0021-972X/05/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 90(3):1414–1419
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doi: 10.1210/jc.2004-0614

Alcohol and Endogenous Sex Steroid Levels in Postmenopausal Women: A Cross-Sectional Study

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Breast cancer risk increases with increased levels of alcohol consumption, potentially through an effect on sex hormone levels. In a cross-sectional study among Dutch participants (n = 17,357) of the European Prospective Investigation into Cancer and Nutrition conducted in Utrecht, The Netherlands (Prospect-EPIC), we investigated the relation between alcohol intake and estrogen and androgen levels. Alcohol intake was calculated from a food frequency questionnaire. Women were included if they were postmenopausal, had donated a blood sample, and did not use hormone replacement therapy or oral contraceptives at the time of blood donation (n = 1093).

Women who consumed more than 25 g of alcohol per day had higher levels of estrone (P_{trend} = 0.001), estradiol (P_{trend} = 0.03), dehydroepiandrosterone sulfate (P_{trend} = 0.18), and higher estrone/estradiol (P_{trend} = 0.14) and estrone/androstenedione (P_{trend} = 0.06) ratios, compared with nondrinkers. Levels of androstenedione, testosterone, and SHBG did not differ between women who consumed alcohol and nondrinkers. Furthermore, there were no differences in the free androgen index or estradiol to testosterone ratio.

In conclusion, levels of estrogens and dehydroepiandrosterone sulfate are higher in women who consume more alcohol. This finding supports the hypothesis that alcohol use may increase breast cancer risk at least partially through an effect on sex steroid levels. (*J Clin Endocrinol Metab* 90: 1414–1419, 2005)

A RECENT META-ANALYSIS showed that the risk of breast cancer increases with 7% for each glass of alcohol consumed per day (1). The underlying mechanism is still unclear. It is now well established that higher levels of circulating estrogens increase breast cancer risk in postmenopausal women (2, 3). Recently, evidence has been accumulating to suggest that higher levels of androgens might increase breast cancer risk as well (2, 4). One possible mechanism for the effect of alcohol on breast cancer risk might be through an increase in endogenous sex hormone levels (5), either through an increase in aromatase activity (6) or an effect on the adrenal gland (7).

In postmenopausal women, estrone sulfate (E1SO4) and estradiol (E2) seem to be elevated among those who drink alcohol (8–17), but the results on estrone (E1) are less consistent (7, 11–13, 15, 16, 18, 19). The effect of alcohol on androgen levels has been studied less extensively and results are not consistent (7, 14–16, 18, 19). These inconsistencies might be due to relatively small sample sizes. Differences in duration and dose of alcohol intake are also mentioned as possible explanations (5). Furthermore, the effects of alcohol may be confounded by other risk factors affecting estrogen metabolism, such as smoking. Five of the above-mentioned studies did not adjust for smoking status (14–16, 19, 20).

In a cross-sectional study, we examined the effect of alcohol use on estrogen as well as androgen levels in postmenopausal women.

Subjects and Methods

Subjects

Women included in this study are Dutch participants of the European Prospective Investigation into Cancer and Nutrition (EPIC), conducted in Utrecht, The Netherlands (Prospect-EPIC) (21). Between 1993 and 1997, 17,357 women, aged 49–70 yr, residing in Utrecht and vicinity were recruited through a regional population-based breast cancer-screening program. All women signed a written informed consent before study inclusion. The study complies with the Declaration of Helsinki and was approved by the Institutional Review Board of the University Medical Center Utrecht.

At recruitment, each participant filled out a general questionnaire on lifestyle factors, reproductive (gynecological and obstetric) history, and past and current morbidity as well as a validated (semi)quantitative food frequency questionnaire (FFQ) aimed at capturing the habitual diet during the year preceding enrollment. In addition, pulse rate, blood pressure, and anthropometric measurements were taken, and a 30-ml nonfasting blood sample was donated. Within 24 h, samples of 4 ml serum, 9 ml citrate plasma, 2 ml white blood cells, and 2 ml red blood cells were fractionated into 0.5-ml aliquots, and stored at −196 C. Blood samples, successfully drawn from 97.5% of the women, were donated between 0800 and 1600 h [the majority (75%) donated before 1410 h] (21).

Of all Prospect-EPIC participants, a 10% random sample (n = 1736) was taken, and for the present study, only those who were postmenopausal at recruitment either through natural (no menstrual periods for at least 12 months after spontaneous cessation of their menses) or surgical (hysterectomy, ovariectomy, or a combination) causes, had donated a plasma sample, and did not use hormone replacement therapy (HRT) or oral contraceptives (OCs) at the time of the blood donation were included. In addition, all subjects should have filled out the FFQ, and only those with nonmissing data on alcohol consumption and with a daily energy intake equal to or higher than 500 kcal and equal to or lower than 6000 kcal were included (n = 1093). Daily energy intake outside this range was considered to be outside the normal range, and we assumed that these women did not correctly fill out the FFQ.

First Published Online November 30, 2004

Abbreviations: A, Androstenedione; BMI, body mass index; CI, confidence interval; DHEAS, dehydroepiandrosterone sulfate; E1, estrone; E1SO4, estrone sulfate; E2, estradiol; FAI, free androgen index; FFQ, food frequency questionnaire; HRT, hormone replacement therapy; OC, oral contraceptive; TST, testosterone; WHR, waist to hip ratio.

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Alcohol intake

Information on current alcohol use was obtained from the FFQ. Total alcohol consumption in grams per day was calculated from questions on the number of glasses of wine, beer, fortified wines, and liqueur/spirits per day, week, month, or year. One glass of any alcoholic beverage was assumed to contain 10 g alcohol. The alcohol intake of women who consumed less than 1 g/d was set to 0. The total alcohol consumption was then classified into five categories: no alcohol consumption (0 g/d), 1–4 g/d, 5–14 g/d, 15–24 g/d, and 25 g/d or more. These categories were based on those used in the recently published, large meta-analyses on alcohol and breast cancer risk (1). By using the same categories, we ensure that the papers are comparable.

Hormone measurements

Levels of E1, E2, androstenedione (A), dehydroepiandrosterone sulfate (DHEAS), testosterone (TST), and SHBG were measured in plasma using commercially available double-antibody RIA kits (Diagnostic System Laboratories Inc., Webster, TX). The following kits were used: E1, DSL-8700; E2, DSL-39100; A, DSL-4200; DHEAS, DSL-2700; TST, DSL-4100; and SHBG, DSL-6300. The intraassay coefficients of variation were 5.6, 3.9, 4.3, 5.2, 7.7, and 3.0%, respectively. The interassay coefficients of variation were 11.1, 4.1, 6.3, 5.3, 8.1, and 4.0%, respectively. Although technically SHBG is not a hormone, for reasons of convenience, it will be referred to as such.

Potential confounders

To be able to adjust for possible confounding by smoking, we calculated the number of pack-years smoked for each woman based on questions on duration and dosage of smoking at different ages. Body mass index and (BMI) waist to hip ratio (WHR) were calculated from the anthropometric measurements, and energy-adjusted total fat intake was calculated from the FFQ. The Voorrips total score was calculated from questions in the general questionnaire on daily physical activity, which has been developed and validated especially for women in this age group (22). Finally, we obtained information on reproductive factors from the general questionnaire.

Data analyses

Means with their SD values, medians, and ranges (for the not normally distributed characteristics) or frequencies (where appropriate) of baseline characteristics were presented for women who did not drink alcohol (0 g/d) and those who consumed 1 g alcohol per day or more. Differences between the two groups were tested with the *t* test (normally distributed continuous variables), Mann-Whitney *U* test (not normally distributed continuous variables), or χ^2 test (proportions).

Concentrations of all hormones were logarithmically transformed to produce approximately normal distributions. To make the results easily interpretable, we transformed the mean and its 95% confidence interval (CI) back to their original scale, resulting in geometric means and 95% CIs. Geometric mean levels and 95% CIs were calculated for each hormone (E1, E2, A, DHEAS, TST, SHBG), the free androgen index (FAI), and the E1 to E2, E1 to A, and E2 to TST ratios. Geometric mean hormone levels were calculated in each category of alcohol consumption, using analysis of covariance models. Results are presented unadjusted and adjusted for age at enrollment, waist to hip ratio, body mass index, number of pack-years smoked, physical activity (tertiles of Voorrips total score), type of menopause (natural or surgical), time since menopause (calculated as age at enrollment minus age at menopause), energy-adjusted total fat intake, total energy intake, age at menarche, parity/age at first full-term pregnancy (20 or less, 21–25, 26–30, older than 30 yr, no children), ever use of oral contraceptives, ever use of hormone replacement therapy, and having a mother or sister with breast cancer.

For each group of alcohol consumption, median alcohol levels in these groups were imputed. Subsequently these values were included as a continuous variable in a linear regression model to test for linear trends.

For all analyses we used the Statistical Package for Social Sciences (version 11.0.1, SPSS, Chicago, IL).

Results

Table 1 shows characteristics of women who do not consume alcohol *vs.* women who do. Approximately 35% of the women included in this study did not consume alcohol (<1 g/d). Among alcohol consumers the median intake was 8.3 g/d, which is slightly less than one glass per day. The most striking difference between the two groups was that in women who do not drink alcohol, the median number of pack-years smoked was zero. Women who consume more alcohol also smoke more cigarettes. Moreover, in the alcohol consumers, energy intake and physical activity were higher. Energy-adjusted total fat intake was lower in the alcohol consumers, compared with the nonconsumers. Table 2 shows the geometric mean hormone levels for all women with 95% CIs of E1; E2; A; DHEAS; TST; SHBG; FAI; and E1 to E2, E1 to A and E2 to TST ratios.

Table 3 shows the unadjusted and adjusted geometric mean hormone levels and 95% CI for each hormone according to the level of alcohol consumption. Compared with nondrinkers, levels for E1 were higher in women who consumed more than 25 g alcohol per day [17.7 pg/ml (95% CI: 15.3–20.6) *vs.* 14.4 pg/ml (95% CI: 13.3–15.5)] [conversion factor to SI units (nanomoles per liter): 0.0037]. This was also observed for E2 [9.5 pg/ml (95% CI: 8.3–10.8) and 8.6 pg/ml (95% CI: 8.0–9.1)] [conversion factor to SI units (nanomoles per liter): 0.0037], and the E1 to E2 ratio [1.9 (95% CI: 1.6–2.1) and 1.7 pg/pg (95% CI: 1.6–1.8)]. Tests for trend were statistically significant for E1 and E2 (P_{trend} = 0.001 and 0.03, respectively). For the E1 to E2 ratio, the trend test was significant only univariately (P_{trend} = 0.03). In the multivariate analyses, the ratio increased in the same amount as in the univariate analyses, but the test for trend was no longer statistically significant (P_{trend} = 0.14).

For the androgens, levels of DHEAS were higher in women with higher alcohol consumption. Geometric mean levels of DHEAS in nondrinkers were 429.7 ng/ml (95% CI: 397.0–465.0), compared with 501.2 ng/ml (95% CI: 428.4–587.0) in the group with the highest alcohol consumption [conversion factor to SI units (nanomoles per liter): 2.71], but after adjustment for confounders, the trend test was no longer statistically significant (P_{trend} = 0.18). We did not find a significant association with higher alcohol consumption levels for A, TST, and SHBG or the FAI.

The E1 to A ratio was also higher in women with higher alcohol consumption levels [25+: 34.1 pg/ng (95% CI: 28.1–41.3) *vs.* 0: 32.0 pg/ng (95% CI: 29.0–35.5)], which is likely to be explained by the increase in E1. The trend test for this ratio was borderline significant (P_{trend} = 0.06). The E2 to TST ratio remained stable over the categories of alcohol consumption.

Discussion

In this cross-sectional study, we found an association between alcohol consumption and sex steroid levels in postmenopausal women. Compared with nondrinkers, levels of E1, E2, and DHEAS and the E1 to A ratio were higher in women who reported consumption of more than 25 g alcohol per day, compared with the nondrinkers.

To our knowledge, this is the largest study on the relation between alcohol consumption and sex steroid levels to date

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TABLE 1. General characteristics of the population for non-alcohol users and women who drink at least 1 g of alcohol/d

Characteristic	Alcohol use		*P*
	0 g/d	≥1 g/d	
n	386	707	
Age at enrollment (yr) [mean (SD)]	60.0 (5.5)	59.2 (5.7)	0.019
Height (cm) [mean (SD)]	163.1 (5.6)	164.3 (6.1)	0.001
Weight (kg) [mean (SD)]	71.7 (13.2)	69.2 (10.9)	0.002
BMI (kg/m^2) [mean (SD)]	26.9 (4.5)	25.6 (3.8)	<0.001
WHR [mean (SD)]	0.80 (0.06)	0.79 (0.06)	<0.001
Age at menopause (yr) [median (IQR)]	49 (45–52)	49 (45–52)	0.496
Time since menopause (yr) [median (IQR)]	12 (8–16)	11 (6–16)	0.004
No. of pack years smoked [median (IQR)]	0.00 (0.0–9.9)	2.84 (0.0–11.9)	<0.001
Energy intake (kcal/d) [mean (SD)]	1741 (424)	1800 (403)	0.024
Total fat intake (g/d), energy adjusted [mean (SD)]	68.7 (10.9)	66.8 (9.99)	0.004
Alcohol total (g/d) [median (IQR)]	Not applicable	8.3 (3.27–18.8)	
Age at menarche (yr)			
≤11	39 (10.1)	61 (8.6)	
12	76 (19.7)	157 (22.2)	
13	89 (23.1)	158 (22.3)	
14	80 (20.7)	149 (21.1)	
≥15	102 (26.4)	182 (25.7)	0.84
Nulliparous	47 (12.2)	82 (11.6)	0.78
Age at first full-term pregnancy (yr)			
≤20	55 (14.2)	52 (7.4)	
21–25	154 (39.9)	290 (41.0)	
26–30	106 (27.5)	233 (33.0)	
≥31	24 (6.2)	50 (7.1)	0.002
Ever pill use	182 (47.2)	416 (58.8)	<0.001
Ever HRT use	44 (11.4)	110 (15.6)	0.06
Surgical menopause	160 (41.4)	308 (43.6)	0.50
Physical activity			
1st tertile (≤3.27)	154 (43.3)	185 (28.0)	
2nd tertile (3.27–7.86)	114 (32.0)	225 (34.0)	
3rd tertile (≥7.86)	88 (24.7)	251 (38.0)	<0.001

Data represent frequency (%), except where designated otherwise. IQR, Interquartile range.

including almost twice the number of women, compared with the largest study reported until now (n = 481) (23). Because we believe that effects of alcohol on endogenous hormone levels may be rather small, we designed our study to have sufficient power (>90%) to detect even small changes (≤5%).

In addition, we included estrogens as well as androgens, which provided the opportunity to investigate the effect of alcohol on both types of hormones thought to be relevant in breast cancer etiology.

Although large in size, our study was limited by the relatively high percentage of nondrinkers and low percentage of women who consume high amounts of alcohol. Thirty-five percent of the women did not drink alcohol, and only 30% reported to drink more than one glass of alcohol per day (>10 g/d).

TABLE 2. Geometric mean serum sex hormone and SHBG levels

Serum hormone	Geometric mean	95% CI
E1 (pg/ml)[a]	15.8	15.1–16.5
E2 (pg/ml)[a]	9.0	8.7–9.4
A (ng/ml)[a]	0.46	0.44–0.49
DHEAS (ng/ml)[a]	458.2	438.2–479.1
TST (ng/ml)[a]	0.25	0.24–0.26
SHBG (μg/ml)[a]	5.9	5.6–6.3
FAI [TST/SHBG*100 (ng/μg*100)]	4.8	4.5–5.0
E1/E2 ratio (pg/pg)	1.8	1.7–1.8
E1/A ratio (pg/ng)	34.0	32.3–35.9
E2/TST ratio (pg/ng)	35.6	34.1–37.1

[a] Conversion factors from metric units to SI units (nanomoles per liter): E1 and E2, 3.7; A, 3.45; DHEAS, 2.71; TST, 3.47; SHBG, 2.87.

In contrast to randomized clinical trials in which known quantities of alcohol were administered (7, 12, 13), self-reported alcohol intake could lead to misclassification because women might tend to underreport. However, it is unlikely that underreporting was related to endogenous hormone levels, and any resulting random misclassification will only underestimate relations. Despite its limitations, however, self-reported alcohol intake is reflected into an increased risk of breast cancer (1), which indicates that variance in self-reported alcohol intake is large enough to detect differences in risk.

Several studies investigated the effects of alcohol use on endogenous sex steroid levels in postmenopausal women, but with conflicting results. Three randomized clinical trials were conducted in which fixed amounts of alcohol were administered and the acute effects on hormone levels were measured (7, 12, 13). One reported increased levels of E2, which peaked approximately 35 min after administering alcohol only in women on estrogen replacement therapy. No effect of alcohol consumption on E1 levels was observed (12, 13). In a randomized cross-over trial, either a placebo drink or 15 or 30 g alcohol per day were administered in an energy-controlled diet. Increasing alcohol dose was associated with increased levels of DHEAS (5.1 and 7.5%, respectively) (7), which was about half the level of increase we observed when comparing the women who consumed more than 25 g alco-

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TABLE 3. Unadjusted and adjusted[a] geometric mean (95% CI) serum hormone and SHBG concentrations in categories of alcohol consumption

	Alcohol intake (g/d)					P_{trend}
	0	1–4	5–14	15–24	25+	
Median alcohol intake level (IQR)	0.06 (0.01–0.34)	2.67 (1.49–3.54)	9.30 (5.73–11.52)	20.13 (17.98–21.85)	31.22 (29.41–40.75)	
n	386	251	244	112	100	
E1 (pg/ml)[b]						
Unadjusted	14.8 (13.8–15.9)	15.7 (14.3–17.1)	16.2 (14.8–17.7)	17.7 (15.5–20.2)	17.3 (15.1–20.0)	0.01
Adjusted[a]	14.4 (13.3–15.5)	15.3 (14.0–16.8)	16.6 (15.1–18.3)	18.3 (15.9–20.9)	17.7 (15.3–20.6)	0.001
E2 (pg/ml)[b]						
Unadjusted	8.8 (8.3–9.4)	8.9 (8.2–9.7)	9.2 (8.5–10.0)	9.5 (8.4–10.7)	9.0 (7.9–10.2)	0.47
Adjusted[a]	8.6 (8.0–9.1)	8.8 (8.1–9.5)	9.5 (8.7–9.8)	9.9 (8.8–11.2)	9.5 (8.3–10.8)	0.03
A (ng/ml)[b]						
Unadjusted	0.45 (0.41–0.49)	0.50 (0.45–0.55)	0.45 (0.40–0.50)	0.44 (0.38–0.51)	0.51 (0.43–0.60)	0.64
Adjusted[a]	0.45 (0.41–0.49)	0.49 (0.44–0.55)	0.45 (0.40–0.50)	0.44 (0.37–0.52)	0.52 (0.44–0.62)	0.50
DHEAS (ng/ml)[b]						
Unadjusted	417.4 (387.2–449.9)	487.8 (444.5–535.4)	477.2 (434.0–524.3)	466.4 (405.5–535.9)	505.7 (436.2–585.8)	0.05
Adjusted[a]	429.7 (397.0–465.0)	473.0 (429.7–520.6)	466.8 (423.2–514.9)	456.2 (395.4–525.8)	501.2 (428.4–587.0)	0.18
TST (ng/ml)[b]						
Unadjusted	0.25 (0.24–0.27)	0.26 (0.24–0.28)	0.24 (0.22–0.26)	0.25 (0.23–0.28)	0.27 (0.24–0.30)	0.55
Adjusted[a]	0.25 (0.23–0.26)	0.26 (0.24–0.28)	0.24 (0.22–0.26)	0.21 (0.23–0.29)	0.28 (0.24–0.31)	0.25
SHBG (μg/ml)[b]						
Unadjusted	5.8 (5.3–6.3)	5.6 (5.1–6.3)	6.3 (5.6–7.0)	6.4 (5.4–7.6)	6.1 (5.1–7.3)	0.20
Adjusted[a]	6.0 (5.5–6.6)	5.5 (4.9–6.2)	5.9 (5.3–6.7)	6.0 (5.1–7.0)	5.9 (4.9–7.0)	0.91
FAI (ng/μg*100)						
Unadjusted	4.9 (4.4–5.3)	5.1 (4.5–5.7)	4.4 (3.9–4.9)	4.6 (3.8–5.5)	4.9 (4.1–5.9)	0.64
Adjusted[a]	4.5 (4.1–5.0)	5.2 (4.6–5.8)	4.6 (4.1–5.2)	4.9 (4.1–5.8)	5.2 (4.3–6.2)	0.43
E1/E2 ratio (pg/pg)						
Unadjusted	1.7 (1.6–1.8)	1.8 (1.6–1.9)	1.8 (1.6–1.9)	1.9 (1.7–2.1)	1.9 (1.7–2.2)	0.03
Adjusted[a]	1.7 (1.6–1.8)	1.7 (1.6–1.9)	1.8 (1.6–1.9)	1.8 (1.6–2.1)	1.9 (1.6–2.1)	0.14
E1/A ratio (pg/ng)						
Unadjusted	32.9 (30.1–36.0)	31.3 (28.1–34.9)	36.1 (32.3–40.3)	40.7 (34.5–48.0)	34.0 (28.6–40.5)	0.10
Adjusted[a]	32.0 (29.0–35.2)	31.2 (27.7–35.1)	37.2 (33.0–41.9)	41.8 (35.1–49.8)	34.1 (28.1–41.3)	0.06
E2/TST ratio (pg/ng)						
Unadjusted	35.1 (32.7–37.6)	34.2 (31.3–37.3)	38.2 (35.0–41.7)	37.3 (32.8–42.5)	33.3 (29.0–38.2)	0.88
Adjusted[a]	34.7 (32.3–37.4)	33.7 (30.8–36.9)	39.2 (35.8–43.0)	38.5 (33.7–44.0)	34.5 (29.8–40.0)	0.39

IQR, Interquartile range.

[a] Adjusted for: age at enrollment, WHR, BMI, number of pack-years smoked, physical activity, type of menopause, time since menopause, energy adjusted total fat intake, total energy intake, age at menarche, parity/age at first full-term pregnancy, ever use of OC, ever use of HRT, and familial history of breast cancer.

[b] Conversion factors from metric units to SI units (nanomoles per liter): E1 and E2, 0.0037; A, 3.45; DHEAS, 2.71; TST, 3.47; SHBG, 2.87.

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hol per day with the nondrinkers. Levels of E1, E2, A, and dehydroepiandrosterone did not increase after alcohol administration (7). These three trials, however, included only a few women (range 7–51), and the statistical power was, therefore, limited. They do provide evidence, however, that alcohol may acutely increase sex steroid levels in postmenopausal women.

Several cross-sectional studies have been reported, mostly of small scale, ranging from 20 to 456 women (8–11, 14–16, 18–20, 23). Most consistently, these studies show that higher alcohol intake was associated with higher E1SO4 (11–15) and E2 levels (8–10, 14, 15), although some studies did not find higher E2 levels (11, 16, 23); Cauley *et al.* (18) even reported decreased E2 levels. The results for the other hormones, such as the estrogens E1 and bioavailable E2, the androgens A, DHEA, DHEAS, and TST, and SHBG are less consistent: some report increased levels (14–16), some report decreased levels (16, 18, 20), whereas others found no effect of alcohol on these hormone levels (11, 14–16, 18, 19, 23).

In premenopausal women, levels of E2, A, and TST seem consistently higher in those who drink alcohol (24–33). The results on E1 and DHEAS are inconsistent in premenopausal women as well: some report increased levels with increased alcohol consumption (24, 32), whereas others find no effect of alcohol consumption on levels of these two hormones (26, 28, 33).

The half-life of hormones can influence whether we will be able to demonstrate an effect of alcohol intake on hormone levels (7). It is likely that in most cross-sectional studies on alcohol and sex steroid levels, blood samples have been taken during the day. Therefore, women probably would have consumed their last glass of alcohol the previous day, which makes it hard to find effects on levels of hormones with short half-lives such as E1 and E2. The half-life of sulfated hormones, such as E1SO4 and DHEAS are much longer (DHEAS: 14 h; E1SO4: 5–7 h) (7). Ginsburg *et al.* (13) showed that levels of E1 and E2 peaked after approximately 30 min after alcohol administration and then started to decrease. Also, plasma levels of E1 and E2 are much lower, compared with levels of E1SO4 and DHEAS, which makes it even harder to find small differences in levels. Our study has sufficient power to find even small increases in hormone levels, which explains why we were able to pick up small differences in E1 and E2 levels, whereas others were not.

Alcohol might affect sex steroid levels through several mechanisms. In the liver, alcohol consumption increases the nicotinamide adenine dinucleotide hydroxide to nicotinamide adenine dinucleotide (oxidized form) ($NADH/NAD^+$) ratio, which leads to a decreased catabolism of sex steroids (29, 30). This decreased catabolism would then lead to increased levels of TST and E1 and decreased levels of A and E2. Furthermore, the E1 to E2 ratio would also decrease (29, 30). In our study we were not able to demonstrate an effect of alcohol on TST and A. If anything, the levels were increased, although not statistically significant. Furthermore, the levels of E2 and also the E1/E2 ratio were increased, rather than decreased. Another possible mechanism is an effect of alcohol consumption on aromatase activity (34). An effect on the aromatase activity would be likely if estrogen levels are increased and androgen levels are decreased. Also, the E1/A and E2/TST ratios are both expected to be increased. We indeed found increased levels of E1 and E2. However, the androgens (A and TST) were not decreased in our study. Also, the E2/TST ratio remained unchanged, and the increase in the E1/A ratio in our study is probably the result of the increase of E1, rather than a combination of an increase in E1 with a decrease in A.

In animal studies, it has consistently been found that alcohol affects the hypothalamic-pituitary-adrenal axis (35). The adrenal gland exclusively produces DHEAS, and increased levels of this androgen suggests that alcohol probably may have its effect primarily on the adrenal gland, as was suggested before (7). We found effects of alcohol intake on estrogens as well as DHEAS. In postmenopausal women, E1 and E2 are formed from androgens in the peripheral tissue by aromatase. Increased production of precursors of androgens by adrenal glands could, therefore, account for increased levels of estrogens. However, the adrenal gland also produces androstenedione. Therefore, we would expect A also to be elevated in these women. In our data the A levels are indeed higher, compared with women who do not drink alcohol, although these trends were not statistically significant. As mentioned above, sulfated hormones have longer half-lives, compared with nonsulfated hormones, which could explain why we were not able to detect higher levels of A but did find increased concentrations of DHEAS (7).

In short, our results seem to provide most support for an effect of alcohol on the adrenal gland, but effects on catabolism of sex steroids in the liver or on aromatase activity cannot be excluded either.

We have tried to translate the magnitude of the observed effect of alcohol drinking on endogenous levels into breast cancer risk. Alcohol drinking as well as endogenous sex steroid levels have recently been related to breast cancer in a large meta-analysis, which showed that with each 10 g of alcohol consumed per day, a woman's breast cancer risk increases approximately 7% (1). In our population women in the highest category of alcohol consumption (>25 g/d) show a median alcohol consumption of 30 g/d. According to the meta-analysis on alcohol and breast cancer risk, these women, compared with nondrinkers, would thus have a 21% increase in breast cancer risk ($3 \times 7\%$).

The same group of investigators also performed a meta-analysis on endogenous sex steroid levels and breast cancer risk (3). Let us take E1 levels as an example. If in our population we divide E1 levels into quintiles, the geometric mean E1 level (14.7 pg/ml; 95% CI: 5.8–48.9) in nondrinkers falls in the second quintile (10.0–14.9 pg/ml). The geometric mean E1 level (17.3 pg/ml; 95% CI: 5.1–59.1) in women in the highest category of alcohol consumption falls in the third quintile (15.0–18.7 pg/ml). According to the meta-analysis results, the increase in alcohol from zero to more than 25 g/d will result in a 22% increase ($RR_{3rd\ quintile} = 1.55$, $RR_{2nd\ quintile} = 1.27$; increase in risk = 1.55/1.27 = 1.22) in breast cancer risk (3).

Although these calculations are based on many assumptions, *i.e.* that increases in risk across quintiles are identical, irrespective of the absolute values or distribution of hormones, they, however, indicate that the magnitude of the effects of alcohol on endogenous levels are compatible with

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observed risks for alcohol and endogenous levels and thus that there is evidence for the proposed pathway of alcohol and breast cancer risk via endogenous sex steroid levels.

In conclusion, levels of estrogens and DHEAS are higher in women who consume more alcohol. This finding supports the hypothesis that alcohol use may increase breast cancer risk, at least partially, through an effect on sex steroid levels.

Acknowledgments

Received April 1, 2004. Accepted November 22, 2004.

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This work was supported by the Dutch Cancer Society (Grant UU1999-1935) and the Dutch Heart Foundation (Grant 99-159).

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