Relationship of alcohol intake and sex steroid concentrations in blood in pre- and post-menopausal women: the European Prospective Investigation into Cancer and Nutrition

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Abstract
Objective Women with a moderate intake of alcohol have higher concentrations of sex steroids in serum, and higher risk of developing breast cancer, compared to non-drinkers. In the present study, we investigate the relationships between alcohol consumption and serum levels of sex steroids and sex-hormone binding globulin (SHBG) in 790 pre- and 1,291 post-menopausal women, who were part of the European Prospective Investigation into Cancer and Nutrition (EPIC).
Methods Serum levels of testosterone (T), androstenedione (Δ_4), dehydroepiandrosterone sulphate (DHEAS), estrone (E_1), estradiol (E_2) and SHBG were measured by direct immunoassays. Free T (fT) and free E_2 (fE_2) were calculated according to mass action laws. Current alcohol intake exposure to alcohol was assessed from dietary questionnaires.
Results Pre-menopausal women who consumed more than 25 g/day of alcohol had about 30% higher DHEAS, T and fT, 20% higher Δ_4 and about 40% higher E_1, concentrations compared to women who were non-consumers. E_2, fE_2 and SHBG concentrations showed no association with current alcohol intake. In post-menopausal women, DHEAS, fT, T, Δ_4, and E_1 concentrations were between 10% and 20% higher in women who consumed more than 25 g/day of alcohol compared to non-consumers. E_2 or fE_2 were not associated with alcohol intake at all. SHBG levels were about 15% lower in alcohol consumers compared to non-consumers.

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Conclusion This study supports the hypothesis of an influence of alcohol intake on sex hormone concentrations in blood.

Keywords Sex steroids · Alcohol intake · Pre-menopausal · Post-menopausal women

Abbreviations

EPIC	European Prospective Investigation into Cancer and Nutrition
T	Testosterone
Δ_4	Androstenedione
DHEAS	Dehydroepiandrosterone sulphate
E_1	Estrone
E_2	Estradiol
SHBG	Sex-hormone binding globulin
BMI	Body mass index
OR	Odds ratio
CI	Confidence interval

Introduction

Moderate alcohol consumption has been shown to increase breast cancer risk in both pre- and post-menopausal women. Recent re-analyses of cohort studies have shown that the risk of breast cancer is increased

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by about 7–9% for an increment of 10 g/day of alcohol [1, 2]. Even though most of the studies have been conducted on post-menopausal women, the increasing risk of breast cancer with increasing alcohol consumption is modest but still present also for pre-menopausal women [3–5]. It has also been suggested in either randomized trials [2, 6–9] or cross-sectional studies [10–19], that alcohol consumption may increase the concentrations of sex steroids in serum and urine in both pre- and post-menopausal women, but the results of these studies have been somewhat inconsistent and the effect of alcohol on circulating sex hormone levels remains incompletely understood.

We recently published results from a large nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort showing an increased risk of breast cancer among women who had elevated pre-diagnostic serum concentrations of androgens (pre- and post-menopausal women), estrogens (post-menopausal women only) and low serum levels of SHBG (post-menopausal women only) [20, 21]. Here, we report about the relationships of current alcohol consumption and serum levels of sex steroids and SHBG, among both pre- and post-menopausal women who were selected as controls in the fore mentioned studies.

Materials and methods

Study population and blood sample collection

The EPIC cohort consists of about 370,000 women and 150,000 men, aged 35–69, recruited between 1992 and 1998 in 23 research centers in 10 Western European countries [22]. Extensive standardized questionnaire were collected on habitual diet and physical activity, lifetime history of tobacco smoking, consumption of alcoholic beverages, history of previous illness and surgical operations, and for women, menstrual and reproductive history, current and past use of oral contraceptives (OC) and post-menopausal hormone replacement therapy (HRT). Blood samples were also collected from about 65% women and 93% of men. Height, weight, waist and hip circumferences were measured for all subjects according to standardized protocols [23].

Blood samples were collected according to a standardized protocol [24]. Thirty milliliters of blood were drawn from each subject, centrifuged and separated into serum, plasma, red cells, buffy coat. Fractions were then aliquoted in plastic straws and stored under liquid nitrogen (–196°C). In Denmark, blood samples were collected in non-fasting blood status, and serum, plasma, red cells or buffy coat were aliquoted into 1-ml tubes, stored in the vapor phase in liquid nitrogen containers (–150°C).

The present study includes subjects from 19 recruitment centers in eight of the participating countries: Denmark, France, Germany, Greece, Italy, the Netherlands, Spain and the United Kingdom [20, 21].

Selection of subjects

Subjects were selected among pre- and post-menopausal women who were part of the EPIC study, and who participated as controls in a nested case–control study on breast cancer and endogenous hormones in pre- and post-menopausal women [20, 21]. Women were excluded from the study if they used any hormone replacement therapy at the time of blood donation, or any exogenous hormones for contraception or medical purposes, if they had previous diagnosis of cancer (except non-melanoma skin cancer), if they had missing values for alcohol intake, or for all hormones, and if they had extreme values for energy intake/energy requirement (energy requirements were computed as 1.55 times the basal metabolic rate; bottom and top 1% were excluded).

A total of 790 pre- and 1,291 post-menopausal women were selected in the study. All participants had given their written consent for future analyses of their blood samples and the Internal Review Board (IRB) of IARC had approved the hormone analyses.

Determination of menopausal status at blood donation, and of phase of menstrual cycle (pre-menopausal women)

Women were considered as pre-menopausal when they reported having had regular menses over the past 12 months, or when they were less than 42 years of age at recruitment. Women were considered as post-menopausal when they reported not having had any menses over the past 12 months, or when they reported bilateral ovariectomy. Women with missing or incomplete questionnaire data, or with reported previous hysterectomy, were considered post-menopausal only if they were older than 55 years.

Women were considered as unknown when they were between 42 years and 55 years of age, and had missing or incomplete questionnaire data, or reported previous hysterectomy (without ovariectomy). Women defined as unknown were excluded from the study.

A detailed description of the determination of the phase of menstrual cycle in pre-menopausal women

has been reported previously [21]. In brief, the phase of a woman's menstrual cycle was determined using two different dating methods: 'forward' dating counted forward from the woman's reported date of the start of her last menses, and/or 'backward' dating counted backward from the date of the start of her next menses after blood donation, which the woman reported on a pre-paid postcard that she sent back to the recruitment center after her visit to donate a blood sample. When both dating methods were available, the backward dating method was used to determine the menstrual cycle phase. In France, the Netherlands, Greece and Germany, data were available only for forward dating of the phase of menstrual cycle at blood donation, whereas for the vast majority of cohort participants in Italy, Spain, and Oxford, data were also available for backward dating. In Denmark and in Cambridge, no information on phase of menstrual cycle was collected. The phase of menstrual cycle was then categorized in ''early follicular'' (days 0–7 of the cycle), ''late follicular'' (days 8–11), ''peri-ovulatory'' (days 12–16), ''midluteal'' (days 20–24) and ''other luteal'' (days 17–19 or 25–40) [21].

Laboratory assays

All hormone assays were performed at the IARC (Nutrition and Hormones Group). Testosterone (T), androstenedione (Δ_4), dehydroepiandrosterone sulphate (DHEAS), estrone (E_1), estradiol (E_2) and sex-hormone binding globulin (SHBG) measurements were performed on serum samples (that have been thawed only once before hormone analyses) by radioimmunoassay, or immunoradiometric assays. Details about the choice of the assays, and their precision and validity have been reported in detail elsewhere [20, 21, 25]. As quality control measure, three re-constituted serum samples containing different hormone concentrations were inserted in each analytical batch. Intra-batch and inter-batch coefficients of variations were: 9.9% and 15.3%, respectively for T, 5.3% and 18.9% for Δ_4, 6.2% and 12.4% for DHEAS, 6.4% and 12.6% for E_1 3.2% and 7.2% for E_2 in pre-menopausal women, and 5.8% and 13.1% for E_2 in post-menopausal women and 7.5% and 16.5% for SHBG.

E_1 and E_2 were not measured on samples from pre-menopausal women from Cambridge and Denmark for lack of information on phase of menstrual cycle. Concentrations of free T (fT) and free E_2 (fE_2) have been calculated by using equations based on mass action law [26]. Even though these equations have been validated for post-menopausal women only, additional theoretical sensitivity analyses in our laboratory showed that the same equations would also provide valid results within the range of pre-menopausal women, provided that their levels of testosterone, estradiol, dehydrotestosterone and SHBG are within the non-pathological range. The validity of the simplified equations in the pre-menopausal range has also been previously reported [27, 28].

Classification of alcohol variables

Diet was measured by country-specific questionnaires, designed to specifically capture local dietary habits, as described in details elsewhere [24]. Extensive self-administered questionnaires, providing data on up to 300–350 food items per country, were used in Italy, The Netherlands, Greece, Germany, Spain and France. A food frequency questionnaire and a 7-day food consumption record were used in the UK (the data on alcohol reported in this paper are from the food frequency questionnaires). In Denmark, semi-quantitative food-frequency questionnaires (with the same standard portions assigned to all subjects) were used [29]. In these questionnaires, alcohol intake was recorded as the average intake of alcohol over the preceding year. The questionnaires used in each country were all validated against reference measurements based on twelve 24-h diet recall interviews throughout 1 year [30].

Detailed descriptions of the questionnaire data on current alcohol intakes, and of average alcohol intakes in each participating country have been given elsewhere [29, 31]. Alcoholic drinks were classified into eight different categories: wines, fortified wines, beer/cider, spirits, aniseed drinks, liqueurs, cocktails/punches and all other alcoholic beverages. The total intake of alcohol was expressed in grams per day (g/day), and it was calculated by multiplying the volume of a drink by the percentage of alcohol content. Total alcohol intake was categorized into five categories: non-consumers (0–1 g/day), 2–4 g/day, 5–14 g/day, 15–24 g/day and 25 g/day or more. These categories were chose with reference to the results of recent large meta-analyses on alcohol intake and breast cancer risk [1, 2], which showed an average increase in risk of about 7–9% per 10 g of alcohol (one drink) per day.

Data analyses

All hormone concentrations were log-transformed to normalize their distributions. Statistical significance of differences between alcohol consumers and non-consumers was evaluated by *t*-tests for continuous

variables, while for categorical variables, a Cochran–Mantel–Haenszel test was used. Geometric means of hormone levels were calculated according the categories of alcohol intake. *F*-tests were used to test for significance of linear trend by assigning ordinal scores to each successive category and treating variables as continuous in the regression model.

Body mass index (BMI) was calculated from the anthropometric values and defined as kilograms divided by the height (expressed in meters) squared. Dietary energy intakes were calculated from the questionnaires. In all models, energy intake was included for making isoenergetic comparisons, together with age (continuous), BMI (continuous), center, previous use of oral contraceptive (ever/never/missing) and smoking status (never/ex/current/missing) as covariates. For analyses on estrogens levels among pre-menopausal women, the phase of the menstrual cycle was also included as covariate. For analyses on post-menopausal women time since menopause was also included as covariate, and categorized as missing, 0–5 years, 5.1–10 years, 10.1–15 years, 15.1–20 years, and above 20.1. Covariates that differed significantly between alcohol users and non-users (age, time since menopause, BMI, energy intake, smoking status, previous pill use and previous HRT use, see Table 1) were included in the model.

An analysis of covariance adjusted for age and batch was used to estimate percentages of between-subject variation in hormone concentrations statistically explained by differences in alcohol consumption.

All statistical analyses were performed using the SAS statistical package, version 9 (SAS Institute, Cary, NC, USA). Tests of statistical significance were two-sided. *P*-values <0.05 were considered statistically significant.

Results

Overall, women reporting not to drink alcohol had a higher BMI but lower total energy intake, had less frequently used oral contraceptives in the past and smoked less (Table 1). In post-menopausal women, non-consumers, on average, were also older, had a longer time since menopause ($p = 0.06$) and were younger at menarche ($p = 0.06$).

In pre-menopausal women, androgens and E_1 concentrations were positively associated with alcohol consumption (Table 2). Compared to women who did not consume alcohol, those who consumed more than 25 g/day had about 30% higher DHEAS, T and fT concentrations, 20% higher Δ_4 concentrations and about 40% higher E_1, concentrations. E_2, fE_2 and SHBG concentrations showed no association with alcohol intake. The association between alcohol intake and E_1 concentrations remained virtually unchanged when the analyses were restricted to women who had data on date of next menses (backward dating) only. In the same subgroup of women, E_2 concentrations showed no association with alcohol intake. The relationship between alcohol intake and estrogens were virtually the same when analysing follicular and luteal phases separately. In post-menopausal women, as in pre-menopausal women, androgens and E_1 concentrations were positively associated with alcohol consumption: DHEAS, fT and E_1 concentrations were about 20% higher in women consuming more than 25 g/day of ethanol compared to non-consumers, while T and Δ_4, were about 10% higher, even though the increase for Δ_4 was of borderline statistical significance (Table 3). E_2 and fE_2 concentrations showed no association with alcohol intake, while SHBG concentrations were about 15% lower in women consuming more than 25 g/day compared to non-users.

The adjustment for smoking status in both pre- and post-menopausal women had only very minor effects on the relationships between alcohol consumption and sex hormone concentrations. In non-smokers, the associations between hormone levels and alcohol consumption were the same as those observed in the whole population for both pre- and post-menopausal women (results not shown). Adjustments for energy intake, BMI, previous use of oral contraceptives and time since menopause changed the hormone geometric means only trivially.

Although the relationship between alcohol intake and hormone concentrations were statistically quite significant, the percentage of variance explained in hormone concentrations by alcohol was very small, less than 4% in pre-menopausal women, and less than 2% for post-menopausal women (results not shown).

Discussion

In the present study, which is the largest cross-sectional study published so far on alcohol intake and sex hormone concentrations in blood, daily alcohol consumption was associated with higher concentrations of serum androgens, as well as E_1 in pre- and post-menopausal women. SHBG was inversely associated to alcohol intake only in post-menopausal women, while no relationship of E_2 (and fE_2) with alcohol intake was observed in both populations.

In pre-menopausal women not taking any exogenous hormones, previous cross-sectional studies on the

Table 1 Selected characteristics of the population for alcohol non-consumers (0–1 g/day) and consumers (>1 g/day), adjusted by center—means (25th–75th percentiles) and numbers (percentages) by menopausal status

	Pre-menopausal women				Post-menopausal women			
	N[a]	Alcohol consumption		p diff[b]	N[a]	Alcohol consumption		p diff[b]
		Non-consumers	Consumers			Non-consumers	Consumers	
Current alcohol intake (g/day)	268/522	0.0 (0.0–0.4)	12.1 (3.7–16.3)	–	533/758	0.0 (0.0–0.4)	10.9 (2.9–14.3)	–
Age (yr)	268/522	45.6 (41.6–48.2)	45.2 (42.9–50.3)	0.29	533/758	60.8 (57.1–64.6)	60.1 (56.6–64.4)	0.02
Age at menopause (yr)[c]	–	–	–	–	494/714	48.7 (47.0–52.0)	48.7 (47.0–52.0)	0.97
Time since menopause (yr)[c]	–	–	–	–	494/714	12.0 (6.4–16.0)	11.2 (6.2–15.1)	0.06
BMI (kg/m^2)	268/522	25.9 (22.9–29.2)	24.8 (22.1–26.5)	0.001	533/758	27.2 (24.2–29.9)	26.6 (23.2–28.6)	0.01
Energy intake (kcal/day)	268/522	1884 (1609–2291)	1992 (1722–2393)	0.008	533/758	1807 (1436–2123)	1956 (1597–2267)	<0.0001
Previous pill use (number, %)	265/517	132 (49.8)	356 (68.9)	0.002	530/747	154 (29.1)	297 (39.8)	0.006
Previous HRT use[c] (number, %)	–	–	–	–	521/736	74 (14.2)	164 (22.3)	0.08
Age at menarche (yr)	268/519	12.9 (12.0–14.0)	13.1 (12.0–14.0)	0.14	521/747	13.2 (12.0–14.0)	13.4 (12.0–14.0)	0.06
Age at first full-term pregnancy (yr)[d]	237/432	24.9 (22.0–27.0)	25.2 (22.0–27.0)	0.40	470/637	24.8 (22.0–28.0)	25.3 (23.0–28.0)	0.08
Smoking status (number, %)	267/517			0.06	529/750			0.16
Never smoker		165 (61.8)	267 (51.6)			350 (66.1)	426 (56.8)	
Former smoker		54 (20.2)	152 (29.4)			105 (19.9)	212 (28.3)	
Current smoker		48 (18.0)	98 (19.0)			74 (14.0)	112 (14.9)	

[a]Non-consumers/consumers

[b]t-test for difference (mean values) and Cochran–Mantel–Haenszel test (categories)

[c]Post-menopausal women only

[d]Parous women only

Table 2 Geometric means (95% CI) of sex steroid and SHBG concentrations by categories of alcohol consumption in pre-menopausal women[a]

	Non-consumers	Alcohol consumption (g/day), pre-menopausal women			25+	Variation[b] (%)	*p* trend
		2–4	5–14	15–24			
DHEAS (μmol/l)	3.17 (2.62–3.85)	3.30 (2.70–4.03)	3.29 (2.71–3.99)	3.67 (2.93–4.60)	4.15 (3.37–5.12)	31	0.0003
Number of subjects	*268*	*144*	*224*	*67*	*86*		
Δ_4 (nmol/l)	3.73 (3.13–4.45)	3.92 (3.26–4.71)	3.81 (3.19–4.55)	4.23 (3.45–5.20)	4.60 (3.80–5.58)	23	0.003
Number of subjects	*268*	*143*	*224*	*68*	*84*		
T (nmol/l)	1.37 (1.15–1.63)	1.47 (1.23–1.77)	1.39 (1.17–1.65)	1.62 (1.32–1.97)	1.80 (1.49–2.18)	31	< 0.0001
Number of subjects	*266*	*142*	*221*	*67*	*83*		
fT (pmol/l)	19.1 (15.4–23.8)	19.9 (15.8–25.0)	19.0 (15.2–23.6)	21.8 (16.9–28.0)	24.6 (19.4–31.3)	29	0.004
Number of subjects	*266*	*141*	*221*	*67*	*83*		
E_1 (pmol/l)[c]	360.4 (280.8–462.5)	415.4 (318.7–541.4)	378.7 (294.3–487.1)	391.4 (292.2–524.3)	498.7 (379.3–655.6)	38	0.001
Number of subjects	*232*	*109*	*150*	*51*	*67*		
E_2 (pmol/l)[c]	234.5 (167.8–327.8)	251.1 (176.1–357.9)	246.3 (174.6–347.3)	224.5 (152.6–330.4)	250.7 (175.0–359.1)	6.9	0.69
Number of subjects	*229*	*110*	*149*	*51*	*68*		
fE_2 (pmol/l)[c]	5.69 (4.07–7.97)	5.86 (4.10–8.37)	5.75 (4.08–8.12)	5.33 (3.61–7.85)	5.98 (4.17–8.58)	5.1	0.90
Number of subjects	*229*	*110*	*148*	*51*	*68*		
SHBG (nmol/l)	42.8 (35.8–51.1)	45.6 (37.9–54.9)	45.0 (37.7–53.9)	45.6 (37.0–56.1)	45.4 (37.5–55.1)	6.1	0.27
Number of subjects	*268*	*143*	*223*	*68*	*86*		

[a]Adjusted for age, center, energy intake, BMI, previous use of oral contraceptives and smoking

[b]Consumers more than 25 g of alcohol per day versus non-consumers

[c]Further adjustment for phase of the menstrual cycle

Table 3 Geometric means (95% CI) of sex steroid and SHBG concentrations by categories of alcohol consumption in post-menopausal women[a]

	Non-consumers	Alcohol consumption (g/day), post-menopausal women			25+	Variation[b] (%)	*p* trend
		2–4	5–14	15–24			
DHEAS (μmol/l)	1.84 (1.56–2.17)	2.04 (1.72–2.43)	2.04 (1.72–2.41)	2.33 (1.91–2.83)	2.23 (1.82–2.75)	21	0.0003
Number of subjects	*527*	*241*	*315*	*106*	*85*		
Δ_4 (nmol/l)	2.92 (2.50–3.41)	3.04 (2.59–3.58)	2.96 (2.53–3.46)	3.33 (2.76–4.01)	3.26 (2.68–3.96)	12	0.08
Number of subjects	*524*	*240*	*318*	*104*	*84*		
T (nmol/l)	1.08 (0.93–1.25)	1.15 (0.98–1.34)	1.16 (1.00–1.35)	1.27 (1.06–1.52)	1.18 (0.98–1.42)	9.3	0.02
Number of subjects	*523*	*241*	*316*	*105*	*85*		
fT (pmol/l)	17.5 (14.8–20.8)	18.3 (15.4–21.9)	18.5 (15.6–22.0)	22.0 (17.9–26.9)	21.0 (17.0–25.9)	20	0.002
Number of subjects	*523*	*241*	*316*	*105*	*85*		
E_1 (pmol/l)	133.4 (119.5–149.0)	133.8 (119.3–150.2)	137.1 (122.5–153.5)	147.2 (129.1–167.9)	166.0 (144.5–190.7)	24	0.0001
Number of subjects	*510*	*240*	*306*	*104*	*84*		
E_2 (pmol/l)	93.7 (83.6–105.0)	91.9 (81.6–103.6)	95.5 (85.1–107.3)	95.0 (82.8–108.9)	93.0 (80.6–107.3)	–0.7	0.72
Number of subjects	*532*	*242*	*321*	*106*	*87*		
fE_2 (pmol/l)	2.42 (2.15–2.73)	2.36 (2.08–2.68)	2.45 (2.17–2.78)	2.54 (2.19–2.94)	2.54 (2.18–2.95)	5.0	0.27
Number of subjects	*532*	*242*	*321*	*106*	*87*		
SHBG (nmol/l)	34.7 (30.4–39.6)	36.1 (31.4–41.4)	36.2 (31.7–41.4)	30.5 (26.0–35.8)	29.5 (25.0–34.9)	–15	0.03
Number of subjects	*533*	*243*	*322*	*106*	*87*		

[a]Adjusted for age, center, energy intake, BMI, previous use of oral contraceptives, time since menopause and smoking

[b]Consumers more than 25 g of alcohol per day versus non-consumers

relationship of alcohol intake and blood hormone concentrations were generally based on a relatively small number of subjects and differed according to the hormones measured. These studies have shown somewhat variable results: some showed a positive association of either T and fT concentrations [17], or Δ_4 and DHEA or DHEAS concentrations [19], or SHBG [13] and estrogens levels [10, 18, 19] with alcohol intake, while some others did not show the same association [10, 13, 19, 32]. Intervention studies in both animals and humans have shown a direct relationship between alcohol intake and circulating androgen and estrogen levels [8, 9, 33–36], as a result of increased ACTH production by the pituitary gland.

In our study, in pre-menopausal women, we observed an overall direct association between alcohol intake and serum levels of androgens, both of adrenal (DHEAS, Δ_4 and T) and ovarian origin (Δ_4 and T), perhaps suggesting an action of alcohol on steroidogenesis in both types of glands. A very strong, significant positive association with alcohol intake was observed for E_1 while no association was observed for E_2 and SHBG. To our knowledge, only one study has explored the relationship between E_1 concentrations in blood in pre-menopausal women and daily alcohol intake [19], but no relationship was observed. The null association between alcohol intake and E_2 levels in the present study might be explained by the fact that E_2 was measured only during one menstrual cycle, so measurement errors due to the low reliability of E_2 measurements over the menstrual cycle might have limited our ability to detect an association [18].

In post-menopausal women not receiving exogenous hormones, previous cross-sectional studies published on the relationship of alcohol intake and hormone concentrations showed inconsistent results: some studies showed a positive association of T, DHEAS and SHBG with alcohol consumption [16, 37], while some other showed a negative association [14, 15], or no association at all [12, 13, 38]. More consistent results were observed for estrogens, where almost all published studies showed a positive association of E_1, E_1 sulfate and E_2 concentrations with alcohol consumption [11, 13–16, 37–40], even though overall results for E_2 have been less robust. Intervention studies in post-menopausal women showed an increase in DHEAS concentrations [6, 7], while no effect was generally observed for T, E_1 or E_2 concentrations [7, 35, 41].

In the present study, androgen levels in post-menopausal women were positively associated with increasing alcohol consumption, as in pre-menopausal women. E_1 levels were also strongly associated with higher alcohol intake, while E_2 and fE_2 levels seemed to have no relationship at all with alcohol consumption. As for pre-menopausal women, the positive association of DHEAS, T and Δ_4 (even though not significant) with alcohol consumption might indicate a direct effect of alcohol on both the adrenal gland and ovaries.

Mechanisms by which alcohol increases sex steroid levels in humans, however, are still not completely elucidated [33, 42]. Those that have been postulated include: stimulation of ovarian theca cells to produce androgens through increased LH secretion [19], a direct action on adrenal androgen productions [1], induction of a different catabolism of androgens in the liver [7, 35], or an increase of aromatase activity in the liver, leading to a higher conversion of androgens into estrogens [9, 19, 43, 44]. It has also been hypothesized that in post-menopausal women, alcohol breakdown in the liver would be accompanied by accumulation of hepatic nicotinamide-adenine dinucleotide (NADH), which inhibits the conversion of E_2 to E_1 [41]. The results of our study do not support this last hypothesis, since in both pre- and post-menopausal women a strong association with alcohol intake has been observed for E_1 but not for E_2 concentrations. When exploring the relationship of alcohol intake and breast cancer risk through a conditional logistic regression analysis in the nested case–controls studies previously published [20, 21], a direct, but not significant relationship of alcohol intake with breast cancer risk was observed on this specific population (OR: 1.26 [0.96–1.67], p trend = 0.24, highest versus lowest quintile of the overall population). However, even though the relationship between alcohol intake and hormone concentrations was statistically quite significant, the percentage of variance explained in hormone concentrations in control subjects by alcohol was very small, less than 4% in pre-menopausal women, and less than 2% for post-menopausal women.

A major strength of the present study is its size. To our knowledge, it is the largest cross-sectional study on alcohol consumption and sex-steroid hormones conducted so far, combining both pre-menopausal and post-menopausal women. The multi-center design of this study allows us to evaluate associations between alcohol consumption and serum hormones over a wide range of diets and lifestyle habits. Dietary questionnaires that ensured the estimate of chronic alcohol exposure were highly standardized. Some limitations of this study, however, were the low overall alcohol intake, and the fact that hormones were measured only on a single serum sample, possibly leading to a misclassification of subjects on their over-time exposure, above all for estrogens in pre-menopausal women. However, androgens and SHBG concentrations have

been shown to be quite reproducible over-time, in both pre- and post-menopausal women [45–47].

In conclusion, in the present study we have observed a significant positive association of current alcohol intake and serum concentrations of androgens and E_1. These results support the hypothesis that the increase in breast cancer risk caused by alcohol consumption is mediated by increases in sex hormone levels.

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