

I.H. Light at Night

Introduction

Breast cancer incidence rates vary dramatically across geographic regions, both internationally and within the U.S..^{1,2} The observation that breast cancer rates are higher in more urbanized areas,^{3,4} coupled with the slow but persistent increase in incidence rates during the latter half of the last century,^{2,4} has led breast cancer researchers to investigate etiologic factors related to industrialization. Electricity is a fundamental hallmark of industrialization and a great deal of interest has focused on the potential health effects of exposures to artificial light at night and electromagnetic fields (EMF) associated with the advent of widespread electrical usage.⁵

Early work in this area was more focused on EMF exposures. Recently, however, attention has been redirected towards the role of exposures to light at night, because epidemiologic studies of shift workers have provided compelling results that light at night may be a risk factor for breast cancer. Laboratory studies have also provided a strong biologic rationale for an effect. Furthermore, the U.S. and other industrialized nations are moving towards ‘24-hour societies.’⁶ As the number of people employed in alternative work schedules increases,⁷ exposures to both occupational and environmental sources of light at night will increase.

Most work to date has been focused on breast cancer incidence, with little attention paid to the other outcomes on the breast cancer continuum. There is, however, evidence emerging that light at night (and corresponding levels of melatonin, the

hormone that mediates the body’s response to light at night), may affect disease progression and thus could provide avenues for new treatment regimens.

Concept/Exposure Definition

The idea that light at night may increase breast cancer risk is predicated on earlier hypotheses surrounding the role of melatonin output by the pineal gland in breast carcinogenesis.⁸ Stevens and colleagues suggested that if high levels of melatonin could decrease breast cancer risk, then light at night, by lowering levels of melatonin, could increase breast cancer risk.^{5,9}

While substantial evidence exists that exposure to light at night suppresses nocturnal production of melatonin by the pineal gland,¹⁰⁻¹⁵ there is uncertainty as to how much and what kind of light is necessary to produce a clinically-relevant reduction in melatonin production. Melatonin suppression will likely depend on the color of the light, its intensity, and the duration of the exposure.¹⁵ Laboratory evidence suggests that even relatively dim light (such as that equal to twice the illumination provided by a full moon) can suppress nocturnal melatonin production in animals.¹⁵ Recent evidence in humans suggests that melatonin production may be especially sensitive to blue light at levels as low, or lower than, those documented in rodents.¹⁶ This is an important area of further inquiry.

Epidemiologic studies have used a wide variety of approaches to characterize exposure in evaluating this hypothesis, some of which focus more on light-at-night exposures (or surrogates thereof), while others focus more directly on melatonin

levels. Incidence studies of breast cancer have been conducted among blind women who lack light perception and thus experience no light at night. Other researchers have relied on occupations that typically involve a great deal of night-shift work, such as nurses and flight attendants. Some of these studies have information on duration and frequency of night-shift work,¹⁷⁻¹⁹ while others have relied on occupational titles as proxies for nighttime work.^{20, 21} Other investigators have used sleep times and durations as a proxy for exposures to light at night.^{17, 22, 23}

A number of epidemiologic studies have aimed to more directly address a link between melatonin levels and breast cancer. Most of the earlier studies were case-control studies that relied on measurements of plasma concentrations of circulating melatonin collected at the time of, or after, diagnosis.²⁴⁻²⁸ Thus, while these studies generally found lower levels of plasma melatonin in cases than in controls, they were unable to distinguish whether such differences were a cause or a consequence of breast cancer.

Recently, two prospective studies have been published that have relied upon urinary, rather than plasma, measures of melatonin.^{29, 30} Sulphatoxymelatonin (aMT6), the primary metabolite of melatonin, is excreted in the urine, and correlates well with plasma melatonin levels (as discussed by Schernhammer et al.³¹). Urinary measures of aMT6 appear to be particularly good at capturing peak nocturnal levels of plasma melatonin,³² which is thought to be the most biologically relevant metric. Furthermore, there is evidence that spot measurements of aMT6 can provide reasonably reliable estimates of chronic

plasma melatonin levels, at least over a span of several years.^{31, 33} These measures have the advantage of being less invasive and easier and less expensive to collect, making them more useful in prospective study designs. The potential disadvantage of using urinary aMT6 levels is that this is a somewhat less direct measure of melatonin output from the pineal gland; aMT6 levels may be affected by individual differences in melatonin metabolism.

Finally, the most comprehensive evaluation of exposures comes from animal studies, which have the ability to measure both light at night and plasma melatonin levels simultaneously. Furthermore, the effect of these exposures can be examined in conjunction with the effects of pinealectomy, blindness, and the administration of exogenous melatonin. It is from these studies that some of the most convincing evidence has arisen (see subsection on biologic plausibility below).

Biologic Plausibility

There are several lines of evidence that support the biologic rationale for a connection between light at night and breast cancer etiology.^{34, 35} Most of this evidence is directed at testing the hypothesis that light at night suppresses the pineal gland's production of melatonin, which, in turn, stimulates mammary carcinogenesis. The most convincing evidence supporting this hypothesis arises from a large body of laboratory studies, mostly in rodents. These studies have shown that both removal of the pineal gland and exposures to constant light can independently result in an increase in mammary carcinogenesis, while administration of exogenous melatonin and light deprivation decrease mammary carcinogenesis.^{10, 12} That these

relationships may also exist in humans is bolstered by the observations that melatonin receptors are present in both normal and tumor tissue in the human breast, that melatonin levels are lower in breast cancer patients than in women without breast cancer, and that nocturnal melatonin levels are suppressed by exposure to light at night.^{36, 37}

In 2005 a landmark paper was published by Blask and colleagues that pulled together these different threads of evidence and provided compelling data to support the hypothesis that light at night, mediated by a suppression of melatonin, promotes carcinogenesis in human breast tissue.³⁸ By measuring the response of rats bearing human breast cancer xenografts to increasing intensities of ocular light exposures during normal periods of darkness, Blask was able to demonstrate a dose-dependent suppression of nocturnal melatonin levels, as well as a dose-related increase in tumor growth rates. Furthermore, the time to tumor onset decreased as the light intensity increased. Perhaps more importantly, though, this study also measured the responses of the human breast cancer xenografts to perfusion *in situ* with blood from pre-menopausal women collected during the day, at night, and at night following 90 minutes of light exposure. As predicted, melatonin levels were substantially higher in the nighttime-collected blood, compared to both the daytime collected blood and the blood collected at night following 90 minutes of light exposure. Furthermore, they found that the tumors perfused with daytime-collected blood exhibited high-proliferative activity, compared to those perfused with nighttime-collected blood. The breast cancer xenografts that were perfused with human blood collected after 90 minutes of light at night

exhibited the same high-proliferative activity as those exposed to daytime-collected blood. Finally, to test whether these effects were mediated by melatonin, the investigators added melatonin to the melatonin-depleted blood that was collected after 90 minutes of light-at-night exposures and found that the high-proliferative activity was prevented. These results, while needing to be replicated, provide some very strong evidence that light at night, through the suppression of melatonin output, stimulates breast carcinogenesis.

Initially, the mechanistic pathway by which melatonin was thought to inhibit breast carcinogenesis was through its ability to reduce levels of circulating estrogens.³⁹ Evidence for this pathway has been somewhat mixed.^{31, 37, 40, 41} Furthermore, recent positive findings for other cancers⁴² suggest effects may not be mediated (or entirely mediated) by melatonin's effect on estrogen levels. Consequently, researchers are now considering a number of other potential pathways for melatonin's inhibitory effect on breast carcinogenesis. These are nicely summarized in four recent review articles.^{12, 34, 43, 44} Mechanisms receiving the most attention include:

1. a direct anti-proliferative effect, mediated by lowered levels of estrogen
2. increased immune response
3. antioxidant activity, scavenging free radicals
4. changes in the metabolism of linoleic acid by tumor cells

5. modulation of cell life cycle length through the p53 pathway

In summary, the biologic plausibility for an etiologic effect of light at night is strong and generally supported by a large body of laboratory evidence. While the data generally support the idea that the effects of light at night are mediated by reduced output of melatonin, the exact pathway by which melatonin exerts its inhibitory effects remains to be determined. There also is growing interest in other hormones that are controlled by circadian rhythms that could be disrupted by exposures to light at night. These include cortisol, dopamine, somatotropin and growth hormones.²² To date, little is known about the role of these hormones in breast cancer etiology. Evaluation of the role of pineal peptides has also been suggested as a course of further study.¹² There is some evidence emerging that these polypeptides, which have been found in the pineal gland and whose biologic function is largely unknown, may have antigonadotropic and tumor-inhibiting activity.¹² Finally, while most rodent studies have shown a strong and positive association between light exposures and mammary tumors, one recent study found the opposite was true.⁴⁵ This recent study began the light exposures later in life (the equivalent of human adolescence), which suggests there may be a window of vulnerability to light exposures. This avenue of inquiry deserves further attention.

Critical Review of the Literature

Literature to date on this topic has almost entirely focused on incidence and etiology. There are only a limited number of epidemiologic studies that have used a wide range of approaches, but these

have produced remarkably consistent results overall. The comparatively large body of laboratory studies also supports a relationship between light at night/melatonin and breast cancer risk. There is some evidence, mostly from laboratory studies, which suggests light at night/melatonin can affect disease progression, and thus may ultimately be useful in breast cancer treatment.

Incidence

Epidemiologic studies addressing this question fall into several categories: occupational studies; studies of blind women; studies of sleep duration/timing; and studies of melatonin levels. Findings for each are summarized below.

Occupational Studies

The majority of occupational studies examining light at night and breast cancer risk are focused on airline flight crews.⁴⁶⁻⁵² Originally the rationale for looking at cancer incidence among flight attendants was based on concern over the elevated levels of cosmic radiation experienced by these workers. After publication of initial findings, it was suggested that the reported increases in risk of breast cancer also could be due to melatonin deficiencies resulting from occupational exposures to light at night.⁵³ A recent meta-analysis of the seven flight attendant studies published up to 2005 reported an elevated risk of breast cancer with a summary standardized incidence ratio (SIR) of 1.44, 95% C.I. 1.36-1.61.⁴³

All of these studies were retrospective cohort studies using linkage of pre-existing data sources. Specific details of night-shift work were not

available, nor was information on most breast cancer risk factors. Thus, the results from these studies are remarkably consistent, but are limited by lack of good ‘exposure’ data, small numbers (the largest study had 60 cases of breast cancer), and inability to completely control for other breast cancer risk factors. Since the publication of this meta-analysis, results have been published from a small nested case-control study which sought to evaluate breast cancer risks associated with lifestyle and occupational factors among a group of airline cabin attendants.⁵⁴ This study, which was relatively unique in its ability to control for other breast cancer risk factors, reported a slightly increased risk of breast cancer in cabin attendants who reported disruption in sleep rhythms (“sometimes or often” compared to “never”), but this finding was not statistically significant.

As a whole, the flight attendant studies have not been able to directly assess the role of light at night on breast cancer risk. They were, however, the first group of occupational studies in women to suggest that disruptions in circadian rhythms may impact risk. The rationale for the early studies of breast cancer in flight attendants was based on putative elevated exposure to cosmic radiation. Pukkala et al.⁴⁹ specifically addressed this possibility. They calculated the cumulative excess radiation exposure for the study subjects on the basis of low-dose extrapolation from the Japanese atomic bomb cohort and estimated that radiation would yield a relative risk of 1.01, not close to the 1.87 they observed.

The other occupational group that has received considerable attention in this arena is nurses. The results from these studies also suggest an

increased risk of breast cancer associated with night-shift work. In both the original Nurses Health Study (NHS), a prospective study of breast cancer in predominantly post-menopausal women, and in the Nurses Health Study II, a companion study focused solely on pre-menopausal women, nurses who worked rotating night shifts for many years had a higher incidence of breast cancer.^{18, 19} In the NHS, which included both pre- and post-menopausal women, a moderate increase in breast cancer risk was observed, with risks increasing with increasing duration of rotating night-shift work (RR = 1.36, 95% CI = 1.04–1.78 for 30+ years of night-shift work, p-trend = 0.02).¹⁸ These results were similar, although no longer statistically significant, when the data were stratified by menopausal status. Case counts, especially for the pre-menopausal group, were very small.

In the NHS II, which was limited entirely to pre-menopausal women, elevated rates of breast cancer were observed for the highest duration of night-shift work (RR = 1.79, 95% CI = 1.79, 1.06–3.01 for 20+ years), but there was no evident trend of increasing risk with increasing years of night-shift work (p-trend = 0.65). These results were based on small numbers, with only 15 cases in the highest exposure category. Similarly, a large prospective study of Norwegian nurses also reported an increased risk of breast cancer associated with working nights for 30+ years.⁵⁵ These prospective studies generally had good information on potential confounding by established risk factors, though no effort was made to investigate possible confounding by exposures to the many chemical agents in medical settings, including sterilants, solvents, and therapeutic

agents, many of which are animal mammary carcinogens or hormonally active. Detailed information on duration and frequency of night-shift work is a strength of these studies.

The evidence of an increased risk of breast cancer associated with night work from population-based studies is a bit more mixed. Both a Danish study²⁰ and a Seattle study¹⁷ reported increased risks of breast cancer among women who worked at night. In contrast, a study conducted among participants of the Electromagnetic Fields and Breast Cancer on Long Island Study reported that women who worked non-day shifts were not at increased risk (OR = 1.04; CI = 0.79–1.38); a post-hoc analysis found that for evening-shift work, the OR was 1.08 (CI = 0.81–1.44) and for night work, the OR was reduced, at 0.55 (CI = 0.32–0.94).⁵⁶ Reasons for these disparate findings are not readily apparent, although each of these studies relied on slightly different definitions of night-shift work and considered different windows of exposure, with the Seattle study considering shift work only within the last ten years, the Long Island study considering shift work within the prior 15 years, and the Danish study considering shift work over a lifetime.

Finally, two studies have approached this issue from an entirely different angle by studying women who are in darkness most of the day. Both of these studies reported reduced risks of breast cancer among photo processors, who typically work in darkness for several hours during the day.^{57, 58}

In summary, although the number of epidemiologic studies conducted to date is limited, there is consistent evidence that women who work

at night are at an increased risk of breast cancer. This is supported by data from a number of occupations that are not likely to share any other common exposures (i.e., nurses, telegraph operators, flight attendants), with the possible exception of ionizing radiation exposures, which are likely to be high in flight attendants and among some nurses. Many of these studies, in particular the flight attendant studies, did not have full information on established breast cancer risk factors. While it is possible that at least some of the excess risk is due to incomplete control for confounding, it is unlikely to fully explain the elevated risks among nighttime workers. In the meta-analysis of 13 studies on night shift work and breast cancer performed by Megdal et al., the summary risk ratio for the seven flight attendant studies was virtually the same as that based on the remainder of the studies, which, for the most part, were adjusted for the main breast cancer risk factors.⁴³

Aside from possible confounding by established breast cancer risk factors, other characteristics of night-shift workers might underlie the associations with breast cancer risk. There has been emerging interest surrounding the hypothesis that vitamin D from sunlight exposure may reduce breast cancer risk (see Section 1, Chapter E). The degree to which night-shift workers may suffer from reduced sunlight exposure has not yet been investigated in this regard. It is possible that women working on a permanent night-shift schedule may be better able to adapt and less likely to experience circadian disruption than women who work rotating night shifts; further evaluation of whether permanent and rotating night-shift work confer the same risk may be

fruitful. The intriguing, although preliminary, findings of reduced risk of breast cancer among photo-processors deserves further attention.

There have recently also been studies of hormone production in shift workers. Four studies have reported melatonin production to be reduced in shift workers.^{31, 59-61} One of the studies also reported elevated estrogen levels,³¹ although the others did not.

Studies of Blind Women

Much of the early interest in light at night was fueled by the observation of lower breast cancer incidence among blind women.⁶²⁻⁶⁵ The reduced risk of breast cancer among blind women appears to be limited to the totally blind and severely visually impaired,^{62, 65} although one study reported reduced risks across most categories of visual impairment and a decreasing trend with greater level of impairment.⁶⁴ Generally, the results from these studies are consistent with a hypothesized reduced risk of breast cancer in blind women due to higher levels of melatonin secretion by the pineal gland in response to the lack of ocular light perception.^{62, 66} These studies, however, tend to be limited by small sample size and lack of information on other breast cancer risk factors that may co-vary with visual impairment. Information on nulliparity, a well-established risk factor for breast cancer was available from one study, which suggested that blind women are much more likely to be nulliparous than sighted women.⁶⁵ This would increase, not decrease, breast cancer risk. Future incidence studies of breast cancer among blind women would be strengthened by incorporation of measured levels of circulating melatonin, greater sample sizes, and information

on age of onset of visual impairment and on other breast cancer risk factors.

Studies of Sleep

Another approach to evaluating the melatonin hypothesis has been to examine sleep habits in relation to breast cancer risk. To date, two case-control studies have been published on the risk of breast cancer associated with sleep habits and the lighting of the bedroom environment, yielding conflicting results.^{56, 67} The study conducted in Seattle found an increased risk of breast cancer among women who frequently experienced ‘non-peak sleep’ (i.e., they did not sleep between 1 and 2 a.m., when nocturnal melatonin levels are typically at their highest). They found no association between breast cancer risk and several measures of bedroom light exposures, including number of times during the night that the subject turned on a light, the percentage of time that a light was on in the bedroom, and reported ambient levels of light in the subjects’ bedrooms.¹⁷ In contrast, the study conducted on Long Island reported no association with non-peak sleep (defined in the same way as the Seattle study), but an increased risk associated with frequency of turning on a light during sleep hours.⁵⁶ Reconciling these findings is difficult, as the exposure definitions used in these two studies are quite similar, although the time period for the Long Island study was more recent and shorter than that examined in the Seattle study.

Using a slightly different approach, a Finnish cohort study examined sleep duration with respect to breast cancer risk.²² The rationale for the study was based on the observation that an increase in sleep duration may be associated with greater

nocturnal melatonin secretion.²² Sleeping habits were ascertained prospectively from questionnaires administered six years apart. While there was no overall effect of sleep duration, when the analysis was restricted to ‘stable sleepers’ (i.e. sleep duration categorization was the same across the two questionnaires), breast cancer risk significantly decreased with increasing sleep (p-value for trend = 0.03), such that those who slept six hours or less had an increased risk (HR = 1.10, 95% CI = 0.59–2.05) and those who slept for nine hours or more had a decreased risk (HR = 0.28, 95% CI = 0.09–0.88), compared to those who slept an average of eight hours a night. While these findings are consistent with an increased risk of breast cancer associated with light at night mediated by melatonin secretion, the authors of this study also note that sleep duration is likely to impact other circadian rhythms, including rhythmic fluctuations in secretion of cortisol, dopamine, somatotropin, and growth hormone. The relation of these hormones to breast cancer is largely unknown.

In contrast to Verkasalo et al.,²² Pinheiro et al.²³ reported on sleep duration and breast cancer risk in the Nurses' Health Study I, and found no overall association. Among women reporting the same sleep duration on questionnaires from 1986 and 2000, there was a modest increased risk in those who slept more than nine hours, compared to those who slept less than seven. There are many differences between these two cohorts, not the least of which is that the NHS cohort is made up of nurses, most of whom currently work, or in the past worked, a non-day shift.

The results from these studies are intriguing and warrant further study. It might be useful to elucidate which characteristics of sleep behavior (i.e., sleep duration, timing, ambient lighting) have the largest impact on the secretion of melatonin and some of the other hormones that are tied to circadian rhythms, particularly cortisol, for which there appears to be growing interest with respect to its role in breast cancer etiology.⁶⁸⁻⁷⁰

Studies of Melatonin Levels

As much as 25 years ago, it was noted that plasma melatonin levels tend to be depressed in women with breast cancer.^{24, 25, 27, 28, 71-74} Because levels were measured at the time of, or after, diagnosis, it was impossible to assess whether this was a cause or a consequence of the disease. With the recent identification of a useful urinary biomarker for plasma melatonin,^{32, 75, 76} it is now possible to prospectively evaluate melatonin levels in relation to breast cancer risk.

To date there have been two epidemiologic studies published that have made use of this biomarker. Both of these studies were large, well-conducted, prospective breast cancer studies that relied on urinary measures of Sulphatoxymelatonin (aMT6) as a marker for plasma melatonin levels. The first of these was a case-control study nested in the Guernsey III study, a large prospective cohort study of hormones and breast cancer conducted in Britain.³⁰ This study, which measured aMT6 in 24-hour urine samples collected at the time of cohort enrollment, found no association between breast cancer and aMT6 levels (OR = 0.99, 95% CI = 0.58–1.70, comparing the highest to lowest categories). In contrast, a nested case-control analysis within the Nurses Health Study II

found lower breast cancer risk associated with higher aMT6 levels as measured in first morning urine (OR = 0.59, 95% CI = 0.36–0.97, comparing the highest to lowest quartiles of aMT6). The design of these two studies was very similar, but the critical difference that may explain the disparate findings is the use of 24-hour versus first-morning urine. Use of a 24-hour urine sample, as was used in the British study, cannot capture nocturnal duration or peak concentrations of melatonin, which are likely to be important in determining cancer risk.⁷⁷ Furthermore, the British study, which did control for many of the important risk factors for breast cancer, did not have information on night-shift work, alcohol consumption, or exposures to light at night, all of which are well-documented determinants of plasma melatonin levels.^{20, 31, 40, 41}

While more research is needed to evaluate the degree to which melatonin levels in 24-hour urine samples correlate to those measured in first morning urine, and the degree to which timing and duration of sleep might affect these levels, this approach holds great promise. The use of a urinary marker for melatonin levels that can be collected prior to the onset of disease is extremely valuable in directly assessing the ‘melatonin hypothesis’ with respect to breast cancer etiology.

Circadian Disruption During Pregnancy

The idea that exposures to a woman during her pregnancy that alter her sex hormone levels could result in increased lifetime risk of breast cancer in her daughters has gained wide interest and mounting scientific support.⁷⁸⁻⁸⁰ The hypothesized mechanism is by altering the normal development of breast tissue. For example, Stevens and

Hilakivi-Clarke⁸¹ proposed that low and moderate alcohol intake during pregnancy would increase risk of breast cancer in the daughters. This idea was based on observations that ethanol can affect estrogen and/or testosterone production^{82, 83} and lower melatonin production.^{84, 85} Hilakivi-Clarke et al.⁸⁶ tested this hypothesis in rats and found that female rats fed low and moderate amounts of ethanol during pregnancy had female offspring that were more susceptible to chemically-induced mammary cancer than offspring from pregnant rats not fed ethanol. The alcohol levels were far below those required to result in fetal alcohol syndrome, being as little as the equivalent human consumption of one drink per day. Alcohol can also be a circadian disruptor.⁸⁷ Similarly, other circadian disruption during pregnancy may affect the lifetime risk of breast cancer in the daughters. Specific tests of this idea are that shift work during pregnancy leads to increased risk in daughters. This could be tested in case-control studies. Prospective studies would be considerably more difficult, but intermediate endpoints might be possible, such as breast density in early adulthood, based on the Child Health and Development Study led by Barbara Cohn at the Public Health Institute in Berkeley (e.g., Cohn, et al.⁸⁸ and Stevens et al.⁸⁹).

In summary, the incidence studies conducted to date are supportive of an association between light at night and breast cancer risk. While the body of literature is still fairly small, the results from occupational studies, which generally report an approximate 50 percent elevated risk of breast cancer among night-shift workers, are extremely consistent. The incidence studies among blind women, while hindered somewhat by lack of

information on breast cancer risk factors and small numbers, also support this hypothesis. The studies of sleep patterns and those using urinary markers of melatonin are too few to draw solid conclusions from at this point, but suggest future research is needed.

Etiology

Studies addressing etiology have already been summarized in the prior subsections on biologic plausibility and incidence. There are also a number of cross-sectional exposure studies focused on identifying determinants of melatonin levels in humans; these studies both indirectly address etiology and raise some methodologic dilemmas in studying this exposure. There have been quite a few studies examining the effects of light at night on melatonin levels in humans, yielding somewhat mixed results, with some studies showing a relationship, while others not.^{31, 37, 40, 41} Differences in results are likely due to inconsistencies in exposure metrics. More research is needed to determine the effects of timing, duration, and intensity of light at night on levels of nocturnal melatonin levels.

Furthermore, melatonin levels appear to be affected by a number of known breast cancer risk factors, including age, alcohol consumption, BMI, physical activity, and height, as well as use of a number of medications (e.g. NSAIDS, psychotropics). A summary of this literature appears in Table 1. Calcification (for example from exposure to fluoride⁹⁰) may also effect on the pineal's ability to produce melatonin, but is difficult to study.⁹¹

Table 1. Factors that have been identified as determinants of melatonin concentrations in humans.

Factor	Direction of relationship associated with an increase in the factor	References
Age	↓	Knight et al., ⁴¹ Travis, et al. ³⁰
BMI	↓	Davis et al., ⁶⁷ Schernhammer et al., ⁴⁰ Travis et al. ³⁰
Height	↑	Knight et al. ⁴¹
Alcohol consumption	↓	Davis et al. ⁶⁷
Smoking	↓	Schernhammer et al. ⁴⁰
Vegetable intake	↑	Nagata et al. ⁹²
Exercise	↑	Knight et al. ⁴¹
Parity	↑	Schernhammer et al. ⁴⁰ Travis et al. ³⁰
Hours of daylight	↓	Davis et al., ⁶⁷ Knight et al. ⁴¹

These results are important for two reasons. First they suggest a potential melatonin-mediated pathway of breast carcinogenesis for these risk factors. Second, they highlight the importance of careful modeling when evaluating the risk of breast cancer associated with melatonin levels. If

these factors are in the causal pathway, then adjustment for them in models examining melatonin and breast cancer could obscure a true association. For example, if light at night disrupts fertility, resulting in nulliparity (which increases breast cancer risk), then controlling for nulliparity in study of melatonin could mask a true effect.

While these exposure studies have identified a number of important predictors of melatonin exposures, overall we have yet to identify the most important predictors, as the percent of variability explained by these studies is quite low. Given the fairly strong evidence that melatonin is likely to play an etiologic role in breast cancer, identifying the determinants of melatonin levels should be a research priority.

Finally, in evaluating the role of melatonin in breast cancer etiology, genetic susceptibility must be considered. The genes that regulate circadian rhythms are emerging as key players in expression of a wide variety of genes that regulate cell cycle length and apoptosis.^{34, 93, 94} A number of genes have been identified that play a critical role in sleep-related conditions and diurnal preferences.⁹³ Diurnal preference (i.e. night owls versus morning larks) predicts tolerance to evening or overnight shift work and may be related to melatonin levels. Of particular interest is new evidence that polymorphisms in the Period (Per) gene family, which is central to regulation of the circadian rhythm, can affect tumor suppression and DNA damage response in mice and may be related to breast cancer (as described by Davis et al.⁹³).

Treatment

Much of the etiologic evidence from animal studies seems to indicate that melatonin/light at night may act during the promotion, rather than the initiation, phase of carcinogenesis.⁹⁵ This suggests melatonin and/or manipulation of the light/dark cycle may be useful in treating breast cancer. Studies to this end, especially in humans, are limited. A number of clinical trials have shown that administration of exogenous melatonin in conjunction with other oncostatic drugs slows disease progression and improves quality of life in patients with a variety of cancers.⁹⁶⁻⁹⁹ Clinical trials of the effectiveness of melatonin alone in the treatment of breast cancer are lacking. While a number of laboratory studies have provided compelling evidence that light at night exposures can affect progression of chemically-induced tumors,⁹⁵ the effectiveness of ‘darkness therapy’ as a treatment for breast cancer has not been evaluated in humans.

A related area of emerging interest in breast cancer treatment that taps into the importance of circadian rhythms is that of ‘chronotherapy.’ Chronotherapy, which aims to administer anticancer drugs at optimal times of the circadian clock, has been extensively evaluated in rodents and has been shown to alter the toxic effects of more than 30 different anti-cancer drugs.¹⁰⁰ Very limited data from clinical trials in humans suggest this may be a promising avenue to pursue. In a recent clinical trial among metastatic colon cancer patients, it was found that patients who received anticancer drugs at selected times considered to be optimal with respect to the circadian clock, instead of the constant-rate infusions typically done,

experienced fewer side effects, more shrinking of tumor size, and increased survival times¹⁰¹ (as described by Ross¹⁰⁰).

Conclusions and Future Directions

There is mounting evidence that disruptions in the circadian rhythm play a role in breast carcinogenesis. This is supported by data from a large body of both laboratory and epidemiologic studies. The timing, duration, and intensity of light-at-night exposures are likely to modify risk and warrant further investigation. There is substantial evidence that these effects are mediated by melatonin, although there are a number of other potential mechanisms that deserve further attention. The recent identification of a urinary marker for melatonin levels in humans offers the opportunity to more directly evaluate the role of melatonin in mediating the effects of light at night. To date, only two studies have utilized this marker, offering conflicting results.

The recent identification of a number of ‘clock genes,’ which regulate the circadian rhythm and appear to be important in cell cycle regulation and apoptosis throughout the body, calls for investigation of how these genes may alter an individual’s susceptibility to disruptions of the circadian clock by exposures to light at night.³⁴ Substantial and provocative findings from laboratory studies on the effectiveness of melatonin in cancer treatments highlights the need to further pursue the usefulness of melatonin/light-dark therapies in breast cancer treatment regimens. Another potentially critical topic is circadian disruption (such as from shift work) during pregnancy and its effect on the daughter's risk of breast cancer later in life.

While the mechanism by which disruptions in circadian rhythm affect breast cancer risk have yet to be fully elucidated, the evidence that nighttime shift work increases breast cancer risk is internally consistent and makes biological sense.

No other occupational exposure with known or potential carcinogenicity is as common as work at night.²⁰ Identifying factors which may limit or reduce the harmful effects of night-shift work should be a research priority.

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