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Sleep duration, melatonin and breast cancer among Chinese women in Singapore

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Background: **Sleep duration has been hypothesized to be inversely associated with breast cancer risk, possibly due to greater overall melatonin production in longer sleepers. However, data are inconclusive from the three studies conducted in Western populations on sleep duration and breast cancer risk.** ***Methods:*** **We investigated the relationship between self-reported usual sleep duration determined at baseline and subsequent risk of breast cancer in the prospective, population-based cohort of the Singapore Chinese Health Study. We excluded from the study women with <2 years of follow-up due to possible change in sleep pattern among breast cancer cases close to the time of diagnosis. Five hundred and twenty-five incident cases of breast cancer were identified among the remaining 33 528 women after up to 11 years of follow-up.** ***Results:*** **Among women postmenopausal at baseline, breast cancer risk decreased with increasing sleep duration (P trend = 0.047); those who reported 9+ h of sleep showed a relative risk of 0.67 (95% confidence interval = 0.4–1.1) compared with women who reported ≤6 h of sleep. This inverse association was observed primarily in lean women [i.e. body mass index below the median value (23.2 kg/m^2)] (P = 0.024). In this study population, irrespective of gender, urinary 6-sulfatoxymelatonin levels increased with increasing self-reported hours of sleep (P trend = 0.035) after adjustment for age and time of day of urine collection. Melatonin levels were 42% higher in those with 9+ versus those with ≤6 h of sleep.** ***Conclusion:*** **Sleep duration may influence breast cancer risk, possibly via its effect on melatonin levels.**

Introduction

Melatonin is synthesized and secreted by the pineal gland in the brain and plays a key role in controlling the body's circadian rhythm (1). Darkness stimulates the release of melatonin and light suppresses its activity. Normal melatonin cycles are disrupted when we are exposed to excessive light in the evening. According to the circadian disruption hypothesis, factors in the environment (e.g. light at night) that might disrupt the endogenous circadian rhythm and that suppress nocturnal melatonin production may increase the risk of breast cancer. The favored hypothesis is that melatonin influences breast cancer risk via its effect on estrogen signaling (2).

Epidemiologic studies have examined the role of circadian disruption and breast cancer risk using both direct measures (urinary melatonin levels) and indirect measures including sleep duration and shift work. Melatonin production may be closely related to sleep duration while night shift work is expected to disrupt sleep pattern and thus decrease melatonin levels (3,4). To date, epidemiologic studies with direct and indirect measures of melatonin have been conducted exclusively in Western populations and the results are suggestive (5,6). Breast cancer risk was significantly and inversely associated with urinary melatonin levels (6-sulfatoxymelatonin) in the Nurses' Health Study II (7) but not in the United Kingdom Guernsey Cohort (8). Breast cancer risk was significantly reduced in association with long sleep duration in Finnish women (9) but not in USA women (10,11). Results from three cohort studies (12–14) and two (15,16) of three case–control studies (15–17) also show higher breast cancer risk in women who work evening or overnight shifts.

Little is known about the impact of melatonin and circadian disruption on risk of breast cancer in Asians and other non-whites. Over the past four decades, Singapore has transitioned from a mainly agricultural society to a highly industrialized metropolis accompanied by significant changes in many aspects of lifestyles. In a recent global survey (http://www2.acnielsen.com/news/20050228_ap.shtml), seven of the top 10 globally ranked night-owl nations were Asian countries, and Singapore was one of these seven nations. Over half of the Singaporeans reported going to bed after midnight. During the same time period, breast cancer incidence has more than doubled in Singapore (18). While the focus of previous studies has been to study the impact of westernization on menstrual and reproductive factors and dietary habits in explaining the increasing incidence trends of breast cancer in Singapore, this investigation represents the first study to also examine the effects of sleep patterns and breast cancer risk in this transitioning population. Exposure to light at night leading to consequent changes in endogenous melatonin/estrogen levels may be highly relevant to the increasing breast cancer incidence in Singapore and other Asian countries.

Materials and methods

The study design and subject recruitment of the Singapore Chinese Health Study have been described (19). Briefly, 35 303 Chinese women and 27 954 Chinese men aged 45–74 years belonging to the Hokkien or Cantonese dialect group were enrolled in the population-based cohort study between April 1993 and December 1998. To date, <1% (n = 17) of cohort members are lost to follow-up (i.e. migration out of Singapore). At recruitment, information on lifestyle factors, usual diet and reproductive history (for women only) was obtained through in-person interviews. In addition, subjects were asked as part of the baseline questionnaire the average number of hours of sleep in a 24 h period. Possible response categories were ≤5, 6, 7, 8, 9 or 10+ h/day.

Information on habitual sleep duration was available on 35 303 women participants. We excluded participants with a history of cancer (except non-melanoma skin cancer) (n = 1275) at baseline. We also excluded 500 women with <2 years of follow-up due to concern that sleep duration may change close to the time of cancer diagnosis in our breast cancer cases. We identified incident breast cancer cases through the population-based cancer registry in Singapore. This nationwide cancer registry has been in place since 1968 and has been shown to be comprehensive and complete in its recording of cancer cases (20). As of 31 December 2005, 525 cases of incident breast cancer had developed among the remaining 33 528 female cohort subjects following an accumulation of 270 627 person-years. We use the tumor-node-metastases staging as defined by the American Joint Committee on Cancer Staging System for breast cancer (21). Histological and staging information on all breast cancer diagnoses were confirmed by manual review of the pathology reports and clinical charts.

Peripheral blood or buccal cells and a randomly timed single-void urine specimen were collected from ~31 000 (roughly 50%) of cohort participants. The protocol for biospecimen collection and processing had been described (22). In this study, we used the first 498 subjects (220 men and 278 women) recruited to the biospecimen subcohort to investigate the relationship between duration of sleep and urinary melatonin levels (23). Urinary 6-sulfatoxymelatonin was measured by competitive enzyme-linked immunosorbent assay with a capture antibody technique, using reagents obtained from Buhlmann (Basel, Switzerland). The assay was carried out on a microtiter plate coated with a polyclonal antibody specific for rabbit immunoglobulin, following a 1:200 dilution of each urine sample. The 6-sulfatoxymelatonin enzyme-linked immunosorbent assay has been shown to be reliable. The intra-assay and interassay coefficient of variations were 7.1 and 11.9%, respectively. Urinary creatinine (Cr) was measured colorimetrically using a standard procedure. Levels of urinary 6-sulfatoxymelatonin was expressed as μg/g Cr.

Abbreviations: BMI, body mass index; Cr, creatinine; RR, relative risk.

Statistical analysis

We calculated person-years from the baseline questionnaire to the date of breast cancer diagnosis, death or 31 December 2005, whichever was sooner. Cox proportional hazards regression methods were used to examine the association between category of sleep duration (≤6, 7, 8 and 9+ h/day) and breast cancer risk, measured by relative risks (RRs) and their corresponding 95% confidence intervals in all subjects and among women who were postmenopausal at baseline. Relevant demographic factors including age (years) at recruitment, year of recruitment (1993–1998), dialect group (Cantonese and Hokkien), education (no formal education, primary school, secondary school or higher) and established risk factors for breast cancer including age when period became regular (<12, 13–14, 15–16, 17+ years or period never became regular), number of live births (none, 1–2, 3–4 and 5+), body mass index (BMI) (continuous, kg/m^2) and menopausal status were adjusted for in the analysis (model A). In addition, factors including tobacco smoking (never and ever), alcohol intake (non-drinker, monthly, weekly and daily) and history of diabetes appear to influence sleep duration and therefore, they also were adjusted for in the analysis (model B). Results are similar using the basic model (model A) or the more elaborate model (model B) and we show the results based on the more elaborate model.

Body size (BMI) is an established risk factor for breast cancer in postmenopausal women, with endogenous estrogen being the hypothesized etiologic underpinning of this anthropometry–risk association (estrogen synthesis takes place in fat cells in a postmenopausal woman) (24). Given that melatonin may affect breast cancer development via an estrogen-driven pathway, we examined whether BMI modified the observed sleep duration–breast cancer risk association in postmenopausal women in the Singapore Cohort (Table IV). To examine the potential effect modification of the sleep duration–breast cancer association by body size, product terms between BMI and sleep duration were tested. We further investigated the influence of sleep duration on breast cancer risk among postmenopausal women without a history of diabetes. This analysis is motivated by the accumulating evidence that history of diabetes increases risk of breast cancer and this may be mediated, in part, via its effects on sex hormones, in particular sex hormone-binding globulin and bioavailable androgen and estrogen (25,26). *P* values <5% are considered statistically significant and all *P* values quoted are two sided.

The distribution of urinary 6-sulfatoxymelatonin levels in our study population was markedly skewed; therefore, formal statistical testing was performed on logarithmically transformed values and geometric (as opposed to arithmetic) mean values are presented. We first investigated whether 6-sulfatoxymelatonin levels varied by time of day of the urine collection, age and gender. Given that all three factors independently affected 6-sulfatoxymelatonin, they were included as adjustment covariates in the analysis of covariance tests used to examine the relationship between 6-sulfatoxymelatonin and self-reported hours of usual sleep. Further inclusion of BMI as adjustment covariate in the analysis of covariance tests yielded similar results.

Results

Table I shows selected demographic and lifestyle characteristics of women in each of the sleep duration categories (≤6, 7, 8 and 9+ h/day). Some factors (age, dialect group and BMI) appeared to have an U-shaped distribution with respect to sleep duration; both short (≤6 h) and long (9+ h) sleepers were more likely to be older, Cantonese and were less likely to have below-median BMI. The prevalence of nulliparity, early age at first birth, tobacco smoking, alcohol drinking, history of diabetes and the premenopausal status tended to be higher among women who reported 9+ h of sleep. Age at menarche, physical activity and use of menopausal hormones were unrelated to duration of sleep.

We found a trend of decreasing breast cancer risk with increasing sleep duration in all subjects; the results were statistically significant in postmenopausal women (*P* trend = 0.047). Postmenopausal women who reported 9+ h of sleep showed a RR of 0.67 (95% confidence interval = 0.4–1.1) compared with women who reported ≤6 h of sleep (Table II). Increasing duration of sleep was not inversely associated with breast cancer risk in premenopausal women (*P* trend = 0.48). However, these differences in RRs by menopausal status were not statistically significant (*P* interaction = 0.10).

Table III shows the relationship between urinary 6-sulfatoxymelatonin and time of urine collection in the 498 cohort subjects. Age, gender and timing of urine collection independently influenced urinary 6-sulfatoxymelatonin levels. Irrespective of gender, melatonin levels decreased with increasing age (*P* trend = 0.04) (data not shown). The mean level in men aged 44–49 years was 2.50 μg/g Cr, whereas levels were 1.81 μg/g Cr in those aged 60+ years. The comparable figures for women were 1.65 and 1.28 μg/g Cr, respectively. After adjusting for age and time of urine collection, men exhibited significantly higher mean 6-sulfatoxymelatonin levels (1.97 μg/g Cr) than women (1.61 μg/g Cr) (*P* = 0.025). All spot urine specimens were collected between 8 a.m. and 4 p.m. on any given day. After adjustment for age and gender, there was a monotonic decrease in levels of 6-sulfatoxymelatonin with increasing time interval between early morning (8 a.m.) and time of urine collection. The trend was highly significant (*P* trend < 0.0001) (Table III).

Table I. Mean age and age-adjusted baseline characteristics by sleep duration

	Sleep duration (h)			
	≤6	7	8	9+
Number of women	11 370	11 039	8835	2284
Mean age (year)	57.0	55.5	55.5	57.1
% secondary school education	19.7	20.9	22.1	21.1
% Cantonese	48.8	46.9	47.6	48.1
% ≤12 age at menarche	14.6	13.8	14.3	15.9
% nulliparous	6.5	6.9	7.7	7.3
% first live birth ≤ age 20 years	19.2	18.6	19.3	22.2
% below-median BMI	53.2	54.4	54.2	52.5
% diabetic	8.6	8.0	9.5	11.9
% postmenopausal	72.8	71.8	70.0	69.3
% naturally menopausal	63.1	63.3	61.0	59.7
% ever smoked	8.4	8.5	8.4	11.2
% weekly alcohol	9.3	8.5	9.4	10.3
% no weekly moderate, vigorous or strenuous physical activity	75.6	75.0	74.7	75.0
% ever use menopausal hormones	5.9	5.0	5.6	6.3

More significantly, melatonin levels increased with increasing duration of sleep in both men and women after adjustment for age and time of specimen collection (Table III). Longer hours of sleep were associated with higher levels of urinary melatonin, regardless of gender; the results for men and women were not statistically different from each other (*P* = 0.51). In either gender group, those reporting 9+ h of sleep showed a mean level of urinary melatonin that was 42% higher than their counterparts with ≤6 h of sleep. Results were essentially unchanged when we further adjusted for BMI in the analysis (data not shown).

Table IV shows that the sleep duration effect on breast cancer risk reduction was twice as strong in lean (below-median BMI value for all women in the cohort) versus those with higher BMI. Relative to lean women reporting ≤6 h of sleep per night, lean women with 9+ h of sleep experienced a 50% reduction in risk (*P* for trend = 0.02), whereas their heavier counterparts showed only a 15%, statistically non-significant reduction in risk (*P* for trend = 0.61) (Table IV, top). The inverse association between sleep duration and risk was even stronger when we further restricted the analysis to women without a history of diabetes. Among postmenopausal women who were lean and without a history of diabetes, the RR for those reporting 9+ versus ≤6 h of sleep was 0.36 (95% confidence interval = 0.13–0.99) (based on 4 women with breast cancers in the 9+ h category versus 63 women with breast cancers in the ≤6 h of sleep category) (data not shown).

Interestingly, the positive association between urinary 6-sulfatoxymelatonin levels and sleep duration also was modified by BMI. Lean (below-median BMI) women with longer (9+) hours of sleep showed >2-fold higher levels of urinary melatonin than those with ≤6 h of sleep (*P* trend = 0.005). In contrast, there was little difference in melatonin levels by duration of sleep among women with above-median BMI (*P* for interaction = 0.075) (Table IV, bottom).

Table II. Breast cancer risk (95% confidence intervals) by sleep hours in all subjects and by menopausal status, excluding subjects with <2 years of follow-up

Sleep hours	All subjects		Premenopausal at baseline		Postmenopausal at baseline	
	Number of cases/ Person-year	RR (95% CI)[a]	Number of cases/ Person-year	RR (95% CI)[a]	Number of cases/ Person-year	RR (95% CI)[a]
≤6	179/90 504	1.00	38/21 681	1.00	141/68 823	1.00
7	186/90 445	1.03 (0.8–1.3)	65/27 495	1.33 (0.9–2.0)	121/62 912	0.94 (0.7–1.2)
8	131/71 814	0.90 (0.7–1.1)	49/23 251	1.19 (0.8–1.8)	82/48 553	0.81 (0.6–1.1)
9+	29/17 865	0.81 (0.6–1.2)	11/5034	1.25 (0.6–2.5)	18/12 821	0.67 (0.4–1.1)
P trend		0.20		0.48		0.047

CI, confidence interval.
[a]Adjusted for age at recruitment, year of recruitment, education, dialect group, age when period became regular, parity, tobacco smoking, alcohol intake, BMI, history of diabetes and menopausal status for the analysis in all subjects (see Materials and methods for details).

Discussion

In this prospective investigation of sleep duration and breast cancer among Chinese women in Singapore, we found a significant decline in postmenopausal breast cancer risk with increasing self-reported hours of sleep. The inverse association was noted mainly in lean (less than or equal to the median BMI of 23.2) women, especially those free of a history of diabetes. It is noteworthy that in the same study population, we observed a significant trend of increasing urinary melatonin levels with increasing sleep duration. Thus, our data are compatible with the notion that modifications in melatonin as a result of variation in sleep duration may play a role in breast cancer development.

Our results show an inverse association between duration of sleep and breast cancer risk in postmenopausal women but there is no evidence that sleep duration is associated with premenopausal breast cancer risk. However, the lower age limit of this cohort study is 45 years and only 26% of the female cohort participants and 140 incident cases of breast cancer were between the ages of 45–50 years. Therefore, the present study cannot meaningfully address the question of whether menopausal status modifies the association between sleep duration. Our overall results on sleep duration and breast cancer risk are compatible with findings from a Finnish cohort study (9). In the Finnish study, sleep duration reported in 1975 was used to predict breast cancer from 1976 to 1996. Compared with those with 7–8 h of sleep, women who reported 9+ h showed a 31% risk reduction that was not statistically significant. A significant risk reduction (RR of 0.28) associated with 9+ h of sleep was reported in an analysis that was restricted to women who reported the same sleep duration in both 1975 and 1981. It is of interest that average BMI was 22.7 for women in the Finnish study, not unlike the average BMI of women in the Singapore Chinese Health Study. In contrast, our results differed from those reported in two studies conducted in the USA (10,11); the average BMI of participants in these two studies was ∼25.0. One was a large population-based case–control study conducted in Massachusetts, New Hampshire and Wisconsin; sleep duration was positively associated with breast cancer risk. Relative to women whose average lifetime sleep hours per night were 7.0–7.9 h, breast cancer risk was below unity for women who reported sleeping <7 h per night and was above unity for those who reported sleeping 8+ h per night (10). In the Nurses' Health Study, sleep duration reported in 1986 was not associated with breast cancer risk (11). Compared with women sleeping 7 h/day, the risk for those sleeping ≤5, 6, 8 and 9+ h were 0.93, 0.98, 1.05 and 0.95, respectively. BMI was adjusted for in both USA studies but it is not known whether results on sleep duration and risk were modified by body size.

Previous studies have found that night/shift workers tend to excrete less melatonin in urine than day workers (27); this difference in melatonin levels has been hypothesized to explain, in part, the higher breast cancer risk found in studies of female night/shift workers and flight attendants (6). Sleep pattern probably influences breast cancer risk via the same melatonin-mediated pathway. In the present study, urinary melatonin levels exhibited a statistically significant, dose-dependent positive association with self-reported number of hours of sleep. Postmenopausal women reporting 9+ h of sleep possessed urinary melatonin levels that were 42% higher than those reporting ≤6 h of sleep (Table III); this association was apparent primarily in leaner women whose BMI was below the median (Table IV). In a cross-sectional analysis conducted in the Nurses' Health Study, urinary melatonin levels were 52% higher among women with 9+ h of sleep compared with those with ≤5 h of sleep after adjusting for BMI and other covariates but it is not known whether body weight modified this association (28).

There is accumulating evidence that the impact of menopausal hormones, history of diabetes and other hormone-related breast cancer risk factors may be modified by body size and that leaner women may be most affected (25,29,30). The inverse association between sleep duration and postmenopausal breast cancer risk was most

Table III. Geometric means (μg/g Cr) of urinary 6-sulfatoxymelatonin by time of specimen collection and by sleep duration

	Urinary 6-sulfatoxymelatonin (μg/g Cr)		
	Number of subjects	Mean	95% CI
Time of specimen collection[a]			
8:00–8:59	11	5.22	2.76–9.85
9:00–9:59	172	2.20	1.88–2.59
10:00–10:59	164	1.77	1.51–2.09
11:00–11:59	83	1.52	1.21–1.92
12:00–12:59	32	1.20	0.83–1.74
13:00–16:00	36	0.81	0.57–1.15
P trend			<0.0001
Hours of sleep—men[a]			
≤6	75	1.69	1.34–2.14
7	75	2.18	1.73–2.75
8	53	2.00	1.51–2.63
9+	17	2.41	1.48–3.92
Hours of sleep—women[a]			
≤6	93	1.37	1.09–1.72
7	99	1.66	1.34–2.06
8	74	1.82	1.41–2.34
9+	12	1.95	1.04–3.65
Hours of sleep—men and women combined[a]			
≤6	168	1.50	1.27–1.76
7	174	1.90	1.62–2.22
8	127	1.88	1.56–2.27
9+	29	2.12	1.44–3.14
P trend			0.035

CI, confidence interval.
[a]Adjusted for age, gender (if applicable) and time of specimen collection.

Table IV. Association between sleep hours and breast cancer risk (95% confidence intervals) and urinary 6-sulfatoxymelatonin levels (μg/g Cr) by BMI in postmenopausal women

Sleep hours	Below-median BMI (≤23.2)			Above-median BMI (>23.2)		
	Person-year	Number of cases	RR (95% CI)[a]	Person-year	Number of cases	RR (95% CI)[a]
≤6	35 933	70	1.00	32 889	71	1.00
7	33 309	62	0.96 (0.7–1.4)	29 603	59	0.93 (0.7–1.3)
8	25 533	35	0.70 (0.5–1.1)	23 021	47	0.94 (0.7–1.4)
9+	6452	6	0.47 (0.2–1.1)	6368	12	0.85 (0.5–1.6)
P trend			0.02			0.61
P interaction = 0.17						
	Number of subjects	Mean[b] (μg/g Cr)	95% CI	Number of subjects	Mean[b] (μg/g Cr)	95% CI
≤6	42	1.48	1.09–2.02	51	1.30	0.93–1.82
7	49	1.94	1.46–2.58	50	1.45	1.04–2.02
8	40	2.60	1.89–3.57	34	1.14	0.76–1.72
9+	6	3.23	1.41–7.39	6	1.21	0.46–3.17
P trend			0.005			0.66
P interaction = 0.075						

CI, confidence interval.
[a]Adjusted for age at recruitment, year of recruitment, education, dialect group, age when period became regular, parity, tobacco smoking, alcohol intake, BMI, history of diabetes and menopausal status for the analysis in all subjects.
[b]Adjusted for age and time of specimen collection.

evident in lean women who were free of a history of diabetes; this is compatible with the hypothesis that melatonin's protective effect on the breast may be mediated through an estrogen-related pathway (2,31,32). High circulating estrogen is a recognized risk factor for breast cancer (24) and high melatonin levels have been linked to low circulating estrogen in women (33), although these results are not all consistent (28). Leaner, and especially non-diabetic, postmenopausal women are known to possess lower levels of circulating estrogen and lower risk for breast cancer than their heavier counterparts (24,25). Therefore, if the melatonin–breast cancer link is at least partly tied to the estrogen–breast cancer pathway and the quantitative estrogen–risk relationship is a non-linear one, then one would predict that any observed melatonin–breast cancer association would be most apparent among women with the lowest levels of endogenous estrogen.

Results from our cross-sectional study on urinary melatonin levels in Singapore Chinese add to the sparse data in Asian populations, particularly from population-based samples. Studies conducted in different populations may be informative because of the differences in baseline estrogen profiles, body size and other lifestyle habits and these factors may influence melatonin levels. Consistent with many previous studies conducted in primarily Western populations (34), we observed a significant decline in melatonin levels with increasing age. We also noted that independent of age and time of urine collection, Singapore Chinese men possessed 22% higher levels of urinary melatonin than women. Overall, the literature on melatonin levels between men and women, based mainly on Caucasian data, does not suggest any consistent differences by gender (34). In the present study, the higher levels noted in men are consistently observed in each age group and each category of specimen collection time between 8 a.m. and 4 p.m.. It is unlikely that this observed gender effect is an artifact. We believe the consistent positive association between melatonin levels and sleep hours in both men and women strengthens the overall observation and demonstrates the utility of this single measurement of melatonin using a spot urine specimen.

Strengths of this study include its prospective design that minimizes recall bias of sleep duration. Information on relevant covariates allows us to adjust for important potential confounders during statistical analysis of study data. Finally, demonstration of a significant positive association between sleep hours and melatonin levels in our study population, in both men and women, provides a biologically sound explanation for our observed sleep duration–breast cancer risk association. A major limitation of the present study is the lack of information on sleep pattern at multiple time points and about lighting conditions during sleep, leading to non-differential misclassification of exposure status in study subjects. Since it is established that such non-differential errors tend to result in a diminution of RR estimates (35), our observed risk reduction in breast cancer risk associated with longer hours of sleep may be an underestimation of the underlying association. We did not have information on work history of rotating shift work. To our knowledge, this is the first study in an Asian population that examines the role of sleep duration–melatonin and breast cancer risk. Our findings provide further indirect support that circadian disruption may be etiologically related to breast cancer and may contribute to the rising incidence of breast cancer in newly affluent societies throughout Asia (18,36). This study represents the strongest evidence to date of an 'environmental' link to the rapidly increasing breast cancer incidence in Singapore and elsewhere in Asia.

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References

1. Brzezinski,A. (1997) Melatonin in humans. *N. Engl. J. Med.*, **336**, 186–195.
2. Sanchez-Barcelo,E.J. *et al*. (2005) Melatonin-estrogen interactions in breast cancer. *J. Pineal Res.*, **38**, 217–222.
3. Erren,T.C. (2002) Does light cause internal cancers? The problem and challenge of an ubiquitous exposure. *Neuro Endocrinol. Lett.*, **23** (suppl. 2), 61–70.
4. Arendt,J. (1998) Melatonin and the pineal gland: influence on mammalian seasonal and circadian physiology. *Rev. Reprod.*, **3**, 13–22.

5. Stevens,R.G. (2006) Artificial lighting in the industrialized world: circadian disruption and breast cancer. *Cancer Causes Control*, **17**, 501–507.
6. Megdal,S.P. *et al*. (2005) Night work and breast cancer risk: a systematic review and meta-analysis. *Eur. J. Cancer*, **41**, 2023–2032.
7. Schernhammer,E.S. *et al*. (2005) Urinary melatonin levels and breast cancer risk. *J. Natl Cancer Inst.*, **97**, 1084–1087.
8. Travis,R.C. *et al*. (2004) Melatonin and breast cancer: a prospective study. *J. Natl Cancer Inst.*, **96**, 475–482.
9. Verkasalo,P.K. *et al*. (2005) Sleep duration and breast cancer: a prospective cohort study. *Cancer Res.*, **65**, 9595–9600.
10. McElroy,J.A. *et al*. (2006) Duration of sleep and breast cancer risk in a large population-based case-control study. *J. Sleep Res.*, **15**, 241–249.
11. Pinheiro,S.P. *et al*. (2006) A prospective study on habitual duration of sleep and incidence of breast cancer in a large cohort of women. *Cancer Res.*, **66**, 5521–5525.
12. Lie,J.A. *et al*. (2006) Breast cancer and night work among Norwegian nurses. *Cancer Causes Control*, **17**, 39–44.
13. Schernhammer,E.S. *et al*. (2006) Night work and risk of breast cancer. *Epidemiology*, **17**, 108–111.
14. Schernhammer,E.S. *et al*. (2001) Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. *J. Natl Cancer Inst.*, **93**, 1563–1568.
15. Davis,S. *et al*. (2001) Night shift work, light at night, and risk of breast cancer. *J. Natl Cancer Inst.*, **93**, 1557–1562.
16. Hansen,J. (2001) Light at night, shiftwork, and breast cancer risk. *J. Natl Cancer Inst.*, **93**, 1513–1515.
17. O'Leary,E.S. *et al*. (2006) Shift work, light at night, and breast cancer on Long Island, New York. *Am. J. Epidemiol.*, **164**, 358–366.
18. Seow,A. *et al*. (1996) Breast cancer in Singapore: trends in incidence 1968–1992. *Int. J. Epidemiol.*, **25**, 40–45.
19. Hankin,J.H. *et al*. (2001) Singapore Chinese Health Study: development, validation, and calibration of the quantitative food frequency questionnaire. *Nutr. Cancer*, **39**, 187–195.
20. Parkin,D.M. *et al*. (2002) *Cancer Incidence in Five Continents*. IARC, Lyon.
21. Singletary,S.E. *et al*. (2002) Revision of the American Joint Committee on Cancer staging system for breast cancer. *J. Clin. Oncol.*, **20**, 3628–3636.
22. Seow,A. *et al*. (1998) Urinary total isothiocyanate (ITC) in a population-based sample of middle-aged and older Chinese in Singapore: relationship with dietary total ITC and glutathione S-transferase M1/T1/P1 genotypes. *Cancer Epidemiol. Biomarkers Prev.*, **7**, 775–781.
23. Saw,S.M. *et al*. (2001) Genetic, dietary, and other lifestyle determinants of plasma homocysteine concentrations in middle-aged and older Chinese men and women in Singapore. *Am. J. Clin. Nutr.*, **73**, 232–239.
24. Key,T.J. *et al*. (2003) Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. *J. Natl Cancer Inst.*, **95**, 1218–1226.
25. Wu,A.H. *et al*. (2007) Diabetes and risk of breast cancer in Asian-American women. *Carcinogenesis*, **28**, 1561–1566.
26. Xue,F. *et al*. (2007) Diabetes, metabolic syndrome, and breast cancer: a review of the current evidence. *Am. J. Clin. Nutr.*, **86**, 823S–835S.
27. Burch,J.B. *et al*. (2005) Melatonin, sleep, and shift work adaptation. *J. Occup. Environ. Med.*, **47**, 893–901.
28. Schernhammer,E.V. *et al*. (2006) Urinary 6-sulfatoxymelatonin levels and their correlations with lifestyle factors and steroid hormone levels. *J. Pineal Res*, **40**, 116–124.
29. Lee,S.A. *et al*. (2005) An overview of menopausal oestrogen-progestin hormone therapy and breast cancer risk. *Br. J. Cancer*, **92**, 2049–2058.
30. Wu,A.H. *et al*. (2007) Body size, hormone therapy and risk of breast cancer in Asian-American women. *Int. J. Cancer*, **120**, 844–852.
31. Cohen,M. *et al*. (1978) Pineal gland and breast cancer. *Lancet*, **2**, 1381–1382.
32. Stevens,R.G. (1987) Electric power use and breast cancer: a hypothesis. *Am. J. Epidemiol.*, **125**, 556–561.
33. Schernhammer,E.S. *et al*. (2004) Epidemiology of urinary melatonin in women and its relation to other hormones and night work. *Cancer Epidemiol. Biomarkers Prev.*, **13**, 936–943.
34. Bojkowski,C.J. *et al*. (1990) Factors influencing urinary 6-sulphatoxymelatonin, a major melatonin metabolite, in normal human subjects. *Clin. Endocrinol. (Oxf.)*, **33**, 435–444.
35. Armstrong,B.K. *et al*. (1992) *Principles of Exposure Measure in Epidemiology.* Oxford University Press, Oxford.
36. Ursin,G. *et al*. (1994) Breast cancer. *Cancer Surv.*, **19–20**, 241–264.

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