Biomonitoring of Estrogen and Melatonin Metabolites Among Women Residing Near Radio and Television Broadcasting Transmitters

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Objectives: *Metabolites of estrogen (estrone-3-glucuronide [E1G]) and melatonin (6-hydroxymelatonin sulfate [6-OHMS]) were characterized among women living in a community with increased radiofrequency (RF) exposure from radio and television transmitters.* ***Methods:*** *RF spot measurements, and personal 60-Hz magnetic field and residential parameters were collected. Overnight urine samples were assayed for E1G and 6-OHMS excretion.* ***Results:*** *Among premenopausal women, there were no associations between RF or 60-Hz nonionizing radiation and E1G or 6-OHMS excretion. Among postmenopausal women, increased residential RF exposures, transmitter proximity and visibility, and temporally stable 60-Hz exposures were significantly associated with increased E1G excretion. This association was strongest among postmenopausal women with low overnight 6-OHMS levels.* ***Conclusions:*** *RF and temporally stable 60-Hz exposures were associated with increased E1G excretion among postmenopausal women. Women with reduced nocturnal 6-OHMS excretion may represent a sensitive subgroup.* (J Occup Environ Med. *2007;49:1149–1156*)

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Concerns about the potential health effects of radiofrequency (RF) nonionizing radiation arise in part because of the pervasiveness of radio and television (TV) broadcasting. Exposure to RF nonionizing radiation has been associated with perturbations in human hematological, neurological, cardiovascular, reproductive, and endocrine systems, and with increased cancer risks.[1,2] Studies examining the relationship between nonionizing radiation and cancer in humans have focused primarily on power frequency (50 to 60 Hz) exposures rather than RF or the combined effects of RF and 60-Hz fields, although both exposures are common.[2–4] Results from these studies have been inconsistent and inconclusive, in part because of the lack of an established biological mechanism. One hypothesized mechanism to explain how nonionizing radiation may increase cancer risks focuses on reductions in melatonin, which have been observed in response to nonionizing radiation exposures in some studies.[3,5–9] There is considerable evidence that melatonin modulates human reproduction, and reduced melatonin levels have been linked with increased estrogen and other reproductive hormones in women.[10–14] Elevated levels of estrogen or its metabolites are a risk factor for breast cancer, particularly among postmenopausal women.[15–19] Therefore, reductions in melatonin are thought to confer an increased breast cancer risk in part because of increased estrogen production.[5,6,20–22] Recently, a reduction in breast cancer risk was observed among women with

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increased nocturnal melatonin excretion.[21] The relationship between melatonin and estrogen has not been well characterized and may be important in elaborating breast cancer susceptibility. There is a need for more research to define the relationship between these hormones in women, and to determine whether they may be altered by exposure to RF or combined RF and 60-Hz fields. To test the hypothesis that RF or 60-Hz nonionizing radiation exposures reduce melatonin and increase estrogen production, we measured a urinary metabolite of each hormone among women living in a community with increased RF power densities from nearby radio and TV broadcasting antennae.[23]

Subjects and Methods

The study area, participant population, and RF exposure characteristics have been previously described.[23] A population census identified 576 homes and 1375 individuals in the study area. A sample of 280 randomly selected male and female participants aged ≥8 years was recruited from 161 residences with high (>4.0 $\mu W/cm^2$), medium (0.5 to 4.0 $\mu W/cm^2$), or low (<0.5 $\mu W/cm^2$) RF exposures.[23] The study participation rate was 64% among eligible persons contacted, and the mean age and mean residential power density did not differ between participants and nonparticipants. A total of 127 postmenarche women aged 12 to 81 years participated in the study, conducted between September 2002 and December 2003.

Each subject was studied for a 2.5-day period beginning in the evening on the initial day and ending in the morning on the final day of participation. Exposure assessment included RF spot measurements inside and outside the home, and continuous personal monitoring for 60-Hz magnetic field (MF) and ambient light exposures. RF power densities were obtained from one exterior and five interior locations (bedroom, kitchen, living room, computer room or office, room most used) using a survey meter (Narda EMR-300 with type 18 isotropic E-field probe, flat response: 0.1 to 3000 MHz, detection limit: 0.2 V/m, Narda Safety Test Solutions, Pfullingen, Germany). RF measurements were converted to $\mu W/cm^2$, nondetectable measurements were assigned a value of one-half the detection limit (0.01 $\mu W/cm^2$), and the five interior readings were averaged to obtain the house average RF power density. A global positioning system identified the geographic coordinates and the elevation of each exterior RF measurement (eTrex Legend, Part No. 190-00,234-00, Garmin International, Olathe, KN). The distance between each transmitter and residence was characterized using a geographic information system (ERSI ArcInfo, Redlands, California). The average distance from each residence to 15 major transmitters in the study area, the home elevation, and the percentage of transmitters visible from each home were used for RF exposure assessment along with the house average power density.[23]

Personal exposure to 60-Hz MFs and ambient light was logged continuously at 15-second intervals using EMDEX II meters (Enertech, Campbell, CA) adapted with a light sensor (Grasby Optronics, Orlando, FL).[24] Each participant wore an EMDEX meter at the waist during the day, and placed it bedside on nonconductive material adjacent to the waist during sleep. MF exposures from the following periods were extracted from each EMDEX recording: sunset until bedtime, and sunrise until 4 hours past sunrise. These periods were chosen *a priori* to characterize times of day (ie, around sunrise and sunset) when MF exposures[24,25] may influence human melatonin production. Time-weighted average MF exposure intensity (geometric mean) and temporal stability (standardized rate of change metric, RCMS) were calculated for each period.[8,24,26] The RCMS estimates first-lag serial autocorrelation of consecutive MF measurements; low values represent temporally stable personal MF exposures.[27] Power frequency exposures summarized using the geometric mean and RCMS have previously been linked with changes in 6-hydroxymelatonin sulfate (6-OHMS) excretion and other biological effects in men.[8,24,26,28]

Each participant collected one overnight urine sample immediately after their first night of participation and a second overnight sample on their final participation night. Samples included the entire urinary output after sleep plus any voids occurring during the sleep period. Samples were transported on ice and frozen at −70°C until analysis. Melatonin was characterized by radioimmunoassay of its major metabolite, 6-OHMS (Stockgrand, Surrey, UK).[29,30] Nocturnal 6-OHMS concentrations were normalized to urinary creatinine and expressed as nanograms per milligram creatinine. The interassay coefficient of variation was 22% for the low control, 10% for the medium control, and 7% for the high control. The average intra-assay coefficient of variation was 13%, and the limit of detection was 0.1 ng/mL.

Estrogen production was assessed using a previously established enzyme immunoassay for estrone-3-glucuronide (E1G).[31] Urinary E1G is a principal estrogen metabolite that is well correlated with circulating 17-β-estradiol; it is frequently used to evaluate ovarian function in women.[32–35] Results were adjusted for nonparallelism,[31] and normalized to specific gravity to account for specimen dilution.[31,36] The method detection limit was 1.45 ng/mL.[31] Inter- and intra-assay coefficients of variation for the high control concentration (3.75 ng/mL) were 3.2% and 3.6%, respectively, and those for the low control (1.51 ng/mL) were 13.7% and 12.2%, respectively.

Self-administered questionnaires were completed at the end of the data collection period to characterize individual (age, race, body mass index

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[BMI], education, occupation, income, sleep quality), medical (current medications and supplements: birth control medication; hormone replacement therapy; melatonin supplementation; use of sleep aids, anti-inflammatory agents, antidepressants, pain killers, β-adrenergic agents), reproductive (age at menarche, menstrual cycle regularity, parity, breastfeeding, menopausal status), and lifestyle factors (amount of exercise; and fruit, vegetable, alcohol, and tobacco consumption) potentially influencing the estrogen or melatonin metabolites. Date of onset of last menstruation was recorded at the time of urine collection, and a coded mailer was used to obtain the next date of menses onset.[17] For premenopausal cycling women, the menstrual cycle was divided into three categories to permit subgroup analyses.[37–39] Menstrual cycle status was categorized as follows: days 0 to 9 (follicular phase), days 10 to 18 (ovulatory estrogen peak), or days 19 to 32 (luteal phase) after onset of bleeding.

Statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC). Women reporting supplemental melatonin consumption ($n = 4$), current use of birth control medications ($n = 16$), breastfeeding ($n = 4$), or hormone replacement therapy ($n = 20$) were excluded. Potential confounders for each biomarker were identified by comparing urinary 6-OHMS or E1G concentrations among subjects with or without a given characteristic. Variables were retained for subsequent multivariable analyses at a $P < 0.10$ level of significance. Study hypotheses were tested among women grouped by menopausal status after adjustment for factors identified using this procedure. Adjustment for the effects of ambient light exposure or self-reported sleep quality did not influence the outcome of these analyses.

The relationship between the estrogen and melatonin metabolites was evaluated using generalized linear models. Women were grouped into quartiles of 6-OHMS and adjusted (least-squares) means of E1G excretion were calculated within each quartile. E1G concentrations in the upper and lower quartiles of 6-OHMS excretion were then compared using the least significant differences statistic based on the type III sums of squares. This relationship was also evaluated using 6-OHMS and E1G as continuous variables. Excretion of 6-OHMS on the initial day (day 0) was compared with E1G measured on the second collection day (day 2). Separate analyses of the relationship between these metabolites were performed for samples collected on the same day.

The hypothesis that RF exposures (house average RF power density, proximity, elevation, transmitter visibility) decreased 6-OHMS or increased E1G excretion was tested. Subjects were grouped into RF exposure quartiles, and adjusted mean metabolite concentrations in the upper and lower quartiles were compared using the least significant differences statistic. The effect of RF exposure as a continuous variable on 6-OHMS or E1G excretion was also evaluated. The effects of 60-Hz MF exposure on 6-OHMS or E1G levels were analyzed in a similar manner. Separate analyses assessed whether women with reduced 6-OHMS excretion were more sensitive to the effects of RF or 60-Hz fields on E1G.[25,28,40–43] Women were first grouped above and below median melatonin metabolite concentrations. Exposure tertiles were used to test potential dose-response relationships, and to ensure that at least several women would be distributed among each exposure category. Adjusted mean E1G concentrations were compared among women in the upper and lower tertiles of RF power density, or the other exposure parameters. The combined effects of RF and 60-Hz exposures on each biomarker were evaluated similarly. Results obtained using 6-OHMS or E1G values averaged over the 2 collection days, or using total overnight 6-OHMS or E1G excretion as the dependent variable were similar to those presented below.

Results

A total of 56 pre- and 27 postmenopausal women were studied. The proportion of women who were of non-Hispanic white race or ethnicity was 90% or greater among both pre- and postmenopausal groups. The median age was 43 years (interquartile range, 18 to 49) among premenopausal, and 59 years (47 to 81) among postmenopausal women. The median BMI was 22 kg/m^2 (20 to 24) among premenopausal, and 25 kg/m^2 (23 to 30) among postmenopausal participants. Average 24-hour ambient light exposures were similar among both groups (premenopausal median, 443 lux [interquartile range, 99 to 1158]; and postmenopausal median, 577 lux [279 to 989]).

The relationship between estrogen and melatonin metabolite excretion is presented in Table 1. There were no statistically significant differences in adjusted mean E1G concentrations among women with increased melatonin metabolite excretion overall (Table 1). Modest reductions in mean E1G relative to increased 6-OHMS excretion were noted among postmenopausal women and among premenopausal women in their follicular phase. Results obtained when comparing mean 6-OHMS and E1G values among urine samples that were collected concurrently (both on day 0 or both on day 2, data not shown) were similar to those obtained when 6-OHMS levels from the initial day were compared with E1G concentrations on the second day.

Among premenopausal women, measures of exposure to RF or 60-Hz fields were not associated with E1G levels after adjustment for use of birth control medication, menstrual cycle stage, fruit intake, exercise, and season of participation (data not shown). There were also no effects of RF or 60-Hz exposures on 6-OHMS

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TABLE 1
Estrogen Metabolite Concentrations by Quartile of Melatonin Metabolite Excretion, Lookout Mountain, Golden, CO, 2002–2003

Mean Urinary E1G Concentration*	Melatonin Metabolite Quartiles†					
	1	2	3	4	1 vs 4 *P*	Continuous Variable *P*
All women ($n = 83$)‡	30.2	29.6	31.0	26.2	0.46	0.67
Premenopausal ($n = 56$)§	34.0	46.0	41.5	38.5	0.65	0.09
Follicular ($n = 12$)	26.9	29.9	19.6	11.4	0.36	0.12
Ovulatory ($n = 13$)	64.6	68.1	62.2	111.4	0.45	0.79
Luteal ($n = 21$)	35.3	56.9	58.0	46.2	0.48	0.47
Perimenopausal‖ ($n = 10$)	136.0	63.1	NA	23.7	0.02	NA
Postmenopausal¶ ($n = 27$)	16.0	11.3	13.5	10.6	0.21	0.39

*Least squares means of estrone-3-glucuronide (E1G) on day 2 (ng/mL).
†6-hydroxymelatonin sulfate (6-OHMS) excretion on day 0 (ng/mg creatinine).
‡Adjusted for menstrual stage and menopausal status.
§Adjusted for menstrual stage, season, fruit intake, and exercise; excludes women taking melatonin supplements, birth control medication, and hormone replacement therapy.
‖Women with a menstrual cycle longer than 32 days.
¶Adjusted for BMI; excludes women taking melatonin supplements or on hormone replacement therapy.

excretion among premenopausal women (adjusted for education, miscarriages, and smoking, data not shown). The response of E1G to RF or 60-Hz exposures did not differ among premenopausal women grouped above and below median 6-OHMS levels (data not shown). When the combined effects of RF and 60-Hz exposures were evaluated, there was no evidence for a reduction in 6-OHMS or an elevation in E1G levels in either pre- or postmenopausal women (data not shown).

Postmenopausal women in the highest quartile of house average RF power density had higher mean E1G concentrations (adjusted for BMI) compared with women in the lowest RF exposure quartile (Table 2). Statistically significant increases in E1G were also associated with increased RF transmitter proximity or visibility (Table 2). Postmenopausal women with increased exposure to temporally stable 60-Hz fields in the evening (sunset to bedtime) had higher adjusted mean E1G levels than those observed in the lowest exposure quartile ($P = 0.03$, Table 2). There was no statistically significant reduction in mean nocturnal 6-OHMS excretion (adjusted for month of participation and eye color) among postmenopausal women with increased exposure to RF or 60-Hz fields (Table 2).

RF-related increases in mean E1G concentrations were more pronounced among postmenopausal women with low 6-OHMS levels. An increase in adjusted mean E1G excretion across tertiles of house average RF power density was observed among women with low 6-OHMS levels ($P < 0.01$, Fig. 1), whereas no RF-related changes in E1G were observed among women with elevated 6-OHMS levels. A similar increase in mean urinary E1G concentrations was observed across tertiles of RF transmitter proximity ($P < 0.01$, Fig. 2), or visibility (least-squares means for tertiles 1, 2, and 3 = 8.7, 23.4, and 17.2, respectively, $P = 0.03$ for tertile 1 vs 3) among women with reduced 6-OHMS excretion, but not among women with increased 6-OHMS excretion (12.4, 12.2, 12.2, $P = 0.97$ for visibility tertile 1 vs 3).

Discussion

To our knowledge, this study was the first to examine the relationship between residential RF and 60-Hz exposures on estrogen and melatonin metabolite excretion in adult women. Previous epidemiologic studies of populations residing near TV and radio transmitters have relied on distance from the RF source as a proxy for exposure.[1,2,44–50] RF exposures in this study were quantified using in-home power density measurements and other RF exposure parameters that were validated using a geographic information system (proximity, transmitter line of sight visibility, elevation).[23] Each RF exposure parameter was associated with an increase in E1G excretion among postmenopausal women, and the effects persisted with adjustment for potential confounding factors. Results from this investigation also indicated that personal exposure to temporally stable 60-Hz MFs increase E1G excretion among postmenopausal women. Use of the RCMS was based on evidence that the temporal aspects of personal MF exposure can elicit changes in biological processes,[27,51] and is supported by other studies linking temporal stability with endocrine alterations in men.[8,24,26]

Studies evaluating the relationship between melatonin and estrogen production have had inconsistent results. Melatonin and its receptors are present in human reproductive tissues and in brain regions that regulate steroid hormone production.[10,52–57] At elevated levels, melatonin reduces estrogen and inhibits ovulation in women.[58–62] Endogenous melatonin secretion has been inversely associated with gonadotrophic hormones[13,63,64] and with estrogen levels,[12–14,61,64] although not consistently.[65,66] Acute melatonin suppression did not alter estrogen secretion among premenopausal women.[65] Nevertheless, long-term night work, which disrupts melatonin production among women,[67–69] has been associated with increased urinary estradiol and androstenedione levels.[14] Recent studies demonstrating melatonin-mediated inhibition of aromatase activity may provide a plausible explanation for reductions in E1G excretion that may be observed among postmenopausal

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TABLE 2
Urinary Estrogen and Melatonin Metabolite Concentrations Among Postmenopausal Women (n = 27), Lookout Mountain, Golden, CO, 2002–2003

	Exposure Quartiles					
Mean Urinary Concentration*	**1**	**2**	**3**	**4**	**1 vs 4 *P***	**Continuous Variable *P***
House average RF power density (μW/cm†)						
Average Exposure within quartile	0.04	0.2	0.4	1.4		
6-OHMS	60.6	62.5	65.2	30.1	0.09	0.31
E1G	8.4	14.2	15.9	17.2	0.02	<0.01
Proximity to RF transmitters (km)†						
Average Exposure within quartile	3.1	2.2	1.6	0.9		
6-OHMS	69.8	37.5	51.4	54.4	0.52	0.51
E1G	8.4	11.2	17.8	18.2	0.01	0.04
Transmitter visibility (percent transmitters visible from home)						
Average Exposure within quartile	0	9	39	83		
6-OHMS	58.6	38.5	41.7	62.4	0.87	0.96
E1G	8.7	12.5	14.6	18.5	0.02	0.02
Elevation of residence (feet above sea level)						
Average Exposure within quartile	7303	7398	7491	7638		
6-OHMS	57.5	38.4	51.4	56.9	0.98	0.75
E1G	11.0	10.7	15.3	17.4	0.15	0.08
Geometric mean 60-Hz magnetic fields (mG)‡						
Average Exposure within quartile	0.2	0.3	0.5	1.3		
6-OHMS	30.7	44.6	82.5	88.8	0.02	0.28
E1G	11.2	17.4	11.8	15.1	0.31	0.24
Temporally stable 60-Hz magnetic fields (RCMS/15 s)‡						
Average Exposure within quartile	0.45	0.68	0.84	0.94		
6-OHMS	39.6	95.6	35.3	74.4	0.10	0.61
E1G	10.1	11.1	16.3	19.6	0.03	<0.01

*Least squares means of urinary 6-hydroxymelatonin sulfate (6-OHMS, ng/mg creatinine) adjusted for month, eye color; estrone-3-glucuronide (E1G, ng/mL) adjusted for body mass index.

†Average distance to 15 nearby radio and television transmitters.

‡Personal exposure to 60-Hz magnetic fields between sunset and bedtime on the evening before overnight urine sample collection.

RF indicates radio frequency (0.1 to 3000 MHz); RCMS, standardized rate of change metric.

women with elevated 6-OHMS levels.[70–72] Future studies of the amplitude, duration, and acrophase of melatonin and estrogen production over time are suggested to help elucidate the relationship between these hormones.

No association between MF exposure and melatonin production was identified among pre- or postmenopausal women in this study. Numerous independent investigators have reported reduced human melatonin production in response to MF exposures, although others have reported no effects or modest reductions at marginal levels of statistical significance.[1,3,7,73,74] The lack of consistency among studies may be due to a true lack of MF effect on melatonin. Alternatively, the inconsistent findings may be due to an undefined critical exposure parameter, complexities involved with the accurate characterization of melatonin, small sample sizes with corresponding low statistical power, an inability or lack of control for confounding factors such as ambient light exposure, or genetic variability.[1,3,7,73,74] Several investigators have suggested that certain individuals may be more sensitive to the effects of nonionizing radiation than others.[28,75–78] Support for this idea has been presented in studies of 60-Hz MF exposure and human melatonin production.[25,40–43] In the present study, associations between nonionizing radiation exposure and estrogen were strongest among postmenopausal women who, for whatever reason, had low 6-OHMS levels. The increased sensitivity to RF and 60-Hz fields among postmenopausal women with reduced melatonin is compatible with our previous study in which 60-Hz associated changes in several immune markers were accentuated among workers with reduced melatonin production on the preceding night.[28]

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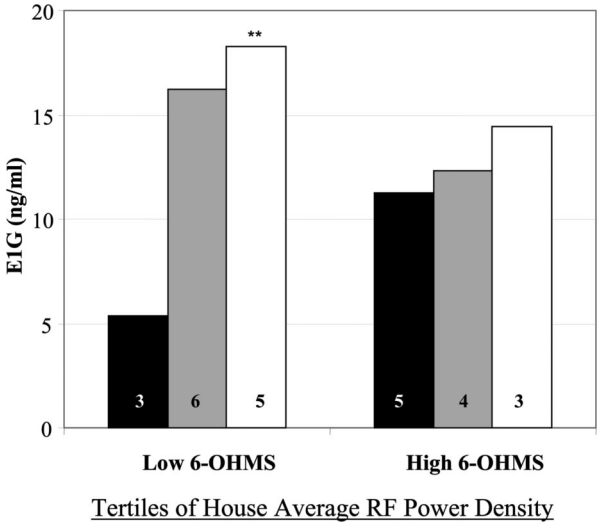


Fig. 1. Least squares means (adjusted for body mass index) of E1G excretion on day 2 by tertile of house average power density (black bars, lower tertile; white bars, upper tertile; number of women per tertile noted inside bar) stratified among postmenopausal women above (right side) or below (left side) median 6-hydroxymelatonin sulfate (6-OHMS) excretion on day 0. Women taking hormone replacement therapy or melatonin supplements were excluded. $^{**}P \leq 0.001$ versus first tertile within 6-OHMS stratum.

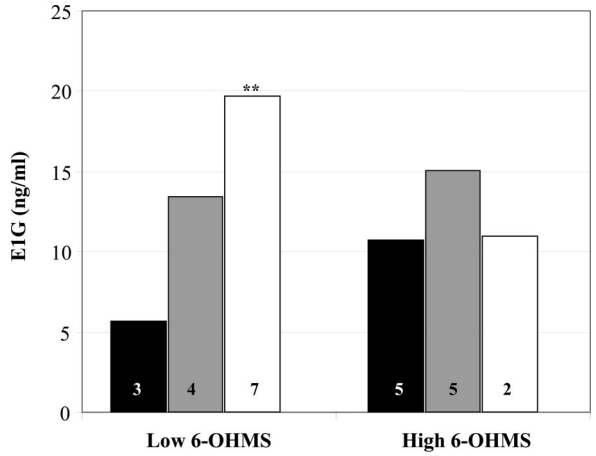


Fig. 2. Least squares means (adjusted for body mass index) of E1G excretion on day 2 by tertile of average proximity to nearby RF transmitters (black bars, lower tertile; white bars, upper tertile; number of women per tertile noted inside bar) stratified among postmenopausal women above (right side) or below (left side) median 6-hydroxymelatonin sulfate (6-OHMS) excretion on day 0. Women taking hormone replacement therapy or melatonin supplements were excluded. $^{**}P \leq 0.001$ versus first tertile within 6-OHMS stratum.

Conclusions

In summary, an association between RF and 60-Hz nonionizing radiation exposures and increased E1G excretion was observed among postmenopausal but not premenopausal women residing on Lookout Mountain in Colorado, a community with a high density of radio and TV transmitters. Interpretation of these results is limited by the number of postmenopausal women available for analysis and by the lack of exposure-related effects among premenopausal women. The ability to detect an effect among premenopausal women may have been obscured by the larger natural variations in estrogen that occur during the menstrual cycle. Also, the possibility that estrogen metabolism was shifted to another metabolite cannot be excluded. Nonetheless, these observations persisted with adjustment for potential confounding, and were consistent when multiple RF exposure metrics (measured power density, distance, proximity to transmitters, elevation) were evaluated. The findings support the hypothesis that RF and temporally stable 60-Hz exposures influence E1G excretion among postmenopausal women. Women with reduced nocturnal 6-OHMS excretion may represent a sensitive subgroup.

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References

1. Krewski D, Byus CV, Glickman BW, et al. Potential health risks of radiofrequency fields from wireless telecommunication devices. *J Toxicol Environ Health B Crit Rev*. 2001;4:1–143.
2. Ahlbom A, Green A, Kheifets L, Savitz D, Swerdlow A. Epidemiology of health effects of radiofrequency exposure. *Environ Health Perspect*. 2004;112:1741–1754.
3. Portier CJ, Wolfe MS, eds. Assessment of Health Effects from Exposure to Power-Line Frequency Electric and Magnetic Fields. Department of Health and Human Services Publication. NIH Publication Number 98-3981. Research Triangle Park, NC: National Institute of Environmental Health Sciences; 1998.

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4. Feychting M, Forssen U. Electromagnetic fields and female breast cancer. *Cancer Causes Control*. 2006;17:553–558.
5. Stevens RG. Electric power use and breast cancer: a hypothesis. *Am J Epidemiol*. 1987;125:556–561.
6. Caplan LS, Schoenfeld ER, O'Leary ES, Leske MC. Breast cancer and electromagnetic fields-a review. *Ann Epidemiol*. 2000;10:31–44.
7. Henshaw DL, Reiter RJ. Do magnetic fields cause increased risk of childhood leukemia via melatonin disruption? *Bioelectromagnetics*. 2005;Suppl 7:S86–S97.
8. Burch JB, Reif JS, Noonan CW, Yost MG. Melatonin metabolite levels in workers exposed to 60-Hz magnetic fields: work in substations and with 3-phase conductors. *J Occup Environ Med*. 2000;42:136–142.
9. Burch JB, Reif JS, Noonan CW, et al. Melatonin metabolite excretion among cellular telephone users. *Int J Radiat Biol*. 2002;78:1029–1036.
10. Cagnacci A, Volpe A. Influence of melatonin and photoperiod on animal and human reproduction. *J Endocrinol Invest*. 1996;19:382–411.
11. Brzezinski A. Melatonin in humans. *N Engl J Med*. 1997;336:186–195.
12. Okatani Y, Morioka N, Wakatsukin A. Changes in nocturnal melatonin secretion in perimenopausal women: correlations with endogenous estrogen concentrations. *J Pineal Res*. 2000;28:111–118.
13. Bellipanni G, Bianchi P, Pierpaoli W, Bulian D, Ilyia E. Effects of melatonin in perimenopausal and menopausal women: a randomized and placebo controlled study. *Exp Gerontol*. 2001;36:297–310.
14. Schernhammer ES, Rosner B, Willett WC, et al. Epidemiology of urinary melatonin in women and its relation to other hormones and night work. *Cancer Epidemiol Biomarkers Prev*. 2004;13:936–943.
15. Kabuto M, Akiba S, Stevens RG, Nerishi K, Land CE. A prospective study of estradiol and breast cancer in Japanese women. *Cancer Epidemiol Biomarkers Prevent*. 2000;9:575–579.
16. Toniolo PG. Endogenous estrogens and breast cancer risk: the case for prospective cohort studies. *Environ Health Perspect*. 1997;105:587–592.
17. Key TJA, Wang DY, Brown JB, et al. A prospective study of urinary oestrogen excretion and breast cancer risk. *Br J Cancer*. 1996;73:1615–1619.
18. Helzlsouer KJ, Alberg AJ, Bush TL, et al. A prospective study of endogenous hormones and breast cancer. *Cancer Detect Prev*. 1994;18:79–85.
19. Rosenberg CR, Pasternack BS, Shore RE, Koenig KL, Toniolo PG. Premenopausal estradiol levels and the risk of breast cancer: a new method of controlling for day of the menstrual cycle. *Am J Epidemiol*. 1994;140:518–525.
20. Sanchez-Barcelo EJ, Cos S, Mediavilla D, et al. Melatonin-estrogen interactions in breast cancer. *J Pineal Res*. 2005;38:217–222.
21. Schernhammer ES, Hankinson SE. Urinary melatonin levels and breast cancer risk. *J Natl Cancer Inst*. 2005;97:1084–1087.
22. Blask DE, Brainard GC, Dauchy RT, et al. Melatonin-depleted blood from premenopausal women exposed to light at night stimulates growth of human breast cancer xenografts in nude rats. *Cancer Res*. 2005;65:11174–11184.
23. Burch JB, Clark M, Yost G, et al. Radio frequency non-ionizing radiation in a community exposed to radio and television broadcasting. *Environ Health Perspect*. 2005;114:248–253.
24. Burch JB, Reif JS, Yost MG, Keefe TJ, Pitrat CA. Nocturnal excretion of a urinary melatonin metabolite among electric utility workers. *Scand J Work Environ Health*. 1998;24:183–189.
25. Wood AW, Armstrong SM, Sait ML. Changes in human plasma melatonin profiles in response to 50 Hz magnetic field exposure. *J Pineal Res*. 1998;25:116–127.
26. Burch JB, Reif JS, Yost MG, Keefe TJ, Pitrat CA. Reduced excretion of a melatonin metabolite in workers exposed to 60 Hz magnetic fields. *Am J Epidemiol*. 1999;150:27–36.
27. Yost MG. Alternative magnetic field exposure metrics: occupational measurements in trolley workers. *Radiation Protection Dosimetry*. 1999;83:99–106.
28. Ichinose TY, Burch JB, Noonan CW, et al. Immune markers and ornithine decarboxylase activity among electric utility workers. *J Occup Environ Med*. 2004;46:104–112.
29. Arendt J, Bojkowski C, Franey C, Wright J, Marks V. Immunoassay of 6-hydroxymelatonin sulfate in human plasma and urine: abolition of the urinary 24-hour rhythm with atenolol. *J Clin Endocr Metab*. 1985;60:1166–1173.
30. Aldous ME, Arendt J. Radioimmunoassay for 6-sulphatoxymelatonin in urine using an iodinated tracer. *Ann Clin Biochem*. 1988;25:298–303.
31. O'Connor KA, Brindle E, Shofer JB, et al. Statistical correction for non-parallelism in a urinary enzyme immunoassay. *J Immunoassay Immunochem*. 2004;25:259–278.
32. Denari JH, Farinati Z, Figueroa Casas PR, Oliva A. Determination of ovarian function using first morning urine steroid assays. *Obstet Gynecol*. 1981;58:5–9.
33. Merlo AB, Farinati Z, Quiroga S, et al. Direct assay of urinary steroid glucuronides for monitoring the approach of ovulation. *Int J Fertil*. 1984;29:189–193.
34. Wilcox AJ, Baird DD, Weinberg CR, et al. The use of biochemical assays in epidemiologic studies of reproduction. *Environ Health Perspect*. 1987;75:29–35.
35. Catalan R, Castellanos JM, Palomino T, et al. Correlation between plasma estradiol and estrone-3-glucuronide in urine during the monitoring of ovarian induction therapy. *Int J Fertil*. 1989;34:271–275.
36. Miller RC, Brindle E, Holman DJ, et al. Comparison of specific gravity and creatinine for normalizing urinary reproductive hormone concentrations. *Clin Chem*. 2004;50:924–932.
37. Harlow SD, Ephross SA. Epidemiology of menstruation and its relevance to women's health. *Epidemiol Rev*. 1995;17:265–286.
38. Windham GC, Elkin E, Fenster L, et al. Ovarian hormones in premenopausal women: variation by demographic, reproductive and menstrual cycle characteristics. *Epidemiology*. 2002;13:675–684.
39. O'Connor KA, Brindle E, Holman DJ, et al. Urinary estrone conjugate and pregnanediol 3-glucuronide enzyme immunoassays for population research. *Clin Chem*. 2003;49:1139–1148.
40. Wilson BW, Wright CW, Morris JE, et al. Evidence for an effect of ELF electromagnetic fields on human pineal gland function. *J Pineal Res*. 1990;9:259–269.
41. Davis S, Kaune WT, Mirick DK, Chen C, Stevens RG. Residential magnetic fields, light-at-night, and nocturnal urinary 6-sulfatoxymelatonin concentration in women. *Am J Epidemiol*. 2001;154:591–600.
42. Levallois P, Dumont M, Touitou Y, et al. Effects of electric and magnetic fields from high-power lines on female urinary excretion of 6-sulfatoxymelatonin. *Am J Epidemiol*. 2001;154:601–609.
43. Davis S, Mirick DK, Chen C, Stanczyk FZ. Effects of 60-Hz magnetic field exposure on nocturnal 6-sulfatoxymelatonin, estrogens, luteinizing hormone, and follicle-stimulating hormone in healthy reproductive-age women: results of a crossover trial. *Ann Epidemiol*. 2006;166:22–31.
44. Maskarinec G, Cooper J, Swygert L. Investigation of increased incidence in childhood leukemia near radio towers in Hawaii: preliminary observations. *J Environ Pathol Toxicol Oncol*. 1994;13:33–37.
45. Hocking B, Gordon IR, Grain HL, Hatfield GE. Cancer incidence and mortality

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and proximity to TV towers. *Med J Aust*. 1996;165:601–605.
46. Dolk H, Shaddick G, Walls P, et al. Cancer incidence near radio and television transmitters in Great Britain. I. Sutton Coldfield transmitter. *Am J Epidemiol*. 1997;145:1–9.
47. Dolk H, Elliott P, Shaddick G, Walls P, Thakrar B. Cancer incidence near radio and television transmitters in Great Britain. II. All high power transmitters. *Am J Epidemiol*. 1997;145:10–17.
48. McKenzie DR, Yin Y, Morrell S. Childhood incidence of acute lymphoblastic leukaemia and exposure to broadcast radiation in Sydney—a second look. *Aust N Z J Public Health*. 1998;22:360–367.
49. Michelozzi P, Capon A, Kirchmayer U, et al. Adult and childhood leukemia near a high-power radio station in Rome, Italy. *Am J Epidemiol*. 2002;155:1096–1103.
50. Park SK, Ha M, Im HJ. Ecological study on residences in the vicinity of AM radio broadcasting towers and cancer death: preliminary observations in Korea. *Int Arch Occup Environ Health*. 2004;77:387–394.
51. Litovitz TA, Penafiel M, Krause D, Zhang D, Mullins JM. The role of temporal sensing in bioelectromagnetic effects. *Bioelectromagnetics*. 1997;18:388–395.
52. Illnerova H, Buresova M, Presl J. Melatonin rhythm in human milk. *J Clin Endocrinol Metab*. 1993;77:838–841.
53. Yie SM, Brown GM, Liu GY, et al. Melatonin and steroids in human pre-ovulatory follicular fluid: seasonal variations and granulosa cell steroid production. *Hum Reprod*. 1995;10:50–55.
54. Van Varuun RJ, Pitout MJ, Van aswegen CH. Putative melatonin receptor in human spermatozoa. *Biochem.* 1992;25: 125–127.
55. Laudon M, Gilad E, Matzkin H, Braf Z, Zisapel N. Putative melatonin receptors in benign human prostate tissue. *J Clin Endocrinol Metab*. 1996;81:1336–1342.
56. Weaver DR, Stehle JH, Reppert SM. Melatonin receptors in human hypothalamus and pituitary: implications for circadian and reproductive responses to melatonin. *J Clin Endocrinol Metab*. 1993;76:295–301.
57. Silman R. Melatonin and the human gonadotrophin-releasing hormone pulse generator. *J Endocrinol*. 1991;128:7–11.
58. Berga SL, Mortola JF, Yen SS. Amplification of nocturnal melatonin secretion in women with functional hypothalamic amenorrhea. *J Clin Endocrinol Metab*. 1988;66:242–244.
59. Brzezinski A, Lynch HJ, Seibel MM, et al. The circadian rhythm of plasma melatonin during the normal menstrual cycle and in amenorrheic women. *J Clin Endocrinol Metab*. 1988;66:891–895.
60. Voordouw BCG, Euser R, Verdonk RER, et al. Melatonin and melatonin-progestin combinations alter pituitary-ovarian function in women and can inhibit ovulation. *J Clin Endocrinol Metab*. 1992; 74:108–117.
61. Okatani Y, Sagara Y. Amplification of nocturnal melatonin secretion in women with functional secondary amenorrhea: relation to endogenous oestrogen concentration. *Clin Endicrinol*. 1994;41:763–770.
62. Kripke DF, Kline LE, Shadan FF, et al. Melatonin effects on luteinizing hormone in postmenopausal women: a pilot clinical trial NCT00288262. *BMC Womens Health*. 2006;6:8.
63. Vakkuri O, Kivela A, Leppaluoto J, Valtonen M, Kauppila A. Decrease in melatonin precedes follicle-stimulating hormone increase during perimenopause. *Eur J Endocrinol*. 1996;135: 188–192.
64. Fernandez B, Malde JL, Montero A, Acuna D. Relationship between adenohypophyseal and steroid hormones and variations in serum and urinary melatonin levels during the ovarian cycle, perimenopause and menopause in healthy women. *J Steroid Biochem*. 1990;35: 257–262.
65. Graham C, Cook MR, Gerkovich MM, Sastre A. Examination of the melatonin hypothesis in women exposed at night to EMF or bright light. *Environ Health Perspect*. 2001;109:501–507.
66. Schernhammer ES, Kroenke CH, Dowsett M, Folkerd E, Hankinson SE. Urinary 6-sulfatoxymelatonin levels and their correlations with lifestyle factors and steroid hormone levels. *J Pineal Res*. 2006;40:116–124.
67. Benhaberou-Brun D, Lambert C, Dumont M. Association between melatonin secretion and daytime sleep complaints in night nurses. *Sleep*. 1999;22:877–885.
68. Hakola T, Harma MI, Laitinen JT. Circadian adjustment of men and women to night work. *Scand J Work Environ Health*. 1996;22:133–138.
69. Burch JB, Yost MG, Johnson W, Allen E. Melatonin, sleep, and shift work adaptation. *J Occup Environ Med*. 2005;47: 893–901.
70. Cos S, Martinez-Campa C, Mediavilla MD, Sanchez-Barcelo EJ. Melatonin modulates aromatase activity in MCF-7 human breast cancer cells. *J Pineal Res*. 2005;38:136–142.
71. Cos S, Gonzalez A, Guezmes A, et al. Melatonin inhibits the growth of DMBA-induced mammary tumors by decreasing the local biosynthesis of estrogens through the modulation of aromatase activity. *Int J Cancer*. 2006;118:274–278.
72. Martinez-Campa C, Gonzalez A, Mediavilla MD, et al. Melatonin enhances the inhibitory effect of aminoglutethimide on aromatase activity in MCF-7 human breast cancer cells. *Breast Cancer Res Treat*. 2005;94:249–254.
73. Warman GR, Tripp HM, Warman VL, Arendt J. Circadian neuroendocrine physiology and electromagnetic field studies: precautions and complexities. *Radiat Prot Dosimetry*. 2003;106:369–373.
74. Touitou Y, Bogdan A, Lambrozo J, Selmaoui B. Is melatonin the hormonal missing link between magnetic field effects and human diseases? *Cancer Causes Control*. 2006;17:547–552.
75. Di Carlo AL, Litovitz TA. Is genetics the unrecognized confounding factor in bioelectromagnetics? Flock-dependence of field-induced anoxia protection in chick embryos. *Bioelectrochem Bioenerg*. 1999;48:209–215.
76. Fedrowitz M, Loscher W. Power frequency magnetic fields increase cell proliferation in the mammary gland of female Fischer 344 rats but not various other rat strains or substrains. *Oncology*. 2005;69:486–498.
77. Altpeter ES, Roosli M, Battaglia M, et al. Effect of short-wave (6–22 MHz) magnetic fields on sleep quality and melatonin cycle in humans: the Schwarzenburg shut-down study. *Bioelectromagnetics*. 2006;27:142–150.
78. Johansson O. Electrohypersensitivity: state-of-the-art of a functional impairment. *Electromagn Biol Med*. 2006;25: 245–258.

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