HHS Public Access

Author manuscript

Hepatology. Author manuscript; available in PMC 2021 August 01.

Published in final edited form as:

Hepatology. 2020 August ; 72(2): 535–547. doi:10.1002/hep.31057.

Associations between Prediagnostic Concentrations of Circulating Sex Steroid Hormones and Liver Cancer Among Post-Menopausal Women

Jessica L. Petrick[1,2], **Andrea A. Florio**[1], **Xuehong Zhang**[3], **Anne Zeleniuch-Jacquotte**[4,5], **Jean Wactawski-Wende**[6], **Stephen K. Van Den Eeden**[7], **Frank Z. Stanczyk**[8], **Tracey G. Simon**[9], **Rashmi Sinha**[1], **Howard D. Sesso**[10,11], **Catherine Schairer**[1], **Lynn Rosenberg**[2], **Thomas E. Rohan**[12], **Mark P. Purdue**[1], **Julie R. Palmer**[2], **Martha S. Linet**[1], **Linda M. Liao**[1], **I-Min Lee**[10,11], **Jill Koshiol**[1], **Cari M. Kitahara**[1], **Victoria A. Kirsh**[13], **Jonathan N. Hofmann**[1], **Chantal Guillemette**[14], **Barry I. Graubard**[1], **Edward Giovannucci**[9], **J. Michael Gaziano**[11,15], **Susan M. Gapster**[16], **Neal D. Freedman**[1], **Lawrence S. Engel**[17], **Dawn Q. Chong**[18], **Yu Chen**[4,19], **Andrew T. Chan**[3,9,20], **Patrick Caron**[14], **Julie E. Buring**[10,11], **Gary Bradwin**[21], **Laura E. Beane Freeman**[1], **Peter T. Campbell**[16], **Katherine A. McGlynn**[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD,

[2]Slone Epidemiology Center, Boston University, Boston, MA,

[3]Channing Division of Network Medicine, Brigham and Women's Hospital, Boston, MA,

[4]Department of Population Health, New York University School of Medicine, New York, NY,

[5]NYU Perlmutter Cancer Center, New York University School of Medicine, New York, NY,

[6]Department of Epidemiology and Environmental Health, University at Buffalo, Buffalo, NY,

[7]Division of Research, Kaiser Permanente Northern California, Oakland, CA,

[8]Departments of Obstetrics and Gynecology and Preventive Medicine, University of Southern California Keck School of Medicine, Los Angeles, CA,

[9]Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School, Boston, MA,

[10]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA,

[11]Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, MA,

[12]Department of Epidemiology & Population Health, Albert Einstein College of Medicine, Bronx, NY,

[13]Epidemiology Division, Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada,

Corresponding author and address: 72 East Concord Street, Boston, MA, 02118. Phone: 617-206-6179. Fax: 617-728-5119. jpetrick@bu.edu.

Disclosure of Potential Conflicts of Interest: No potential conflicts of interest were disclosed.

[14]Pharmacogenomics Laboratory, Centre Hospitalier Universitaire de Québec - (CHU de Québec) Research Center - Université Laval and Faculty of Pharmacy, Laval University, Québec, Canada,

[15]VA Boston Healthcare System, Boston, MA,

[16]Epidemiology Research Program, American Cancer Society, Atlanta, GA,

[17]Department of Epidemiology, University of North Carolina, Chapel Hill, NC,

[18]Division of Medical Oncology, National Cancer Centre Singapore, Singapore,

[19]Department of Environmental Medicine, New York University School of Medicine, New York, NY,

[20]Clinical and Translational Epidemiology Unit, Massachusetts General Hospital, Boston, MA,

[21]Clinical and Epidemiologic Research Laboratory, Department of Laboratory Medicine, Boston Children's Hospital, Boston, MA

Abstract

Background: In almost all countries, incidence rates of liver cancer are 100–200% higher in males than in females. However, this difference is predominantly driven by hepatocellular carcinoma (HCC), which accounts for 75% of liver cancer cases. Intrahepatic cholangiocarcinoma (ICC) accounts for 12% of cases and has rates only 30% higher in males. Hormones are hypothesized to underlie observed sex differences. We investigated whether prediagnostic circulating hormone and sex hormone binding globulin (SHBG) levels were associated with liver cancer risk, overall and by histology, by leveraging resources from five prospective cohorts.

Methods: Seven sex steroid hormones and SHBG were quantitated using gas chromatography-tandem mass spectrometry (GC-MS/MS) and competitive electrochemiluminescence immunoassay, respectively, from baseline serum/plasma samples of 191 post-menopausal female liver cancer cases (HCC n=83, ICC n=56) and 426 controls, matched on sex, cohort, age, race/ethnicity, and blood collection date. Odds ratios (ORs) and 95% confidence intervals (CIs) for associations between a one-unit increase in $\log_2$ hormone value (approximate doubling of circulating concentration) and liver cancer were calculated using multivariable-adjusted conditional logistic regression.

Results: A doubling in the concentration of 4-androstenedione was associated with a 50% decreased liver cancer risk (OR=0.50,95%CI=0.30–0.82), while SHBG was associated with a 31% increased risk (OR=1.31,95%CI=1.05–1.63). Examining histology, a doubling of estradiol was associated with a 40% increased risk of ICC (OR=1.40,95%CI=1.05–1.89), but not HCC (OR=1.12,95%CI=0.81–1.54).

Conclusions: This study provides the first evidence that higher levels of 4-androstenedione may be associated with lower, and SHBG with higher, liver cancer risk in women. However, this study does not support the hypothesis that higher estrogen levels decrease liver cancer risk. Indeed, estradiol may be associated with an increased ICC risk.

Keywords

cohort study; mass spectrometry; sex hormones; hepatocellular carcinoma; intrahepatic cholangiocarcinoma; human

Introduction

Liver cancer incidence rates have notable sex differences worldwide, with rates among males being 100–200% higher than those among females. However, these differences are largely determined by the dominant histologic type of liver cancer, hepatocellular carcinoma (HCC), which accounts for approximately 75% of cases. The second most common histologic type of liver cancer, intrahepatic cholangiocarcinoma (ICC), accounts for approximately 12% of cases and has incidence rates that are only 30% higher in men (1, 2). Established risk factors that vary in prevalence by sex, including hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, alcohol consumption, smoking, and obesity, cannot fully explain the male predominance of liver cancer (3).

Sex steroid hormones have been proposed as an explanation for the sex differences, with a hypothesized beneficial role for estrogens and a detrimental role for androgens in liver cancer pathogenesis (4, 5). This hypothesis is supported by epidemiologic studies, which have reported an association between oophorectomy and increased liver cancer risk (6, 7). Similarly, animal models have reported that dosing female rodents with testosterone or removing their ovaries increases liver cancer development, while dosing male rodents with estrogen or castrating them reduces liver cancer development (8–10). Several small studies have investigated circulating hormones, primarily testosterone or estradiol, in relation to HCC risk in males (11–15). These studies have reported that testosterone levels are higher in HCC cases. Only one prospective study, has examined this hypothesis in a population that included females, concluding that sex hormone binding globulin (SHBG), but not testosterone, was associated with increased HCC risk (16). However, the study was not powered to examine the associations separately for females.

To date, no prospective study has examined circulating sex steroid hormones in relation to liver cancer risk among female HCC or ICC cases. Additionally, all prior epidemiologic studies of sex steroid hormones and liver cancer have been conducted outside the US (11–16), and endogenous hormone levels have been shown to vary by geography (17). Thus, we investigated whether prediagnostic circulating sex steroid hormone concentrations were associated with liver cancer risk in US females.

Methods

Study Population.

The current study was nested in the Liver Cancer Pooling Project (7) and leveraged the resources from five prospective parent cohort studies: Multiphasic Health Checkup Study (MHC) (18); New York University Women's Health Study (NYUWHS) (19); Nurses' Health Study (NHS) (20); Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) (21); and Women's Health Initiative (WHI) (22) (Supplemental Table S1). All studies received Institutional Review Board and data sharing approvals from their host institutions and the NCI.

The current study was restricted to post-menopausal women at blood draw, as there are known differences in circulating sex steroid hormones between pre- and post-menopausal

women (23). Menopausal status was determined through a combination of self-report, age at blood draw, and levels of estrone and estradiol. Liver cancer, defined as incident primary liver cancer (International Classification of Diseases, 10th edition [ICD-10] diagnostic code C22), was ascertained by linkage to state cancer registries or medical/pathology record review. Cases were further classified using International Classification of Diseases for Oncology, 3rd edition (ICD-O-3) morphology codes 8170–8175 for HCC and 8032–8033, 8041, 8050, 8070–8071, 8140–8141, 8160, 8260, 8480, 8481, 8490, and 8560 for ICC.

Controls were individually matched to cases using incidence-density sampling based on parent cohort, age, race/ethnicity, and date of baseline blood collection (±3-months). A total of 191 post-menopausal cases and 426 controls were included in the analysis: 101 participants from MHC, 44 from NYUWHS, 40 from NHS, 80 from the PLCO, and 352 from WHI.

Laboratory Methods.

Steroid hormone assays were performed at the Pharmacogenomics Laboratory of Laval University (Quebec, Canada) (24). All samples were quantitated for estradiol and testosterone, using a gas chromatography selected reaction monitoring–tandem mass spectrometry assay (GC-MS/MS; Figure 1). In studies with sufficient volume available, samples were also quantitated for estrone, dehydroepiandrosterone (DHEA), 4-androstenedione, and Δ−5-androstenediol using GC-MS/MS (Supplemental Table S2). For hormone values below the lower limit of quantification (LLOQ), a value of ½ LLOQ was assigned. Blinded quality control (QC) replicate samples were included in each batch (n=81 total QC samples). Within and between batch coefficients of variation (CVs) were <17%, and the intraclass correlation coefficient (ICC) was >0.80 for all hormones.

SHBG was quantitated in all samples at the Clinical and Epidemiologic Research Laboratory of Boston Children's Hospital (Boston, MA) using a competitive electrochemiluminescence immunoassay on the Roche E Modular system (Roche Diagnostics, Indianapolis, IN). For SHBG, the within and between batch CVs were <3%, and the ICC was 0.99.

In addition to the individual hormones, testosterone:estradiol ratio, androstenedione:estrone ratio, free estradiol (25), and free testosterone (26) were calculated. Circulating hormone concentrations were categorized in quartiles, based on the control distribution. Tests of linear trend were performed using quartile-specific hormone concentration medians. As continuous hormone values were right skewed, values were also $\log_2$ transformed, which corresponds to a doubling of circulating hormone concentration per unit change.

To determine HBV status, hepatitis B surface antigen (HBsAg) was assayed using the Bio-Rad GS HBsAg 3.0 enzyme immunoassay (Bio-Rad Laboratories, Redmond, WA, USA). For determination of HCV status, antibody to HCV (anti-HCV) was assessed using the Ortho HCV Version 3.0 ELISA test system (Ortho-Clinical Diagnostics, Inc.).

Data and Sample Collection.

MHC collected non-fasting blood samples from Kaiser Permanente Northern California members who underwent a multiphasic health checkup in Oakland or San Francisco. Serum samples were initially stored at −23°C, and then since 1980, stored at −40°C (18). NYUWHS collected non-fasting blood samples from during the baseline visit; serum samples were stored at −80°C (19). NHS collected blood samples via mail, and participants reported fasting status. Plasma samples were stored at −130°C (20). PLCO collected blood samples from participants in the screening intervention study arm during baseline visit. Fasting status was not ascertained; serum samples were stored at −70°C (21). WHI collected overnight fasting blood samples during the baseline visit. Serum samples were initially frozen at −70°C; after arriving at the biorepository, samples were stored at −80°C (22). Prior studies have shown long-term stability of sex steroid hormones at ≤−20°C (27–29).

Statistical Analysis.

Geometric mean hormone concentration levels were adjusted for parent study and age; ANOVA was used to compare cases and controls. Differences in potential covariates between cases and controls were assessed by either the chi-square or Fisher's exact test for categorical variables and the Wilcoxon-Mann-Whitney test for continuous variables. Odds ratios (ORs) and 95% confidences intervals (CIs) were estimated using conditional logistic regression to examine the association between circulating hormone concentrations and liver cancer risk. Liver cancer cases were also stratified by histology (HCC vs. ICC), where sample size permitted (n>5 cases in each quartile).

Values for missing covariates were derived using a single imputation: race (5.2% of cases, 6.1% of controls), BMI (4.2%, 3.5%), smoking status (5.8%, 6.8%), alcohol consumption (6.3%, 7.0%), HBV status (0.5%, 0.2%), HCV infection (0.5%, 0.2%), diabetes (4.2%, 3.8%), and menopausal hormone therapy (MHT) use (7.3%, 6.8%). We also examined results utilizing a complete case analysis (i.e., whereby only observations with complete covariates were maintained) or creating a 'missing' covariate category, and results did not differ (data not shown). Confounding was assessed by first determining whether covariates were associated with exposure among the controls and with the outcome among the unexposed (i.e., the lowest quartile of each hormone concentration) (30). If a potential confounder was associated with the exposure and outcome, then each covariate was removed from the full model one at a time to evaluate if the log odds ratio changed by ≥10%. If the change occurred for any hormone, the variable was considered a confounder and remained in the model. All potential covariates met these criteria and were included in the final models: age (continuous), BMI (<25, 25-<30, ≥30 kg/m^2), smoking (never, former, current), current alcohol use, HBsAg, anti-HCV, self-reported diabetes, and current MHT use. Using likelihood ratio tests, effect measure modification of the relationships between log_2 transformed hormones and liver cancer were assessed by the covariates included in the final models. There was no evidence of effect measure modification by any of the covariates (all Ps≥0.05). All tests were 2-sided. Analyses were conducted using SAS, version 9.4 (SAS Institute, Cary, NC).

Sensitivity Analysis.

In addition to calculating quartiles based on the overall control distribution, we calculated study-specific quartiles, and tests of linear trend were performed based on the quartile score. To evaluate the possibility of reverse causation, we conducted three lag analyses excluding case-control pairs whose case was diagnosed within 2, 5, and 10 years of the date of blood donation. As WHI accounted for roughly half of the participants, we examined associations between estradiol, testosterone, and SHBG excluding these participants. To examine if there was residual confounding by BMI, we examined BMI as a continuous variable. Finally, we excluded individuals with hormone concentrations below the LLOQ.

Results

As shown in Table 1, controls were more likely than cases to have a BMI <25 kg/m^2 (43.0% vs. 34.0%) and to drink alcohol at study baseline (60.1% vs. 48.7%). Controls were less likely to be anti-HCV(+) (2.1% vs. 18.8%) and have diabetes (5.2% vs. 13.1%). Cases had higher mean concentrations of estrogens than controls (e.g., estradiol 7.46 vs. 6.70 pg/mL), while controls had higher mean concentrations of most androgens (e.g., testosterone 0.17 vs. 0.18 ng/mL).

Estradiol was not associated with liver cancer or HCC risk. However, a doubling in the concentration of estradiol was associated with a 40% increased ICC risk (OR per one unit increase in $\log_2$ estradiol=1.40,95%CI=1.05–1.89;P=0.02;Table 2). Results were consistent when we examined quartiles of estradiol concentration (OR quartile 4 vs. 1=5.72,95%CI=1.18–27.76;P_{trend}=0.04), although the referent quartile only included five cases. The association between free estradiol and ICC was similar to the estradiol-ICC association. No associations were seen with estrone.

A doubling in the concentration of 4-androstenedione was associated with a 50% reduction in liver cancer risk (OR=0.50,95%CI=0.30–0.82;P=0.006;Table 2). The highest quartile of 4-androstenedione was associated with a 73% reduced liver cancer risk (OR=0.27,95%CI=0.09–0.80;P_{trend}=0.01). Similar inverse trends were observed for HCC and ICC, although neither association attained statistical significance. A doubling in the concentration of free testosterone was modestly associated with a decreased liver cancer risk (OR=0.81,95%CI=0.68–0.96;P=0.01). Similar, although not statistically significant, associations with free testosterone were observed for both HCC and ICC. No other associations were observed between androgens and liver cancer risk.

For SHBG, a doubling in the concentration was associated with a 31% increased liver cancer risk (OR=1.31,95%CI=1.05–1.63;P=0.02;Table 2). A similar association was also observed for SHBG and HCC risk (OR=1.60,95%CI=1.14–2.22;P=0.006). The highest quartile of SHBG was associated with a 4.8-times increased HCC risk (OR=4.84,95%CI=1.31–17.82;P_{trend}=0.01). There was little to no association with ICC risk (OR per one unit increase in $\log_2$ SHBG=1.13,95%CI=0.72–1.78;P=0.6).

Androgen:estrogen ratio metrics were associated with a decreased ICC risk (Table 2). A doubling of the testosterone:estradiol ratio was associated with 30% decreased ICC risk

(OR=0.70,95%CI=0.55–0.90;P=0.009). Results were similar for the ratio of free testosterone:free estradiol.

Demographics by parent study are shown in Supplemental Table S3. To examine potential differences by parent study, we conducted a sensitivity analysis using study-specific quartiles of hormone concentrations; results were similar (Supplemental Table S4). Adjustment for covariates had little effect on the observed estimates, as evidenced in the minimally adjusted models (Supplemental Table S5). Follow-up in the parent cohorts varied from 16–45 years (Supplemental Table S1); the mean duration between blood draw and liver cancer diagnosis was 7.7 years.

Having an undiagnosed liver cancer at baseline could, in theory, affect hormone concentrations. Thus, we conducted lag analyses excluding case-control pairs whose case was diagnosed within 2, 5, and 10 years from parent study baseline; results were similar (Supplemental Table S6). When we excluded WHI, adjusted for BMI as continuous, or excluded hormone values below the LLOQ, results were similar (data not shown).

Conclusion

In an analysis combining data from five large prospective cohort studies, higher prediagnostic concentrations of circulating 4-androstenedione were associated with 50% reduction in liver cancer risk in post-menopausal females, while higher levels of SHBG were associated with a 31% increased risk. Additionally, higher levels of estradiol and free estradiol were associated with a 40–43% increased risk of ICC, but not HCC.

This is the first study to examine the association between prediagnostic sex steroid hormone levels and liver cancer risk in post-menopausal women. Prior studies conducted in Asian populations reported that testosterone levels were higher in male HCC cases compared to controls (11–14). However, a primary risk factor for liver cancer in Asia is HBV, and it has been reported that HBV X protein enhances androgen receptor-responsive gene expression in a manner that is dose-dependent on circulating androgen levels, possibly accounting for a portion of the male-predominance of HBV-related HCC (31). In a study from Shanghai, China, the highest level of serum testosterone was associated with a 13-fold increased liver cancer risk among HBsAg-positive male participants, but there was no association among HBsAg-negative participants (11). Two European studies have investigated hormones and SHBG, one in a population of males with cirrhosis and one in a general population study that included females (15, 16). Similar to the current report, these studies found that SHBG, but not testosterone, was associated with increased HCC risk.

4-androstenedione is the only circulating androgen that has higher concentrations in pre-menopausal women than in men (23). In pre-menopausal women with oophorectomy or in post-menopausal women, circulating 4-androstenedione is decreased by 50% (32). 4-androstenedione serves as a prohormone and can be converted to testosterone by 17β-hydroxysteroid dehydrogenase or to estrone by aromatase. Additionally, 4-androstenedione itself has weak androgenic activity, which is approximately 10% that of testosterone (23). Reasons for an inverse association between circulating 4-androstenedione concentration and

liver cancer risk are not completely understood. In experimental studies of both male and female rodents, hepatocarcinogenesis has been promoted by 4-androstenedione, which is thought to cause positive regulation of male gene expression through STAT5b (33–35). However, the animal studies administered exogenous androstenedione compounds via oral gavage. Thus, the relevance to humans is questionable. Alternatively, androgens are known to induce immunosuppression, which is thought to be related to the female preponderance of autoimmune diseases (36). In a study examining modulation of thymocyte (i.e., precursor of T lymphocytes) proliferation, testosterone and DHT inhibited, while androstenedione increased, thymocyte proliferation (37). Taken together, these studies suggest that testosterone and DHT promote immunosuppression, which is typically seen in males, while androstenedione enhances immunostimulation, suggesting a female-type immune response that may be beneficial in suppressing liver cancer development.

SHBG levels are typically twice as high in women as in men, due to estrogen promotion and androgen inhibition of SHBG production (23). Two prior studies have found similar associations to those reported herein between circulating SHBG and liver cancer risk (15, 16), but the mechanisms underlying this association are unclear. Studies of HCV-infected individuals reported that SHBG levels were positively associated with severity of liver disease in men (38), but this association was not observed in women (39). However, it has been hypothesized that SHBG may be a marker of liver damage. The mechanism underlying this correlation between SHBG and liver damage has been suggested to be through IGF-1, which downregulates SHBG production (40). Concentrations of IGF-1 have been shown to decrease preceding HCC diagnosis (41, 42). In our lag analysis, the association between SHBG and liver cancer risk was robust, even after excluding participants that developed liver cancer within 10 years of blood draw. However, this could suggest that IGF-1 and SHBG are dysregulated early in hepatocarcinogenesis.

In contrast to the hypothesis that higher levels of estrogens decrease liver cancer risk, we report that higher levels of estradiol and free estradiol were associated with an increased ICC risk and had no association with HCC. A prior study from Thailand similarly reported higher estradiol in male cholangiocarcinoma patients (n=54) compared to controls (n=68) (43) but did not examine women and nor stratify by cholangiocarcinoma topography (i.e., intrahepatic versus extrahepatic). Additional experimental studies have implicated factors that affect estrogenic regulation in ICC development. In a normal liver, cholangiocytes have undetectable levels of estrogen receptors, but ICC tumors express both estrogen receptor-α and -β (44–46). Experimental evidence suggests that estrogen, potentially mediated through interleukin-6 or vascular endothelial growth factor, promotes cholangiocarcinogenesis, while selective estrogen receptor modulators can inhibit growth (47–49). Taken together, these observations raise the provocative hypothesis that estrogen could partially explain the sex differences reported in incidence rates of ICC and HCC.

Strengths of this study include use of state-of-the-art quantitation of sex steroid hormones, serum samples collected prior to incident diagnosis of cancer, and large sample size. Prior studies utilized radioimmunoassay for hormone quantitation (11–16, 43). While correlations between radioimmunoassay and mass spectrometry technologies are high, mass spectrometry is considered to be the gold standard for hormone quantification, as it has

greater sensitivity and accuracy of detection (50). The study design, which entailed pooling data across five prospective cohort studies, allowed for the first study of the association between hormones and liver cancer risk in a female population. The sample size also allowed for stratification by liver cancer type – HCC and ICC. However, the samples sizes for HCC and ICC were still somewhat limited, which made it difficult to determine differences in associations by liver cancer histology.

Limitations of the current study include lack of information on preexisting liver disease and generalizability. We utilized information and serum samples collected prior to diagnosis among women enrolled in prospective cohort studies. However, none of these studies were specifically designed to assess liver cancer outcomes and provided no information on possible preexisting liver diseases. As underlying disease processes might affect hormone concentrations, we conducted a lag analysis. Results were similar, suggesting that preexisting liver disease did not substantially affect our findings. However, we cannot definitively say that potential preexisting liver disease was excluded in these lag analyses. Further, this study is only among post-menopausal females, thus may not be generalizable to men and pre-menopausal women. The great majority of liver cancer among women in the US, however, occurs among post-menopausal women. Studying risk factors for liver cancer among women is of vital as the forecast rates of liver cancer in women are increasing faster than among men (51). Thus, women will share a larger burden of liver cancer in the future.

In summary, this study does not support the hypothesis that higher levels of estrogens decrease, or androgens increase, liver cancer risk among postmenopausal women. We report that among women, higher 4-androstenedione levels were associated with a decreased liver cancer risk, while higher SHBG levels were associated with an increased risk. Further, higher levels of circulating estradiol and free estradiol were associated with a significantly increased risk of ICC, but not HCC. These results provide evidence of etiological heterogeneity by liver cancer histologic type with regard to the roles of estrogens and androgens. While intriguing, these findings need to be replicated in additional populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

For the Nurses' Health 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 authors assume full responsibility for analyses and interpretation of these data.

For the Women's Health Initiative, the full list of investigators that have contributed can be found on the following website: https://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf.

Funding: NIH Intramural Research Program, National Cancer Institute (JL Petrick, AA Florio, LE Beane-Freeman, JN Hofmann, CM Kitahara, ND Freedman, BI Graubard, J Koshiol, LM Liao, MS Linet, MP Purdue, C Schairer, R Sinha, KA McGlynn). National Institutes of Health grants CA047988 (I Lee, JE Buring), CA182913 (I Lee, JE Buring), HL043851 (I Lee, JE Buring), HL080467 (I Lee, JE Buring), HL099355 (I Lee, JE Buring), K07 CA188126 (X Zhang), DK098311 (AT Chan), CA186107 (AT Chan), CA87969 (AT Chan), CA167552 (AT Chan), UM1 CA182934 (A Zeleniuch-Jacquotte), P30 CA016087 (A Zeleniuch-Jacquotte), P30 ES000260 (A Zeleniuch-

Jacquotte), U01 CA164974 (L Rosenberg, JR Palmer), and R01 CA058420 (L Rosenberg, JR Palmer). American Cancer Society Research Scholar Grant RSG NEC-130476 (X Zhang). The WHI program (J Wactawski-Wende, TE Rohan) is funded by the National Institutes of Health contracts HHSN268201600018C, HHSN268201600001C, HHSN268201600002C, HHSN268201600003C, and HHSN268201600004C. The HPFS and NHS programs (X Zhang) were support by the National Cancer Institute, National Institutes of Health grant numbers UM1CA186107, P50CA127003, P01CA87969, and UM1CA167552.

Abbreviations:

MHC	Multiphasic Health Checkup Study
NYU	New York University
NHS	Nurses' Health Study
PLCO	Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial
WHI	Women's Health Initiative
NCI	National Cancer Institute
HCC	hepatocellular carcinoma
ICC	intrahepatic cholangiocarcinoma
OR	odds ratio
CI	confidence interval
DHEA	dehydroepiandrosterone
4-dione	4-androstenedione
5-diol	Δ−5-androstenediol
T	testosterone
DHT	dihydrotestosterone
ADT	androsterone
3β-diol	5-α -androstane-3β,17β-diol
E1	estrone
E2	estradiol
SHBG	sex hormone binding globulin
p	progesterone
LLOQ	lower limit of quantification
HBsAg	hepatitis B surface antigen
anti-HCV	antibody to HCV
MHT	menopausal hormone therapy

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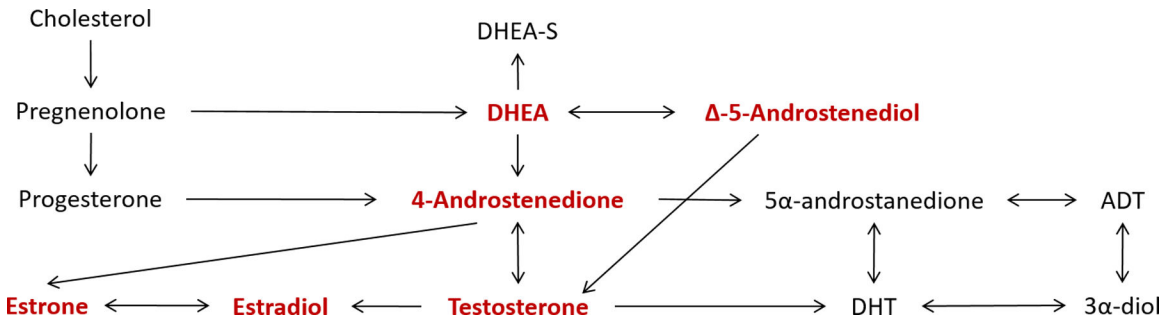

Figure 1.

Schematic of sex steroid hormone metabolism. Quantitated sex steroid hormones are highlighted in red. Sex hormone binding globulin (SHBG) is not shown, as it is not part of the sex steroid metabolism pathway.

Table 1.

Characteristics of study participants by case-control status.

Characteristic	Controls (n=426) N (%)		Cases (n=191) N (%)		P-value
Mean age at baseline (SD), years	62.5	(6.9)	62.8	(7.2)	0.5
Race					
White	324	(76.1)	141	(73.8)	
Black	47	(11.0)	22	(11.5)	
Asian/Pacific Islander	27	(6.3)	14	(7.3)	
American Indian/Alaska Native	2	(0.5)	1	(0.5)	
Other	26	(6.1)	13	(6.8)	1.0
Body mass index (kg/m^2)					
<25.0	183	(43.0)	65	(34.0)	
25.0 – <30.0	151	(35.5)	72	(37.7)	
≥ 30.0	92	(21.6)	54	(28.3)	0.07
Smoking status					
Never	237	(55.6)	98	(51.3)	
Former	146	(34.3)	71	(37.2)	
Current	43	(10.1)	22	(11.5)	0.6
Current alcohol drinker					
No	170	(39.9)	98	(51.3)	
Yes	256	(60.1)	93	(48.7)	0.008
Chronic hepatitis B virus infection (HBsAg)					
No	420	(98.6)	186	(97.4)	
Yes	6	(1.4)	5	(2.6)	0.3
Chronic hepatitis C virus infection (anti-HCV)					
No	417	(97.9)	155	(81.2)	
Yes	9	(2.1)	36	(18.8)	<0.001
Diabetes					
No	404	(94.8)	166	(86.9)	
Yes	22	(5.2)	25	(13.1)	<0.001
Current menopausal hormone therapy use					
No	283	(66.4)	128	(67.0)	
Yes	143	(33.6)	63	(33.0)	0.9
Parent study					
Multiphasic Health Checkup Study (MHC)	71	(16.7)	30	(15.7)	
New York University Women's Health Study (NYUWHS)	33	(7.8)	11	(5.8)	
Nurses' Health Study (NHS)	28	(6.6)	12	(6.3)	
Prostate, Lung, Colorectal, and Ovarian Cancer Trial (PLCO)	60	(14.1)	20	(10.5)	
Women's Health Initiative (WHI)	234	(54.9)	118	(61.8)	0.5
Hormone Concentrations, *age- and study-adjusted mean (95% CI)* [1]					
Estradiol (pg/mL)	6.70	(6.52, 6.88)	7.46	(6.90, 8.07)	0.2

Characteristic	Controls (n=426) N (%)		Cases (n=191) N (%)		P-value
Estrone (pg/mL)	31.29	(30.34, 32.26)	39.04	(36.13, 42.18)	0.4
Testosterone (ng/mL)	0.18	(0.17, 0.18)	0.17	(0.16, 0.17)	0.4
Dehydroepiandrosterone (ng/mL)	1.71	(1.68, 1.74)	1.40	(1.37, 1.43)	0.05
4-Androstenedione (ng/mL)	0.46	(0.46, 0.47)	0.41	(0.41, 0.42)	0.04
Δ−5-androstenediol (pg/mL)	272.76	(264.97, 280.82)	276.80	(268.43, 285.43)	0.8
Sex hormone binding globulin (nmol/L)	78.47	(77.53, 79.41)	96.66	(95.26, 98.07)	<0.001
Free estradiol (pg/mL)	0.12	(0.12, 0.12)	0.12	(0.11, 0.13)	1.0
Free testosterone (pg/mL)	1.67	(1.62, 1.72)	1.33	(1.29, 1.36)	<0.001
Testosterone:Estradiol (pg/mL)	26.67	(25.51, 27.88)	21.78	(20.01, 23.71)	0.04
Free testosterone:Free estradiol (pg/mL)	13.86	(13.23, 14.53)	10.67	(9.81, 11.61)	0.01
4-Androstenedione:Estrone	0.01	(0.01, 0.01)	0.02	(0.01, 0.02)	0.03

[1] Standardized to 62.5 years, mean age among controls.

Table 2.

Adjusted[1] odds ratios (OR) and 95% confidence intervals (CI) for associations between circulating sex steroid hormone concentrations and liver cancer risk.

		Liver Cancer				Hepatocellular Carcinoma[2]				Intrahepatic Cholangiocarcinoma[2]			
		Controls	Cases	OR	95% CI	Controls	Cases	OR	95% CI	Controls	Cases	OR	95% CI
Estradiol (pg/mL)													
Q1	0 to 3.22	108	38	1.00		34	12	1.00		26	5	1.00	
Q2	>3.22 to 6.25	105	48	1.18	(0.63, 2.22)	44	23	2.28	(0.59, 8.86)	31	15	2.87	(0.72, 11.53)
Q3	>6.25 to 14.58	107	54	1.39	(0.71, 2.72)	53	24	1.13	(0.28, 4.62)	33	17	4.61	(0.97, 21.94)
Q4	>14.58	106	51	1.50	(0.73, 3.06)	49	24	2.52	(0.57, 11.22)	29	17	5.72	(1.18, 27.76)
p-value for trend[3]					*0.2*				*0.5*				*0.04*
Continuous (log_2)		426	191	1.08	(0.93, 1.25)	180	83	1.12	(0.81, 1.54)	119	54	1.40	(1.05, 1.89)
Estrone (pg/mL)													
Q1	0 to 13.64	48	17	1.00		7	2	—		6	1	—	
Q2	>13.64 to 23.86	48	20	1.18	(0.50, 2.77)	11	6	—		7	3	—	
Q3	>23.86 to 45.13	48	17	0.87	(0.32, 2.34)	18	4	—		11	4	—	
Q4	>45.13	48	19	0.92	(0.32, 2.63)	14	6	—		14	5	—	
p-value for trend[3]					*0.8*				*0.7*				*0.9*
Continuous (log_2)		192	73	1.11	(0.83, 1.49)	50	18	1.32	(0.29, 5.90)	38	13	1.26	(0.58, 2.71)
Testosterone (ng/mL)													
Q1	0 to 0.12	107	50	1.00		50	20	1.00		32	21	1.00	
Q2	>0.12 to 0.17	107	52	1.23	(0.71, 2.13)	47	21	1.95	(0.74, 5.13)	37	11	0.31	(0.10, 0.95)
Q3	>0.17 to 0.25	106	40	0.79	(0.44, 1.42)	50	19	1.28	(0.42, 3.87)	24	11	0.56	(0.19, 1.64)
Q4	>0.25	106	49	0.99	(0.57, 1.74)	33	23	2.44	(0.77, 7.68)	32	13	0.51	(0.19, 1.38)
p-value for trend[3]					*0.6*				*0.3*				*0.4*
Continuous (log_2)		426	191	0.90	(0.75, 1.08)	180	83	1.34	(0.93, 1.94)	125	56	0.74	(0.49, 1.12)
Dehydroepiandrosterone (ng/mL)													
Q1	0 to 1.16	48	20	1.00		11	6	—		12	3	—	
Q2	>1.16 to 1.82	48	21	1.57	(0.66, 3.76)	13	7	—		12	6	—	
Q3	>1.81 to 2.82	48	19	1.10	(0.45, 2.67)	15	4	—		6	2	—	

		Liver Cancer				Hepatocellular Carcinoma[2]				Intrahepatic Cholangiocarcinoma[2]			
		Controls	Cases	OR	95% CI	Controls	Cases	OR	95% CI	Controls	Cases	OR	95% CI
Q4	>2.82	48	13	0.64	(0.24, 1.70)	11	1	—		11	3	—	
p-value for trend[3]					*0.3*				*0.2*				*0.2*
Continuous (log_2)		192	73	0.75	(0.52, 1.08)	50	18	0.14	(0.01, 2.77)	41	14	2.98	(0.48, 18.68)
4-Androstenedione (ng/mL)													
Q1	0 to 0.36	48	25	1.00		12	5	—		11	4	—	
Q2	>0.36 to 0.51	48	23	1.04	(0.46, 2.39)	15	7	—		11	5	—	
Q3	>0.51 to 0.69	48	13	0.54	(0.22, 1.32)	15	4	—		11	3	—	
Q4	>0.69	47	12	0.27	(0.09, 0.80)	8	2	—		9	2	—	
p-value for trend[3]					*0.01*				*0.7*				*0.4*
Continuous (log_2)		191	73	0.50	(0.30, 0.82)	50	18	0.69	(0.07, 6.68)	42	14	0.48	(0.09, 2.69)
Δ−5-androstenediol (pg/mL)													
Q1	0 to 194.30	48	20	1.00		16	7	—		14	3	—	
Q2	>194.30 to 355.56	48	17	1.32	(0.50, 3.47)	15	4	—		11	5	—	
Q3	>355.56 to 497.24	48	18	1.20	(0.44, 3.24)	12	7	—		10	2	—	
Q4	>497.24	47	18	0.83	(0.28, 2.46)	7	0	—		6	4	—	
p-value for trend[3]					*0.8*				*0.4*				*0.5*
Continuous (log_2)		191	73	0.95	(0.64, 1.40)	50	18	15.33	(0.22, 1078.12)	41	14	1.64	(0.54, 4.94)
Sex hormone binding globulin (nmol/L)													
Q1	0 to 53.76	107	43	1.00		38	12	1.00		34	21	1.00	
Q2	>53.76 to 81.61	106	30	0.78	(0.43, 1.42)	41	10	1.11	(0.31, 4.01)	32	8	0.33	(0.11, 0.96)
Q3	>81.61 to 130.10	107	46	1.24	(0.70, 2.21)	48	19	2.54	(0.76, 8.57)	33	12	0.76	(0.26, 2.22)
Q4	>130.10	106	72	1.81	(0.94, 3.47)	53	42	4.84	(1.31, 17.82)	26	15	1.27	(0.39, 4.18)
p-value for trend[3]					*0.06*				*0.01*				*0.9*
Continuous (log_2)		426	191	1.31	(1.05, 1.63)	180	83	1.60	(1.14, 2.22)	125	56	1.13	(0.72, 1.78)
Free estradiol (pg/mL)													
Q1	0 to 0.06	106	37	1.00		30	16	1.00		28	2	—	
Q2	>0.06 to 0.13	107	51	1.52	(0.78, 2.96)	51	23	1.32	(0.36, 4.80)	32	17	—	
Q3	>0.13 to 0.23	106	47	1.36	(0.69, 2.69)	57	20	0.70	(0.20, 2.47)	29	14	—	

		Liver Cancer				Hepatocellular Carcinoma[2]				Intrahepatic Cholangiocarcinoma[2]			
		Controls	**Cases**	**OR**	**95% CI**	**Controls**	**Cases**	**OR**	**95% CI**	**Controls**	**Cases**	**OR**	**95% CI**
Q4	>0.23	107	56	1.88	(0.91, 3.90)	42	24	1.28	(0.31, 5.36)	36	23	—	
p-value for trend[3]					*0.2*				*1.0*				*0.04*
Continuous (log_2)		426	191	1.01	(0.87, 1.17)	180	83	0.89	(0.62, 1.26)	125	56	1.43	(1.04, 1.95)
Free testosterone (pg/mL)													
Q1	0 to 0.94	107	64	1.00		47	35	1.00		34	16	1.00	
Q2	>0.94 to 1.56	106	49	0.86	(0.50, 1.49)	56	16	0.45	(0.17, 1.17)	29	17	1.39	(0.50, 3.88)
Q3	>1.56 to 2.60	106	33	0.57	(0.32, 1.03)	42	14	0.50	(0.17, 1.47)	29	9	0.66	(0.20, 2.15)
Q4	>2.60	107	45	0.69	(0.38, 1.23)	35	18	0.59	(0.19, 1.87)	33	14	0.88	(0.32, 2.44)
p-value for trend[3]					*0.1*				*0.3*				*0.5*
Continuous (log_2)		426	191	0.81	(0.68, 0.96)	180	83	0.90	(0.66, 1.23)	125	56	0.76	(0.53, 1.08)
Testosterone:Estradiol													
Q1	0 to 12.67	105	55	1.00		53	24	1.00		33	26	1.00	
Q2	>12.67 to 27.26	105	54	0.93	(0.52, 1.65)	53	22	1.40	(0.52, 3.78)	34	18	0.47	(0.17, 1.27)
Q3	>27.26 to 56.71	105	38	0.60	(0.32, 1.12)	41	20	1.59	(0.51, 5.02)	34	7	0.25	(0.09, 0.76)
Q4	>56.71	104	41	0.78	(0.39, 1.56)	27	15	2.02	(0.56, 7.21)	24	5	0.29	(0.07, 1.09)
p-value for trend[3]					*0.2*				*0.3*				*0.01*
Continuous (log_2)		419	188	0.89	(0.78, 1.02)	174	81	1.09	(0.82, 1.44)	125	56	0.70	(0.55, 0.90)
Free testosterone:Free estradiol													
Q1	0 to 6.52	104	58	1.00		54	25	1.00		33	25	1.00	
Q2	>6.52 to 15.49	105	52	0.82	(0.46, 1.47)	49	25	1.27	(0.46, 3.48)	37	17	0.54	(0.20, 1.45)
Q3	>15.49 to 31.02	106	46	0.60	(0.32, 1.11)	45	21	0.94	(0.31, 2.83)	32	9	0.24	(0.08, 0.75)
Q4	>31.02	104	33	0.56	(0.27, 1.15)	26	10	1.09	(0.29, 4.13)	23	5	0.36	(0.10, 1.28)
p-value for trend[3]					*0.07*				*0.9*				*0.02*
Continuous (log_2)		419	189	0.87	(0.76, 0.99)	174	81	0.99	(0.76, 1.29)	125	56	0.72	(0.56, 0.91)
Androstenedione:Estrone													
Q1	0 to 0.01	47	25	1.00		17	9	—		16	7	—	
Q2	>0.01 to 0.02	48	18	0.63	(0.26, 1.57)	17	6	—		10	5	—	
Q3	>0.02 to 0.03	47	18	0.61	(0.22, 1.64)	8	2	—		9	1	—	

		Liver Cancer				Hepatocellular Carcinoma[2]				Intrahepatic Cholangiocarcinoma[2]			
		Controls	**Cases**	**OR**	**95% CI**	**Controls**	**Cases**	**OR**	**95% CI**	**Controls**	**Cases**	**OR**	**95% CI**
Q4	>0.03	48	12	0.27	(0.08, 0.91)	8	2	—		6	1	—	
p-value for trend[3]					*0.05*				*0.2*				*1.0*
Continuous (log_2)		190	73	0.75	(0.55, 1.02)	50	19	0.03	(0.00, 200.30)	41	14	0.78	(0.38, 1.62)

[1]Models are adjusted for age (continuous), matching factors, BMI, smoke status, alcohol status, HBV, anti-HCV, diabetes, and current menopausal hormone therapy use.

[2]Models with categories of <5 cases are suppressed.

[3]P-values for linear trend were calculated using the Wald test.