Moderate Alcohol Consumption and 24-Hour Urinary Levels of Melatonin in Postmenopausal Women

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Context: Low overnight urinary melatonin metabolite concentrations have been associated with increased risk for breast cancer among postmenopausal women. The Postmenopausal Women's Alcohol Study was a controlled feeding study to test the effects of low to moderate alcohol intake on potential risk factors for breast cancer including serum and urinary levels of hormones and other biomarkers. Previously, we observed significant increases in concentrations of serum estrone sulfate and dehydroepiandrosterone sulfate in participants after consumption of 15 or 30 g (one or two drinks) of alcohol per day.

Objective: In the present analysis, we evaluated the relationship of alcohol consumption with 24-h urinary 6-sulfatoxymelatonin (6-SMT) concentration (micrograms per 24 h).

Design and Participants: Healthy postmenopausal women (n = 51) consumed a controlled diet plus each of three treatments (a nonalcoholic placebo beverage or 15 or 30 g alcohol/d) during three 8-wk periods in random order under conditions of weight maintenance.

Measures: 6-SMT was measured in 24-h urine samples that were collected at entry into the study (baseline) and at the midpoint (4 wk) and end (8 wk) of each of the three diet periods.

Results: Concentration of 6-SMT was not significantly modified by the alcohol treatment after adjustment for body mass index, hours of sleep, daylight hours, and baseline level of 6-SMT.

Conclusions: These results suggest that low to moderate daily alcohol consumption does not significantly affect 24-h urinary levels of melatonin among healthy postmenopausal women. **(*J Clin Endocrinol Metab* 97: E65–E68, 2012)**

Prospective epidemiological studies support a positive relationship between alcohol consumption and breast cancer incidence (1). Some evidence, but not all, suggests that estrogens play a role in breast cancer etiology (2). We reported significantly increased serum estrone sulfate and dehydroepiandrosterone sulfate concentrations after postmenopausal women consumed 15 or 30 g alcohol daily over 8 wk (3, 4).

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Abbreviations: BMI, Body mass index; 6-SMT, 6-sulfatoxymelatonin.

Cohen *et al.* (5) first suggested a role for melatonin, a pineal hormone secreted in response to darkness, in the etiology of breast cancer in 1978. Stevens and Hiatt (6) later hypothesized that melatonin may play a role in breast cancer through links with both estrogens and alcohol. Alcohol is known to disturb sleep; thus, ingestion might lower melatonin production and release, resulting in increased ovarian estrogen production and elevated circulating estradiol levels, which in turn would increase breast cancer risk. A significant body of literature supports this hypothesis; however, some inconsistencies remain. Under experimental conditions in animals (7) and humans (8, 9), alcohol may modify estrogen levels and influence nocturnal melatonin metabolism. Stevens *et al.* (10) observed a significant inverse association between self-reported alcohol consumption and urinary 6-sulfatoxymelatonin (6-SMT) concentrations when women ages 20–74 reported consuming at least two drinks per day (n = 203). Melatonin has been inversely associated with serum and urinary estrogen concentrations in some (11), but not all studies (11–13) among women. Nighttime shift work (14–16) and low overnight urinary melatonin concentrations (17, 18) have been associated with increased risk for breast cancer. In contrast, a prospective study of 127 incident postmenopausal breast cancer cases reported no association for 24-h urinary melatonin (19), whereas another reported a positive association between overnight urinary melatonin and cancer risk (20).

The Women's Alcohol Study (WAS) was designed to evaluate the effect of moderate alcohol consumption on serum hormones and other potentially important biomarkers related to breast cancer risk. We tested the hypothesis that moderate alcohol consumption would significantly decrease 24-h urinary melatonin levels.

Subjects and Methods

Subjects

The WAS was conducted at the U.S. Department of Agriculture's Beltsville Human Nutrition Research Center (BHNRC) in 1998–1999. The institutional review boards at the National Cancer Institute (Bethesda, MD) and the Johns Hopkins University Bloomberg School of Public Health (Baltimore, MD) approved the study, and all participants signed informed consent. Participants were healthy, nonsmoking, postmenopausal women, at least 50 yr of age who reported not using hormone replacement therapy. Women had to be willing to adhere to the study diet and could not be an alcohol abstainer or abuser. Of the initial 57 women, four did not complete the study, and two were excluded for glucocorticoid use during follow-up, leaving 51 for analysis.

Study design

The study design and methods have been reported (3). Participants consumed diets prepared by the BHNRC plus each of three alcohol treatments (0, 15, and 30 g alcohol/d) in random order for three 8-wk periods separated by 2- to 5-wk alcohol-free washout periods. Breakfast and dinner were consumed onsite; carryout foods and beverages were provided for weekday lunches and weekend meals. The diets provided 15% energy as protein, 33% as fat, and the remainder as carbohydrate and alcohol. Alcohol was provided as 95% ethanol (Everclear) in 12 ounces of orange juice. Participants were not informed of the alcohol content of the beverages and were asked to consume them 1 to 2 h before bedtime with the study snack. For the 0- and 15-g alcohol periods, energy from alcohol was replaced with carbohydrates (Polycose and soft drinks). Participants' weights were measured daily. Sleep time was self-reported and collected only at baseline. Data from the National Naval Observation Station (Washington, DC) was used to estimate daylight exposure for participants on the days of the 24-h urine collections.

Urine assays

All urine was collected into tared plastic jugs over a 24-h period, returned to the BHNRC on ice, weighed, aliquoted, and stored at −70 C. Each participant collected one 24-h sample at baseline and at 4-wk into each period. At the end of each 8-wk period, three 24-h urine samples were collected, volume was measured, and aliquots were pooled for analysis. Because samples were 24-h urine collections, creatinine was not measured. Samples for individuals were grouped in random order for analysis within the same batch and assayed in duplicate. The melatonin metabolite, 6-SMT, was measured using a competitive ELISA (EK-M6S; Bühlmann Laboratories, distributed by ALPCO Diagnostics, Salem, NH). Samples were centrifuged at 12,000 × *g* for 1 min, then assayed at 1:200 dilution. We were not able to reliably estimate two very high and random values that were outside of our standard curve and likely due to melatonin use; these were omitted from the analyses. A standard curve (0.8–40 ng 6-SMT/ml), including five points and a zero, was included with each microtiter plate. Two Bühlmann human control pools (lots 2512 and 0913A) with low and high levels of 6-SMT were assayed in duplicate at the front and rear of each microtiter plate. The coefficient of variation for the 6-SMT assay was 5.8%.

Statistical analyses

We used SAS software (SAS/Stat version 9.1; SAS Institute, Cary, NC) for all analyses. A two-sided *P* value of 0.05 was considered statistically significant. Linear mixed models were used to test for differences in biomarker concentrations across the treatment groups (0, 15, 30 g alcohol) on two occasions (4 and 8 wk) in repeated measures ANOVA. Participant (subject) was treated as a random effect (*i.e.* a single random intercept), and time (4 and 8 wk) and alcohol levels were treated as fixed effects designated by indicator variables. 6-SMT levels were analyzed as untransformed and $\log_e$-transformed with similar results; results are reported for untransformed data. We evaluated the effects of other variables including age, race, body mass index [BMI = weight (kilograms)/height (meters)^2], baseline (pretreatment) biomarker level, treatment period, and order for contribution to overall fit or improvement in precision of the model. Likelihood ratio tests were used to evaluate whether covariates improved model fit and also to test for treatment effect. Because

there were no significant time effects (4 *vs.* 8 wk), data were combined to test the treatment effect. Treatment effect was tested by simultaneously testing whether the fixed effects covariates corresponding to the two treatment effect indicators were zero (χ^2 2 degrees freedom). We tested for significant differences between treatment levels and no alcohol by individually testing whether these two coefficients were zero. SE values of alcohol estimates from simple models, including characteristics potentially associated with 6-SMT concentration, were compared to evaluate the effect of adjustment on precision. The final models included baseline level of the biomarker of interest, hours of sleep time and daylight, and BMI. Covariates including age, race, energy intake and expenditure, concentrations of other hormones, period, and treatment order had no effect. Effect modification by treatment order, BMI, baseline biomarker concentration, and age was assessed after addition of the interaction to models that included the main effects for alcohol and the characteristic of interest. Final results are reported as least square means with 95% confidence intervals. Lastly, in *post hoc* analyses with our data, we estimate that we had 80% power (two-sided paired test at 0.05 significance) to detect a 10% reduction in melatonin levels between the zero and two drinks per day treatments.

Results

Baseline participant characteristics have been reported (3). Mean age was approximately 60 yr (range, 49–79), and BMI was 27 kg/m^2 (range, 17.3–41.8). The participants were primarily Caucasian (73%); 22% were African-American, and 4% were Asian. Participants reported about 7 h of sleep, and daylight for 24-h urine collection days was approximately 13.3 h. Table 1 shows mean 6-SMT levels after consumption of the placebo and mean change for the alcohol treatments after adjusting for covariates. We saw no statistically significant changes in 6-SMT when women consumed either 15 or 30 g alcohol/d.

Discussion

Investigations of the association between urinary melatonin and alcohol have used a variety of designs and methods for alcohol exposure and urine collection. Ekman *et al.* (8) evaluated the acute effects of alcohol on melatonin metabolism among nine healthy volunteers (five females, four males) ages 21–23 yr. Participants received 0, 0.5, and 1 g ethanol/kg body weight. Melatonin secretion was significantly depressed in serum; however, no differences were observed for 12-h urinary excretion. These levels of alcohol exposure, if applied to the median-weight woman in our study (73.2 kg), would have resulted in consumption of 37 g and 73 g alcohol/d, more than double our highest dose. Röjdmark *et al.* (9) provided either a placebo or alcohol at two doses (0.34 and 0.52 g alcohol/kg) three times over 6 h to young participants. The goal was to achieve "moderate intoxication." Nocturnal melatonin secretion in blood was measured hourly and increased by 33% with alcohol consumption; however, a 9-h urinary sample showed no significant differences in melatonin concentrations across groups. In this scenario, the median-size woman in our study would have received 74 g and 114 g alcohol/d, two to four times our highest dose. Lastly, a cross-sectional sample of 203 women ages 20–74 were observed on multiple days and collected nocturnal urine samples (after retiring through first morning) for three consecutive nights on two occasions (10). Alcohol consumption (servings of 12-ounce beer, 4-ounce wine, or 1.5-ounce spirits) was reported for the preceding 24-h periods. In models adjusted for age, hours of darkness, BMI, and medication use, 9, 15, and 17% reductions in urinary melatonin concentrations were observed with reports of two, three, and at least four drinks per day, respectively. There were no significant differences observed for one drink per day. Given the difficulty in accurately reporting portion sizes and the tendency of individuals to underestimate intakes of "undesirable" foods/beverages, it is conceivable that alcohol intake was underreported.

There are strengths and limitations to this research. We provided a known quantity and type of alcohol to postmenopausal women consuming a controlled diet under conditions of weight maintenance. The crossover design allows for participants to serve as their own controls. Women used a standardized method for the collection and storage of 24-h samples, and all of a participant's samples were randomly ordered and assayed together within the same batch. In addition, we tested an aliquot from 24-h urine samples pooled over 3 d (rather than spot, first morning, or nocturnal samples) to assess daily melatonin

TABLE 1. Mean 24-h urinary melatonin metabolite (6-SMT) levels (95% confidence intervals) for women consuming no alcohol and those consuming 15 and 30 g alcohol/d[a]

	No alcohol	15 g/d[b]	30 g/d[b]	Overall alcohol *P* value
6-SMT (μg/24 h)	9.367 (8.487, 10.247)	9.345 (8.468, 10.222)	9.043 (8.167, 9.918)	0.58
Difference (μg/24 h)[b]		−0.022 (−0.710, 0.666)	−0.324 (−1.011, 0.362)	
		$P = 0.95$	$P = 0.35$	

[a] Model adjusted for baseline 6-SMT, BMI, sleep (hours), time (4 *vs.* 8 wk), and daylight (hours) (n = 51).

[b] These differences and *P* values are for the respective comparisons with the no-alcohol treatment.

production. There are potential weaknesses in our study design. The duration of each treatment period was limited to 8 wk. We did not observe the timing or the consumption of alcohol, and we provided only a limited range of alcohol to participants. We collected only a "usual" sleep time variable. The 24-h sample may have diluted any treatment effect on nocturnal melatonin levels expected to manifest during sleep; it is possible that both daily quantity and pattern of melatonin production are important considerations.

In summary, our results suggest that decreasing total daily melatonin is not an important mechanism through which moderate alcohol consumption increases postmenopausal breast cancer risk.

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