Alcohol Consumption and Urinary Concentration of 6-Sulfatoxymelatonin in Healthy Women

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Consumption of alcoholic beverages may suppress circulating melatonin levels at night, possibly resulting in an increase in circulating estrogen. An increased estrogen burden could increase the risk of breast cancer. This study was designed to investigate whether alcohol consumption is associated with a decrease in nighttime melatonin levels in a group of healthy women. A total of 203 randomly selected healthy women between the ages of 20 and 74 years were recruited for a broader study of the effects of exposure to power-frequency magnetic fields on nocturnal levels of urinary 6-sulfatoxymelatonin. For the purposes of this analysis, data collection consisted of the following during two seasons of the year: (1) an in-person interview, (2) a daily activity diary, and (3) nocturnal urine collection for each of 3 consecutive nights. We found that the nocturnal urinary concentration of the primary metabolite of melatonin (6-sulfatoxymelatonin) decreased in a dose-dependent manner with increasing consumption of alcoholic beverages in the preceding 24-hour period, after taking into account the independent effects on melatonin of age, hours of darkness, use of medications that affect melatonin levels, and body mass index. A categorical analysis revealed no effect of one drink, but a 9% reduction with two drinks, a 15% reduction with three drinks, and a 17% reduction with four or more drinks. It remains unknown whether such a change could affect estrogen levels or breast cancer risk. (Epidemiology 2000;11:660–665)

Keywords: breast neoplasms, alcohol drinking, nocturnal variation, melatonin levels, melatonin metabolites, gender.

There is mounting evidence that alcohol consumption increases the risk of breast cancer in humans and that alcohol acts at a late stage in breast carcinogenesis.[1–4] A biological mechanism has been proposed that consumption of alcoholic beverages may suppress the normal nocturnal rise in melatonin production and release from the pineal gland,[5] which in turn could increase the release of estrogen by the ovaries.[6] There is now evidence that alcohol intake can result in an increase in estrogen production in women.[7–9] A chronic increase in estrogen might result in an increased risk of breast cancer, at least postmenopausally.[10–12]

There is relatively little known about the relation between alcohol consumption and circulating melatonin levels at night in humans. The first reports that pineal function might be affected by alcohol consumption appeared in the late 1970s. Alcoholics have depressed melatonin peaks at night.[13] In small experimental studies, alcohol administration has been reported to reduce the nocturnal rise in serum melatonin in both rats[14,15] and humans.[16,17] The purpose of this study was to evaluate the relation between alcohol consumption and nighttime pineal melatonin production in a group of healthy women. The concentration of 6-hydroxymelatonin sulfate in a morning-void urine sample accurately reflects total nocturnal melatonin production during the night.[18]

Subjects and Methods

The analyses reported here were part of a broader investigation designed to determine whether residential exposure to power-frequency (60-Hz) magnetic fields and/or light at night is associated with a decrease in nocturnal levels of the primary urinary metabolite of melatonin, 6-sulfatoxymelatonin, in women without a history of breast cancer. Additional details describing the methods of the full study are described elsewhere.[19,20] In brief, eligible subjects were women between the ages of 20 and 74 years who were randomly selected from the populations of King and Snohomish counties in Washington state, who participated as age-matched controls in a parent case-control study of breast cancer, and who agreed to be recontacted for future studies (N = 591). A protocol was developed to select a sample of approximately 200 women from this group with roughly equal

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representation of the highest and lowest magnetic field exposures. Of 241 women recruited to participate, 203 (84%) agreed.

Each participant was sent an introductory letter explaining the study, followed within 1 week by a telephone call to schedule an in-home visit. Data collection consisted of (1) a brief in-person interview focused on factors that might affect normal melatonin production at night; (2) 72-hour measurement of magnetic field levels and light intensity in the participant's bedroom, measured at 30-second intervals; (3) 72-hour measurement of personal exposure to magnetic fields, using a small meter worn by the participant, measured at 30-second intervals; (4) a daily activity diary corresponding to the 72-hour personal measurement period; and (5) nocturnal urine collection for each of the 3 consecutive nights corresponding to the 72-hour measurement period. Each participant was assigned at random to complete the entire data collection process again either 3 or 6 months later to achieve approximately even representation of measurements across seasons of the year.

Participants were instructed to void their bladder just before retiring for the evening on each of the 3 nights of urine collection. Any urine passed after that time, including the first morning void just after rising, was collected in a toilet-mounted collection device and transferred into an opaque collection bottle. Each participant was instructed to complete a protocol-adherence form, which documented (1) the time of urination before going to bed; (2) the time of first void the next morning; (3) alcohol consumption during the prior 24 hours, including the number of servings each of beer (12 oz), wine (4 oz), and liquor (1–1.5 oz); and (4) any problems associated with the collection of the sample, such as contamination or spillage. Each morning the participant placed the sample and completed form into a polystyrene cooler containing an ice pack for pickup and delivery to the laboratory. Upon receipt, the volume of urine was determined, and each sample was assayed for creatinine concentration using a kinetic modification of the Jaffe reaction with the Roche reagent for creatinine (Roche Diagnostic Systems, Nutley, NJ). Urinary concentrations of the primary metabolite of melatonin, 6-sulfatoxymelatonin, were determined using commercially available radioimmunoassay kits (CIDtech Research Inc., Mississauga, Ontario, Canada). The assay was run in duplicate with 500 ml of diluted sample and included a kit control and in-house urine control for each assay. Assay sensitivity was 0.5 ng/ml urine, and intra- and interassay percentage coefficients of variation were approximately 9% and 13%, respectively.

Statistical Methods

Given that urine samples and exposure data were collected from each participant for 3 consecutive days in each of two separate sessions, each participant contributed up to 6 days of alcohol consumption and melatonin data. Individual observations were highly correlated, and thus a repeated-measures analysis was required. Additionally, alcohol consumption and other factors that could affect nocturnal melatonin levels could change with each measurement day and/or session, thus introducing the additional requirement that such variables be considered as time dependent in the repeated-measures setting. All analyses used the SAS MIXED procedure to fit linear regression models with correlated error structure to account for the correlation of the repeated measurements on each subject.[21–24] The error structure had restricted maximum likelihood estimation of a six-by-six covariance matrix with three unknown parameters: day-to-day variation, session-to-session variation, and residual error.[25]

Three variables were defined before analysis to characterize a participant's alcohol consumption during the 24 hours before sample collection: an indicator of whether any alcohol was consumed (yes/no) and total quantity of all types of alcoholic beverages consumed (drinks/day), which were treated as both a continuous and a five-level categorical variable (no alcoholic beverages consumed, three categories representing one to three alcoholic beverages respectively, and four or more drinks per day). Additionally, several factors that can affect nocturnal melatonin levels were specified for use in covariate adjustment: (1) participant age; (2) season of the year, as measured by hours of darkness; (3) body mass index (BMI); and (4) use of certain medications that can affect melatonin levels.[6,27,28] Age was defined as the participant's age (in years) at the first data collection session. Duration of darkness was computed for each nighttime period as the number of hours between sunset the night urine collection began and the following sunrise (data obtained from the U.S. Naval Observatory for the Seattle metropolitan area for the specific days during which urine specimens were collected). BMI was computed as subject-reported weight (kg) per height squared (m^2) and categorized according to the Surgeon General's Report on Nutrition and Health[28] (category 1 = 15–23, category 2 = 23.1–27.2, category 3 = 27.3–32.1, and category 4 = 32.2–49.9). Medication use was defined as taking at least one medication that could be classified as a beta blocker, calcium channel blocker, or psychotropic medication in the 24 hours before the first morning void.

For all analyses, the natural log transformation of urinary 6-sulfatoxymelatonin level normalized to creatinine (nanograms 6-sulfatoxymelatonin per milligram creatinine) was used as the response variable of interest. All analyses investigated the effects of alcohol consumption on 6-sulfatoxymelatonin without adjustment for any covariates and with adjustment for age, hours of darkness, BMI, and medication use as a group. Using parameter estimates from the models, crude (unadjusted for the covariates) and adjusted least-squares means of natural log 6-sulfatoxymelatonin were computed at each level of total number of alcoholic beverages consumed per day and at yes/no categories of any alcohol consumption. The means were back-transformed to present results in the original scale of nanograms 6-sulfatoxymelatonin per milligram creatinine. Least-squares means could not be computed from regression models, which

TABLE 1. Median Mean Concentration of Nocturnal Urinary 6-Sulfatoxymelatonin by Level of Alcohol Consumption (Drinks per Day)

Alcohol Consumption (Drinks Per Day)	Days of Observation		Median Nighttime Melatonin (ng/mg Creatinine)	Range Nighttime Melatonin (ng/mg Creatinine)
	No.*	%†		
0	811	73.3	16.6	0.4–86.2
1	125	11.3	18.0	0.7–76.1
2	98	8.9	16.6	0.6–70.1
3	47	4.2	14.4	2.0–97.7
4 or more	25	2.3	12.1	0.8–39.9

* Number of days includes up to 6 per individual study participant.
† Percentage of total number of days of observation (1,106) from 203 participants.

used the continuous-variable number of alcoholic beverages consumed per day. Thus, the parameter estimates from these models were back-transformed and are presented in the text.

There were eight samples from five subjects whose concentrations of 6-sulfatoxymelatonin were below the detectable limits of the assay (0.5 ng melatonin/ml urine). For these samples, we assigned half the value of the detectable limit of the assay (0.25 ng melatonin/ml urine), and then we normalized to each sample's creatinine level (mg creatinine/dl urine). Additionally, there were three subjects who reported taking melatonin supplements. We excluded these subjects because melatonin supplementation resulted in urinary 6-sulfatoxymelatonin levels that were elevated more than 100 times over unsupplemented values.

Results

There were 1,218 individual days of data collection, consisting of a urine sample and its corresponding protocol adherence form. Of these, a complete and uncontaminated urine sample was obtained on 1,106 days (91%). Three women who participated in the first session of data collection changed residences before the second session and were consequently omitted from the second session.

There were approximately equal numbers of women according to 5-year age groups between the ages of 40 and 74 years (that is, a virtually uniform distribution across age groups). Only 15 of the 203 participants were under age 40. A slightly greater number of the total sample days occurred at the extremes of daily darkness (8.0 hours at summer solstice and 15.5 hours at winter solstice) than at either equinox. BMI was slightly skewed to the low end of the scale, with 35% in the lowest category and only 15% in the highest category. Medication use was reported by 29% of the participants, with 15 women reporting using beta blockers, 16 using calcium channel blockers, and 34 using psychotropics.

The distribution of 6-sulfatoxymelatonin concentration among the 1,106 complete samples was slightly skewed to the right (mean = 19.3 ng/mg creatinine; standard deviation = 12.8 ng/mg creatinine). Melatonin levels were highly correlated from day to day within each measurement session (Spearman rank correlation coefficients 0.90 and 0.85, respectively, for the two sessions). The concentration of 6-sulfatoxymelatonin varied according to season of the year, defined as the 3-month period centered on the respective equinox or solstice. Highest values were observed in winter (mean = 22.5 ng/mg creatinine) and lowest in the summer (mean = 16.8 ng/mg creatinine). Spring and fall values were intermediate (mean = 20.1 and 18.1 ng/mg creatinine, respectively).

Age, hours of darkness, BMI category, and medication use were each strongly associated with urinary 6-sulfatoxymelatonin levels. Age was inversely associated with 6-sulfatoxymelatonin; each increase of 1 year in age was associated with a 1% decrease in nocturnal urinary 6-sulfatoxymelatonin level [95% confidence level (CL) = –2%, 0%]. Including a quadratic term for age did not alter the estimated effect of participant age on 6-sulfatoxymelatonin and was therefore not included in any subsequent analyses. Nocturnal 6-sulfatoxymelatonin levels were related in a direct manner to hours of darkness. Each 1-hour increase of darkness was associated with approximately a 2% increase in 6-sulfatoxymelatonin concentration (95% CL = 1%, 4%). BMI was inversely associated with 6-sulfatoxymelatonin: more obese women had lower 6-sulfatoxymelatonin levels. Relative to the lowest BMI category, each higher category was associated with a decrease of approximately 7% in nocturnal 6-sulfatoxymelatonin concentration (95% CL = –15%, 0%). Those subjects who reported using beta blockers, calcium channel blockers, or psychotropic medications had lower 6-sulfatoxymelatonin levels. Use of these medications was associated with approximately a 28% decrease in nocturnal urinary 6-sulfatoxymelatonin concentration (95% CL = –38%, –17%).

Table 1 summarizes nocturnal urinary 6-sulfatoxymelatonin levels in relation to reported daily alcohol consumption among study participants. Forty-seven per cent of the participants reported consuming at least one alcoholic beverage on at least 1 night of the up to 6 nights of data and sample collection. Approximately 73% of the urine samples were collected on nights when the study participant reported consuming no alcohol, and only 25 were collected on nights when four or more alcoholic drinks were consumed. There is some indication that the median concentration of 6-sulfatoxymelatonin decreases with increasing alcohol consumption when two or more drinks are consumed. Individual participants are represented up to six times in Table 1, and urinary 6-sulfatoxymelatonin measurements within each subject are highly correlated from day to day. These characteristics of the data are not accounted for in the descriptive results shown in Table 1.

Results of the analyses of any alcohol consumption and category of number of alcoholic beverages consumed per day are presented in Table 2. Crude analyses (unad-

TABLE 2. Estimated Nocturnal Urinary 6-Sulphatoxymelatonin by Any Alcohol Consumption and Category of Number of Alcoholic Drinks Per Day

Alcohol Consumption Variable	Estimated Mean Nighttime Melatonin (ng/mg Creatinine)*	% Change from Referent Level	95% Confidence Limits of % Change from Referent Level
Any alcohol consumption			
Crude (unadjusted)			
No	15.2		
Yes	14.4	−5%	−10%, +2%
Adjusted†			
No	15.2		
Yes	14.5	−5%	−11%, +1%
Alcohol consumption (drinks per day)			
Crude (unadjusted)			
None	15.2		
1	15.4	+1%	−6%, +9%
2	13.8	−9%	−17%, −1%
3	13.0	−14%	−24%, −4%
4 or more	12.7	−16%	−29%, −2%
Adjusted†			
None	15.3		
1	15.4	+1%	−6%, +8%
2	13.9	−9%	−17%, −1%
3	13.1	−15%	−24%, −4%
4 or more	12.7	−17%	−29%, −3%

* Least-squares means computed using parameter estimates from regression models.
† Adjusted for the effects of age (years), hours of darkness, medication use, and body mass index category.

justed for the effects of any covariate) indicated that drinking at least one alcoholic beverage in the 24 hours before urine collection was associated with a 5% decrease in the urinary level of 6-sulfatoxymelatonin. The estimate was unchanged after adjusting for the effects of age, hours of darkness, BMI, or medication use. Table 2 also indicates that a relation exists between decreasing 6-sulfatoxymelatonin and each increment in alcohol consumption level when two or more drinks are consumed per day. Relative to the baseline level (no alcohol consumption), there was little change in 6-sulfatoxymelatonin concentration with the consumption of one alcoholic beverage per day, a 9% reduction with two drinks per day, a 14% reduction with three drinks per day, and a 16% reduction associated with consuming four or more drinks per day. Results were similar when adjusted for the effects of other factors that affect melatonin levels. When the number of alcoholic drinks consumed per day was treated as a continuous variable in a linear model, each drink consumed was associated with a 5% reduction in 6-sulfatoxymelatonin (95% CL = −8%, −3%). There were relatively few individual participant days when more than three alcoholic beverages were consumed and only 8 days when more than four drinks were consumed. Thus, in treating number of drinks per day as a continuous variable, the estimated effect of each drink consumed may be unduly influenced by those few participant days with high alcohol consumption. The association of alcohol consumption with 6-sulfatoxymelatonin was stronger among postmenopausal than among premenopausal women, although in the same direction.

Discussion

Interest in the potential role of melatonin in breast cancer etiology began in the 1960s (reviewed in Ref [29]) and has increased in recent years.[30,31] Chronic disruption of the normal daily cycle of melatonin by aspects of modern life has been suggested as a factor that may account in part for the high risk of breast cancer in industrialized societies.[32,33]

Melatonin injection has been reported to inhibit chemically induced mammary tumor development in rats, and pinealectomy to enhance it in both the 7,12-dimethylbenz(*a*)anthracene model[34] and the nitrosomethylurea (NMU) model,[35] although there has also been a report of no effect of melatonin injection in the NMU model.[36] Two mechanistic interpretations of these observations are that melatonin may slow development and turnover of the normal mammary cells at risk of malignant transformation and/or that melatonin may be directly oncostatic.[37] These two mechanisms act at the opposite ends of the carcinogenic process. *In vitro*, melatonin has been shown to slow the growth of some subclones of the estrogen-responsive human breast cancer cell line MCF7 in culture.[38,39] In chemoprevention assays, melatonin has been shown to inhibit alveolar nodule formation in mouse mammary organ cultures, to inhibit transformation of rat tracheal epithelial cells, and to inhibit anchorage independence in human lung tumor cells and mouse JB6 cells.[40] Shah *et al*[41] used a different approach to investigate the effect of melatonin on tumorigenesis by using constant light as a "functional pinealectomy" to increase 7,12-dimethylbenz(*a*)anthracene-induced mammary tumor yield, an effect that was reversed by melatonin administration.

Nighttime plasma melatonin levels have been reported to be lower in women with estrogen receptor-positive breast cancer than in estrogen receptor-negative breast cancer and in healthy control women[42] and lower in cases of primary breast cancer than in women with benign breast disease.[43] In contrast, daytime melatonin was found to be higher in breast cancer patients in one report.[44] It is difficult to assess the meaning of these findings owing to the presence of disease and its possible effect on melatonin levels. Whether low nocturnal melatonin predisposes to increased risk of breast cancer in women is difficult to determine. Elucidating the role of melatonin in normal and malignant growth of breast tissue may provide a better understanding of the role of estrogen in the etiology of breast cancer.

The majority of breast cancer cases occur after menopause when ovarian estrogen production has effectively ceased. Consequently, it should be irrelevant that alco-

hol consumption after menopause might lead to a suppression of melatonin and an increase in estrogen production. On the other hand, alcohol consumption beginning before menopause may lead to elevated estrogen production in the early years, leading to increased turnover of the normal breast epithelial cells at risk for malignant transformation. If cancer results from mutations in the two homologues of a single gene (for example, tumor-suppressor gene) or the constellation of several mutations at different genes (for example, proto-oncogenes), then early increases in numbers of intermediate cells containing some of the required mutations are expected to elevate risk for the remaining years of life.[45] In addition, if melatonin has an oncostatic capability, particularly in estrogen receptor-positive tumor cells, then an alcohol-mediated melatonin reduction could be relevant to breast cancer etiology both pre- and postmenopausally.

We found a reduction of urinary 6-sulfatoxymelatonin associated with alcohol consumption in the preceding 24-hour period. There appeared to be little or no effect of one drink per day, but reductions occurred with two or more. It is interesting to consider the association of alcohol consumption and risk of breast cancer in light of these findings; in a pooled analysis of large cohort studies, Smith-Warner *et al*[2] reported that women consuming two or more drinks per day had an elevated risk, but for women consuming about one drink per day it was unclear whether risk was elevated. In a large case-control study, Swanson *et al*[4] reported an elevated risk of breast cancer in women who drink, but this elevation was restricted to two or more drinks per day. On the other hand, Bowlin *et al*[3] did report an elevated risk in very light drinkers.

It has been hypothesized that alcohol consumption may reduce nocturnal melatonin levels by affecting sleep patterns in some manner. We used participant-reported hours of sleep for each night of urine collection to investigate this possibility in the present study. Controlling for number of hours of sleep did not materially change the effect of alcohol consumption on nocturnal urinary 6-sulfatoxymelatonin levels. There was an association, however, between increasing consumption of alcoholic beverages in the 24-hour period preceding urine collection and decreasing reported number of hours of sleep that night. There was no information available on participant-reported quality of sleep, and it is possible that sleep quality could be a factor in determining how alcohol consumption might influence nocturnal melatonin levels.

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