Effect of nighttime magnetic field and other exposures on sleep quality in young women

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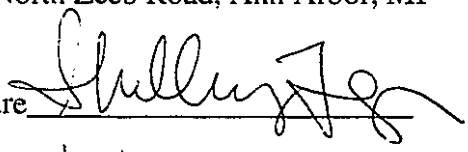
Signature 

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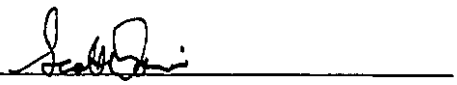

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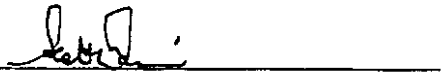

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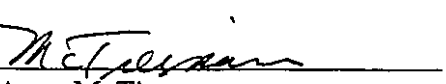

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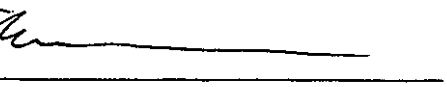

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Abstract

Effect of nighttime magnetic field and other exposures on sleep quality in young women

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The purpose of this study was to: 1. describe sleep patterns in menstruating women sleeping at home, 2. determine the effect of a nighttime magnetic field and other exposures on sleep, and 3. examine the association between sleep and urinary sex hormones during the luteal menstrual phase. Data are from a randomized crossover design trial, comparing an intervention magnetic field (0.5 to 1.0 microTesla above ambient levels) to ambient levels, during two 5-night measurement periods. Subjects collected an overnight urine sample on the fifth night. Subjects were not taking oral contraceptives, 20-40 years old, not pregnant/breast-feeding during the previous year, and had regular menstrual cycles. Sleep outcomes were measured via actigraphy. Of 640 nights, 91 (14%) were missing some data. A wide range of sleep patterns was observed. The intra-class correlation was low ($\rho < 0.21$) for total sleep time, sleep onset, and time in bed, but higher ($\rho = 0.40 - 0.51$) for sleep efficiency, total wake time, wake after sleep onset, awakenings, and awakenings ≥3 minutes. Sleep outcomes were not significantly different between intervention and ambient measurement periods.

However, higher ambient magnetic field exposure was significantly associated with less total sleep time, lower sleep efficiency, and more awakenings ≥3 minutes. Unusual bed or rise times, medication use, employment, day of week, daylight hours, menstrual cycle length, and body mass index were associated with sleep patterns, while age, alcohol consumption, exercise over the previous month, and perceived stress were not. We found few associations between sleep and urinary sex hormone concentrations. However, increased awakenings were modestly associated with higher urinary luteinizing hormone concentrations. Anovulatory cycles were significantly associated with lower sleep efficiency and more awakenings ≥3 minutes. Our results suggest that actigraphy is a feasible method of measuring sleep patterns and assessing associations with various exposures in menstruating women sleeping at home. The intervention exposure had no effect on sleep patterns, however other exposures were associated with worse sleep. Our results also support the hypothesis that sleep patterns and hormonal regulation processes are interrelated. More research is necessary to elucidate the complex relationships between these exposures and sleep.

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Preface

This dissertation consists of three chapters, each of which are stand alone papers that have been or will be submitted to peer-reviewed journals. Each chapter has a background, methods, results, and discussion section with tables for that paper at the end of the corresponding chapter.

Acknowledgements

The author would like to sincerely thank Dr. Scott Davis and Dr. Anne McTiernan not only for their time in assisting in the preparation of this dissertation, but also for their continued mentoring and support in all aspects of my graduate student experience. I would also like to thank my other dissertation committee members, Dr. Martha Lentz, Dr. Scott Emerson, and the Graduate Student Representative Dr. Brian Leroux, for their time and assistance.

Chapter 1: The effect of a nighttime magnetic field exposure on sleep patterns in young women

Background

Poor sleep quality is associated with decrements in memory and learning, and in shift workers is associated with gastrointestinal disorders, depression, and exacerbation of existing chronic disease (Scott AJ, 1990; Dotto L, 1996). Also a recent study suggested that too little or too much sleep might increase risk of cardiovascular disease in women (Ayas NT, 2003). Thus it is important to determine factors that can affect sleep patterns.

Recently, several studies reported that extremely low frequency magnetic field exposure might disrupt normal sleep (Akerstedt T, 1999; Graham C, 1999; Graham C, 2000; Li CY, 2002). Among 5,078 Taiwanese women, ambient magnetic field exposures of > 0.2 microTesla (μT) were associated with disrupted sleep initiation and maintenance (Li CY, 2002). Experimental studies, conducted in sleep laboratories, have reported that exposure to nighttime magnetic fields was associated with less total sleep time (Akerstedt T, 1999; Graham C, 1999; Graham C, 2000), more wake time (Graham C, 1999; Graham C, 2000), and lower sleep efficiency (Akerstedt T, 1999; Graham C, 1999; Graham C, 2000), compared to sham-exposure, among young men (Akerstedt T, 1999; Graham C, 1999) and older women (Graham C, 2000). One study used a continuous, 1 μT, 50Hz magnetic field (Akerstedt T, 1999), while the other two used an intermittent 28.3 μT, 60 Hz magnetic field (Graham C, 1999; Graham C, 2000).

Conversely, a randomized trial of a continuous, 5 μT, 4 Hz magnetic field in 101 insomniacs sleeping at home, reported that those in the exposed group had significant improvements in self-reported sleep outcomes compared to placebo (Pelka RB, 2001).

Magnetic field exposure may reduce nighttime concentrations of melatonin, a hormone that entrains various circadian rhythms to the light-dark cycle (Davis S, 2001; Levallois P, 2001). Habitual sleep hours are closely synchronized with melatonin rhythms (Haimov I, 1994; Czeisler CA, 1999). It is possible that magnetic field exposure could disrupt sleep by reducing melatonin concentrations at night.

The purpose of this study was to determine the effect of a continuous, 60 Hz, nighttime magnetic field exposure (0.5 to 1.0 μT above ambient levels) on sleep outcomes in young women. This study is unique in that it measures women while sleeping at home, using actigraphy, which is an objective measure of sleep outcomes.

Methods

Subjects

Subjects were identified from participants in a randomized crossover trial investigating the effect of a nighttime magnetic field on melatonin and reproductive hormone levels in premenopausal women. Eligibility criteria included not taking oral contraceptives or other hormones in the past six months, being 20 to 40 years old, having regular menstrual cycles, not pregnant or breast-feeding during the previous

year, not a shift worker, and not taking melatonin supplements. Subjects lived in the greater Seattle, Washington area. Between March and September 2001, 85 women from the primary study were asked to participate in an additional component to measure sleep.

Subjects who contacted the study telephone line in response to posted advertisements completed an initial screening interview and, if eligible, were scheduled for a home visit. Written consent, approved by the Institutional Review Board at the Fred Hutchinson Cancer Research Center, was obtained at this visit.

At the first home visit, a technician taught subjects to determine their ovulation date using a menstruation calendar and a commercial ovulation kit (Assure LH Ovulation Predictor, Conception Technologies, San Diego, CA), which detects the luteinizing hormone (LH) surge 24 to 48 hours before ovulation. Subjects tracked one complete menstrual cycle, including detecting an LH surge, before proceeding to the intervention phase. After the next two detectable LH surges, the subject was scheduled for the two measurement periods in which the study intervention was applied. Both measurement periods were five nights and started two days after the LH surge. Half the women were randomly assigned to the intervention and half to the sham-exposure (ambient) during the first measurement period; exposure status was switched at the second period.

The intervention

The intervention consisted of a continuous, 60-Hz magnetic field, 0.5 to 1.0 μT above the ambient levels, at the subject's normal head location on the bed. The exposure was administered by placing a common, household appliance underneath the bed, which was plugged into a power strip that was in the off or on position. There was no indication of whether it was on or off; thus, subjects were blinded to exposure status.

Measurements of magnetic field exposure were collected at 30-second intervals using an EMDEX II meter (Enertech Consultants, Campbell, CA). The meter was placed under the bed, in a position where the magnetic field reading was <0.05 μT different from the reading at the subject's normal head location on the bed.

Sleep data

Subjects wore an actigraph (Actiwatch-16, Mini Mitter Company, Inc., Bend, OR) on their non-dominant wrist (Chung L, 1995) from bedtime to rise time all nights of both measurement periods. On the first day of the measurement periods, subjects completed the Pittsburgh Sleep Quality Index questionnaire (Buysse DJ, 1989) and were instructed on the use of the actigraph.

The actigraph is designed for long-term monitoring of gross motor activity in humans and has an accelerometer capable of sensing motion with a minimal resultant force of 0.01g (Actiwatch 16 / Actiwatch 64 / Actiwatch-L / Actiwatch-Score Instruction Manual, 2001). The actigraph collected data in one-minute sampling periods. When possible, the same actigraph was used at both measurement periods.

Actigraph data were downloaded onto a PC computer, and analyzed using a FORTRAN program with the method of Cole *et al.* (Cole RJ, 1992). Using polysomnographic validation data provided by Mini Mitter Company, Inc. (Actiwatch 16 / Actiwatch 64 / Actiwatch-L / Actiwatch-Score Instruction Manual, 2001), we assessed the sleep/wake status of each minute of the night as follows:

$$D = 0.025 * (0.04A_{-2} + 0.20A_{-1} + 1.0A_{0} + 0.20A_{+1} + 0.04A_{+2})$$

where A_x is the number of detectable motions in that minute. If $D \geq 1$ then the subject was considered to be awake during minute A_0, otherwise the subject was considered to be asleep. We applied the 5 rescoring rules and the 20-minute criterion for determining sleep onset outlined by Cole, *et al.* (Cole RJ, 1992).

Other data collection

At both measurement periods, a technician collected height to the nearest 0.1 cm, weight to the nearest pound, and administered a structured interview collecting demographic information, job status, and exercise habits. Subjects also completed a nightly diary, including bedtime, rise time, medication use, and alcohol consumption, each night of the measurement period. Hours of daylight were determined via sunrise/sunset tables calculated by the National Research Council (Herzberg Institute of Astrophysics, Victoria, BC). On the last night of the period, subjects collected all urine excreted during the night after sleep onset plus the first morning void. To determine whether the cycle was ovulatory, pregnanediol-3-glucuronide was measured in urine by

enzyme immunoassay (Munro CJ, 1991) at the University of Southern California under the direction of Dr. Frank Stanczyk.

Data Analysis

The primary purpose of this study was to determine the effect of the intervention magnetic field versus ambient exposure on total sleep time, sleep efficiency, total wake time, minutes awake after sleep onset, the number of awakenings, number of awakenings ≥ 3 minutes, and sleep onset latency, using linear regression. Sleep efficiency is the total sleep time divided by the time in bed. We also analyzed outcomes based on clinically relevant cut-points: total sleep time (≤6 hr vs. >6hr), sleep efficiency (≤80% vs. >80%), number of awakenings ≥ 3 minutes (>5 vs. ≤5), and sleep onset latency (>30 min vs. ≤30 min), using logistic regression. Patients presenting below these cutoffs are considered to be at risk for sleep problems in clinical settings (Dew MA, 1994; Sateia MJ, 2000; Dew MA, 2003). Due to the correlated nature of the data, we used generalized estimating equations with an independent working correlation matrix (Zeger SL, 1986).

The primary model regressed each sleep outcome on exposure (intervention exposure vs. ambient), night in intervention period as indicator variables (1, 2, 3, 4, 5), order of exposure (ambient-exposed, exposed-ambient), and measurement period (1, 2). In a separate model, we included a linear interaction term between night and exposure

to determine whether the magnetic field effect on sleep changed across nights. However, we found no significant interactions, so data from all nights are combined.

We also examined the relationship between mean nighttime magnetic field exposure as a continuous measure and sleep outcomes in a cross-sectional analysis, adjusting for night in intervention period, order of exposure, and measurement period. Since the distributions of the mean magnetic field during the ambient and exposed periods did not substantially overlap, we examined the effect of mean magnetic field exposure on sleep outcomes stratified by exposure period.

Given that sleep patterns can be affected by other factors, we also adjusted for the following to increase precision in a secondary analysis: job status (yes, no), body mass index (linear), usual hours of sleep over previous month (linear), daylight hours (linear), alcohol servings (0, 1, ≥2), bedtime (linear), rise time (linear), age (linear), took a sedative (yes, no), and took another prescription or over-the-counter medication (yes, no). Medications were originally classified with respect to their potential effect on sleep (sedative, no effect, could cause drowsiness, could cause insomnia, could cause drowsiness or insomnia, unknown), using the reported side effect information in the 1999 Physician's Desk Reference. However, with the exception of taking a sedative, all other categories of medication use had similar associations with sleep patterns and were therefore combined.

Imputation of missing outcome data

Some subjects did not wear the actigraph for the entire night, put it on >30 minutes after bedtime, or took it off >30 minutes before getting up, resulting in at least partially missing data for 88 (14%) nights. Missingness was nominally associated with order of exposure, and removing the actigraph before the reported rise time was associated with significantly worse sleep outcomes compared to nights with complete data. Thus, removing these records from the analysis could bias the results. Therefore, we performed multiple imputation using the regression method (described below) to estimate the uncertainty caused by the missing data (Rubin DB, 1986; Schafer JL, 1999).

We imputed 10 data sets by generating a unique linear regression prediction model for each missing data point, chosen at random. The prediction model contained: exposure, order of exposure and their interaction, night of the measurement period, subject id, job status, minutes per week of exercise in the previous month, took a sedative, took an unknown medication, took another prescription or over-the-counter medication, daylight hours, weekday/weekend day, number of alcohol servings, body mass index, and usual hours of sleep over the previous month. If the subject wore the actigraph for part of the night, the sleep outcomes during that time were also included (by thirds of the night). Each missing data point was replaced by the predicted mean value based on the covariate data for the corresponding record plus a randomly-sampled residual, determined from the prediction model. We assumed the data were missing at random (Rubin DB, 1986; Schafer JL, 1999).

Point estimates were determined by fitting the model in all 10 imputed datasets and taking the mean of the resultant point estimates. Standard errors were determined by taking the square root of the sum of the within- and between-imputation variances (Rubin DB, 1986; Schafer JL, 1999). All statistical tests were two-sided.

We had missing covariate data for usual hours of sleep over the previous month for 3 subjects at one of the measurement periods and body mass index for 1 subject at one period. Since both of these covariates are highly correlated between periods we replaced the missing data with information obtained at the other measurement period.

Results

Of the 85 eligible women, 77 (91%) consented. Nineteen subjects completed one measurement period and 58 completed both. Reasons for not completing the second measurement period included: became ineligible (n=10), dropped the study (n=6), and no actigraph available (n=3). No significant differences existed between subjects who contributed data for two (n=46) versus one measurement period (n=25) (data not shown). We excluded measurement periods in which the subject used an electric blanket (n=1), started menstruating (n=1), took emergency contraceptive hormones (n=1), outcome data for 4 or 5 nights were missing (n=6), or the cycle was anovulatory (pregnanediol-3-glucuronide <1.25 μg/mg creatinine) (n=9). Thus, 117 measurement periods from 71 subjects were available for analysis.

On average, subjects were 31 years of age and had a normal body mass index (Table 1). Subjects reported sleeping about seven hours per night over the previous month, had an average bedtime of 11:18 PM, and a rise time of 7:18 AM during the measurement periods. About 88% of subjects were employed, approximately a third took a medication, and around 60% consumed alcohol on at least one of the measurement nights. Randomization yielded approximately equal numbers of subjects in each exposure order.

On average, subjects slept 6:58 hours, with a sleep efficiency of 88.3% during the ambient period and slept 7:01 hours, with a sleep efficiency of 88.4% during the intervention period (Table 2). The mean magnetic field was about 0.7 μT higher during the intervention versus ambient period. One subject had ambient magnetic fields higher than the minimum measured level during an intervention period. Sleep outcomes were not significantly different between nights with the intervention magnetic field exposure and ambient nights. However, when examining dichotomous sleep outcomes, we found that subjects were nearly significantly less likely (OR: 0.7, 95% CI: 0.4, 1.0) to have more than 5 awakenings ≥ 3 minutes during the intervention versus ambient period (Table 3). However, no other associations were found with intervention period in this analysis.

The effect of increasing mean nighttime magnetic field on sleep outcomes differed between the ambient and exposed periods (Table 4). We observed modest associations between measured magnetic fields and sleep outcomes during the ambient but not the intervention period when only adjusting for order of exposure, night, and

period (data not shown). However, after adjustment for multiple factors affecting sleep, we found that a 0.1 μT increase in magnetic field during the ambient period was associated with 8.9 minutes less total sleep time (95% CI: -16.3, -1.5), 1.3% lower sleep efficiency (95% CI: -2.2, -0.4), 5.2 minutes more total wake time (95% CI: 0.5, 9.8), 4.5 minutes more wake after sleep onset (95% CI: 1.2, 7.7), and 0.6 more awakenings ≥ 3 minutes (95% CI: 0.1, 1.2). In general, there was little association between mean magnetic field levels and sleep outcomes during the intervention period.

Similarly, little or modest associations were observed between dichotomized sleep outcomes and measure magnetic field when only adjusting for order of exposure, night, and period (data not shown). However, after adjusting for multiple factors affecting sleep patterns, we found that a 0.1 μT increase in magnetic field was associated with an increased risk of having a sleep efficiency ≤ 80% (OR: 4.0, 95% CI: 2.0, 8.2) and a modestly increased risk of having more than 5 awakenings of ≥ 3 minutes (OR: 1.9, 95% CI: 1.0, 3.7) during the ambient period only (Table 5). We found no association between total sleep time or sleep onset and measured magnetic field in this analysis.

Discussion

The purpose of this study was to determine the effect of a continuous, 60 Hz, nighttime magnetic field exposure on sleep outcomes in young women sleeping at home. Our results suggest that the intervention had no effect on sleep outcomes.

However, an increased ambient magnetic field exposure was associated with modestly disrupted sleep in this population.

Our finding that the intervention had no effect on sleep outcomes is inconsistent with results from laboratory-based studies, which reported significant reductions of total sleep time (range: 16 – 29 minutes) and sleep efficiency (range: 3% - 7%) during the exposed period (Akerstedt T, 1999; Graham C, 1999; Graham C, 2000), and from one home-based study, which reported significant improvements in self-reported sleep outcomes for exposed insomniacs (Pelka RB, 2001). Disparities between study results may be due to differing exposure protocols, settings, subject populations, or statistical variation. The exposure protocols varied in the magnetic field frequency (4 - 60 Hz), level (0.5 – 28.3 μT), and intermittency (continuous versus intermittent). Such properties may have differing effects on sleep. For example, one study reported that sleep outcomes worsened when subjects were exposed to an intermittent, but not a continuous, magnetic field (Graham C, 1999).

Setting also may be important. The three laboratory-based studies controlled for the temperature, humidity, light exposure, bedtime, and rise time, while our study and the other home-based study did not. One study reported that young, normal sleepers have longer and more consolidated sleep in a laboratory versus home environment (Means MK, 2002), suggesting that additional factors affecting sleep in a home environment could overshadow a potential magnetic field effect.

Further, different populations may not have the same sensitivity to magnetic field exposure. For example older women, but not older men, had reduced sleep quality

during magnetic field versus sham-exposure (Graham C, 2000). The authors theorized that the particularly poor sleep quality of the older men during the placebo condition might have masked a potential magnetic field effect. However, magnetic field exposure improved subjective sleep quality in insomniacs, a population with extreme sleep disruptions (Pelka RB, 2001). It is possible that the effect of a nighttime magnetic field exposure on sleep differs between populations.

Although we found no effect of the intervention exposure, higher ambient magnetic fields were associated with worse sleep. There was an almost 0.5 μT range in ambient magnetic fields, such that women with the highest levels may have about 40 minutes less total sleep time and 9% lower sleep efficiency than those with the lowest levels. Our results are consistent with a study in Taiwan, which reported that increased ambient magnetic fields in the bedroom were associated with self-reported disruptions in sleep initiation and maintenance (Li CY, 2002). Further, higher ambient magnetic field exposure was significantly associated with lower overnight urinary 6-sulfatoxymelatonin, a metabolite of melatonin, in 203 young women (Davis S, 2001). Since melatonin is involved in circadian control, changes may be associated with lowered sleep quality (Czeisler CA, 1999).

Although ambient magnetic fields are highly correlated over time (Davis S, 2001), we found little association between measured magnetic fields and sleep outcomes during the intervention period. Since the amount of the intervention exposure varied, measured magnetic fields during that period may not correctly classify subjects with respect to their ambient exposure. The Spearman rank correlation between the

intervention and ambient periods was 0.23. This could explain the attenuated associations observed between measured magnetic field and sleep during the intervention period.

Our results therefore appear discrepant: the high intervention exposure had no effect on sleep, while increased ambient exposure, which was lower than the intervention exposure, was associated with disrupted sleep. Several reasons may explain these results. First, the ambient and intervention magnetic fields may have different properties. The intervention provided a continuous magnetic field, while ambient fields can fluctuate. Graham *et al.* reported that an intermittent, but not a continuous, magnetic field exposure disrupted sleep (Graham C, 1999). We measured magnetic field variability using the mean rate-of-change metric, which is the root-mean-square of the absolute difference between consecutive pairs of magnetic field measurements (Yost MG, 1999). We found a large positive correlation (ρ=0.71) between the mean rate-of-change metric and ambient magnetic field levels. Second, a confounding factor may be associated with both increased ambient magnetic field and worse sleep quality. For example, family income may be inversely associated with both ambient magnetic field levels (Gurney JG, 1995; Schuz J, 2000) and poor sleep quality (Sickel AE, 1999; Moore PJ, 2002). Other possible confounders are noise and light. Third, the observed association between ambient magnetic field levels and sleep may be spurious; significant results in multiple sleep outcomes could be due to the high correlation between the outcomes themselves.

The current study has several strengths. The sample size is larger than previous laboratory-based studies. We also accounted for the correlated nature of the sleep outcome data, which has the benefit of eliminating between-subject confounding. Our study examined young women, a group that was underrepresented in previous studies. Measurement periods were conducted during the same menstrual phase, thus eliminating the possibility that observed associations were due to differing sleep patterns across the menstrual cycle (Parry BL, 1989; Driver HS, 1996). Although the residential setting may increase sleep variability, it is important to understand the effect of magnetic field exposures outside of a laboratory environment. This will better help in understanding whether such exposures pose a potential public health problem.

The present study has several weaknesses. First, since women were measured while sleeping at home, we could not control for exposures, such as light or bedtime, that can affect sleep. We also did not collect information about whether women slept alone or with another person or pet. However, the randomized nature of the trial should remove the confounding effects of these factors. Second, we did not exclude women with medical conditions or who took medications known to affect sleep. This could affect the results if magnetic fields have a different effect in such individuals. Third, although actigraphy is an objective measure of sleep, it overestimates total sleep time compared to polysomnography because it cannot detect wake time in which no movement occurs (Cole RJ, 1992; Babin L, 1997; Blood ML, 1997). This overestimation may be larger among those with poor sleep quality (Cole RJ, 1992; Hauri PJ, 1992; Kushida CA, 2001), possibly causing an attenuation of the observed

associations. Fourth, 14% of the nights had at least some missing data, which may limit the generalizability of our results. Rather than exclude these nights, possibly leading to biased results, we used a multiple imputation method that produces valid answers with respect to the uncertainty introduced by the missing data (Schafer JL, 1999; Longford NT, 2001).

Our study suggests that a continuous, 60 Hz nighttime magnetic field, 0.5 to 1.0 μT above ambient levels, has no effect on sleep patterns in young women sleeping at home. However, increased ambient magnetic field exposure was associated with worse sleep for women with the highest exposures. The discrepant findings between analyses using the randomized versus cross-sectional data highlight the importance of conducting randomized trials. Overall, more research is necessary to elucidate the complex relationship between magnetic field exposure and sleep patterns.

Table 1. Characteristics of women (n=71) who have complete data for the first (n=67) and second (n=50) data collection periods.

	Completed 1st period Mean (sd[1])	Completed 2nd period Mean (sd[1])
Age (years)	30.5 (5.4)	30.7 (5.2)
BMI (kg/m^2)	23.9 (3.7)	24.0 (3.5)
Usual hours of sleep	7.2 (0.9)	7.3 (0.8)
Daylight hours	14.4 (1.1)	14.5 (1.4)
Bedtime (min)	11:18 PM (82.1)	11:17 PM (79.0)
Rise time (min)	7:19 AM (90.4)	7:17 AM (84.7)
	n (%)	**n (%)**
Employed	59 (88.1)	44 (88.0)
Consumed alcohol[2]	41 (61.2)	28 (56.0)
Took a prescription sedative[2]	2 (3.0)	1 (2.0)
Took another medication[2,3]	23 (34.3)	13 (26.0)
Order of exposure		
Exposed during 2nd period	34 (50.8)	26 (52.0)
Exposed during 1st period	33 (49.2)	24 (48.0)

[1]Standard deviation
[2]At least once during the measurement period
[3]Any prescription or over-the-counter medication, except sedatives

Table 2. Sleep outcomes and mean magnetic field exposure for all nights[1], stratified by intervention period, and the adjusted[2] difference in sleep outcomes between the intervention and ambient periods.

	Ambient (n=251)[3]		Intervention (n=255)[3]		Adjusted[2] difference (n=585)	
	Mean (sd[4])	Range	Mean (sd[4])	Range	β[5] (95% CI[6])	p
Total sleep time (min)	417.9 (69.2)	226 – 689	421.2 (72.5)	175 – 669	**3.8** (-8.1, 15.7)	0.53
Sleep efficiency (%)	88.3 (4.7)	62.6 – 97.3	88.4 (4.6)	73.0 – 98.7	**0.0** (-0.9, 1.0)	0.97
Total wake time (min)	55.4 (24.9)	11 – 163	55.1 (23.1)	7 – 150	**0.2** (-4.5, 4.8)	0.95
Wake after sleep onset (min)	40.6 (18.3)	6 – 109	39.6 (19.1)	4 – 118	**-1.2** (-4.8, 2.5)	0.53
Awakenings	22.3 (8.0)	3 – 51	22.1 (7.7)	3 – 51	**-0.2** (-1.6, 1.3)	0.82
Awakenings ≥ 3 min	4.2 (2.9)	0 – 14	4.1 (3.0)	0 – 15	**-0.2** (-0.7, 0.4)	0.55
Sleep onset (min)	12.4 (13.9)	1 – 70	13.3 (16.3)	1 – 89	**1.4** (-1.1, 4.0)	0.27
Mean magnetic field[7] (μT)	0.08 (0.08)	0.001 – 0.50	0.76 (0.17)	0.41 – 1.21	---	---

[1]Only includes nights with no missing sleep outcome data; means including the imputed data did not significantly change the results.
[2]Adjusted for order of exposure, night, and period; analysis uses imputed data
[3]Number of nights with non-missing sleep outcome data, for sleep onset $n_{ambient}$=263 and $n_{intervention}$=262
[4]Standard deviation
[5]Average difference in sleep outcomes between the intervention exposure versus ambient exposure only.
[6]Confidence interval
[7]Includes 270 measured nights during the ambient period and 260 measured nights during the intervention period

Table 3. Adjusted odds ratios (OR) for clinically relevant sleep cut-points comparing nights with the intervention magnetic field exposure to nights with ambient exposure only.

	Primary Analysis[1]		Secondary Analysis[2]	
Measure	**OR (95% CI[3])**	**p**	**OR (95% CI[3])**	**p**
Total sleep time (≤ 6 hr vs. > 6 hr)	**0.8** (0.5, 1.4)	0.48	**0.6** (0.3, 1.2)	0.17
Sleep efficiency (≤ 80% vs. > 80%)	**0.8** (0.3, 2.0)	0.66	**0.8** (0.3, 1.8)	0.58
Number of awakenings ≥ 3 min (> 5 vs. ≤ 5)	**0.8** (0.5, 1.2)	0.29	**0.7** (0.4, 1.0)	0.08
Minutes to sleep onset (> 30 min vs. ≤ 30 min)	**1.4** (0.9, 2.1)	0.15	**1.4** (0.8, 2.2)	0.22

[1]Adjusted for order of exposure, night, and period
[2]Adjusted for order of exposure, night, period, age, body mass index, servings of alcohol, job status, usual sleep hours in previous month, took a sedative, took any other prescription or over the counter medication, number of daylight hours, bedtime, and rise time.
[3]Confidence interval

Table 4. Average difference[1] in sleep outcomes for a 0.1 microTesla increase in the mean level of magnetic field exposure, stratified by intervention period.

	Ambient		Intervention	
	Difference in sleep outcome[2] (95% CI[3])	**p**	**Difference in sleep outcome[2] (95% CI[3])**	**p**
Total sleep time (min)	**-8.9** (-16.3, -1.5)	0.02	**-2.1** (-5.5, 1.4)	0.24
Sleep efficiency (%)	**-1.3** (-2.2, -0.4)	0.01	**-0.2** (-0.8, 0.3)	0.42
Total wake time (min)	**5.2** (0.5, 9.8)	0.03	**1.0** (-1.5, 3.6)	0.43
Wake after sleep onset (min)	**4.5** (1.2, 7.7)	0.01	**1.7** (-0.3, 3.8)	0.10
Number of awakenings	**0.9** (-0.5, 2.3)	0.21	**0.5** (-0.3, 1.3)	0.23
Number of awakenings ≥ 3 min	**0.6** (0.1, 1.2)	0.03	**0.3** (-0.1, 0.6)	0.14
Minutes to sleep onset	**-1.1** (-3.2, 0.9)	0.26	**0.8** (-0.4, 1.9)	0.21

[1]Adjusted for order of exposure, night, period, age, BMI, servings of alcohol, job status, usual sleep hours in previous month, took a sedative, took any other prescription or over the counter medication, number of daylight hours, bedtime, and rise time.
[2]Difference in sleep outcomes for a 0.1 microTesla increase in magnetic field exposure.
[3]Confidence interval

Table 5. Adjusted odds ratios (OR) for a 0.1 microTesla increase in the mean level of magnetic field exposure, stratified by intervention period.

	Ambient		Intervention	
	OR[2] (95% CI[3])	p	OR[2] (95% CI[3])	p
Total sleep time (≤ 6 hr vs. > 6 hr)	**1.4** (0.6, 3.0)	0.39	**1.2** (0.9, 1.6)	0.19
Sleep efficiency (≤ 80% vs. > 80%)	**4.0** (2.0, 8.0)	<0.001	**1.3** (0.9, 2.0)	0.13
Number of awakenings ≥ 3 min (> 5 vs. ≤ 5)	**1.9** (1.0, 3.7)	0.07	**1.2** (0.9, 1.5)	0.20
Minutes to sleep onset (> 30 min vs. ≤ 30 min)	**0.7** (0.4, 1.4)	0.34	**1.1** (0.8, 1.3)	0.57

[1]Adjusted for order of exposure, night, period, age, BMI, servings of alcohol, job status, usual sleep hours in previous month, took a sedative, took any other prescription or over the counter medication, number of daylight hours, bedtime. and rise time.
[2]Odds ratio for a 0.1 microTesla increase in magnetic field exposure.
[3]Confidence interval

Chapter 2: Sleep patterns, as measured by actigraphy, in menstruating women, aged 20 to 40 years

Background

Sleep quality is associated with memory and learning, gastrointestinal disorders, and potentially immune function (Scott AJ, 1990; Dotto L, 1996). A recent study suggested that too little or too much sleep might increase risk of cardiovascular disease in women (Ayas NT, 2003). Thus it is important to understand sleep patterns in a natural environment and potential risk factors for poor sleep. Both subjective and objective measures should be considered, since each modality may assess different aspects of the sleep experience (Coates TJ, 1982; Buysse DJ, 1989; Vitiello MV, 1997; Vitiello MV, 2002). Actigraphy is a validated, objective method to monitor sleep and other activity patterns over long periods in the home (Sadeh A, 1995; Sadeh A, 2002; Ancoli-Israel S, 2003). Normative sleep data using actigraphy in a natural environment is available for children and adolescents (Acebo C, 1999; Sadeh A, 2000) and the elderly (van Hilten JJ, 1993), however little information is available for menstruating women (Sadeh A, 2002).

Collecting normative data is important for understanding the variability of sleep patterns and their stability across multiple nights. For example, several studies suggested that 5 to 7 days of data were needed to appropriately describe sleep patterns in young children and adolescents because there was considerable variability across nights (Acebo C, 1999; Sadeh A, 2000). Among 99 subjects aged 50 to 98 years, the

intra-subject variability in movement parameters was large (van Hilten JJ, 1993). However two small studies ($n \leq 20$) of young and middle-aged subjects reported consistently high correlations between two consecutive nights for sleep efficiency ($\rho \geq 0.70$) and awakenings ≥ 3 minutes ($\rho \geq 0.69$), but not for total sleep time ($\rho = 0.60$ and $\rho = 0.05$, respectively) (Jean-Louis G, 1995b; Jean-Louis G, 1995a).

The purpose of this study was to examine sleep patterns across multiple nights in 20 to 40 year-old menstruating women during the luteal phase of the menstrual cycle, using actigraphy. Specifically we assessed the within- and between-subject variability, the reliability of sleep measures over multiple nights, and what exposures were associated with sleep patterns. We also examined associations between some exposures and self-reported sleep quality to compare whether these associations might differ from those found when using actigraphic outcomes. This study provides the first normative, actigraphic data about sleep patterns in a natural environment for young women and will be useful for planning future studies.

Methods

Subjects

Subjects were identified from participants in a randomized crossover trial investigating the effect of a nighttime magnetic field on melatonin and reproductive hormone levels in premenopausal women. Eligibility criteria included not taking oral

contraceptives or other hormones in the past six months, being 20 to 40 years old, having regular menstrual cycles, a body mass index ≤ 30.0 kg/m^2, not pregnant or breast-feeding during the previous year, not a shift worker, and not taking melatonin supplements. Subjects lived in the greater Seattle, Washington area.

Subjects who contacted the study telephone line in response to posted advertisements completed an initial screening interview and, if eligible, were scheduled for a home visit. Between March and September 2001, 85 women from the primary study were asked to participate in an additional component to measure sleep. Written consent, approved by the Institutional Review Board at the Fred Hutchinson Cancer Research Center, was obtained at this visit.

At the first home visit, a technician taught subjects to determine their ovulation date using a menstruation calendar and a commercial ovulation kit (Assure LH Ovulation Predictor, Conception Technologies, San Diego, CA), which detects the luteinizing hormone (LH) surge 24 to 48 hours before ovulation. Subjects tracked one complete menstrual cycle, including detecting an LH surge, before proceeding to the intervention phase. After the next two detectable LH surges, the subject was scheduled for the two measurement periods in which the study intervention was applied. Both measurement periods were five nights long and started approximately two days after the LH surge. Half the women were randomly assigned to the intervention and half to ambient exposure during the first measurement period; exposure status was switched at the second period.

The intervention

The intervention consisted of a continuous, 60-Hz magnetic field, 0.5 to 1.0 μT above the ambient levels, at the subject's normal head location on the bed. The exposure was administered by placing a common, household appliance underneath the bed, which was plugged into a power strip that was either off or on. There was no indication of whether it was off or on; thus, subjects, but not technicians, were blinded to exposure status.

Sleep data

Subjects wore an actigraph (Actiwatch-16, Mini Mitter Company, Inc., Bend, OR) on their non-dominant wrist (Chung L, 1995) from bedtime to rise time all nights of both measurement periods. Written and oral instructions for using the actigraph were provided at each measurement period.

The actigraph is designed for long-term monitoring of gross motor activity in humans and has an accelerometer capable of sensing motion with a minimal resultant force of 0.01g (Actiwatch 16 / Actiwatch 64 / Actiwatch-L / Actiwatch-Score Instruction Manual, 2001). The actigraph collected data in one-minute sampling intervals. When possible, the same actigraph was used at both measurement periods.

Actigraph data were downloaded onto a PC computer, and analyzed via a FORTRAN program using the method of Cole *et al.* (Cole RJ, 1992). Using polysomnographic validation data provided by Mini Mitter Company, Inc. (Actiwatch

16 / Actiwatch 64 / Actiwatch-L / Actiwatch-Score Instruction Manual, 2001), the sleep/wake status of each minute of the night was assessed as follows:

$$D = 0.025 * (0.04A_{-2} + 0.20A_{-1} + 1.0A_0 + 0.20A_{+1} + 0.04A_{+2})$$

where A_x is the number of detectable motions in that minute. If $D \geq 1$ then the subject was considered to be awake during minute A_0, otherwise the subject was considered to be asleep. To increase accuracy, we applied the 5 rescoring rules outlined by Cole, *et al.*, which correct for the problem that subjects falling asleep after waking up during the night tend to stop moving a few minutes before polysomnography indicates the onset of sleep (Cole RJ, 1992). Initial sleep onset was considered to be at the beginning of the first 20-minute interval in which no more than one minute was scored as awake; this criterion had the highest correlation with polysomnography (Cole RJ, 1992).

Other data collection

Age and self-reported menstrual cycle length were collected during the screening interview. On the first day of each measurement period, a technician measured height to the nearest 0.1 cm; weight to the nearest pound; administered a structured interview collecting demographic information, job status, exercise habits, premenstrual syndrome symptoms, and the Perceived Stress Scale (Cohen S, 1983); and asked subjects to complete the Pittsburgh Sleep Quality Index (PSQI) questionnaire (Buysse DJ, 1989). Usual bedtime and rise time were abstracted from the PSQI and the global index assessing sleep quality was calculated. Subjects also completed a nightly diary during both measurement periods, recording their bedtime, rise time, medication

use, and alcohol consumption. Hours of daylight were determined via sunrise/sunset tables calculated by the National Research Council (Herzberg Institute of Astrophysics, Victoria, BC). On the last night of the measurement period, subjects collected all urine excreted during the night after sleep onset plus the first morning void. To determine whether the cycle was ovulatory, pregnanediol-3-glucuronide was measured in urine by enzyme immunoassay (Munro CJ, 1991) at the University of Southern California under the direction of Dr. Frank Stanczyk.

Statistical methods

The primary purpose of this study was to describe sleep patterns in young women. We considered seven actigraphic sleep measures (total sleep time, sleep efficiency, total wake time, minutes awake after sleep onset, the number of awakenings, number of awakenings ≥3 minutes, sleep onset latency) and three measures abstracted from the sleep diary (time in bed, bedtime, rise time). Sleep efficiency is the total sleep time divided by the time in bed. The within- and between-subject standard deviations of each measure were determined using a random effects model to assess the variance components, controlling for exposure status, exposure order, and month as fixed effects (Rosner B, 1995). We also considered whether the within-subject variance differed for subjects who had different actigraphs at the two measurement periods. The intraclass correlation, an indication of within-subject sleep stability across nights, was calculated by dividing the between-subject variance by the total variance (Armstrong BK, 1992). The reliability of 2 to 5 nights of aggregated sleep data was determined using the

Spearman-Brown formula (Armstrong BK, 1992). This measure predicts how many nights of data are needed to obtain a representative sample of a subject's sleep patterns; a reliability ≥0.70 was considered adequate based on previous literature (Acebo C, 1999). We used data from both the intervention and ambient measurement periods, since the intervention was not associated with sleep patterns (See Chapter 1).

Using linear regression we assessed whether sleep outcomes with an intraclass correlation > 0.30 were associated with the following exposures: 1. bedtime differed from usual bedtime on the PSQI (within 1hr, ≥ 1hr later, ≥ 1hr earlier); 2. rise time differed from usual rise time on the PSQI (within 1hr, ≥ 1hr later, ≥ 1hr earlier); 3. alcohol servings (none, one, two or more); 4. minutes/week of exercise during the previous month (linear); 5. took a prescription or over-the-counter medication other than a sedative (no vs. yes); 6. job status (employed vs. unemployed); 7. Perceived Stress Scale in quartiles (≤12, 13-18, 19-24, ≥25); 8. day of week (weekday vs. weekend); 9. daylight hours (linear); 10. age (linear); 11. menstrual cycle length (linear); and 12. body mass index (linear). All exposure variables were included in one model that was adjusted for intervention status (exposed, not exposed), exposure order (exposed-ambient, ambient-exposed), measurement period (1, 2), night in the period (1, 2, 3, 4, 5), and ovulatory status (ovulatory, anovulatory, unknown). Due to the correlated nature of the data, we used generalized estimating equations with an independent working correlation matrix (Zeger SL, 1986). Medications were originally classified with respect to their potential effect on sleep (sedative, no effect, could cause

drowsiness, could cause insomnia, could cause drowsiness or insomnia, unknown), using the 1999 Physician's Desk Reference. However, with the exception of taking a sedative, all medication categories had similar associations with sleep patterns and were therefore combined. We did not consider sedative use or chronic premenstrual syndrome due to the small number of women reporting these exposures. We excluded six records with extreme bedtimes (>03:00) or rise times (>14:00).

Using logistic regression we determined whether self-reported poor sleep quality, defined as having a score >5 on the PSQI, was associated with the following exposures measured before or at the same time as the PSQI: age (20-24, 25-29, 30-34, 35-40); minutes/week of exercise during previous month (≤139, 140-249 250-359, ≥360); menstrual cycle length; body mass index; employment status; and Perceived Stress Scale. All exposure variables were included in one model that was adjusted for measurement period and ovulatory status. Due to the correlated nature of the data, we used generalized estimating equations with an independent working correlation matrix (Zeger SL, 1986). Age and minutes of exercise were categorized for this analysis because their association with self-reported sleep quality was not linear.

Some subjects did not wear the actigraph for the entire night, put it on >30 minutes after bedtime, or took it off >30 minutes before rise time, resulting in at least partially missing data for 91 (14%) of 640 nights. Removing the actigraph early was associated with significantly worse sleep outcomes compared to nights with complete data. Thus, to avoid having biased results and to estimate the uncertainty caused by the missing data, we used multiply imputed data, described previously, for the regression

analyses (See Chapter 1). Briefly, we performed multiple imputation using the regression method, assuming that the data were missing at random (Rubin DB, 1986; Schafer JL, 1999). We imputed ten data sets by generating a unique linear regression prediction model for each missing data point, chosen at random. Inference was made by running the regression models on all imputed datasets. Point estimates were determined by taking the mean of the resultant point estimates and standard errors were determined by taking the square root of the sum of the within- and between-imputation variances. All statistical tests were two-sided.

Results

Of the 85 eligible women from the parent trial, 77 (91%) consented to participate in the sleep ancillary study. Nineteen subjects completed one measurement period and 58 completed both. Reasons for not completing the second measurement period included: became ineligible (n=10), dropped the study (n=6), and no actigraph available (n=3). We excluded measurement periods in which the subject started menstruating (n=1) or sleep data for 4 or 5 nights were missing (n=6). Thus, 128 measurement periods from 74 subjects were available for analysis. No significant differences existed between subjects who contributed data for two (n=54) versus one measurement period (n=20) (data not shown), except that women with one measurement period reported significantly more exercise during the previous month than women with two measurement periods (p=0.004). Of the 640 nights of data collection, 91 (14%) were missing some sleep data.

On average, subjects were 31 years old, had a normal body mass index, and a menstrual cycle length of nearly 29 days (Table 6). The usual bed and rise times reported on the PSQI were about 22:53 and 06:58, respectively, nearly a third of women had a global score >5 on the PSQI, indicating poor sleep, and almost 90% were employed. Eight women reported having severe premenstrual syndrome at the first measurement period, but only one reported it again at the second period. Slightly fewer women were in the highest quartile of perceived stress during the second measurement period. Some sort of prescription or over-the-counter medication was used on nearly a quarter of the nights; however sedative use was low. Alcohol was consumed on about 25% of nights. Bed and rise times differed by over an hour from the usual times reported on the PSQI for over a third of the nights.

The mean total sleep time was almost 7 hours per night, but ranged from about 2.5 to 11.5 hours (Table 7). Similarly, mean sleep efficiency was 88.3%, but varied from 60.5% to 98.7%. The mean number of awakenings was 22.2 per night, with an average of 4.2 awakenings being ≥3 minutes long. The median sleep onset was 7 minutes. Bedtimes and rise times spanned a wide range. One woman went to bed at 07:00 and got up at 16:00 hours on one night; excluding this night the latest bedtime was 04:00 and the latest rise time was 14:00 hours. For all sleep measures, the within-subject standard deviation was nearly the same as or larger than the between-subject standard deviation. The within-subject standard deviation was slightly lower for sleep efficiency, total wake time, wake after sleep onset, awakenings, and awakenings ≥3

minutes, and sleep onset when excluding nine subjects who had different actigraphs at their two measurement periods (data not shown).

The intraclass correlation was low for total sleep time (ρ=0.19), sleep onset (ρ=0.10), and time in bed (ρ=0.14) (Table 8). Most other sleep outcomes had an intra-class correlation ≥0.40. When aggregating multiple nights of data, four to five nights are needed to reach a predicted reliability of ≥ 0.70 (Acebo C, 1999) for most sleep measures. However, even after five nights, those measures with a low intraclass correlation – total sleep time, sleep onset, and time in bed – did not meet an adequate reliability of ≥0.70.

No difference in sleep efficiency was detected for nights in which the subject's bedtime or rise time was more than an hour different versus within an hour of usual (Table 9). However, going to bed ≥1 hour later than usual was associated with decreased total wake time, wake after sleep onset, and awakenings. Going to bed ≥1 hour earlier or rising ≥1 hour later than usual was associated with increased total wake time, wake after sleep onset, and awakenings. Going to bed ≥1 hour later than usual was associated with a 53.4-minute later rise time (95% confidence interval (CI): 34.1, 72.6).

Using any non-sedative medication was associated with a 1.8% lower sleep efficiency (95% CI: -3.7, 0.1), 11.7 more minutes of total wake time (95% CI: 2.0, 21.3), and a 22.6-minute earlier bedtime (95% CI: -44.3, -0.8) (Table 9). Compared to employed women, unemployed women had a 2.5% higher sleep efficiency (95% CI:

1.0, 4.0), 12.8 minutes less total wake time (95% CI: -20.2, -5.4), 1.4 fewer awakenings ≥3 minutes (95% CI: -2.4, -0.4), and a 39.4 minute later bedtime (95%CI: 4.3, 74.4). On weekend nights, subjects went to bed and rose significantly later (10.5 minutes and 17.0 minutes, respectively) and had 0.8% lower sleep efficiency (95% CI: -1.5, -0.2) than on weeknights. An hour increase in daylight was associated with lower sleep efficiency, more total wake time, and more time awake after sleep onset.

A day increase in menstrual cycle length was significantly associated with a 0.4% lower sleep efficiency (95% CI: -0.7, -0.1) and 1.8 minutes more total wake time (95% CI: 0.3, 3.3) (Table 9). Increasing body mass index was modestly associated with lower sleep efficiency (-0.2% for each kg/m^2 increase), more total wake time, and more minutes awake after sleep onset. Older age (range was from 20 to 40 years old) was associated with earlier bedtimes, but no other sleep parameters. Alcohol consumption, exercise during the previous month, and perceived stress were not significantly associated with sleep outcomes (data not shown).

Self-reported poor sleep (a score >5 on the PSQI) was not significantly associated with menstrual cycle length, body mass index, or exercise during the previous month (Table 10). However, 25-29 and 30-34 year olds were at an increased risk of reporting poor sleep compared to 20-24 year olds; the oldest women were not significantly different from the youngest. Unemployed women had a nearly significantly increased risk (OR: 4.2, 95% CI: 0.8, 20.9) of reporting poor sleep compared to employed women. There was a nearly significant trend of increased risk for reporting poor sleep with increasing quartiles of the Perceived Stress Scale.

Discussion

The purpose of this study was to describe sleep patterns at home, using actigraphy, for 20 to 40 year-old menstruating women. Overall, actigraphy was an acceptable modality for measuring sleep in this population as over 90% of subjects consented to participate. However, during 6 (4.5%) of 134 possible measurement periods, the subject did not wear the actigraph at all or did so for only one night. After excluding these periods, actigraphic data were missing, at least in part, for 14% of nights. Thus missing data is potentially a serious problem for actigraphic studies in this population. We hypothesize that most missing data was due to the subject forgetting to put on the actigraph when going to bed. Asking subjects to wear the actigraph 24 hours a day could ameliorate this problem. For about 25% of the missing nights the subject took off the actigraph early, i.e. before getting out of bed. Sleep measures on these nights were significantly worse than nights with complete data, even when delineating all missing minutes as sleep. This suggests that if a subject is sleeping poorly, she may remove the actigraph in an effort to improve sleep. Thus it is important to query subjects about whether they removed the actigraph during the night.

About 30% of our subjects had a score >5 on the PSQI, indicating a perception of poor sleep quality; only 2 subjects (2.4%) reported taking a sedative. This is consistent with surveys of sleep problems in this age group. Among 2,782 young adults (aged 17 to 30 years), only 36% reported being completely free of seven common sleep disturbance patterns (Coren S, 1994). Among 2,714 adults aged 20 to 45 years from Brighton, 24% reported delayed sleep onset, 21% were dissatisfied with their sleep, and

8% took medication as a sleep aid (Mniszek DH, 1988). Among Japanese women over the age of 20 years, 20% reported poor sleep quality and 5% took medication as a sleep aid (Doi Y, 2000). Another study of 2,202 persons aged 20 to 45 years from four European cities reported that the prevalence of hypnotic use ranged from 0.4 to 2.0% (Janson C, 1995). Thus, although young people have fewer sleep problems than the elderly (Doi Y, 2000), there is a substantial proportion with at least some sleep disturbances, indicating that it is important to study sleep patterns in this population.

We observed a wide range of sleep patterns, even though we excluded women who did shiftwork, had irregular menstrual cycles, or had recently had or breastfed a child. This suggests that the stringent eligibility criteria did not lead to the exclusion of all women with poor sleep. We also found that the within-subject variability was nearly the same as or more than the between-subject variability, signifying that sleep patterns in a natural environment may not be stable across nights. Several other studies also have reported a large within-subject variability when studying sleep in a natural environment for both children (Acebo C, 1999) and the elderly (van Hilten JJ, 1993).

Further, relatively low intraclass correlations were observed, particularly for total sleep time, sleep onset, and time in bed, such that 5 nights of measurement may not result in a representative sampling of sleep outcomes. Other measures, including sleep efficiency, total wake time, awakenings, and awakenings $\geq$ 3 minutes were more stable, so that four to five nights of data collection should result in a representative picture of an individual's sleep. Our results are consistent with those found in children and adolescents, in which 5 nights of data were needed to obtain stable measures of

total wake time and sleep efficiency, but seven or more nights were needed for total sleep time and time in bed (Acebo C, 1999; Sadeh A, 2000). However, two small studies that collected two consecutive nights of sleep data reported higher correlations for sleep efficiency and awakenings ≥ 3 minutes than our study (Jean-Louis G, 1995b; Jean-Louis G, 1995a). Despite this, the low intraclass correlations and high within-subject standard deviations suggest that at least five nights of data should be collected when using actigraphy in a natural setting among menstruating women.

We also examined various exposures that may be associated with sleep patterns in this population. Medication use, but not alcohol consumption or exercise over the past month, was associated with worse sleep patterns. Taking a medication may reflect the presence of an underlying chronic disease, such as asthma or gastrointestinal problems, that could affect sleep (Orr WC, 2001; Bohadana AB, 2002). Although we did not find that alcohol was associated with sleep patterns, other studies suggest that it may initially improve sleep, but then disrupt sleep during the second half of the night (Roehrs T, 2001b; Roehrs T, 2001a). We may not have observed an association with alcohol because we only considered sleep patterns across the entire night. Other research suggests that exercise may moderately improve sleep (Youngstedt SD, 1997; Driver HS, 2000), however our measure of exercise may not have been sensitive enough to observe an association, since we only asked about exercise over the month before the measurement period.

Sleep hygiene recommendations suggest that it is important to establish a routine bed and rise time (Morin CM, 1999). In our study, nearly 40% of bedtimes and

a third of rise times were more than 1 hour different than usual, suggesting that changes in routine bed and rise times are common. We found that restricting time in bed by either going to bed later or getting up earlier than usual was associated with less total wake time and fewer awakenings. Conversely, increasing time in bed by either going to bed earlier or getting up later than usual was associated with more total wake time and awakenings. However, changes in the sleep routine were not associated with lower sleep efficiency. The associations with total wake time and awakenings could be due to the fact that these measures are not adjusted for the amount of time spent in bed. Interestingly, going to bed late was associated with a later rise time, but going to bed early was not associated with an earlier rise time.

We also observed that although perceived stress was not associated with actigraphic sleep measures, those with high perceived stress were more likely to have a poor score on the PSQI. Two studies comparing good and poor sleepers reported no differences in perceived stress between the two groups (Friedman L, 1995; Shaver JL, 2002). Further, in one study, perceived stress was associated with subjective, but not objective (actigraph), sleep measures (Friedman L, 1995). Similarly, we found that unemployment was associated with better actigraphic sleep measures but worse subjective sleep. Likewise, age was not associated with actigraphic sleep measures, but some age groups were more likely to have an increased risk of poor subjective sleep than others. These data indicate that subjective sleep reports may partly reflect an individual's perspective or state of mind as well as their objective sleep patterns. Thus it is important to assess both the subjective and objective aspects of sleep, if possible.

As expected, we found that subjects went to bed and got up later on weekends versus weekdays. This is consistent with a study of 266 healthy subjects, aged 20 to 50 years, which reported a 26 minute later bedtime and a 53 minute later wake time on weekends (Monk TH, 2000). We also found that sleep efficiency was worse on weekends. This suggests that the sleep experience on weekend days may differ from that on weekdays; thus sleep on both types of days should be collected. We also observed that increasing number of daylight hours was associated with worse sleep, despite the fact that data collection periods only occurred between March and September. This is consistent with a study of 982 Finlanders, in which 20.4% of the sample said that their sleep was worse in the summer (Ohayon MM, 2002). These data suggest that studies measuring subjects at multiple time points should either ensure similar hours of daylight between measurement periods or adjust for this factor at the analytic phase.

Finally we found that increased body mass index and menstrual cycle length were associated with slightly worse sleep efficiency, despite the limited range of these exposures in our study. Obesity, defined as a body mass index > 30 kg/m^2, is associated with sleep apnea and other sleep disorders (Khaodhiar L, 1999). Our data suggest that even women who are overweight (body mass index between 25 and 30) may experience reductions in sleep quality. To our knowledge, no other studies have examined menstrual cycle length and sleep, however previous research suggests that sleep patterns change across the menstrual cycle (Parry BL, 1989; Driver HS, 1996; Manber R, 1997) and by ovulation status (Lee KA, 2000).

The current study has several strengths. We examined young women, a group for which there is little normative sleep data in a natural or home environment, especially using actigraphy as a measurement modality. Another unique aspect of the study is that we did not place restrictions on participants with respect to various parameters that may affect sleep, such as medication use and alcohol consumption. This allowed us to consider the variability of sleep patterns in a natural environment and look at multiple exposures that may affect sleep. Also, measurement periods were conducted during the same menstrual phase, thus eliminating the possibility that observed associations were due to differing sleep patterns across the menstrual cycle (Parry BL, 1989; Driver HS, 1996; Manber R, 1997). Finally, our analytic technique accounted for the correlated nature of the data, which has the benefit of providing valid standard error estimates.

The present study, however, has several weaknesses. First, because the parent trial was not designed specifically to measure sleep patterns, we did not collect information on some important exposures, such as caffeine consumption, pets in bed, the presence of small children in the home, or bed partners. Although this does not affect the validity of the reliability analysis, these exposures could potentially confound the observed associations between other exposures and sleep. Second, we had some exclusion criteria (e.g. no shiftwork) that may have excluded women with the worst sleep. Thus our results may not be generalizeable to all women in this age group. Third, although actigraphy is an objective measure of sleep, it overestimates total sleep time compared to polysomnography because it cannot detect wake time in which no

movement occurs (Cole RJ, 1992; Babin L, 1997; Blood ML, 1997). This overestimation may be larger among those with poor sleep quality (Cole RJ, 1992; Hauri PJ, 1992; Kushida CA, 2001), possibly causing an attenuation of the observed associations. Fourth, 14% of the nights had some missing data, which may limit the generalizability of our results. Rather than exclude these nights, possibly leading to biased results, we used a multiple imputation method that produces valid answers with respect to the uncertainty introduced by the missing data (Schafer JL, 1999; Longford NT, 2001).

In conclusion, we found that sleep patterns varied widely in menstruating women ages 20 to 40 years sleeping at home. Our results suggest that multiple nights of data collection, including weekend and weeknights, are necessary to obtain a reliable representation of an individual's sleep patterns. We also reported that unusual bed or rise times, medication use, employment, day of week, daylight hours, menstrual cycle length, and body mass index were associated with sleep patterns in this population. Associations of perceived stress, employment, and age with objective and subjective sleep patterns differed, suggesting that these two modalities may measure different aspects of the sleep experience. Finally, actigraphy is an acceptable and feasible method of measuring sleep patterns in a natural environment among menstruating women, which could be used for either observational or intervention studies.

Table 6. Characteristics of 74 women who provided 640 nights of data collection.

	First period (n=73 subjects, 365 nights) Mean (SD[1])	Second period (n=55 subjects, 275 nights) Mean (SD[1])
Age (years)	30.7 (5.4)	31.1 (5.3)
Body mass index (kg/m^2)	23.9 (3.7)	24.0 (3.7)
Menstrual cycle length (days)	28.8 (2.1)	28.6 (1.9)
Daylight (hours)	14.4 (1.1)	14.5 (1.3)
Exercise in past month (min/wk)	269.8 (199.2)	258.9 (152.8)
Usual bedtime[2] (min)	22:51 (57.7)	22:54 (52.3)
Usual rise time[2] (min)	06:55 (72.0)	07:00 (77.9)
	n (%)	**n (%)**
Number of subjects:		
Self-reported poor sleep[3]	21 (29.2)	17 (32.7)
Exposed to magnetic field	35 (48.0)	28 (50.9)
Employed	65 (89.0)	48 (87.3)
Severe premenstrual syndrome	8 (11.0)	1 (1.8)
Perceived Stress Scale[4]		
≤ 12	18 (24.7)	17 (30.9)
13 – 18	17 (23.3)	17 (30.9)
19 – 24	17 (23.3)	13 (23.6)
≥ 25	21 (28.8)	8 (14.6)
Number of nights in which subjects:		
Took a sedative	5 (1.4)	2 (0.7)
Took another medication[5]	89 (24.4)	64 (23.3)
Weekend day	112 (30.7)	78 (28.4)
Consumed		
One alcoholic drink	54 (14.8)	29 (10.6)
Two or more alcoholic drinks	50 (13.7)	38 (13.8)
Bedtime[2]		
≥ 1 hour later than usual	108 (30.7)	86 (31.4)
≥ 1 hour earlier than usual	30 (8.2)	22 (8.0)
Rise time[2]		
≥ 1 hour later than usual	90 (24.7)	63 (23.0)
≥ 1 hour earlier than usual	40 (11.0)	36 (13.1)

[1]Standard deviation.
[2]Usual bed and rise times were abstracted from the Pittsburgh Sleep Quality Index.
[3]Poor sleep was defined as having a global score > 5 on the Pittsburgh Sleep Quality Index.
[4]Scale presented in approximate quartiles.
[5]Any non-sedative, prescription or over-the-counter medication.

Table 7. Mean, median, between- and within-subject standard deviation, and range for sleep parameters measured via actigraphy or abstracted from the sleep diary.

	n[1]	Mean	Median	Standard deviation		Range
				Between subject	Within subject	
Total sleep time (min)	549	418.4	419	31.5	64.0	143 – 689
Sleep efficiency (%)	549	88.3	89.2	3.6	3.5	60.5 – 98.7
Total wake time (min)	549	55.5	51	17.7	17.5	7 – 163
Wake after sleep onset (min)	549	40.2	37	13.5	14.4	4 – 118
Awakenings	549	22.2	22	5.1	6.3	3 – 51
Awakenings ≥ 3 minutes	549	4.2	4	2.2	2.3	0 – 17
Sleep onset (min)	572	12.8	7	4.7	14.2	1 – 89
Time in bed (min)	639	480.4	481	30.6	74.4	181 – 871
Bedtime (min)	639	23:19	23:00	49.7	62.3	19:00 – 07:00
Rise time (min)	639	07:19	07:10	52.0	72.4	03:30 – 16:00

[1]Number of nights with non-missing data

Table 8. Intraclass correlation coefficient and estimated reliability for aggregating two to five nights of sleep parameters.

	n[1]	Intraclass correlation[2]	Estimated reliability[3] for Two nights	Three nights	Four nights	Five nights
Total sleep time (min)	549	0.19	0.33	0.42	0.49	0.55
Sleep efficiency (%)	549	0.51	0.68	**0.76**	**0.81**	**0.84**
Total wake time (min)	549	0.51	0.67	**0.75**	**0.80**	**0.84**
Wake after sleep onset (min)	549	0.47	0.64	**0.73**	**0.78**	**0.82**
Awakenings	549	0.40	0.57	0.67	**0.73**	**0.77**
Awakenings ≥ 3 minutes	549	0.48	0.65	**0.74**	**0.79**	**0.82**
Sleep onset (min)	572	0.10	0.18	0.25	0.30	0.35
Time in bed (min)	639	0.14	0.25	0.34	0.40	0.46
Bedtime (min)	639	0.39	0.56	0.66	**0.72**	**0.76**
Rise time (min)	639	0.34	0.51	0.61	0.67	**0.72**

[1]Number of nights with non-missing data

[2]The intraclass correlation is equivalent to the reliability for one night of data collection.

[3]Determined using the Spearman-Brown Formula; bolded numbers indicate an estimated reliability greater than 0.70.

Table 9. Average[1] difference (95% CI[2]) in selected sleep parameters by various exposures that may affect sleep.

	Sleep efficiency (%)	Total wake time (min)	Wake after sleep onset (min)	Awakenings	Awakenings ≥ 3 minutes	Bedtime (min)	Rise time (min)
Bedtime ≥ 1 hr later than usual[3]	0.6 (-0.5, 1.7)	**-8.4**** **(-13.6, -3.2)**	**-5.5**** **(-9.5, -1.4)**	**-3.2**** **(-4.7, -1.7)**	-0.4 (-1.1, 0.3)	---	**53.4**** **(34.1, 72.6)**
Bedtime ≥ 1 hr earlier than usual[3]	-0.6 (-2.2, 1.0)	**8.0*** **(-0.3, 16.4)**	**7.4**** **(0.2, 14.6)**	**3.8**** **(0.5, 7.2)**	0.9 (-0.5, 2.3)	---	2.8 (-28.7, 34.3)
Rise time ≥ 1 hr later than usual[3]	-0.1 (-1.2, 1.1)	**6.0**** **(0.8, 11.1)**	**5.9**** **(2.4, 9.5)**	**2.7**** **(1.1, 4.3)**	**0.7*** **(0.0, 1.3)**	**52.3**** **(38.9, 65.6)**	---
Rise time ≥ 1 hr earlier than usual[3]	-0.3 (-1.9, 1.4)	-5.3 (-13.6, 3.1)	-4.1 (-10.4, 2.2)	-2.5 (-5.5, 0.6)	-0.1 (-1.2, 1.0)	**35.2**** **(10.1, 60.4)**	---
Used a medication[4] vs. not	**-1.8*** **(-3.7, 0.1)**	**11.7**** **(2.0, 21.3)**	**7.4*** **(-0.3, 15.1)**	**3.2**** **(0.0, 6.5)**	0.8 (-0.4, 2.1)	**-22.6**** **(-44.3, -0.8)**	4.0 (-20.2, 28.2)
Unemployed vs. employed	**2.5**** **(1.0, 4.0)**	**-12.8**** **(-20.2, -5.4)**	**-7.7**** **(-13.7, -1.6)**	-2.5 (-5.8, 0.8)	**-1.4**** **(-2.4, -0.4)**	**39.4**** **(4.3, 74.4)**	24.9 (-11.7, 61.6)
Weekend vs. week day	**-0.8**** **(-1.5, -0.2)**	**4.1**** **(0.8, 7.3)**	**2.7*** **(-0.1, 5.5)**	**1.2**** **(0.0, 2.3)**	**0.5*** **(0.0, 0.9)**	**10.5**** **(0.2, 20.1)**	**17.0**** **(5.4, 28.6)**
Daylight hours	**-0.6**** **(-1.2, -0.1)**	**3.2**** **(0.5, 6.0)**	**2.2**** **(0.1, 4.4)**	**0.8*** **(-0.1, 1.8)**	0.2 (-0.2, 0.5)	1.3 (-6.5, 9.1)	-1.5 (-9.8, 6.8)
Menstrual cycle length[5] (day)	**-0.4**** **(-0.7, -0.1)**	**1.8**** **(0.3, 3.3)**	**1.3**** **(0.0, 2.7)**	0.3 (-0.4, 1.0)	**0.2*** **(0.0, 0.4)**	-2.7 (-7.8, 2.3)	-3.0 (-9.3, 3.2)
Body mass index[5] (kg/m^2)	**-0.2*** **(-0.4, 0.0)**	**1.0**** **(0.0, 2.1)**	**0.9**** **(0.1, 1.8)**	0.1 (-0.2, 0.5)	**0.1*** **(0.0, 0.3)**	0.1 (-2.9, 3.1)	1.3 (-2.1, 4.7)
Age (years)[5]	0.1 (-0.05, 0.3)	-0.5 (-1.3, 0.3)	-0.3 (-0.9, 0.3)	-0.1 (-0.3, 0.2)	-0.1 (-0.2, 0.1)	**-2.0**** **(-4.0, 0.0)**	-1.6 (-3.8, 0.6)

[1]All parameters were included simultaneously in the model, adjusting for intervention status, exposure order, measurement period, night, and ovulatory status.
[2]95% confidence interval.
[3]Usual bed/rise times were abstracted from the Pittsburgh Quality Sleep Index; reference group is bed or rise times are within 1 hour of usual.
[4]Any non-sedative, prescription or over-the-counter medication.
[5]Included as a linear variable, such that presented coefficients are the change in sleep parameters for a one-unit increase in the exposure.
*$p \leq 0.10$ **$p \leq 0.05$

Table 10. Adjusted[1] odds ratios for self-reported poor sleep[2] by selected exposures that may affect sleep.

	Odds Ratio (95% CI[3])	p-trend[4] (if applicable)
Unemployed vs. employed	4.2 (0.8, 20.9)*	---
Exercise in past month (min/wk)[5]		
≤ 139	Ref.[6]	
140 – 249	1.6 (0.5, 5.6)	0.86
250 – 359	1.4 (0.3, 6.2)	
≥ 360	0.4 (0.1, 2.4)	
Perceived Stress Scale[5]		
≤ 12	Ref.[6]	
13 – 18	3.1 (1.0, 10.2)*	0.08
19 – 24	4.1 (1.0, 17.5)**	
≥ 25	5.2 (0.9, 32.3)*	
Age (years)		
20 – 24	Ref.[6]	
25 – 29	5.6 (0.9, 32.8)*	0.28
30 – 34	7.7 (1.3, 45.5)**	
35 – 40	1.9 (0.3, 14.4)	
Menstrual cycle length[7] (days)	0.9 (0.7, 1.2)	---
Body mass index[7] (kg/m^2)	1.0 (0.8, 1.1)	---

[1]All parameters were included simultaneously in the model, adjusting for measurement period and ovulatory status.
[2]Poor sleep was defined as having a global score > 5 on the Pittsburgh Sleep Quality Index.
[3]95% confidence interval.
[4]The p-value for a linear trend across categories.
[5]Groups presented are approximate quartiles.
[6]Reference category.
[7]Included as a linear variable, such that presented values are the odds ratio of poor sleep for a one-unit increase in the exposure.
*$p \leq 0.10$ **$p \leq 0.05$

Chapter 3: Association between sleep and sex hormones in premenopausal women with regular menstrual cycles

Background

Sleep may be involved in sex hormone regulation (Czeisler CA, 1999), including luteinizing hormone (LH), follicle stimulating hormone (FSH), estrogens, and pregnanediol-3-glucuronide, a urinary metabolite of progesterone. It is important to understand factors that influence sex hormones, because they are associated with breast cancer risk (Key TJ, 1999), fertility (Findlay JK, 2001; Li H, 2001; Beckers NG, 2002), and migraine headaches (Silberstein SD, 2000) in premenopausal women.

Current evidence suggests that sleep slows the pulse frequency of luteinizing hormone during the follicular (Soules MR, 1985; Rossmanith WG, 1988) and possibly the luteal phases of the menstrual cycle (Filicori M, 1986; Rossmanith WG, 1988; Rossmanith WG, 1991). In healthy women, forced partial sleep deprivation led to increased LH concentrations compared to a normal night (Baumgartner A, 1993); similarly transient awakenings during the night may trigger an LH surge (Filicori M, 1986; Van Cauter E, 1990b). The association of sleep with FSH, estrogens, and pregnanediol-3-glucuronide is less clear. One study of 323 menstrual cycles among 106 healthy women reported that long sleepers ($\geq$ 8 hours) had 20% higher urinary FSH levels than short sleepers ($p=0.008$) during all menstrual phases; however, no associations were found with estrone-3-glucuronide (a urinary metabolite of estradiol) and pregnanediol-3-glucuronide concentrations (Touzet S, 2002). Conversely, forced

partial sleep deprivation was associated with increased estradiol concentrations and no change in FSH concentrations, compared to a normal sleep night (Baumgartner A, 1990; Baumgartner A, 1993).

Sleep may influence sex hormone concentrations by either exerting some type of direct control or enhancing existing circadian rhythms, however, little is known about the actual mechanisms (Czeisler CA, 1999). Gonadatropins, such as LH and FSH, have a pulsatile secrtory pattern (Catt KJ, 1991). Sleep may influence these hormones either by changing the pulse frequency or amplitude, while the transition from sleep to awakening may activate or suppress the pulse generators (Van Cauter E, 1990a; Van Cauter E, 1990b). Alterations in these gonadatropins could ultimately lead to changes in circulating estrogen and progesterone concentrations (Adashi EY, 1991).

The current study examined the association between sleep patterns and nighttime urinary excretion of LH, FSH, conjugated estrogens (estradiol, estrone, and estriol), and pregnanediol-3-glucuronide in premenopausal women during the luteal menstrual phase. Specifically we examined measures of sleep disruption (number of awakenings and number of awakenings ≥3 minutes) and duration (total sleep time). In an exploratory analysis, we considered whether anovulatory cycles were associated with different sleep patterns than ovulatory cycles.

Methods

Subjects

Subjects were identified from participants in a randomized crossover trial investigating the effect of a nighttime magnetic field on melatonin and reproductive hormone levels in premenopausal women. Eligibility criteria included not taking oral contraceptives or other hormones in the past six months, being 20 to 40 years old, a body mass index ≤ 30.0 kg/m^2, having regular menstrual cycles, not pregnant or breast-feeding during the previous year, not a shift worker, and not taking melatonin supplements. Subjects lived in the greater Seattle, Washington area. Between March and September 2001, 85 women from the primary study were asked to participate in an additional component to measure sleep.

Subjects who contacted the study telephone line in response to posted advertisements completed an initial screening interview and, if eligible, were scheduled for a home visit. Written consent, approved by the Institutional Review Board at the Fred Hutchinson Cancer Research Center, was obtained at this visit.

At the first home visit, a technician taught subjects to determine their ovulation date using a menstruation calendar and a commercial ovulation kit (Assure LH Ovulation Predictor, Conception Technologies, San Diego, CA), which detects the LH surge 24 to 48 hours before ovulation. Subjects tracked one complete menstrual cycle, including detecting an LH surge, before proceeding to the intervention phase. After the next two detectable LH surges, the subject was scheduled for the two measurement periods in which the study intervention was applied. Both measurement periods were

five nights long and started two days after the LH surge. Half the women were randomly assigned to the intervention and half to the sham-exposure (ambient) during the first measurement period; exposure status was switched at the second period.

The intervention

The intervention consisted of a continuous, 60-Hz magnetic field, 0.5 to 1.0 μT above the ambient levels, at the subject's normal head location on the bed. The exposure was administered by placing a common, household appliance underneath the bed, which was plugged into a power strip that was in the off or on position. There was no indication of whether it was on or off; thus, subjects were blinded to exposure status. Study technicians were not blinded.

Hormone data

On the last night of the period, subjects collected all urine excreted during the night after sleep onset plus the first morning void. A technician collected the sample the same morning and took it to the Specimen Processing Core Laboratory at the Fred Hutchinson Cancer Research Center where it was processed, aliquotted into ten 2-mL aliquots, which were then stored at -80° Celsius.

Assays were performed at the Reproductive Endocrine Research Laboratory (University of Southern California), directed by Dr. Frank Stanczyk. Urinary conjugated estrone, estradiol, and estriol were measured following hydrolysis, using high-performance liquid chromatography combined with radioimmunoassays (RIAs)

(Gentschein E, 1997; Ursin G, 1999). Samples were acidified and subjected to ß-glucuronidase/aryl sulfatase hydrolysis before the chromatography, which separated estrone, estradiol, and estriol, and subsequent quantification by specific RIAs. LH was determined by the DELFIA LH Spec assay, which is a solid phase, two-site fluoroimmunometric assay based on the direct sandwich technique. FSH was determined by enzyme-linked immunosorbent assay (Qiu Q, 1997; Qiu Q, 1998). This assay is based on the dissociation of the FSH heterodimer, and measures the beta subunit of urinary FSH as a representation of total urinary FSH. Pregnanediol-3-glucuronide was measured in urine by enzyme immunoassay (Munro CJ, 1991). Urinary creatinine was determined using the modified Jaffe method. All hormones were adjusted for creatinine levels.

For quality control, we created a urine pool from subjects who completed one measurement period. Two specimens of the pooled sample were placed into 5 batches of each assay. Laboratory personnel were blinded with regard to subject and QA sample identity. The intra- and inter-assay coefficients of variation were: estrone 12.8% and 18.4%; estradiol 13.4% and 22.1%; estriol 13.2% and 16.7%; LH 5.5% and 14.7%; FSH 5.2% and 9.6%; and pregnanediol-3-glucuronide 5.9% and 7.3%.

Sleep data

Subjects wore an actigraph (Actiwatch-16, Mini Mitter Company, Inc., Bend, OR) on their non-dominant wrist (Chung L, 1995) from bedtime to rising time all nights of both measurement periods. On the first day of the measurement periods, subjects

completed the Pittsburgh Sleep Quality Index questionnaire (Buysse DJ, 1989) and were instructed on the use of the actigraph.

The actigraph is designed for long-term monitoring of gross motor activity in humans and has an accelerometer capable of sensing motion with a minimal resultant force of 0.01g (Actiwatch 16 / Actiwatch 64 / Actiwatch-L / Actiwatch-Score Instruction Manual, 2001). The actigraph collected data about the number of movements made during a one-minute sampling period. When possible, the same actigraph was used at both measurement periods.

Actigraph data were downloaded onto a PC computer, and analyzed using a FORTRAN program with the method of Cole *et al.* (Cole RJ, 1992). Using polysomnographic validation data provided by Mini Mitter Company, Inc. (Oakley NR, 1997; Actiwatch 16 / Actiwatch 64 / Actiwatch-L / Actiwatch-Score Instruction Manual, 2001), we assessed the sleep/wake status of each minute of the night as follows:

$$D = 0.025 * (0.04A_{-2} + 0.20A_{-1} + 1.0A_{0} + 0.20A_{+1} + 0.04A_{+2})$$

where A_x is the number of detectable motions in that minute. If $D \geq 1$ then the subject was considered to be awake during minute A_0, otherwise the subject was considered to be asleep. We applied the 5 rescoring rules and the 20-minute criterion for determining sleep onset outlined by Cole, *et al.* (Cole RJ, 1992).

Other data collection

At both measurement periods, a technician measured height to the nearest 0.1 cm, weight to the nearest pound, and administered a structured interview collecting demographic information, job status, and exercise habits. Subjects also completed a nightly diary, including bedtime, rising time, medication use, and alcohol consumption, each night of the measurement period. Hours of daylight were determined via sunrise/sunset tables calculated by the National Research Council (Herzberg Institute of Astrophysics, Victoria, BC).

Data Analysis

The primary purpose of the analysis was to determine whether total sleep time (linear), the number of awakenings (linear), or the number of awakenings ≥3 minutes (linear) on the fourth night of each measurement period was associated with log-transformed urinary hormone concentrations collected on the last night. We used the fourth night of the measurement period because urinary hormone concentrations track changes in serum concentrations with about a one-day lag time (Munro CJ, 1991; Stanczyk FZ, 1997). Nine anovulatory cycles (pregnanediol-3-glucuronide <1.25 mg/g creatinine) were removed from the primary analyses (Haiman CA, 2002).

We first assessed the correlation between sex hormones using the Spearman correlation coefficient. Then we determined the percent change in hormone concentrations and 95% confidence intervals (95% CI) for a one-unit increase in sleep parameters using linear regression, adjusting for the following *a priori* potential

confounders: exposure status (intervention, ambient), order of exposure (ambient-intervention, intervention-ambient), measurement period (1, 2), alcohol servings on the fourth night (0, 1, 2 or more), body mass index in quartiles (≤ 20, >20 – 23, >23 – 26, >26), average cycle length in days (linear), age in 5 year intervals (20 – 24, 25 – 29, 30 – 34, 35 – 40), and over-the-counter or prescription medication use, including sedatives, on the fourth night (yes, no). We considered adjusting for alcohol consumption and medication use on the fifth night of the measurement period as these could affect the metabolism and excretion of sex hormones in the urine. Since adjustment for these variables did not substantially change effect estimates, they were not included in the final model. Due to the correlated nature of the data, we used generalized estimating equations with an independent working correlation matrix (Zeger SL, 1986).

In an exploratory analysis, we considered whether sleep patterns (total sleep time, sleep efficiency, total wake time, number of awakenings, and number of awakenings ≥3 minutes) differed by ovulatory status (anovulatory vs. ovulatory). Sleep efficiency is the total sleep time divided by the time in bed. We used linear regression, adjusting for exposure, order of exposure, measurement period, night of the measurement period (1, 2, 3, 4, 5), alcohol servings, body mass index (linear), average cycle length, age (linear), job status (yes, no), hours of daylight (linear), usual hours of sleep over the previous month (linear), bedtime (linear), rising time (linear), sedative use (yes, no), and other over-the-counter or prescription medication use (yes, no). In addition, we included all nights of sleep data for this analysis

We were missing sleep data for 81 (14%) of 580 eligible nights, including 22 measurement periods on 20 subjects for the fourth night. Since missing sleep data was associated with order of exposure and some hormone concentrations, we used multiple imputed data described in a previous analysis (See Chapter 1), to avoid having biased results and to estimate the uncertainty caused by the missing data. Briefly, we performed multiple imputation using the regression method, assuming that the data were missing at random (Rubin DB, 1986; Schafer JL, 1999). We imputed 10 data sets by generating a unique linear regression prediction model for each missing data point, chosen at random. Inference about the primary and secondary data analyses was made by running the model on the 10 imputed datasets. Point estimates were determined by fitting the model in all 10 imputed datasets and taking the mean of the resultant point estimates. Standard errors were determined by taking the square root of the sum of the within- and between-imputation variances. All statistical tests were two-sided.

Results

Of the 85 eligible women, 73 (86%) consented and wore the actigraph during at least one measurement period. Hormone data were available for 62 of these subjects. Eight measurement periods were excluded because no actigraph was available (n=2), the subject took emergency contraceptive hormones (n=1), and sleep data was missing for 4 or more nights of the period (n=5). Of the 116 eligible measurement periods, nine anovulatory cycles were excluded, leaving a total of 107 measurement periods from 61 subjects for the primary analyses.

Subjects on average were 31 years old, with a normal body mass index, and an average menstrual cycle length of 28.8 days (Table 11). Subjects slept 7 hours and 12 minutes and had about 23 awakenings of which about 4 were ≥3 minutes. Fifty-four (87%) women completed 2 measurement periods; approximately half of the subjects had the intervention exposure during the first period. Almost a quarter of the subjects took a prescription or over-the-counter medication and 40% consumed alcohol on the fourth night of either measurement period.

Estrone was significantly correlated with estradiol (ρ=0.79), estriol (ρ=0.24), and β-FSH (ρ=0.21) among ovulatory cycles (Table 12). Estradiol was also significantly correlated with estriol (ρ=0.33) and β-FSH (ρ=0.23). Neither LH nor pregnanediol-3-glucuronide was significantly correlated with the other hormones.

We found little association between sleep patterns and urinary concentrations of β-FSH, estradiol, estriol, and pregnanediol-3-glucuronide (Table 13). However, an increase of one awakening was associated with 2.2% higher urinary LH concentrations (95% CI: 0, 4.4). Although not statistically significant, an increase of one awakening ≥3 minutes was modestly associated with 3.9% higher urinary estrone concentrations (95% CI: -1.2, 9.4), and a one-hour increase in total sleep time was modestly associated with 8.9% lower estrone concentrations (95% CI: -18.8, 2.3).

Anovulatory cycles were associated with 2.8% lower sleep efficiency (95% CI: -5.5, -0.1) and 2.0 more awakenings ≥3 minutes (95% CI: 0.1, 4.0), compared to ovulatory cycles (Table 14). Similarly, anovulatory cycles were modestly associated

with 10.4 more minutes of total wake time (95% CI: -0.7, 21.5) and 12.7 fewer minutes of total sleep time (95% CI: -27.8, 2.4), compared to ovulatory cycles. Ovulation status not associated with the number of awakenings.

Discussion

The purpose of this study was to determine whether measures of sleep disruption and duration were associated with urinary hormone concentrations in premenopausal women. We found that sleep outcomes were possibly associated with concentrations of LH and estrone during the luteal phase of the menstrual cycle, and in an exploratory analysis, anovulatory cycles were associated with worse sleep patterns. Our results provide modest support for the hypothesis that sleep patterns are associated with hormonal regulation processes (Czeisler CA, 1999).

As expected, we found that estrone, estradiol, and estriol were highly correlated (Adashi EY, 1991; Catt KJ, 1991). Estrone and estradiol were also modestly correlated with FSH concentrations; this is consistent with the observation that FSH pulses result in up-regulated estrogen production in the ovaries (Adashi EY, 1991). However we did not find a strong correlation between LH and pregnanediol-3-glucuronide, which is inconsistent with the observation that LH pulses stimulate progesterone production during the luteal phase of the menstrual cycle (Adashi EY, 1991). It is possible that the metabolism of progesterone to pregnanediol-3-glucuronide is regulated independently of LH concentrations.

Our results suggest that an increased number of awakenings are associated with elevated LH concentrations. This is consistent with previous studies, which reported that most awakenings during sleep were followed by a serum LH pulse (Filicori M, 1986; Van Cauter E, 1990b), and that forced partial sleep deprivation was associated with increased serum LH concentrations compared to a normal night in healthy, young women (Baumgartner A, 1990; Baumgartner A, 1993). Further, two studies reported that sleep was modestly associated with a slowing of the LH pulse frequency during the luteal menstrual phase, although the results were not statistically significant (Filicori M, 1986; Rossmanith WG, 1988; Rossmanith WG, 1991). This may be due to the already low LH pulse frequency during the luteal phase and the small sample sizes in the studies ($n \leq 25$). Overall, these data suggest that disrupted sleep patterns, especially waking up during the night, may be associated with increased serum and urinary LH concentrations in premenopausal women.

We found that one awakening was associated with a 2.2% increase in LH concentrations. This means, for example, that 5 more awakenings during the night could be associated with as high as an 11% average increase in LH concentrations. Although the exact biological effects of this increase are unclear, LH surges during the luteal phase stimulate both progesterone and androgen production, which could ultimately lead to higher estrogen concentrations (Adashi EY, 1991; Catt KJ, 1991).

There was little evidence to suggest that sleep patterns were associated with urinary β-FSH concentrations. This is consistent with two studies that reported no effect of forced partial sleep deprivation on serum FSH concentrations in both pre- and

postmenopausal women (Baumgartner A, 1990; Baumgartner A, 1993). However, Touzet *et al.* reported that long sleepers (≥8 hours) had significantly higher urinary FSH concentrations than short sleepers during 323 menstrual cycles from 106 women (Touzet S, 2002). These inconsistent results may be explained by the small sample sizes ($n < 21$) in serum FSH studies and the high variability of urinary FSH concentrations in our study, both of which may have resulted in low power to detect an association.

We found very modest associations between total sleep time and awakenings ≥3 minutes with urinary estrone, but not estradiol and estriol concentrations. This is inconsistent with results from Touzet, *et al.*, who reported that long sleep was not associated with urinary estrone-3-glucuronide concentrations (Touzet S, 2002). Also, partial sleep deprivation was associated with a significant increase in serum estradiol concentrations (estrone was not measured) compared to a normal night in 10 healthy women (Baumgartner A, 1990), but not among 21 pre- and post-menopausal women (Baumgartner A, 1993). Neither study controlled for menstrual phase. Inconsistent results between studies, possibly due to small sample sizes or lack of control for menstrual phase, preclude a definitive conclusion about the association between sleep patterns and estrogen concentrations.

We found no association between sleep patterns and pregnanediol-3-glucuronide concentrations in our study. This is consistent with one other study that reported that long and short sleepers had similar urinary concentrations of this hormone (Touzet S, 2002).

Our data show that, compared to ovulatory cycles, anovulatory cycles were associated with lower sleep efficiency, more total wake time, less total sleep time, and more awakenings ≥3 minutes during the luteal phase of the menstrual cycle. These results should be considered preliminary since subjects in our study were specifically selected to have regular menstrual cycles and they reported detecting an LH surge before the measurement period. Our results however are consistent with a study of 34 women that reported that those with anovulatory cycles had significantly more wake time, a longer REM latency, and less REM sleep compared to ovulatory cycles during the luteal menstrual phase (Lee KA, 2000). Further, other data suggest that sleep patterns vary across the menstrual cycle (Parry BL, 1989; Driver HS, 1996). If hormones and sleep patterns are interrelated, it is possible that the differing hormone milieu following an anovulatory vs. ovulatory cycle may be associated with different sleep patterns. Further research in this area may provide important information about the sleep-hormone relationship.

The current study has several strengths. This was the first study to examine objectively measured sleep patterns and hormone associations among women sleeping at home. Previous studies primarily have been laboratory-based, using polysomnography to measure sleep and intravenous catheters to collect serum. These invasive techniques may influence hormone concentrations regardless of the sleep profile. Another study that did allow women to sleep at home extracted sleep duration estimates from a questionnaire item; self-reported usual sleep duration may not be as accurate of a predictor as objectively measured sleep (Kushida CA, 2001). Another

strength was that our subjects had regular menstrual patterns. This may have reduced the hormonal variability compared to previous studies, and increased power to detect an association. However, if disrupted sleep and hormone regulation are interrelated, it is possible that by excluding women with non-regular menstrual cycles we inadvertently excluded women with very poor sleep. Despite this, we did observe a wide range of sleep patterns in our study. Measurement periods were conducted during the same menstrual phase, thus eliminating the possibility that observed associations were due to differing sleep patterns across the menstrual cycle (Parry BL, 1989; Driver HS, 1996).

Our study has several limitations. First, we were only able to measure urinary sex hormone concentrations, even though serum concentrations are of more biological importance. Although they are correlated, urinary concentrations tend to be lower than serum concentrations (Catt KJ, 1991). Also the coefficients of variation were somewhat high, especially for the estrogens, possibly attenuating the observed associations. Second, although actigraphy is an objective measure of sleep patterns, it overestimates total sleep time compared to polysomnography because it cannot detect wake time in which no movement occurs (Cole RJ, 1992; Babin L, 1997; Blood ML, 1997). This overestimation may be larger among those with poor sleep quality (Cole RJ, 1992; Hauri PJ, 1992; Kushida CA, 2001), possibly causing an attenuation of the observed associations. Third, 14% of the nights had at least some missing data, which may limit the generalizability of our results. Rather than exclude these nights, possibly leading to biased results, we used a multiple imputation method that produces valid answers with respect to the uncertainty introduced by the missing data (Schafer JL, 1999; Longford

NT, 2001). Fourth, our study cannot determine whether changes in sleep patterns led to hormone fluctuations or vice versa.

Our results provide some support that sleep patterns and sex hormones are interrelated among premenopausal women not taking oral contraceptives. Of particular interest is the possibility that anovulatory cycles are associated with worse sleep. Since a normal hormonal milieu is necessary for fertility and increased exposure to estrogens can increase risk of breast cancer, it is important to understand factors that affect sex hormone concentrations. Further studies are needed with larger sample sizes and including women with irregular menstrual cycles to elucidate these complex relationships.

Table 11. Characteristics of the premenopausal women in the study (n=62)

	Mean (SD)	Range
Age (years)	31.1 (5.2)	21 – 40
BMI (kg/m^2)	24.0 (3.6)	17.3 – 31.7
Cycle length (days)	28.8 (2.2)	25 – 35
LH[1] (U/g creatinine)	1.9 (3.0)	0.1 – 20.2
β-FSH[2] (μg/g creatinine)	0.37 (0.39)	0.02 – 2.89
Estrone (μg/g creatinine)	12.7 (8.2)	1.1 – 46.5
Estradiol (μg/g creatinine)	5.3 (3.2)	1.4 – 17.9
Estriol (μg/g creatinine)	21.4 (13.9)	2.1 – 67.8
Pregnanediol-3-glucuronide (mg/g creatinine)	5.3 (3.2)	0.5 – 20
Total sleep time (hr)[3,4]	7:12 (1.2)	4:24 – 11:06
Awakenings[3,4]	22.7 (7.2)	6 – 51
Awakenings ≥ 3 min[3,4]	4.1 (3.0)	0 – 15
	n (%)	
Completed both intervention periods	54 (87.1)	
Exposed during first period	29 (46.8)	
Took any medication[3]	15 (24.2)	
Consumed alcohol[3]	25 (40.3)	

[1]Luteinizing hormone
[2]The β subunit of follicle stimulating hormone
[3]On the fourth night of either measurement period
[4]Only includes non-imputed data (n=94 measurement periods)

Table 12. Spearman rank correlation coefficients between overnight urinary hormone concentrations among premenopausal women with ovulatory cycles[1].

	LH[2]	FSH[3]	Estrone	Estradiol	Estriol	Pregnandiol-3-glucuronide
LH (U/g creatinine)	1.00					
β-FSH (μg/g creatinine)	0.11	1.00				
Estrone (μg/g creatinine)	0.15	0.21	1.00			
Estradiol (μg/g creatinine)	0.02	0.23	0.79	1.00		
Estriol (μg/g creatinine)	0.02	0.05	0.24	0.33	1.00	
Pregnanediol-3-glucuronide (mg/g creatinine)	-0.14	-0.09	-0.04	-0.02	-0.06	1.00

[1]Correlations above 0.20 or below –0.20 are significant at the p=0.05 level

[2]Luteinizing hormone

[3]The β subunit of follicle stimulating hormone

Table 13. Percent change[1] in overnight urinary hormone concentrations for a unit increase in total sleep time, the number of awakenings, or the number of awakenings ≥ 3 minutes during the previous night of sleep among ovulatory cycles

	Total Sleep Time (hr)		Awakenings		Awakenings ≥ 3 min	
	Percent change (95% CI)[2]	p	Percent change (95% CI)[2]	p	Percent change (95% CI)[2]	p
LH[3] (U/g creatinine)	-8.0 (-21.4, 7.6)	0.29	2.2 (0.0, 4.4)	0.06	4.2 (-2.3, 11.1)	0.21
β-FSH[4] (μg/g creatinine)	9.4 (-6.5, 28.0)	0.26	1.1 (-1.6, 3.9)	0.44	3.5 (-2.8, 10.2)	0.28
Estrone (μg/g creatinine)	-8.9 (-18.8, 2.3)	0.12	0.8 (-1.1, 2.8)	0.40	3.9 (-1.2, 9.4)	0.13
Estradiol (μg/g creatinine)	-4.1 (-12.0, 4.5)	0.34	0.8 (-0.5, 2.2)	0.23	2.6 (-1.7, 7.1)	0.24
Estriol (μg/g creatinine)	4.6 (-6.6, 17.2)	0.43	0.6 (-1.4, 2.5)	0.59	3.2 (-1.4, 7.9)	0.18
Pregnanediol-3-glucuronide (mg/g creatinine)	-6.2 (-14.8, 3.2)	0.19	-0.3 (-1.7, 1.7)	0.69	0.1 (-3.9, 4.3)	0.95

[1]Adjusted for exposure status, order of exposure, measurement period, alcohol consumption on the 4th night, body mass index, age, average cycle length, any over-the-counter or prescription medication use on the 4th night.
[2]95% Confidence interval
[3]Luteinizing hormone
[4]The β subunit of follicle stimulating hormone

Table 14. Average difference[1] in sleep parameters during the luteal phase of the menstrual cycle comparing anovulatory versus ovulatory cycles.

	Difference in sleep parameter[2] (95% CI[3])	p
Total sleep time (min)	-12.7 (-27.8, 2.4)	0.10
Sleep efficiency (%)	-2.8 (-5.5, -0.1)	0.04
Total wake time (min)	10.4 (-0.7, 21.5)	0.07
Number of awakenings	1.2 (-2.4, 4.9)	0.52
Number of awakenings ≥ 3 min	2.0 (0.1, 4.0)	0.04

[1]Adjusted for exposure, order of exposure, measurement period, night, alcohol servings, body mass index, average cycle length, age, job status, usual hours of sleep over previous month, hours of daylight, bedtime, rising time, sedative use, and other over-the-counter or prescription medication use.
[2]Average difference in sleep parameters comparing anovulatory versus ovulatory cycles.
[3]Confidence interval

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Vita

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