunoassay on the Roche E Modular system (Roche Diagnostics, Indianapolis, IN, USA) was used to determine SHBG levels at the Clinical and Epidemiologic Research Laboratory of Boston Children's Hospital (Boston, MA, USA). Within-batch CVs were 1.7%. Additionally, free testosterone, free oestradiol, the testosterone/oestradiol ratio, and the free testosterone/free oestradiol ratio were calculated.[25,26] Serum hormone values measured below the lower limit of quantification (LLOQ) were assigned a value of half the LLOQ. Spearman correlation coefficients were calculated and examined for each sex steroid hormone pair (Table S3).

Among the PLCO samples with sufficient volume, dehydroepiandrosterone (DHEA), 4-androstenenedione (4-dione), Δ5-androstenediol (5-diol), dihydrotestosterone (DHT), androsterone (ADT), and oestrone were measured using GC–MS/MS (Fig. 1). Within-batch CVs ranged from 6.7% to 13.8% (Table S2).

HBsAg was assayed using the Bio-Rad GS HBsAg 3.0 enzyme immunoassay (Bio-Rad Laboratories, Redmond, WA, USA). Antibody to HCV (anti-HCV) was assessed using the Ortho HCV Version 3.0 ELISA test system (Ortho-Clinical Diagnostics, Inc., Raritan, NJ, USA).

Statistical analysis

Participant characteristics were quantified with frequencies or means. The differences between cases and controls were assessed using the chi-square test for categorical variables and the Wilcoxon–Mann-Whitney *U* test for continuous variables. Mean hormone concentration levels were adjusted for parent study and age.

The associations between quartiles of sex steroid hormone concentration levels and risk of liver cancer were examined using conditional multivariable logistic regression to estimate odds ratios (ORs) and 95% CIs. Values of *p* for significance of linear

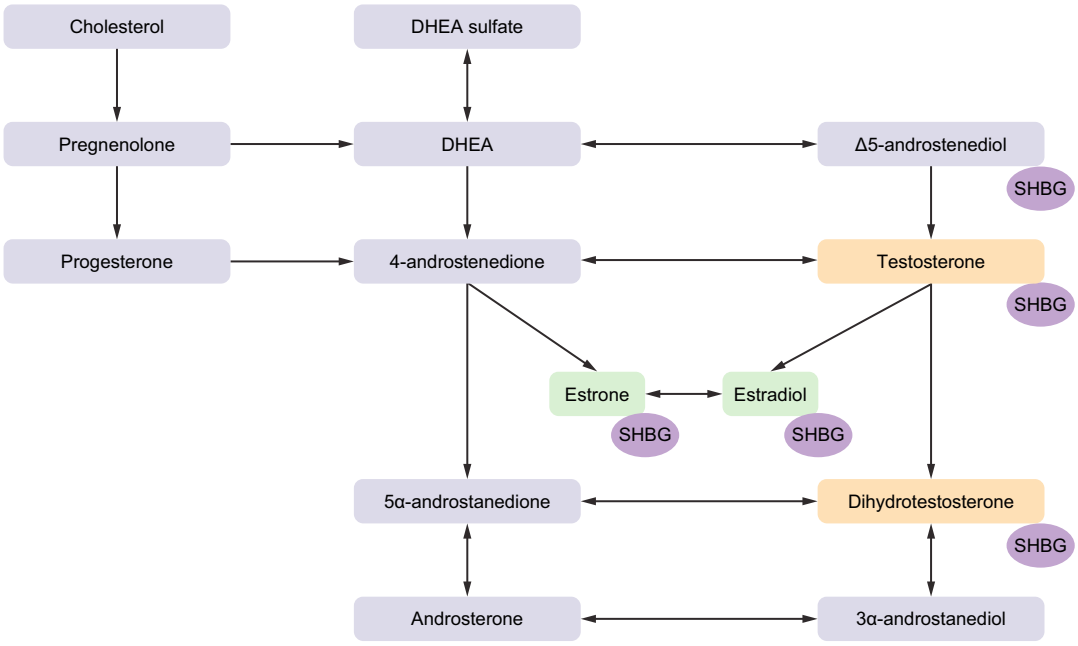

Fig. 1. Schematic of sex steroid hormone metabolism. Quantitated sex steroid hormones/binding globulin are highlighted. Active oestrogens are shown in green. Active androgens are shown in yellow. DHEA, dehydroepiandrosterone; SHBG, sex hormone-binding globulin.

trends were based on the quartile-specific medians of the hormone concentration levels and were estimated using the Wald test. Additionally, hormone concentration values were $\log_2$-transformed (*i.e.* a one-unit increase in $\log_2$ of the transformed is a doubling of circulating concentration) to examine the association between continuous hormone concentration levels and liver cancer risk.

The following covariates, known to be associated with liver cancer, were selected *a priori* to be included in the models: age (continuous in years), BMI (<25, 25 to <30, ≥30 kg/m^2), smoking (never, former, current), current alcohol use (yes, no), positivity for HBsAg (yes, no), positivity for anti-HCV (yes, no), and type 2 diabetes (yes, no). A single imputation using the chained equations method based on multinominal multivariable logistic regression imputed missing values for the variables of race/ethnicity, BMI, smoking status, current alcohol use, positivity for HBsAg, positivity for anti-HCV, and type 2 diabetes. These logistic regression imputation models also included as predictors the variables age and parent study that had no missing values. Imputed values for race/ethnicity (9.9% of controls, 10.9% of cases), BMI (6.9%, 6.9%), smoking status (17.2%, 20.4%), current alcohol use (16.1%, 22.9%), positivity for HBsAg (0.9%, 0.7%), positivity for anti-HCV (0.9%, 0.7%), and diabetes status (10.3%, 14.2%) were used for all main analyses. There was no evidence of effect modification by any covariate after assessing likelihood ratio tests. Mutually adjusting for total testosterone and SHBG or total oestradiol and SHBG in the model did not substantially change the results.

Sensitivity analysis

We conducted an analysis excluding samples with a hormone concentration level measured below the LLOQ. We also stratified cases by histologic type (HCC vs. ICC), where sample size was sufficient. Finally, to examine potential effects on the analyses of latent liver cancer or reverse causation, two lag analyses were conducted by excluding case-control matched sets whose case was diagnosed within 2 and 5 years of parent study enrolment.

Analyses were conducted using SAS, version 9.4 (SAS Institute, Cary, NC, USA). All tests were two-sided and the displayed p values are not adjusted for multiple comparisons. Results for Bonferroni-adjusted p values for multiple comparisons are described in the footnote to Table 1. Owing to the reduced sample sizes with less statistical power, no multiple comparison adjustment was made for the results displayed in Table 2.

Results

Characteristics of the participants at baseline are presented in Table 3. Compared with the controls, cases were less likely to be current alcohol drinkers, and more likely to be HBsAg (+), anti-HCV (+), and to have diabetes.

The age and study-adjusted means of the hormones and hormone ratios are shown in Table 4. Cases had a lower mean concentration of DHEA and higher mean concentrations of testosterone, free testosterone, DHT, oestrone, oestradiol, and SHBG than controls. Cases also had higher testosterone/oestradiol and free testosterone/free oestradiol ratios than did controls.

All cases and controls were included in the examinations of testosterone, oestradiol, SHBG, and the calculations of their free components and ratios of one to the other. As shown in Table 1, testosterone was associated with a 77% increased risk of liver cancer (per one-unit increase in $\log_2$, OR = 1.77, 95% CI = 1.38–2.29). Results were consistent when examined by quartile (quartile 4 *vs.* 1, OR = 3.27, 95% CI = 1.89–5.66, p_{trend} <0.01). In addition, oestradiol was associated with a 58% increased risk (OR = 1.58, 95% CI = 1.22–2.05) and the results were consistent when examined by quartile (OR = 2.62, 95% CI = 1.56–4.41, p_{trend}

Table 1. Characteristics of study participants by case-control status.

Characteristic	Controls (n = 768) n (%)	Cases (n = 275) n (%)	*p* value[1]
Mean age at enrollment (SD), years	53.2 (13.1)	54.2 (12.7)	0.31
Age at enrollment			
<35	80 (10.4)	24 (8.7)	
35–44	138 (18.0)	43 (15.6)	
5–54	145 (18.9)	55 (20.0)	
55–64	242 (31.5)	94 (34.2)	
≥65	163 (21.2)	59 (21.5)	0.77
Race/ethnicity[2]			
White	534 (69.5)	185 (67.3)	
Black	93 (12.1)	39 (14.2)	
Asian/Pacific Islander	116 (15.1)	38 (13.8)	
American Indian/Alaska Native	3 (0.4)	1 (0.4)	
Other	22 (2.9)	12 (4.3)	0.65
Body mass index (kg/m^2)[3]			
<25.0	318 (41.4)	99 (36.0)	
25.0–< 30.0	328 (42.7)	121 (44.0)	
≥30.0	122 (15.9)	55 (20.0)	0.17
Smoking status[4]			
Current	258 (33.6)	84 (30.5)	
Former	285 (37.1)	102 (37.1)	
Never	225 (29.3)	89 (32.4)	0.55
Current alcohol drinker[5]			
Yes	642 (83.6)	210 (76.4)	
No	126 (16.4)	65 (23.6)	0.008
HBsAg[6]			
Yes	9 (1.2)	45 (16.4)	
No	759 (98.8)	230 (83.6)	<0.001
Anti-HCV[7]			
Yes	16 (2.1)	55 (20.0)	
No	752 (97.9)	220 (80.0)	<0.001
Diabetes[8]			
Yes	54 (7.0)	40 (14.5)	
No	714 (93.0)	235 (85.5)	<0.001
Parent Study			
PLCO	263 (34.3)	89 (32.4)	
PHS	80 (10.4)	40 (14.5)	
HPFS	27 (3.5)	9 (3.3)	
CPS-II	18 (2.3)	9 (3.3)	
MHC	380 (49.5)	128 (46.5)	0.37

Abbreviations: kg, kilogram; m, meter; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus.
[1] *p* values for the differences between cases and controls were assessed using the chi-square test for categorical variables and the Wilcoxon-Mann-Whitney test for continuous variables. *p* values <0.05 was considered statistically significant.
[2] 9.9% of values for controls and 10.9% of value for cases imputed.
[3] 6.9% of values for controls and 6.9% of value for cases imputed.
[4] 17.2% of values for controls and 20.4% of value for cases imputed.
[5] 16.1% of controls and 22.9% of value for cases imputed.
[6] 0.9% of value for controls and 0.7% of value for cases imputed.
[7] 0.9% of value for controls and 0.7% of value for cases imputed.
[8] 10.3% of value for controls and 14.2% of value for cases imputed.

<0.01). SHBG was also positivity associated with risk. A doubling in concentration was associated with a 63% increased risk (OR = 1.63, 95% CI = 1.27–2.11). The testosterone/oestradiol ratio was positively associated with risk (OR = 1.30, 95% CI = 1.04–1.64). Results were similar for the free testosterone/free oestradiol ratio (OR = 1.14, 95% CI = 0.94–1.38), although not statistically significant (Table S4).

The analysis of the PLCO samples that were tested for additional hormone concentrations are shown in Table 2. DHT was associated with a 76% increased risk of liver cancer (per one unit increase in $\log_2$, OR = 1.76, 95% CI = 1.21–2.57). Results were consistent when examined by quartile (DHT OR = 2.63, 95% CI = 1.14–6.08, p_{trend} <0.01). None of the other androgens, 4-dione, 5-diol, and ADT, were significantly associated with risk. Oestrone was associated with 74% increased risk (OR = 1.74, 95% CI = 1.08–2.79) and results were consistent when examined by quartile (quartile 4 *vs.* 1, OR = 1.67, 95% CI = 0.70–3.99, p_{trend} = 0.13) although the association did not attain statistically significance. DHEA was associated with a 53% reduced risk of liver cancer (OR = 0.47, 95% CI = 0.33–0.68). Results were consistent when examined by quartile (quartile 4 *vs.* 1, OR = 0.17, 95% CI = 0.06–0.45, p_{trend} <0.01) (Table 2).

In sensitivity analyses that excluded persons with hormone concentrations below the LLOQ and excluded case-control pairs whose case was diagnosed within 2 and 5 years from parent study baseline, the results were similar (Tables S5–S7). When stratifying liver cancer cases by histologic type, results for HCC were similar for most sex steroid hormones except free testosterone, which showed an inverse association with the risk of liver cancer with a doubling in concentration (OR = 0.50, 95% CI =

Table 2. Age- and study-adjusted means[1] and 95% confidence intervals for circulating sex steroid hormone concentrations.

	Overall		Controls		Cases		
Hormone	**Mean**	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**	***p value*[2]**
Dehydroepiandrosterone (DHEA, ng/ml)	2.05	(1.99, 2.12)	2.26	(2.18, 2.33)	1.57	(1.47, 1.68)	*<0.01*
4-Androstenedione (4-dione, ng/ml)	0.91	(0.90, 0.92)	0.88	(0.87, 0.88)	1.02	(0.98, 1.06)	*0.42*
Δ5-androstenediol (5-diol, pg/ml)	654	(644.19, 663.88)	577.67	(572.42, 582.98)	913.88	(853.21, 978.48)	*0.14*
Testosterone (ng/ml)	3.87	(3.81, 3.92)	3.45	(3.41, 3.49)	5.33	(5.25, 5.41)	*<0.01*
Dihydrotestosterone (DHT, pg/ml)	300.34	(298.27, 302.35)	265.49	(263.64, 267.33)	420.27	(411.95, 428.75)	*<0.01*
Androsterone (ADT, pg/ml)	122.18	(121.47, 122.89)	124.34	(123.64, 125.06)	115.86	(113.98, 117.77)	*0.30*
Estrone (pg/ml)	35.17	(34.96, 35.38)	32.16	(32.12, 32.19)	44.85	(43.69, 46.03)	*<0.01*
Estradiol (pg/ml)	21.34	(21.22, 21.45)	20.32	(20.26, 20.37)	24.46	(24.25, 24.67)	*<0.01*
Sex hormone binding globulin (SHBG, nmol/L)	40.52	(39.82, 41.24)	36.61	(35.97, 37.26)	54.27	(52.95, 55.63)	*<0.01*
Free Testosterone (pg/ml)	67.35	(66.23, 68.48)	62.86	(61.70, 64.04)	80.97	(77.99, 84.07)	*<0.01*
Free Estradiol (pg/ml)	0.50	(0.50, 0.51)	0.50	(0.50, 0.50)	0.52	(0.51, 0.52)	*0.18*
Testosterone/Estradiol Ratio	181.18	(179.41, 182.95)	169.61	(167.63, 171.62)	217.81	(217.39, 221.30)	*<0.01*
Free Testosterone/Free Estradiol Ratio	133.62	(131.51, 135.77)	126.27	(124.00, 128.57)	156.34	(150.61, 162.27)	*<0.01*

[1] Standardized to 53.2 years, mean age of controls.
[2] *p* value calculated using the analysis of variance test for the difference in means between cases and controls.

0.30–0.82); results for ICC were generally similar, although the confidence intervals were wider because of the smaller sample size, and most associations did not attain statistical significance (Tables S8).

Discussion

In this nested case-control study of five large prospective cohorts, higher pre-diagnostic concentrations of circulating testosterone, DHT, oestrone, oestradiol, and SHBG were associated with increased risk of liver cancer among men. Conversely, higher levels of DHEA were associated with decreased risk.

Because of the predominance of liver cancer among men, it has been hypothesised that testosterone, the primary active androgen along with DHT, induces or promotes hepatic carcinogenesis, whereas oestradiol, the primary active oestrogen, has a protective effect on liver cancer.[27] Several epidemiology studies have investigated the associations of oestradiol, testosterone,

Table 3. Minimally- and fully-adjusted odds ratios and 95% confidence intervals for associations between quartiles of circulating sex steroid hormone concentrations and liver cancer risk in five U.S. cohorts[1].

			Minimally-Adjusted[2]		Fully-Adjusted[3]	
	Controls	**Cases**	**OR**	**95% CI**	**OR**	**95% CI**
Testosterone (ng/ml)						
0.0–2.985	191	30	1.00		1.00	
2.985–4.199	191	50	1.57	(0.95, 2.61)	1.97	(1.10, 3.52)
>4.199–5.513	191	67	2.22	(1.36, 3.61)	2.09	(1.20, 3.62)
>5.513	191	127	4.41	(2.75, 7.08)	3.27	(1.89, 5.66)
p value for trend[4]				<0.01		<0.01
Continuous (log_2)	764	274	2.35	(1.85, 2.99)	1.77	(1.38, 2.29)
Estradiol (pg/ml)						
0.0–15.813	192	40	1.00		1.00	
>15.813–20.476	191	44	1.09	(0.67, 1.78)	1.01	(0.57, 1.79)
>20.476–26.201	191	70	1.66	(1.06, 2.61)	1.56	(0.92, 2.65)
>26.201	192	120	3.21	(2.08, 4.94)	2.62	(1.56, 4.41)
p value for trend[4]				<0.01		<0.01
Continuous (log_2)	766	274	1.77	(1.42, 2.20)	1.58	(1.22, 2.05)
Sex hormone binding globulin (SHBG, nmol/L)						
0.0–27.920	174	27	1.00		1.00	
>27.920–38.820	174	35	1.38	(0.78, 2.43)	1.19	(0.63, 2.24)
>38.820–55.120	174	55	2.13	(1.24, 3.67)	1.63	(0.89, 2.96)
>55.120	173	120	5.00	(2.99, 8.36)	2.72	(1.52, 4.87)
p value for trend[4]				<0.01		<0.01
Continuous (log_2)	695	237	2.37	(1.90, 2.95)	1.63	(1.27, 2.11)
Testosterone/estradiol Ratio						
0.0–148.059	191	39	1.00		1.00	
>148.059–202.998	191	72	1.67	(1.07, 2.61)	1.57	(0.95, 2.59)
>202.988–261.203	191	68	1.66	(1.06, 2.60)	1.58	(0.93, 2.66)
>261.203	191	95	2.29	(1.48, 3.54)	1.73	(1.01, 2.97)
p value for trend[4]				<0.01		0.07
Continuous (log_2)	764	274	1.47	(1.21, 1.79)	1.30	(1.04, 1.64)

[1] The *p* values displayed in this Table are not adjusted for multiple comparisons. However, the *p* values <0.01 for the fully-adjusted models would be statistically significant even after applying the Bonferroni multiple comparison adjustment for the 4 comparisons displayed.
[2] Minimally adjusted models are adjusted for age (continuous) and matching factors.
[3] Fully adjusted models are adjusted for age (continuous), matching factors, BMI, smoke status, alcohol status, HBV, anti-HCV, and diabetes.
[4] *p* values for linear trend were calculated using the Wald test.

Table 4. Minimally- and fully-adjusted odds ratios and 95% confidence intervals for associations between quartiles of circulating sex steroid hormone concentrations and liver cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO)[1].

			Minimally-Adjusted[2]		Fully-Adjusted[3]	
	Controls	Cases	OR	95% CI	OR	95% CI
Dehydroepiandrosterone (DHEA, ng/ml)						
0.0–0.968	65	42	1.00		1.00	
>0.968–1.469	65	25	0.54	(0.29, 1.01)	0.60	(0.30, 1.21)
>1.469–2.230	66	11	0.23	(0.10, 0.50)	0.26	(0.11, 0.65)
>2.230	65	10	0.19	(0.08, 0.43)	0.17	(0.06, 0.45)
p value for trend[4]				<0.01		*<0.01*
Continuous (log_2)	261	88	0.47	(0.34, 0.65)	0.47	(0.33, 0.68)
4-Androstenedione (4-dione, ng/ml)						
0.0–0.623	65	23	1.00		1.00	
>0.623–0.822	67	23	1.03	(0.53, 1.98)	0.87	(0.41, 1.83)
>0.822–1.108	66	25	1.08	(0.54, 2.14)	1.09	(0.50, 2.39)
>1.108	65	17	0.71	(0.33, 1.54)	0.46	(0.19, 1.12)
p value for trend[4]				0.43		*0.13*
Continuous (log_2)	263	88	0.83	(0.54, 1.29)	0.69	(0.42, 1.15)
Δ5-androstenediol (5-diol, pg/ml)						
0.0–385.926	66	20	1.00		1.00	
>385.926–532.504	65	18	0.95	(0.46, 1.95)	0.71	(0.31, 1.63)
>532.504–733.703	64	19	1.05	(0.51, 2.15)	1.11	(0.51, 2.45)
>733.703	66	31	1.64	(0.84, 3.20)	1.29	(0.60, 2.76)
p value for trend[4]				0.11		*0.27*
Continuous (log_2)	261	88	1.13	(0.85, 1.50)	1.01	(0.75, 1.36)
Dihydrotestosterone (DHT, pg/ml)						
0.0–211.998	65	18	1.00		1.00	
>211.998–288.113	65	9	0.56	(0.24, 1.32)	0.57	(0.21, 1.53)
>288.113–432.840	65	23	1.39	(0.68, 2.83)	1.63	(0.71, 3.74)
>432.840	66	38	2.33	(1.17, 4.64)	2.63	(1.14, 6.08)
p value for trend[4]				<0.01		*<0.01*
Continuous (log_2)	261	88	1.67	(1.22, 2.29)	1.76	(1.21, 2.57)
Androsterone (ADT, pg/ml)						
0.0–94.337	66	31	1.00		1.00	
>94.337–128.956	64	18	0.46	(0.20, 1.03)	0.52	(0.21, 1.28)
>128.956–169.484	66	17	0.41	(0.18, 0.92)	0.50	(0.20, 1.28)
>169.484	65	22	0.53	(0.24, 1.16)	0.50	(0.20, 1.26)
p value for trend[4]				0.16		*0.19*
Continuous (log_2)	261	88	0.79	(0.57, 1.10)	0.71	(0.48, 1.05)
Estrone (pg/ml)						
0.0–24.587	66	16	1.00		1.00	
>24.587–31.738	65	18	1.19	(0.55, 2.57)	0.87	(0.36, 2.10)
>31.738–40.989	64	23	1.65	(0.77, 3.54)	1.20	(0.50, 2.90)
>40.989	66	31	2.08	(1.00, 4.34)	1.67	(0.70, 3.99)
p value for trend[4]				0.03		*0.13*
Continuous (log_2)	261	88	1.90	(1.27, 2.85)	1.74	(1.08, 2.79)

[1] The *p* values for trend are not adjusted for multiple comparisons.
[2] Minimally adjusted models are adjusted for age (continuous) and matching factors.
[3] Fully adjusted models are adjusted for age (continuous), matching factors, BMI, smoke status, alcohol status, HBV, anti-HCV, and diabetes.
[4] *p* values for linear trend were calculated using the Wald test.

and the testosterone/oestradiol ratio with liver cancer in men. Most of the studies have been conducted among Asian populations where either HBV or HCV were the dominant risk factors. These studies include a nested case-control study in Taiwan in which the relative risk of HCC among men with testosterone levels in the upper tertile was 4.1-fold higher than among men with testosterone levels in the middle or lower tertiles.[8] Another nested case-control study by the same group in Taiwan also found that higher levels of testosterone, but not oestradiol or the testosterone/oestradiol ratio, were associated with increased risk of HCC.[28,29] In a prospective study of Japanese men with cirrhosis, higher levels of testosterone (hazard ratio [HR] for upper *vs.* lower tertiles 2.9) and the testosterone/oestradiol ratio (HR for upper *vs.* lower tertiles 4.0) were associated with increased risk of HCC, but no association was found for oestradiol.[30] In another nested case-control study in Shanghai, China, men who were HBsAg-positive and had testosterone levels in the highest tertile had a relative risk for HCC of 12.5 compared with men who were HBsAg-negative and had testosterone levels in the lowest tertile, but no association was found between testosterone levels and HCC risk among HBsAg-negative individuals, suggesting a potential interaction between HBV and testosterone in HCC development.[31] It has been reported that HBV enhances hepatic androgen receptor activity in an androgen-dependent manner and thus amplifies the sex difference in HBV-infected male liver tissues.[32] Men who are HBV carriers tend to have higher testosterone levels than men who are not carriers, which may affect the relationship between testosterone and liver cancer.[31,33]

Fewer studies have been conducted among men in Western populations. A small prospective study of men with cirrhosis in Europe found that testosterone levels were not associated with

HCC risk.[9] A case-control analysis nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort also reported no association between testosterone and HCC risk.[33] However, a recent large prospective study of 182,608 men in the UK Biobank reported that higher total testosterone was associated with an elevated risk of liver cancer.[34] In general, our testosterone results are consistent with several previous studies in that higher testosterone levels were associated with increased risk of liver cancer. One potential mechanism underlying this finding is that the activation of the androgen receptor plays an important role in liver carcinogenesis.[35] This idea is supported by our findings that DHT, the most potent natural androgen that is largely generated from circulating testosterone,[36] was positively associated with risk. In regard to oestrone and oestradiol levels, derived from precursors testosterone and 4-dione, previous studies among men with higher oestradiol levels have not shown a statistically significant increased risk of liver cancer, although most estimates of the association were positive. In contrast, the oestrogen associations were robust in our study, with higher levels of both oestrone and oestradiol being significantly associated with risk. The positive association between oestrogen and liver cancer in our study is in contrast with the hypothesis that oestrogens play a protective role in hepatocarcinogenesis.

SHBG is a circulating protein that binds to sex steroid hormones and modulates their bioavailability.[12] Several prospective studies have reported that SHBG levels were positively associated with liver cancer risk. In the study of men with cirrhosis in Europe, a greater than 3-fold increase in the risk of HCC was observed in association with elevated SHBG levels.[9] Similarly, the EPIC cohort and UK Biobank both reported that higher SHBG levels were associated with elevated risk of liver cancer.[33,34] The study of Japanese men with cirrhosis was the only study to find no association between SHBG and HCC.[30] Because of the limited sample size of the study, however, there may have been insufficient power to detect an association.

DHEA and its plasma reservoir dehydroepiandrosterone sulfate (DHEAS) are the most abundant circulating steroid hormones in humans.[37] DHEA can be converted to DHEAS by DHEA sulfotransferase, whereas DHEAS can be metabolised back to DHEA by steroid sulfatases in circulation.[38] No prior prospective studies of DHEA and liver cancer have been reported. An examination of DHEAS and liver cancer within the EPIC cohort, however, found that DHEAS was inversely associated with risk of HCC.[39] Although the EPIC study included both men and women, the findings were generally consistent with the results of the current study. DHEA is a precursor of both androgens and oestrogens.[36] DHEA can be transformed to oestradiol and DHT, by aromatisation and 5α-reduction of testosterone produced from the transformation of 4-dione, or by directly transformation of 4-dione without testosterone as an intermediate.[40] Our results suggest that a lower capacity to convert the adrenal precursor DHEA to potent androgens and oestrogens could conceivably confer a lower risk of liver cancer, whereas greater capacity to convert DHEA could, in theory, confer increased risk.

Strengths of the current study include that it was a well-characterised investigation nested within five prospective cohorts with large sample sizes and blood samples collected before the diagnosis of liver cancer. In addition, mass spectrometry which is considered to be the gold standard for hormone quantification with well-demonstrated reliability, sensitivity, and accuracy, was used to determine levels of a variety of sex steroid hormones. A limitation was that concentrations of DHEA, 4-dione, 5-diol, DHT, ADT, and oestrone were only tested in samples from one cohort. Although a sensitivity analysis that used study-specific quartiles found that the estimates for testosterone, oestradiol, and SHBG were similar to the main results (data not shown), the possibility that there may be study-specific effects for the other hormones could not be ruled out. In addition, although five cohorts contributed samples to the analysis, the sample size was still somewhat limited to assess associations by liver cancer histology (HCC [n = 98] *vs.* ICC [n = 15]). Nevertheless, this is the first study to explore the association between hormones and ICC risk in men. We used the value of half the LLOQ to impute hormone values below the LLOQ, which may introduce bias into the analysis.[41] However, as the proportion of hormone values below the LLOQ of most hormones are <1%, the impact of the imputation would be limited. In addition, the sensitivity analysis restricted to persons with hormone concentrations above the LLOQ, found results which were similar to the overall results. Finally, as the cases were identified in general cohorts, clinical data on the individuals were not available. As oestradiol and DHEA levels can be affected among men with advanced fibrosis or cirrhosis, the lack of detailed information on any underlying liver disease could have introduced bias. It should be noted, however, that lag analyses that dropped cases in the earlier years of follow-up were conducted and no differences in overall results were identified. Nevertheless, replication of these findings in settings where clinical information is available would be highly desirable.

In conclusion, the current study of US men found that higher levels of androgens and oestrogens were associated with increased risk of liver cancer, findings which do not fully support the hormone hypothesis as currently stated. The inverse association of DHEA, a precursor of both androgens and oestrogens, suggests that a lower capacity to convert DHEA to androgens and oestrogens may confer a lower risk of liver cancer, whereas a greater capacity to convert DHEA could confer increased risk. Future studies with sample sizes sufficiently large to identify differences by liver cancer histology, and in diverse populations, are clearly warranted.

Abbreviations

4-dione, 4-androstenedione; 5-diol, Δ5-androstenediol; ADT, androsterone; anti-HCV, antibody to HCV; CPS-II, Cancer Prevention Study-II Nutrition Cohort; CV, coefficient of variation; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; DHT, dihydrotestosterone; EPIC, European Prospective Investigation into Cancer and Nutrition; GC–MS/MS, gas chromatography–mass spectrometry; HCC, hepatocellular carcinoma; HPFS, Health Professionals Follow-Up Study; HR, hazard ratio; ICC, intrahepatic cholangiocarcinoma; LCPP, Liver Cancer Pooling Project; LLOQ, lower limit of quantification; MHC, Multiphasic Health Checkup Study; NAFLD, non-alcoholic fatty liver disease; NCI, National Cancer Institute; OR, odds ratio; PHS, Physicians' Health Study; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; QC, quality control; SHBG, sex hormone-binding globulin.

Financial support
This work was supported by the Intramural Research Program of the NIH, the National Cancer Institute.

Conflicts of interest
The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions
Conducting the analyses: ZW, AAF. Writing the manuscript: ZW, AAF. Data acquisition: CG, LEBF, JEB, GB, PC, YC, AHE, LSE, NDF, JMG, ELG, JNH, WYH, VAK, CMK, JK, IML, LML, CCN, JRP, MPP, TER, LR, HDS, RS, MJS, CYU, SKVDE, KV, JWW, AZJ, XZ. Critically reviewed manuscript and provided critical feedback: CG, LEBF, JEB, GB, PC, YC, AHE, LSE, NDF, JMG, ELG, JNH, WY, VAK, CMK, JK, IL, LML, CCN, JRP, MPP, TER, LR, HDS, RS, MJS, CYU, SKVDE, KV, JW, AZ, XZ. Study conception and design: JLP, BIG, PTC, KAM. Supervised the project: JLP, BIG, PTC, KAM. Revised the manuscript: JLP, BIG, PTC, KAM.

Data availability statement
Data are available from the corresponding author upon request.

Acknowledgements
For the Health Professionals Follow-up Study, we would like to thank the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The study protocol was approved by the institutional review boards of the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health, and those of participating registries as required. The authors assume full responsibility for analyses and interpretation of these data. The Physicians' Health Study was supported by the National Institutes of Health (Bethesda, MD USA) [Grants CA 097193, CA 34944, CA 40360, HL 26490, and HL 34595]. The study protocol was approved by the institutional review board at Brigham and Women's Hospital.

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2023.100742.

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Author names in bold designate shared co-first authorship

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