Low estradiol levels in women of reproductive age having low sleep variation

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Higher exposure to light at night, for example, owing to night shift work or decrease in sleep duration, may suppress melatonin production, which in turn may increase the reproductive hormone levels. High levels of steroid hormones, especially estrogens, may be associated with an increase of the breast cancer risk. This study investigated whether variation in the sleep duration during one entire menstrual cycle corresponds to estradiol saliva concentrations in healthy, urban women of reproductive age. During 2000–2001, 95 regularly menstruating women aged 24–36 in Poland collected daily saliva samples for one entire menstrual cycle. Saliva samples were analyzed for concentration of 17-β estradiol (E2) using radio immunoassay. Information on the number of hours of sleep per night (sleep duration) was collected daily by questionnaires for one entire menstrual cycle. Using covariance analysis, after adjustments for sleep duration, we documented a positive relationship between the sleep variation (sleep coefficient of variation) and E2 levels in women of reproductive age. Mean levels of E2 differed significantly in women from the lowest sleep coefficient of variation quartile (13.93 pmol/l) in comparison with other quartiles (22.35 pmol/l), ($P < 0.001$). The low sleep variation group, that is, the women who sleep regularly, had mean E2 levels 60% lower than other groups. These results suggest that sleep variation significantly correlates with E2 levels, whereas sleep duration does not show a statistically significant relationship. This study suggests that sleep variation may influence endogenous estrogens, which is of importance for risk of breast cancer. *European Journal of Cancer Prevention* 17:467–472 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Sleep duration and night shift work are related to many aspects of health, possibly including reproduction and risk of breast cancer in women (Davis *et al.*, 2001; Hansen, 2001; Schernhammer *et al.*, 2001). Higher exposure to light at night per se and through decrease in sleep duration and night shift work may suppress serum melatonin levels, which in turn may increase the reproductive hormone levels (Okatani *et al.*, 2000; Schernhammer *et al.*, 2004). Women exposed to steroid hormones, especially estrogens, may be associated with an increase of the breast cancer risk (Hankinson *et al.*, 1998; Cauley *et al.*, 1999; Jasienska and Thune, 2001; Yu *et al.*, 2003).

Melatonin is a hormone released mainly by the pineal gland, whose synthesis and secretion are stimulated by darkness and inhibited by light (Malpaux *et al.*, 2001). The connection between melatonin production and estradiol (E2) levels has been well established (Yie, 1995a; Okatani *et al.*, 2000; Schernhammer *et al.*, 2004). Seasonal variation in daylight has been shown to relate with ovarian function. When melatonin and ovarian activities were measured in a region with a strong seasonal contrast in luminosity (Kauppila *et al.*, 1987), the daytime 12-h melatonin index and daytime urinary melatonin excretion were significantly higher in the dark season than during the light season, and were accompanied by decreased mean serum E2 concentration at the time of ovulation and during the luteal phase of the cycle, indicating lowered ovarian activity. Seasonal difference in ovarian hormone concentrations was also reported by a study on joggers and runners (Ronkainen *et al.*, 1985). Women had lower levels of E2, progesterone and testosterone in the dark season than in the light season. Similarly, E2 concentrations were significantly lower during autumn and winter months than during light months (264.7 ± 44.1 and 661.8 ± 55.1 nmol/l, respectively) when measured in follicular fluid samples that were obtained from the largest preovulatory follicle of 120 women undergoing in-vitro fertilization (Yie *et al.*, 1995a, b). The melatonin concentration in follicular fluid was also higher during the dark season than during the light season (Ronnberg *et al.*, 1990).

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We investigated whether variation in the sleep duration during one entire menstrual cycle corresponds to E2 (E2) saliva concentrations in healthy urban women of reproductive age.

Materials and methods

Study participants

One hundred and thirty-six Polish urban women between 24 and 35 years of age (mean age: 29.5 years, SD: 3.13) were recruited for the study by advertisements between June 2001 and June 2003. Participants in the study were selected if they met the following criteria: regular menstrual cycles and no fertility problems, no gynecological and chronic disorders (i.e. diabetes, hypo/hyperthyroidism), not taking any hormonal medication or using hormonal contraception during the 6 months before recruitment and not having been pregnant or lactating during the 6 months before recruitment. Mean length of menstrual cycle during which saliva samples were collected was 28.9 days (SD: 3.83, range: 22–39 days).

Of 136 urban women who collected saliva samples for an entire menstrual cycle, 95 participants were included in the main analysis: sleep duration data were not collected in 32 women, and reliable identification of the day of the mid-cycle E2 drop could not be made in nine participants. The research protocol was approved by the Jagiellonian University Bioethical Committee.

Sleep and estradiol measurements

Information on the average number of hours of sleep per night (sleep duration) was collected daily by questionnaires for one entire menstrual cycle. During the same menstrual cycle, women collected daily morning saliva samples. Saliva samples from 20 days (reverse cycle days −5 to −24) of each cycle were analyzed for the concentration of E2 using an I-125-based radio immunoassay kit (#39100, Diagnostic Systems Laboratories, Webster, Texas, USA) with published (Jasienska *et al.*, 2004) modifications to the manufacturer's protocol. The sensitivity of E2 assay is 4 pmol/l. Average intra-assay variability was 9% and interassay variability ranged from 23% for lower (15 pmol/l) to 13% for higher (50 pmol/l) values. Before statistical analyses, cycles were aligned on the basis of identification of the day of the midcycle E2 drop (day 0), which provides a reasonable estimate of the day of ovulation, according to the published methods (Lipson and Ellison, 1996). The mean E2 values from 18 consecutive days of each cycle aligned on day 0 were used in analysis.

Anthropometric measurements, physical activity, dietary and general questionnaires

A detailed description of anthropometric measurements and assessment of physical activity were published previously by Jasienska *et al.* (2004, 2006). Average daily energy intake (kcal/day) was assessed using a 24-h precoded food diary, in which women recorded the type and the portion size of every food item consumed during 24 h on 7 selected days in the menstrual cycle (days: 3–6 and days: 21–23) (Furberg *et al.*, 2005). An album showing portions of products and meals was used by each woman to estimate consumed amounts. From the 24-h food diary, the total energy content of the daily food rations was calculated using the Dieta 2 (version 1.1.) computer software (Institute of Food and Nutrition, Warsaw, Poland).

Information on birth weight, education, reproductive history, past use of hormonal medication and tobacco was collected by a general questionnaire (partly administered by an interviewer and partly self-reported).

Statistical analysis

Variation in sleep duration for each woman individually was calculated as the coefficient of variation in sleep duration (sleep CV). Low sleep CV value means that a similar number of hours of sleep each night was recorded during menstrual cycle, whereas high sleep CV value represents irregularity in circadian rhythm.

Women were divided into quartiles based on the CV. Differences among sleep CV groups in potentially confounding factors, such as age, birth weight, height, energy intake, physical activity, body composition variables (body weight, body mass index and percentage of body fat) and mean duration of daylight for sample collection month were tested in separate one-way analysis of variance (ANOVA) with sleep CV as grouping variable. Potential differences among the study groups in the mean duration of daylight were tested to control for the confounding effect of seasonality on E2 levels. Differences between parous and nulliparous women in sleep CV were tested by Student's *t*-test (sleep CV were logarithmically transformed to correct the skewness of distributions and used as dependent variable in Student's *t*-test).

To test if quartiles of sleep CV differ in sleep duration, we performed ANOVA with group division criterion as one factor and mean sleep duration as the dependent variable, followed by contrasts analysis. An α level of 0.0083 (with the Bonferroni correction) was used to indicate statistical significance. The effect of sleep CV on E2 levels was tested by one-way ANOVA with the same group division criterion as one factor and mean E2 levels as the dependent variable (E2 concentrations were logarithmically transformed to correct the skewness of distributions). As it was noticed that sleep quality is associated with E2 (Hollander *et al.*, 2001) and sleep duration may have an influence on the breast cancer risk (Verkasalo *et al.*, 2005; McElroy *et al.*, 2006), possibly by affecting levels of ovarian hormones, we repeated analysis of covariance with mean sleep duration as the covariate.

Covariance analysis was followed by contrasts analysis with an α level of 0.0125 (with the Bonferroni correction) used to indicate statistical significance. For other analyses, the null hypothesis was rejected at the 0.05 level. Statistical analyses were performed with Statistica (version 7.1) computer software (StatSoft, Poland).

Results

General characteristics of the sample

General characteristics of all study participants and the four groups divided by sleep CV are shown in Table 1. The women from groups with low, moderate, high and very high sleep CV did not differ in age, birth weight, education, energy intake, physical activity, smoking, height, body composition (weight, body fat and BMI), reproductive factors (menarcheal age and length of menstrual cycle) and mean duration of daylight. Similarly, there was no statistically significant difference in sleep CV between parous ($n = 41$) and nulliparous ($n = 62$) women (t-test $= -0.863$, $P = 0.390$). Four groups of women with different sleep CV, however, show significant variation in sleep duration [$F(3,100) = 7.210$, $P < 0.001$]. The group with very high sleep CV had shorter sleep duration than low and moderate sleep CV groups [$F(1,100) = 17.591$, $P < 0.001$; $F(1,100) = 13.294$, $P < 0,001$; respectively], (Fig. 1).

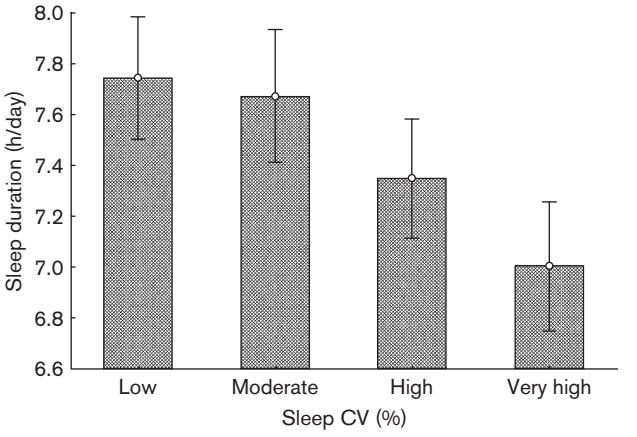
Sleep coefficient of variation and mean sleep duration

Average sleep CV among study participants was 16% (SD: 5.46) and sleep CV ranged from 7.54 to 37.45%. The sleep duration varied from 4.76 to 9.28 h. The average sleep duration was 7.44 h/day (SD: 0.69) and was comparable to the average duration of sleep noted in a representative Polish population sample of 47 924 adults over 15 years of age, which was 7.7 h (7.61–7.73 h) (Kiejna *et al.*, 2004). The majority of participants in our study (63 of 104, or 60.6%) reported sleep duration between 7–8 h/day. Average sleep duration of lesser than or equal to 7 h/day was reported by 24.03% of women, whereas 15.38% of women reported sleeping above 8 h/day.

Estradiol levels and sleep coefficient of variation

One-way ANOVA with mean E2 levels as the dependent variable revealed that four groups of women with different sleep variation differ in mean E2 levels [$F(3,91) = 5.938$, $P < 0.001$]. As sleep duration may also influence the E2 levels, we conducted the covariance analysis stratified by sleep CV with mean sleep duration as the covariate. Variation in E2 levels among the four quartiles of sleep CV remained statistically significant [$F(3,87) = 3.102$, $P < 0.05$]. The impact of sleep duration on mean E2 levels was not significant [$F(1,87) = 2.462$, $P = 0.12$], whereas the interaction between sleep CV and

Fig. 1



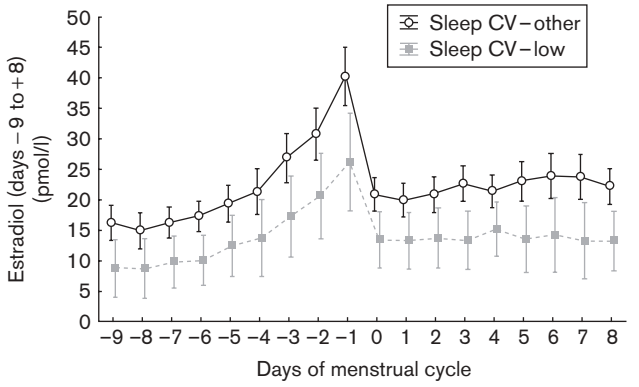
Mean (with 95% confidence interval) sleep duration (h/day) in groups of women with low, moderate, high and very high sleep duration.

Table 1 Characteristics of all study participants and of four groups differing in sleep CV

	All women		Low sleep CV group $N=25$		Moderate sleep CV group $N=22$		High sleep CV group $N=25$		Very high sleep CV group $N=23$		Significance P for trend	F
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Age (years)	29.48	3.131	31.04	3.180	28.95	3.373	30.17	3.036	29.48	3.016	0.113	$F(3,99)=2.045$
Birth weight (g)	3320.02	627.809	3246.67	534.353	3181.68	807.492	3391.11	512.305	3323.33	755.552	0.723	$F(3,81)=0.443$
Education total (years)[a]	16.60	2.720	16.57	2.701	16.89	3.266	16.21	2.516	16.32	2.850	0.928	$F(3,98)=0.150$
Energy intake (kcal)[a]	1937.25	508.472	1985.77	566.829	1994.03	575.674	1809.90	403.033	2004.09	312.444	0.317	$F(3,97)=1.190$
Physical activity (MET hour/day)[a]	30.56	8.185	28.71	5.016	31.34	8.406	31.55	9.877	30.72	8.719	0.671	$F(3,100)=0.520$
Body height (cm)[a]	164.24	6.07	163.14	5.671	163.85	5.664	164.71	6.057	165.22	6.939	0.629	$F(3,100)=1.000$
Body weight (kg)[a]	60.06	8.867	57.69	7.500	60.72	9.171	61.91	8.694	59.94	10.019	0.347	$F(3,99)=1.110$
Body fat (%)	25.78	6.822	24.87	6.408	25.99	7.374	26.94	6.34	25.26	7.458	0.703	$F(3,98)=0.471$
Body mass index (kg/m^2)[a]	22.18	2.885	21.71	2.938	22.61	3.239	22.78	2.67	21.93	3.110	0.428	$F(3,100)=0.930$
Age at menarche (years)[a]	13.30	1.384	13.24	1.422	13.61	1.447	13.22	1.265	13.44	1.502	0.742	$F(3,97)=0.420$
Length of menstrual cycle during sample collection (days)[a]	28.88	3.831	27.41	3.153	29.30	3.878	28.59	4.247	29.84	3.496	0.098	$F(2,101)=0.240$
Mean duration of daylight (% of 24 h)	0.56	0.058	0.56	0.050	0.57	0.050	0.55	0.063	0.56	0.068	0.870	$F(3,98)=0.238$
Sleep CV (%)	16.00	5.460	10.09	1.358	13.78	0.775	16.81	1.333	23.44	4.588	<0.001	$F(3,100)=132.775$
Sleep duration (h/day)	7.44	0.689	7.74	0.497	7.67	0.671	7.35	0.610	7.00	0.749	<0.001	$F(3,100)=7.210$
Mean E2 (from −9 to +8) (pmol/l)[a]	20.10	11.318	13.93	6.626	25.11	10.617	21.92	11.639	20.03	13.111	<0.001	$F(3,91)=5.938$

CV, coefficient of variation; MET, the metabolic equivalent.
[a]Variable log-transformed and used as dependent variable in one-way Anova.

Fig. 2



Mean (with 95% confidence interval) profile of estradiol for group of women with low sleep variation and other (moderate, high and very high) groups combined.

sleep duration approached borderline significance [$F(3,87) = 2.582$, P for interaction $= 0.059$]. Contrast analysis indicated that the group of women characterized by low sleep variation had significantly lower E2 levels [$F(1,87) = 15.970$, $P < 0.001$] than all other groups of women (Fig. 2).

Discussion

We documented, after adjustments for sleep duration, a positive relationship between the sleep variation and E2 levels in women of reproductive age. Mean levels of E2 differed significantly in women from the lowest sleep CV quartile in comparison to other quartiles. The low sleep variation group, that is, the women who sleep regularly, had mean E2 levels 60% lower than other groups. We also noticed that the low sleep variation group slept 10.6% longer than the very high variation group (7.7 vs. 7 h/day).

These results suggest that sleep duration is not as important as variation in sleep duration. Irregularity in sleep duration is associated with an increase in the E2 levels. According to the breast cancer development hypothesis, increasing the lifetime exposure to endogenous estrogens could result in higher risk of breast cancer.

Furthermore, there is widespread agreement for the idea of circadian disruption associated with artificial lighting as the risk factor of breast cancer incidence in the industrialized world (Stevens, 2006). Observational studies on shift workers having elevated breast cancer risk (Davis *et al.*, 2001; Hansen, 2001; Schernhammer *et al.*, 2001) suggest that not only sleep duration, but also sleep variation may play a role in breast cancer etiology. The association between variation in sleep duration and E2 levels has, however, not been previously documented. One study documented the association of lower E2 levels with poor sleep (Hollander *et al.*, 2001). Poor sleep quality among women aged 45–49 years (late reproductive age) may not be comparable with sleep irregularity of women aged 24–36 years, and answers from a self-reported questionnaire used to describe insomnia may not correspond to the sleep CV.

Besides ovarian steroids, we did not measure other hormones in this study, hence we were not able to test the mechanisms responsible for the link between sleep CV and E2 concentrations. Melatonin secretion, which decreases when people are exposed to light at night (Lewy *et al.*, 1980) may, however, play a role in the observed relationship. Switching human volunteers from 8 to 14-h night results in longer duration of nocturnal melatonin secretion (Wehr, 1991), but it is not known whether melatonin production is dependent on individual sleep variation.

Other mechanisms may also be responsible for the observed relationship between sleep and E2 concentrations. Short sleep duration was found to be associated with the reduction of leptin and elevation of ghrelin (Taheri *et al.*, 2004), which likely increase appetite. Leptin, besides its role in body weight (body fat stores) regulation, may be considered an endocrine mediator. Leptin itself exerts effects on different endocrine axes, mainly on the hypothalamic–pituitary–gonadal axis (Wauters *et al.*, 2000). Both in-vitro and in-vivo experimental evidence indicates that a link between leptin and ovarian steroids exists, but the mechanisms have not been clearly understood. Nonetheless, it was found that in normal weight premenopausal women, serum leptin concentrations positively correlated with E2 and were higher in the luteal than in the follicular phase, with a significant preovulatory peak (Mannucci *et al.*, 1998; Cella *et al.*, 2000).

Moreover, sleep disturbances may lead to other metabolic and hormonal changes, such as impairment in glucose tolerance (Spiegel *et al.*, 1999). Insulin sensitivity is closely linked to the serum levels of sex steroids. Women with impairment in glucose tolerance or type 2 diabetes had significantly higher total and bioavailable E2 levels than those with normal glucose tolerance (Goodman-Gruen and Barrett-Connor, 2000).

Furthermore, the sleep-debt condition in comparison to the fully rested condition contributes to a disruption (diminishing) of thyrotropin concentrations (Spiegel *et al.*, 1999). It was noticed that thyroid-stimulating hormone was lower in fertile than in infertile patients and low thyroid-stimulating hormone concentrations negatively correlated with E2 in the luteal phase (Gerhard

et al., 1991). In addition, thyroid hormones were found in human follicular fluid and thyroid hormone receptors in human granulosa cells (Wakim *et al.*, 1993), suggesting that they might participate in direct regulation of reproductive function in women. It is well known that thyroid disorders may be connected with menstrual abnormalities (Krassas, 2000) and reproductive failure such as infertility, pregnancy wastage and stillbirths (Joshi *et al.*, 1993).

The results of our study suggest that lower levels of E2 in women who sleep regularly may be one of the mechanisms responsible for the observed relationship between night shift work and breast cancer in women. Therefore, sleep variation may represent one of the modifiable risk factors for breast cancer. It may be possible to lower the individual risk of this disease by maintaining a more regular circadian rhythm pattern, which may lead to lower lifetime levels of E2 and thus reduce risk of breast cancer.

The strength of our study is that E2 levels were measured in saliva samples collected daily for one entire menstrual cycle by each participant, together with information on number of sleeping hours per night. That methodology allows for the precise and reliable assessment of steroid levels (Jasienska and Jasienski, 2007) and fluctuations in sleep duration.

As this is the first study that has shown a positive association between sleep variation and E2 levels in healthy women of reproductive age, after controlling for sleep duration, further studies are needed to confirm these findings. Additional basic science research is also required to identify and determine the mechanisms between sleep irregularity and E2 levels.

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