

Examination of the Melatonin Hypothesis in Women Exposed at Night to EMF or Bright Light

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It has been hypothesized that the increased incidence of breast cancer in industrial societies is related to greater exposure to power-frequency electric and magnetic fields (EMF) and/or the presence of high levels of light at night (LAN). EMF and LAN are said to reduce circulating levels of the hormone melatonin which, in turn, allows estrogen levels to rise and stimulate the turnover of breast epithelial stem cells and increase the risk for malignant transformation. Three laboratory-based studies, in which a total of 53 healthy young women were exposed at night to EMF or to LAN under controlled exposure conditions, were performed to determine whether such exposures reduce melatonin and are associated with further alterations in estrogen. All-night exposure to industrial-strength magnetic fields (60 Hz, 28.3 μ T) had no effect on the blood levels of melatonin or estradiol. In contrast, nocturnal melatonin levels were profoundly suppressed, and the time of peak concentration was significantly delayed in women exposed to LAN, regardless of whether they were in the follicular or luteal phase of the menstrual cycle. These changes, however, were not associated with alterations in point-for-point matching measures of estradiol. Women who chronically secrete high or low amounts of melatonin each night (area-under-curve range: 86–1,296 pg/mL) also did not differ in their blood levels of estradiol. Taken together, these results are consistent with a growing body of evidence which generally suggests that environmental EMF exposure has little or no effect on the parameters measured in this report. **Key words** breast cancer, electromagnetic fields, estrogen levels, light at night, melatonin, neuroendocrine, reproductive hormones, women's health. *Environ Health Perspect* 109:501–507 (2001). [Online 9 May 2001] <http://ehpnet1.niehs.nih.gov/docs/2001/109p501-507graham/abstract.html>

Breast cancer is the most common cause of cancer morbidity and mortality in women. For unknown reasons, incidence rates continue to rise and to exhibit significant variation across international boundaries (1–4). Risks are highest in North America and northern Europe and lowest in Asia and Africa (2). This pattern has led to the view that environmental factors associated with industrialization and urbanization may be involved in the etiology of this disease (5). Stevens (3) noted that a key characteristic of modern industrial society is the increased use of electric power, with its associated exposure to power-frequency electric and magnetic fields (EMF) and the presence of high levels of light at night (LAN). Stevens proposed, and later refined (3,6), a biologically based explanation of how increased exposure to EMF and/or LAN might increase the risk for hormone-sensitive cancers, particularly breast cancer. Now widely known as the melatonin hypothesis, it has continued to capture the attention of researchers because its focus on a single hormone promises a parsimonious explanation for a health problem of great public concern.

Melatonin is the chief secretory product of the pineal gland. It exhibits a marked circadian rhythmicity in all species studied, including humans, with circulating levels being high at night and low during the daylight hours. Because of its capability to signal daily and seasonal changes in the light/dark cycle, it is best

known as the primary regulator of reproductive physiology in seasonally breeding species (7). Melatonin synchronizes the ebb and flow of reproductive hormones such as estrogen so that mating and birth in these species occur at times that are optimal for survival. According to the melatonin hypothesis, environmental factors that reduce melatonin increase breast cancer risk in humans because the reduction of melatonin allows circulating levels of estrogen to rise. This rise, in turn, stimulates the turnover of breast epithelial stem cells and increases the risk for malignant transformation. Thus, this line of reasoning holds that melatonin acts indirectly on cancer risk by modulating the secretion of hormones implicated in carcinogenesis.

Melatonin is reported to block the estrogen-induced proliferation of human breast cancer cells *in vitro* and to suppress mammary tumorigenesis in rats (8). Of 71 small-animal exposure studies recently reviewed by Brainard et al. (4), 48 studies report that EMF exposure is associated with alterations in important aspects of melatonin biosynthesis, secretion, or metabolism. The literature on exposure of small animals is mixed, however, with numerous instances of inconsistent findings and failures to replicate findings, both within and between laboratories. Nighttime plasma melatonin levels are also reported to be lower in women with estrogen-receptor positive breast cancer compared to healthy women (9), and in women with

primary breast cancer compared to women with benign breast disease (10). These reported differences, however, often disappear under more controlled conditions (11). It is also difficult to assess the meaning of these findings in clinical patients due to the presence of disease and its possible effect on melatonin levels (12).

In contrast to the results with small animals, controlled studies with large animals (sheep, baboons, and humans) have reported uniformly negative results (13–16). For example, no effects on melatonin or its major urinary metabolite, 6-hydroxymelatonin-sulfate (6-OHMS), were found in human exposure studies that varied such key magnetic field parameters as frequency (50 Hz or 60 Hz), intensity [1–127.3 microTesla (μ T)], duration (1–4 consecutive nights), polarity (linear or circular polarization), and exposure pattern (continuous or intermittent) (17–22). Why are exposure effects evident in small but not large animals? Brainard et al. (4) suggested various possibilities: testing of nocturnal versus diurnal and seasonally versus nonseasonally breeding species; substantial species differences in the anatomical location, cellular structure, and morphology, of the pineal gland itself; marked variation in the quantity, substructure, and homogeneity of the tissues actually exposed to the magnetic field; and the present uncertainty regarding the actual locus of action of the magnetic field.

Whether reduced melatonin levels predispose individuals to increased risk of cancer is unknown. Published reports have evaluated the effects of EMF exposure on melatonin, but not on the cascading sequence of changes in melatonin and estrogen proposed to occur in women. It is also important to note that almost all of the above human exposure studies were performed on healthy young men. Thus, little is known about EMF exposure effects on

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women or about how such effects might be influenced by the large changes that naturally occur in estrogen as a function of the menstrual cycle. Finally, there are no studies in women of the relationship between LAN and the sequence of hormonal events described in the melatonin hypothesis, or between LAN and breast cancer. In this report, we address two key issues: first, whether exposure to EMF or LAN reduces nocturnal levels of melatonin in women, and second, whether these reductions are associated with alterations in blood levels of estrogen.

Materials and Methods

Common procedures. The effects of nocturnal exposure to EMF on melatonin and estradiol were evaluated in two studies; exposure to LAN was assessed separately in a third study. Some methods and procedures were common across studies and are described here. In each study, the test protocol received prior review and approval by the Institutional Review Board at the Midwest Research Institute (MRI), and we obtained written informed consent from each volunteer before her participation in a study. All volunteers met the following eligibility criteria for study participation: regular sleep/dietary habits, no evening/night work, no medications particularly hormonal birth control, not pregnant, and regular (26–32 days) and predictable (≤ 2 days) menstrual cycles. All studies were performed using a crossover experimental design in which subjects were assigned randomly to conditions and served as their own controls. The two EMF exposure studies were performed double-blind. All participants refrained from consuming alcohol for 24 hr before a test session, and had no caffeine after 1700 hr on the day of a session.

EMF exposure facility. The two EMF exposure studies were performed in the magnetic field exposure test facilities at the MRI. Exposure characteristics have been documented by the U.S. Department of Energy (23) as part of the U.S. National Electric and Magnetic Field Research and Public Information Dissemination program directed by the National Institute of Environmental Health Sciences (NIEHS) (16). The facilities have been described by Cohen et al. (24) and by Doynov et al. (25). In the control condition of each EMF study, the women were exposed overnight to the ambient 60-Hz background magnetic field measured in the laboratory ($\leq 0.2 \mu\text{T}$). This intensity is characteristic of residential exposures. In the test condition for each study, subjects were exposed to a circularly polarized, 60-Hz sinusoidal magnetic field generated at a resultant flux density of $28.3 \mu\text{T}$. This intensity is within the range of exposures associated with electric utility

operations and the use of industrial machinery or power equipment.

Each volunteer slept on a bed oriented north–south in a sound-attenuated and air-conditioned exposure test room (a cube approximately 2.4 m on each side). The field-generation coils were located out of sight behind the walls, ceiling, and floor of the exposure room. The horizontal axis of the field was oriented north–south, and the vertical axis was perpendicular to the floor. To produce the circularly polarized field, one axis of the field was phase-shifted 90° with respect to the other axis. Magnetic field generation at the selected frequency, intensity, waveform, pattern, and duration was controlled by software operating in conjunction with power generation systems.

The subjects were exposed to the generated magnetic field using the intermittent exposure protocol described by Graham et al. (18). This consisted of alternating 1-hr field-on and field-off periods. During field-off hours, the coils were not energized. During field-on hours, the field cycled on and off at 15-sec intervals. A zero-current crossing technique allowed the magnetic fields to be switched without introducing artifacts because of the generation of high-frequency magnetic field transients at the switch points. The decision to emphasize intermittent exposure and circular polarization was based on research indicating that such exposure is associated with alterations in human physiology (26–28), and also on rodent research reporting that circularly polarized fields are more effective than linearly polarized fields in reducing nocturnal melatonin levels (29).

Measures. Melatonin was assayed using the Bühlmann radioimmunoassay (RIA) distributed by ALPCO, Ltd. (Windham, NH). The detection limit for this assay is 0.3 pg/mL. The inter- and intra-assay coefficients of variation (CVs) are routinely below 10% throughout the assay range (30,31). First-void morning urine samples were assayed for the melatonin metabolite (6-OHMS) by RIA (Stockgrand, Guildford, Surrey, UK). The lower limit of quantitation for this RIA is 3.1 pg/mL, and the inter- and intra-assay CVs are routinely below 10% in our laboratory (30,31). Unlike melatonin, which is an uncharged compound that readily passes through and equilibrates across plasma and tissue membranes, 6-OHMS is a charged metabolite that appears in urine by filtration. Thus, the volume of urine an individual produces can influence the values obtained for 6-OHMS. To correct for this, urinary creatinine was also assayed using a COBAS MIRA chemistry analyzer (Roche, Montclair, NJ; kinetic Jaffe reaction). The 6-OHMS results were normalized to creatinine concentration and expressed as nanograms

6-OHMS per milligram creatinine. We measured estradiol using a double-antibody ^{125}I RIA (Diagnostic Products Corporation, Los Angeles, CA). The sensitivity of this assay is 5 pg/mL, and the inter- and intra-assay CVs were each below 10%.

Study 1

The 22 healthy women volunteers (mean age 24 years, range 19–35 years) in this study came to the laboratory for two test sessions spaced about a month apart. For each individual, the test sessions occurred at the same time point in the menstrual cycle between days 3–8 after the onset of menstruation. We restricted testing to this period because it is a time of relative hormonal stability, and this enhanced our capability to detect even small field-related changes, should they occur. In one session, the women were exposed all night (2300–0700 hr) to the generated 60-Hz magnetic fields. The other session was a control condition similar in all respects, except that it did not involve exposure to the magnetic fields. Half of the study group first participated in the control session, and the remaining subjects participated in the reverse order.

Procedures. On arrival at the laboratory, the volunteers changed into sleepwear and the study nurse inserted an indwelling butterfly catheter into a vein in the arm or back of the hand to minimize disturbance during the subsequent collection of hourly blood samples. After the subject got into bed in the exposure test room, the first blood sample was obtained from the subject at 2255 hr. The automated, double-blind/field-control system was activated, and subjects remained in bed until 0700 hr. During the night, the laboratory was darkened and ambient lighting in the exposure room was maintained at less than 10 lux (lx), a level known not to interfere with human melatonin secretion (32). The nurse entered the exposure room each hour on the hour to collect blood samples. These were drawn from the catheter into EDTA vacutainer tubes, centrifuged, and aliquots of the extracted plasma were frozen at -20°C for later assay of melatonin and estradiol.

If a subject needed to use the bathroom during the night, we were concerned that the attendant light exposure might inhibit nocturnal melatonin secretion. Light-induced inhibition of melatonin is both intensity dependent and spectrally sensitive, with green light being most effective and red light almost completely ineffective (33). We simultaneously controlled for both of these factors by asking subjects to wear fluoroscopic goggles equipped with red lenses (Model 502300, Cone Instruments, Solon, Ohio) if they needed to get up during the night. The goggles allowed night vision to be

maintained while reducing (~ 97%) the photopic transmittance of the incident light, and limiting it to the red portion of the visible light spectrum.

We used analysis of variance (ANOVA) as the primary statistical technique to test for differences in melatonin and estradiol between exposure and control conditions. Analysis variables included the two orders of testing, control versus exposure test conditions, and the hourly blood values for the outcome measures. Probability values were corrected for lack of sphericity using the Huynh-Feldt epsilon technique. Statistical significance was set at $p \leq 0.05$.

Results Melatonin. Figure 1 plots hourly mean values for plasma melatonin in the control and magnetic field exposure test conditions. The typical nocturnal secretion curve was observed in both conditions. Melatonin levels rapidly increased in the early portion of the night, peaked between 0200 and 0400 hr, and then slowly declined as morning approached. Although these changes across the night were highly significant ($F_{7,140} = 23.72$, $p = 0.0001$), ANOVA indicated that exposure to the magnetic field had no effect on the melatonin secretion curve. As clearly illustrated in Figure 1, magnetic field exposure did not alter the area under the curve (AUC), the timing or amplitude of the melatonin peak, the slope of the increase in melatonin between midnight and the peak, or the slope of the decrease from the peak to 0700 hr.

Estradiol. Estradiol does not have a circadian-type nocturnal secretion curve. Thus, we measured plasma concentrations of estradiol at the start of the control or exposure session (2300 hr), at the approximate time when melatonin reached its peak concentration in the circulation (0300 hr), and at the end of a session (0700 hr). ANOVA performed on

these data over all 22 subjects did not reveal any exposure-related differences in estradiol compared to control conditions. Interpretation of this result, however, was complicated by the fact that estradiol levels for a number of subjects were different in their first session compared to their second session, regardless of the type of exposure the subjects had received. In a preliminary examination of this issue, we deleted the five subjects who showed the greatest differences in estradiol between sessions and performed the analysis again on the remaining 17 subjects. This analysis revealed a significant exposure effect ($F_{2,32} = 5.67$, $p = 0.012$). Women had higher mean blood levels of estradiol when exposed to the magnetic field compared to control conditions (24.2 vs. 18.2 pg/mL, respectively). This effect was spurious, however, as indicated by the fact that the difference in estradiol levels was apparent at 2300 hr before magnetic field exposure began. Given the excessive intersession variability we observed under the present experimental design, we had little confidence in these results for estradiol. We therefore decided to change the design to reduce the variability in estradiol and to repeat the study with a new group of women.

Study 2

This study followed the design of study 1, with one major exception. To reduce the variability in estradiol measures, we scheduled the counterbalanced control and exposure test sessions for 2 consecutive nights between days 3 and 8 of each individual's menstrual cycle, rather than 1 month apart as in the earlier study. We also measured blood levels of melatonin and estradiol every hour from 2300 hr to 0700 hr to provide a point-by-point match. In addition, the subjects were asked to provide first-void morning

urine samples after each nocturnal test session (these included all urine voided after 2300 hr) to determine if magnetic field exposure altered 6-OHMS, the major urinary metabolite of melatonin. Fifteen women volunteers (mean age 20 years, range 18–24 years) participated in study 2.

Results Melatonin and 6-OHMS. Figure 2 plots the hourly mean values for melatonin in the control and exposure test conditions. As in the earlier study, the nocturnal secretion curve for melatonin was similar in both conditions. Statistical analysis by ANOVA did not reveal any difference in plasma measures of melatonin secretion as a function of exposure to the magnetic field compared to the control condition ($F_{7,91} = 1.43$, $p = 0.22$). Magnetic field exposure also had no effect on the urinary measure of melatonin metabolism. 6-OHMS levels in first-void morning urine were essentially the same in control and exposure conditions (mean \pm SE = 31.1 ± 4.1 and 31.6 ± 4.2 ng/mg/creatinine, respectively). As expected, the Pearson correlation (r) between the AUC for plasma melatonin and the creatinine-corrected 6-OHMS values was positive, statistically significant, and not differentially altered by magnetic field exposure ($r = 0.70$, $p = 0.004$, 48% of variance), compared to control conditions ($r = 0.73$, $p = 0.002$, 53% of variance).

Estradiol and melatonin. The change in experimental design had the desired effect of reducing session-to-session variance in estradiol measures. The Pearson correlation between the AUC for estradiol in sessions 1 and 2 was significant ($r = 0.727$, $p < 0.002$, 53% of variance), as was the correlation between the initial values obtained for estradiol at the start (2300 hr) of each session ($r = 0.83$, $p < 0.003$, 69% of variance). The AUC values obtained for estradiol in the control and exposure sessions were also

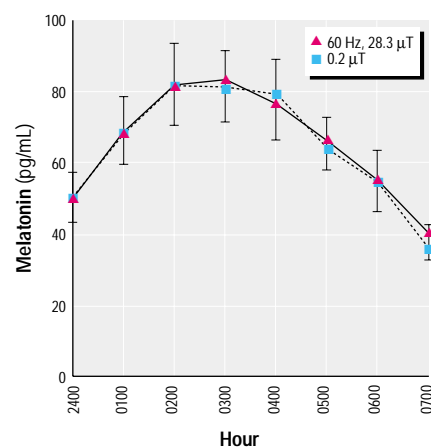


Figure 1. Melatonin (mean \pm SE) was not reduced in 22 women by all-night magnetic field (60 Hz, 28.3 μ T) exposure compared to equivalent no-exposure (0.2 μ T) control conditions (study 1).

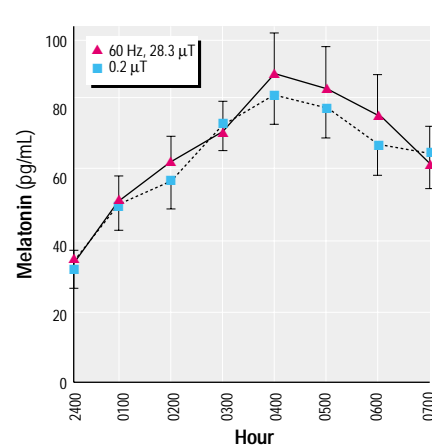


Figure 2. Melatonin (mean \pm SE) was not reduced in 15 women by all-night magnetic field (60 Hz, 28.3 μ T) exposure compared to equivalent no-exposure (0.2 μ T) control conditions (study 2).

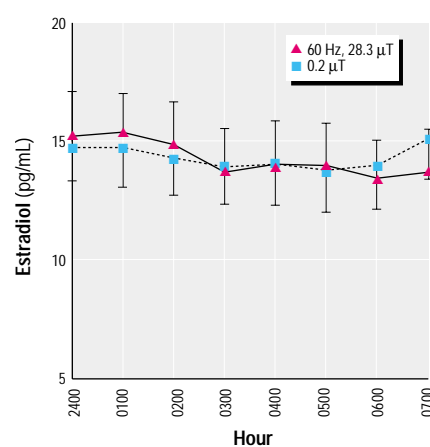


Figure 3. Estradiol (mean \pm SE) did not increase in 15 women during all-night magnetic field (60 Hz, 28.3 μ T) exposure compared to equivalent no-exposure (0.2 μ T) control conditions (study 2).

similar (114.72 and 114.56 pg/mL, respectively). Figure 3 plots hourly mean blood levels of estradiol to illustrate the nocturnal secretion pattern of this hormone in the control and exposure test sessions. The pattern is essentially flat across the night, with mean values fluctuating within a limited range (~2 pg/mL). Thus, although blood levels of melatonin changed significantly across the night regardless of exposure (Figure 2; $F_{7,91} = 9.2$, $p = 0.001$), neither these circadian-type changes nor exposure to the magnetic field had any apparent influence on the nocturnal secretion pattern of estradiol.

Study 3

Exposure to the magnetic field in the above two studies did not result in a reduction of melatonin. Thus, the studies did not allow us to address our second key issue of interest; namely, whether reductions in melatonin are followed by alteration in estrogen, and whether any effects found differ as a function of time in the menstrual cycle. In this study, we used the known suppressive effects of exposure to bright light at night to cause a profound reduction in the total amount of melatonin secreted over the night, and also to shift (by hours) the time when melatonin normally reaches peak concentration in the circulation. The study participants included women in the luteal as well as the follicular phase of their menstrual cycle.

The study group consisted of 16 healthy women volunteers (mean age 22 years, range 19–26 years). The women came to the laboratory on two consecutive nights (2030–0700 hr). Half of the subjects were tested between days 3 and 8 of their menstrual cycle (follicular group). The remaining subjects were tested between days 18 and 23 of their menstrual cycle (luteal group). Within each group, half of the subjects were exposed to bright light on their first night in the laboratory, followed by exposure to dim light on the second night. The remaining subjects participated in the reverse order.

Procedures. The women were scheduled in groups of two and arrived at the laboratory at 2030 hr. They changed into sleepwear, and the study nurse inserted an indwelling butterfly catheter into a vein in the arm or back of the hand for the collection of blood samples each hour on the hour from 2100 hr to 0700 hr. For the first 4 hr (2100–0100 hr), the subjects sat in comfortable chairs at a table positioned in the center of the light exposure room (6.1 m × 6.1 m × 2.75 m). They watched movies displayed on a combination television/videocassette recorder (TV/VCR) located in front of them, and were monitored to assure they did not fall asleep or close their eyes for extended periods of time.

In the dim-light control condition, the women wore the fluoroscopic goggles described earlier. We switched on the ceiling fluorescent lighting fixtures, which produced a measured (Digital Light Meter, Model LX-101; Edmonds Scientific Co., Barrington, NJ) illuminance level of 575 lx on the table top in front of the subjects. When viewed through the goggles, however, the illuminance level was reduced to 25 lx. The subjects did not wear the goggles in the bright-light exposure condition. In that condition, we switched on the ceiling lights as well as an additional six banks of fluorescent lighting, each containing eight SYLVANIA cool-white, 48-inch, 40-watt tubes (OSRAM SYLVANIA, Danvers, MA). Track lighting attached to the ceiling around the room was also turned on. This arrangement produced a bright, evenly lit area in which subjects were able to watch the TV/VCR screen for the 4-hr exposure period without reported eye strain. The illuminance level in the bright-light test condition was 5,200 lx at the measurement location on the table top in front of the subjects. Light exposure ended at 0100 hr, and the subjects went to sleep for the remainder of the night in the magnetic field exposure test rooms described earlier. The field generators were not switched on, however, and the subjects were not exposed to the magnetic fields used earlier. The laboratory was darkened at night, and ambient lighting in the sleeping rooms was maintained at < 10 lx. Blood samples were collected each hour and the subjects awakened at 0700 hr the next morning.

Results

Figure 4 illustrates the effect of light exposure on melatonin. Hourly mean values from 2100 hr to 0700 hr are plotted for the follicular and luteal groups under bright- and dim-light test conditions. Exposure to the bright light significantly reduced the total amount (AUC) of melatonin the women secreted over the entire night ($F_{1,14} = 41.99$; $p < 0.0001$). This effect was not different in the follicular and luteal phases of the menstrual cycle (reductions in AUC of 39% and 35%, respectively). An even more profound reduction of melatonin was observed during the initial 4-hr light exposure period. Exposure to the bright light from 2100 hr to 0100 hr reduced melatonin secretion by more than 90%, compared to the dim-light control condition over the same time period. Further examination indicated that exposure to the bright light also caused a significant delay in the time when melatonin reached peak concentration in the circulation. Mean blood levels of melatonin peaked at 0100 hr in the dim-light control

sessions. Peak concentrations of melatonin occurred at 0500 hr in the bright-light exposure condition, a delay of 4 hr. This figure illustrates two important aspects of the data. First, the rise and fall of melatonin under dim-light control conditions, as well as the response of this hormone to the suppressive effects of bright light, are essentially identical in the early and late phases of the menstrual cycle. Second, once the suppressive effects of bright light are removed, blood levels of melatonin in both menstrual phases recover and peak at the same level observed for subjects unexposed to light, illustrating the power of the endogenous circadian rhythms that control this hormone. The slope of the increase after bright-light exposure is not different from the slope of the increase seen at the start of the dim-light control condition in either group.

As shown in Figure 5, the marked changes observed in melatonin were not associated with any apparent alteration in point-by-point, matching hourly mean blood levels of estradiol. AUC measures for estradiol were not different in bright- versus dim-light exposure sessions ($F_{1,13} = 0.08$, $p = 0.78$). Phase of the menstrual cycle was the single factor found to influence estradiol. As expected, mean blood levels of estradiol were lower in the follicular group compared to the luteal group (26 vs. 62 pg/mL, $F_{1,13} = 8.17$, $p = 0.01$), as was the AUC ($F_{1,13} = 5.20$, $p = 0.04$).

We performed one additional analysis using the combined data from the above three studies. The present studies examined the effects of acute exposure to EMF and LAN on

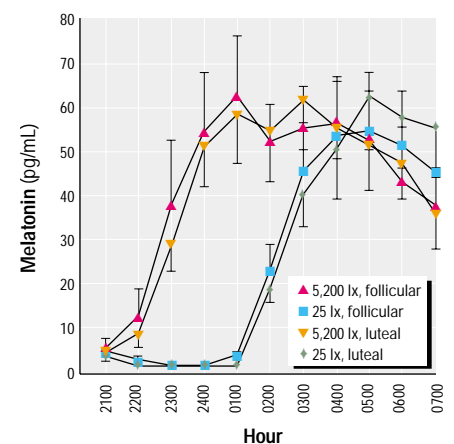


Figure 4. Mean (\pm SE) melatonin levels are plotted from 2100 hr to 0700 hr for eight women in the follicular menstrual phase (days 3–8) initially exposed for 4 hr to bright (5,200 lx) light or to dim (25 lx) light (study 3). Similar data are shown for eight women in the luteal phase (days 18–23) exposed to the same bright and dim light conditions. Bright light reduced the total amount of melatonin secreted ($p < 0.0001$) and delayed peak blood concentrations by 4 hr.

melatonin and estradiol. It has been known for some time, however, that there are large differences between individuals in the amount of melatonin they chronically secrete each night (7). For example, in previous research on a sample of more than 250 volunteers, we found that AUC values over the night ranged from 62 to 854 pg/mL (30,31). In view of the relationships proposed by the melatonin hypothesis, we were curious whether blood levels of estradiol might also naturally differ in women with chronically high versus low daily secretions of melatonin.

Of the 53 women who participated in the above studies, 44 women could provide the complete set of data needed to perform this analysis. All of these women were in the follicular phase of the menstrual cycle during their participation. Their melatonin levels had been measured each hour from 2300 hr to 0700 hr in the no-exposure control condition of the two magnetic-field exposure studies or in the dim-light control condition of the LAN study. Estradiol levels had also been measured in these individuals at three common time points across studies (2300, 0300, and 0700 hr) under the same control conditions. The mean level of estradiol was computed for each woman using the data collected at these three time points. We considered this measure to be a good representation of the total nocturnal output of estradiol for an individual because it correlated highly with the AUC measures of estradiol calculated over the full night in studies 2 and 3 ($r = 0.934$, $p < 0.00001$, 87% of variance accounted for). We performed regression analysis to examine the relationship between

each woman's mean estradiol level (range: 4–60 pg/mL) and her melatonin AUC calculated over the entire night (range: 86–1296 pg/mL). There was no relationship between estradiol and melatonin in this data set ($r = 0.058$, $p = 0.71$, < 0.01% of the variance accounted for).

Discussion

Our results indicate that acute, all-night exposure to industrial-strength, power-frequency magnetic fields has no effect on the nocturnal secretion patterns of melatonin and estradiol in healthy, young women tested in the follicular phase of the menstrual cycle. These results are consistent with the negative findings in regard to melatonin obtained in earlier research performed with healthy, young men exposed under similar magnetic field test conditions (18–22,34). In contrast, when the women volunteers were exposed to bright light at night, nocturnal melatonin levels were profoundly suppressed and the time when melatonin reached its peak concentration in the blood was significantly delayed. These changes in melatonin were equivalent for women in the follicular and the luteal phases of the menstrual cycle. The significant light-induced suppression of melatonin, however, was not accompanied by any change in point-by-point comparison measures of estradiol in either phase of the menstrual cycle. An analysis of women who differed approximately 15-fold in the total amount of melatonin they chronically secrete each night showed no associated differences in blood levels of estradiol.

Most of the research to determine if EMF exposure reduces melatonin has been performed on rodents and other small animals. Contrary to popular opinion, this research has not focused on the effects of chronic exposure. Brainard et al. (4) characterized 71 animal EMF/melatonin studies performed since 1981. Exposure duration in most (~70%) of the studies was 1 day or less, and often it was 1 hr or less. It is important to note, however, that melatonin (pineal, plasma, or serum) suppression was reported in approximately 40% of these acute exposure studies, and that studies with longer (≥ 30 days) exposures did not consistently produce positive results. Field intensity in the animal studies was typically less than 100 μ T, with many studies using intensities in the 30–50 μ T range. Brainard et al. (4) also describe 10 controlled EMF exposure studies performed on large animals. At intensities ranging from 0.6 to 100 μ T, and at durations of minutes to months, EMF exposure had no effect on melatonin or 6-OHMS in large animals. Thus, although positive effects have been reported at durations and intensities comparable to the present study, these

effects have been limited to small nocturnal creatures whose reproductive and endocrine function are intimately tied to the chronobiological rhythms of melatonin.

The recent NIEHS EMF Working Group report (14) concluded that only weak evidence exists for EMF exposure effects on melatonin in small animals, and no evidence exists that it does so in large animals. In line with these conclusions, we recently found that 4 consecutive nights of exposure to industrial-intensity magnetic fields had no effect on melatonin or 6-OHMS in humans (20), nor did continuous exposure for 8 hr to very strong (127.3 μ T) fields (22). We have also tested older women (40–60 years) in a separate study (21) using the same EMF exposure protocol described here, and again found no effects on melatonin or 6-OHMS. Various physiologically based explanations, other than exposure duration or age, have been put forth to account for effects being found in small but not large animals (4).

Previous field studies of people exposed to EMF while at home or at work do examine the effects of chronic exposure, and some have reported suppression of melatonin and/or 6-OHMS (e.g., 35–38). Thus, the possibility exists that disturbances of the melatonin rhythm may be related to as-yet unknown aspects of the more complex magnetic fields found in the man-made environment. The evident weakness here is that unknown elements have to be evoked to justify this possibility. There is also an inherent lack of control over potentially confounding variables in field studies, which makes it difficult to reliably link the EMF exposure to the reported positive effects. For example, Arnetz and Berg (35) reported positive effects. But this was based on a later analysis of uncorrected urine samples stored for more than 5 years after being collected from people who sat before a computer terminal on one day but not on another. EMF measurements were not taken in the original study, and EMF exposure is not now considered a likely factor (15). Similarly, Pfluger and Minder (36) reported reduced 6-OHMS in train drivers exposed to 16.7-Hz magnetic fields compared to a control group. The drivers, however, worked shifts that advanced each day by 15–60 min. This changed their exposure to light and their daily melatonin rhythms. Effects were not found in morning urine, which represents the integrated nocturnal accumulation of melatonin during the time in the 24-hr day when it is actively being produced, but in early evening urine collected when melatonin levels are rising at variable rates across individuals. The chronically exposed (50 Hz, 1 μ T) control group showed no effects, and the authors indicate their results may be due to light exposure.

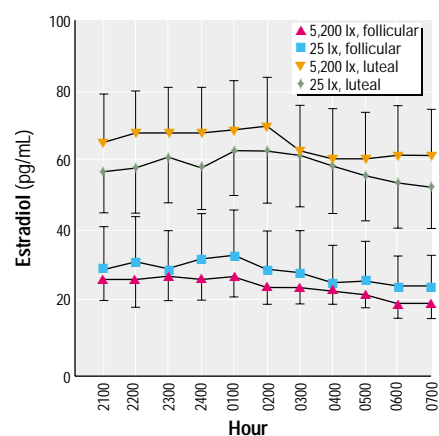


Figure 5. Mean (\pm SE) estradiol levels are plotted from 2100 hr to 0700 hr for the luteal group ($n = 8$) initially exposed for 4 hr to bright (5,200 lx) light or to dim (25 lx) light (study 3). Similar data are presented for the follicular group ($n = 8$) exposed to the bright and dim light conditions. Unlike the profound changes observed in melatonin (Figure 4), no alterations in point-by-point matching measures of estradiol were found in either phase of the menstrual cycle.

Juutilainen et al. (39) reported suppression of 6-OHMS in female garment workers compared to office workers. They later reported that 6-OHMS did not increase or rebound over the nonworking weekends and that no dose-response curve was present in the workplace data (39). In an unpublished study, Kaune et al. (37) found no relationship to the magnetic fields measured by the personal dosimeters worn by the women in their study, and a weak suppressive effect of magnetic fields in the bedroom on morning 6-OHMS. The generalizability of this effect is unknown, however, as it was present in summer and limited to women who used medications known to reduce melatonin (e.g., beta blockers). It has now been determined that the women in this cohort also exhibit an alcohol-related, dose dependent reduction in morning 6-OHMS levels (40). Burch et al. (38) reported 6-OHMS suppression in electric utility workers. This effect was not related to intensity or cumulative exposure in the workplace, however, but to a derived measure of field stability in the home. In this same cohort of workers, 6-OHMS levels are now reported to vary with changes in the geomagnetic field of the earth as measured in Boulder, Colorado (41). Field studies have important limitations that must be taken into account when evaluating reported claims for positive effects.

The search for a plausible biological mechanism by which EMF exposure might alter human physiology has been a central research issue for some time (14,42). The cascade of hormonal events postulated in the melatonin hypothesis is often described as providing such a mechanism (3), although this postulated mechanism owes more to rodent than to human physiology. A key question here is how exposure to the magnetic field comes to initiate this chain of hormonal events in the first place. The synthesis and release of melatonin from the pineal gland is under the control of the suprachiasmatic nuclei (SCN) located in the hypothalamus, and the superior cervical ganglion (SCG) located in the neck region (43). The SCN are the pacemakers that generate the endogenous circadian melatonin rhythm, and the SCG is the main relay station for melatonin release. The SCN are further entrained to the 24-hr day by the eyes, which relay light and dark signals via the retinohypothalamic tract (RHT). Once produced by the pineal, melatonin is released immediately into the circulation, where it has a short half-life in humans because more than 90% of it is cleared from the circulation after a first pass through the liver. For example, bolus injection studies show that plasma melatonin displays a bimodal decay pattern with a first distribution half-life of 2 min, and a second

metabolic half-life of 20 min (44). Thus, for magnetic field exposure to suppress blood levels of melatonin, it would have to exert a "real-time" influence on cellular activity or function somewhere in the region of the producer (pineal gland), the rhythm generator (SCN), the flow regulator (SCG), or the entrainment pathway (RHT).

By Faraday's law, exposure to a time-varying magnetic field will induce an electric field inside a conducting body. If the induced field is of sufficient intensity, it could provide a plausible initiating mechanism to modulate cellular activity or function. In recent comprehensive reviews (14,42), the value of 1 mV/m has been identified as the threshold intensity required for documented electric field-induced alterations in cellular activity. Detailed dosimetric analyses with anatomically correct human models (45,46) indicate that the present, industrial-strength (resultant flux density = 28.3 μ T) magnetic field exposure conditions are capable of inducing peak electric fields in human cortical brain areas on the order of 1.8 mV/m. According to the same dosimetric model, however, it would require an exposure to 60-Hz magnetic fields at an intensity almost 10 times higher than that used here (~300 μ T) to induce a 1 mV/m electric field in the central region of the human brain where the pineal and the SCN are located. Calculations based on this model also indicate that the present exposure conditions would induce an electric field of only 0.1 mV/m at the retina, and a field of approximately 0.56 mV/m at the SCG in the neck region. Thus, there appears to be little biophysical support for the hypothesis that melatonin may be suppressed in humans by nocturnal exposure to power-frequency magnetic fields at the low field intensities (\leq 0.3 μ T) typically found in residences, or even at the somewhat higher field intensities (\leq 1.0 μ T) characteristic of average exposure in the electric utility workplace.

The physiological relevance of the small changes in melatonin and/or 6-OHMS observed in the above field studies is also unknown because the typical nocturnal concentration of melatonin greatly exceeds the amount needed for human melatonin receptors to be activated. Even in EMF exposure studies with seasonal breeders such as the Djungarian hamster, where reproductive function is exquisitely tuned to seasonal changes in the duration of the nocturnal rise in circulating blood levels of melatonin, the typical reduction reported is less than 25%, not nearly enough to trigger changes in reproductive hormonal activity (15,47).

In contrast, during exposure to bright light at night, melatonin was suppressed in women to a much greater degree (> 90%) than reported to date in EMF exposure studies. To

our knowledge this is the first published study to simultaneously examine the effects of variation in LAN on melatonin and estradiol in women. Although we found no relationship between LAN and estradiol in either phase of the menstrual cycle, two previous reports do provide indirect evidence for a possible link between LAN and breast cancer through the melatonin hypothesis. Hahn (48) found that breast cancer incidence rates were lower in blind women (increased melatonin, less sensitivity to LAN) compared to sighted women. Tynes et al. (49) also reported that rates were higher in a postmenopausal subsample of female marine radio operators doing shift work while at sea (less melatonin, greater exposure to LAN). Thus, there may be circumstances under which long-term exposure to LAN could be consequential in terms of hormone levels and human health; however, no epidemiological studies have directly examined the relationship between LAN and breast cancer.

The three studies described here demonstrate that circulating blood levels of melatonin and estrogen are not altered in women exposed at night to EMF at industrial intensities, or when melatonin levels are profoundly suppressed by LAN. Taken together, our findings are consistent with a growing body of evidence which generally suggests that environmental EMF exposure has little or no effect on the endocrine parameters measured in this report.

REFERENCES AND NOTES

1. Stevens RG. Electric power use and breast cancer: a hypothesis. *Am J Epidemiol* 125:556-561 (1987).
2. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int J Cancer* 54:594-606 (1993).
3. Stevens RG, Wilson BE, Anderson LE, eds. *The Melatonin Hypothesis: Breast Cancer and the Use of Electricity*. Columbus, OH: Battelle Press, 1997.
4. Brainard GC, Kavet R, Kheifets LI. The relationship between electromagnetic field and light exposures to melatonin and breast cancer risk: a review of the relevant literature. *J Pineal Res* 26:65-100 (1999).
5. Wolff MS, Coleman GW, Barrett JC, Huff J. Breast cancer and environmental risk factors: epidemiological and experimental findings. *Annu Rev Pharmacol Toxicol* 36:573-596 (1996).
6. Stevens RG, Davis S. The melatonin hypothesis: electric power and breast cancer. *Environ Health Perspect* 104:135-140 (1996).
7. Arendt J. *Melatonin and the Mammalian Pineal Gland*. London: Chapman and Hall, 1995.
8. Blask DE. Melatonin in oncology. In: *Melatonin: Biosynthesis, Physiological Effects, and Clinical Applications* (Yu HS, Reiter RJ, eds). Boca Raton, FL: CRC Press, 1993:447-475.
9. Tamarkin I, Danforth D, Lighter A. Decreased nocturnal plasma melatonin peak in patients with estrogen receptor positive breast cancer. *Science* 216:1003-1005 (1989).
10. Bartsch C, Bartsch H, Fuchs U. Stage-dependent depression of melatonin in patients with primary breast cancer. *Cancer* 64:426-433 (1989).
11. Skene DJ, Bojkowski CJ, Currie JE, Wright J, Boulter PS, Arendt J. 6-Sulphatoxymelatonin production in breast cancer patients. *J Pineal Res* 8:269-276 (1990).
12. Panzer A, Viljoen M. The validity of melatonin as an oncostatic agent. *J Pineal Res* 22:184-202 (1997).

13. Lambrozo J, Touitou Y, Dab W. Exploring the EMF-melatonin connection: a review of the possible effects of 50/60-Hz electric and magnetic fields on melatonin secretion. *Int J Occup Environ Health* 2:37–247 (1996).
14. Portier CJ, Wolfe MS, eds. *Assessment of Health Effects from Exposure to Power-Line Frequency Electric and Magnetic Fields*. NIH Publication no. 98-3981. Research Triangle Park, NC:National Institute of Environmental Health Sciences, 1998.
15. Portier CJ, Wolfe MS, eds. *EMF Science Review Symposium: Breakout Group Reports for Clinical and In Vivo Laboratory Findings*. NIH Publication no. 98-4400. Research Triangle Park, NC:National Institute of Environmental Health Sciences, 1998.
16. Olden K. NIEHS Report on Health Effects from Exposure to Power-Line Frequency Electric and Magnetic Fields. NIH Publication no 99-4493. Research Triangle Park, NC:National Institute of Environmental Health Sciences, 1999.
17. Akerstedt T, Arnetz B, Ficca G, Paulsson L-E, Kallner A. A 50-Hz electromagnetic field impairs sleep. *J Sleep Res* 8:77–81 (1999).
18. Graham C, Cook MR, Riffle DW, Gerkovich MM, Cohen HD. Nocturnal melatonin levels in human volunteers exposed to intermittent 60-Hz magnetic fields. *Bioelectromagnetics* 17:263–273 (1996).
19. Graham C, Cook MR, Riffle DW, Gerkovich MM, Cohen HD. Human melatonin during continuous magnetic field exposure. *Bioelectromagnetics* 18:166–171 (1997).
20. Graham C, Cook MR, Sastre A, Riffle DW, Gerkovich MM. Multi-night exposure to 60-Hz magnetic fields: effects on melatonin and its enzymatic metabolite. *J Pineal Res* 28:1–8 (2000).
21. Graham C, Sastre A, Cook MR, Gerkovich MM. All-night exposure to EMF does not alter urinary melatonin, 6-OHMS, or immune measures, in older men and women. *J Pineal Res* (in press).
22. Graham C, Cook MR, Gerkovich MM, Sastre A. Melatonin and 6-OHMS in high-intensity magnetic fields. *J Pineal Res* (in press).
23. Dietrich F. Synopsis topic #5: quality assurance. In: *EMF Engineering Review Symposium*, 28–29 April 1998, Charleston, SC. Available: <http://www.emf-data.org/symposium98.html> [cited 19 November 2000].
24. Cohen HD, Graham C, Cook MR, Phelps JW. ELF exposure facility for human testing. *Bioelectromagnetics* 13:169–182 (1992).
25. Doynov P, Cohen HD, Cook MR, Graham C. Test facility for human exposure to AC and DC magnetic fields. *Bioelectromagnetics* 20:101–111 (1999).
26. Cook MR, Graham C, Cohen HD, Gerkovich MM. A replication study of human exposure to 60-Hz fields: effects on neurobehavioral measures. *Bioelectromagnetics* 13:261–285 (1992).
27. Graham C, Cook MR, Cohen HD, Gerkovich MM. A dose response study of human exposure to 60-Hz electric and magnetic fields. *Bioelectromagnetics* 15:447–463 (1994).
28. Sastre A, Cook MR, Graham C. Nocturnal exposure to intermittent 60-Hz magnetic fields alters human cardiac rhythm. *Bioelectromagnetics* 19:98–106 (1998).
29. Kato M, Honma K, Shigemitsu T, Shiga Y. Effects of exposure to a circularly polarized 50-Hz magnetic field on plasma and pineal melatonin levels in rats. *Bioelectromagnetics* 14:97–106 (1993).
30. Graham C, Cook MR, Kavet R, Sastre A, Smith DK. Prediction of nocturnal plasma melatonin from morning urinary measures. *J Pineal Res* 24:230–238 (1998).
31. Cook MR, Graham C, Kavet R, Stevens G, Kheifets LI, Davis S. Morning urinary assessment of nocturnal melatonin secretion in older women. *J Pineal Res* 28:41–47 (2000).
32. Trinder J, Armstrong SM, O'Brien C, Luke D, Martin MJ. Inhibition of melatonin secretion onset by low levels of illumination. *J Sleep Res* 5:77–82 (1996).
33. Goeffriau M, Brun J, Chazot, G, Claustrat B. The physiology and pharmacology of melatonin in humans. *Horm Res* 49:136–141 (1998).
34. Selmaoui B, Lambrozo J, Touitou Y. Magnetic fields and pineal function in humans: evaluation of nocturnal acute exposure to ELF magnetic fields on serum melatonin and urinary aMT6s circadian rhythms. *Life Sci* 58:1539–1549 (1996).
35. Arnetz BB, Berg M. Melatonin and adrenocorticotrophic hormone levels in video display unit workers during work and leisure. *J Occup Environ Med*, 38:1108–1110 (1996).
36. Pfluger DH, Minder CE. Effects of exposure to 16.7 Hz magnetic fields on urinary 6-hydroxymelatonin sulfate excretion of Swiss railway workers. *J Pineal Res* 21:91–100 (1996).
37. Kaune W, Davis S, Stevens RG. Relation between residential magnetic fields, light-at-night, and nocturnal urine melatonin levels in women (Report TR-107242). Palo Alto, CA:EPRI, 1997.
38. Burch JB, Reif JS, Yost MG, Keefe TJ, Pitrat CA. Nocturnal excretion of a urinary melatonin metabolite in electric utility workers. *Scand J Work Environ Health* 24:183–189 (1998).
39. Juutilainen J, Stevens RG, Anderson LE, Hansen NH, Kilpelainen M, Kumlin T, Laitinen JT, Sobel E, Wilson BW. Nocturnal 6-hydroxymelatonin sulfate excretion in female workers exposed to magnetic fields. *J Pineal Res* 28:97–104 (2000).
40. Stevens RG, Davis S, Mirick DK, Kheifets L, Kaune W. Alcohol consumption and urinary concentration of 6-hydroxymelatonin in healthy women. *Epidemiology* 11:660–665 (2000).
41. Burch JB, Reif JS, Yost MG. Geomagnetic disturbances are associated with reduced nocturnal excretion of a melatonin metabolite in humans. *Neurosci Lett* 266:209–212 (1999).
42. Gailey P, Sastre A, McGaughy RE. Internal electric fields produced by EMF, dosimetry, and endogenous fields. In: *EMF Science Review Symposium Breakout Group Reports for Theoretical Mechanisms and In Vitro Research Findings* (Portier C, Wolfe M, eds). Research Triangle Park, NC:National Institute of Environmental Health Sciences, 1997:55–70.
43. Klein DC, Moore RY, Reppert SM, eds. *Suprachiasmatic Nucleus: The Mind's Clock*. New York:Oxford University Press, 1991.
44. Mallo C, Zaidan R, Galy G, Vermeulen E, Brun J, Chazot J, Claustrat B. Pharmacokinetics of melatonin in man after intravenous infusion and bolus injection. *Eur J Clin Pharmacol* 38:297–301 (1990).
45. Dawson TW, Caputa K, Stuchly MA. Influence of human model resolution on computed currents induced in organs by 60-Hz magnetic fields. *Bioelectromagnetics* 18:478–490 (1997).
46. Dawson TW, Caputa K, Stuchly MA. Magnetic induction at 60 Hz in the human heart: a comparison between the in situ and isolated scenarios. *Bioelectromagnetics* 20:233–243 (1999).
47. Yellon SM. 60-Hz magnetic field exposure effects on the melatonin rhythm and photo period control of reproduction. *Am J Physiol* 270:E816–E821 (1996).
48. Hahn R. Profound bilateral blindness and the incidence of breast cancer. *Epidemiology* 2:208–210 (1991).
49. Tynes TA, Andersen A, Langmark F. Incidence of cancer in Norwegian workers potentially exposed to electromagnetic fields. *Am J Epidemiol* 136:81–88 (1992).