Rotating Night Shifts and Risk of Breast Cancer in Women Participating in the Nurses' Health Study

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Background: **Melatonin shows potential oncostatic action, and light exposure during night suppresses melatonin production. There is little information, however, about the direct effect of night work on the risk of cancer. We investigated the effect of night work in breast cancer.** ***Methods:*** **We examined the relationship between breast cancer and working on rotating night shifts during 10 years of follow-up in 78 562 women from the Nurses' Health Study. Information was ascertained in 1988 about the total number of years during which the nurses had worked rotating night shifts with at least three nights per month. From June 1988 through May 1998, we documented 2441 incident breast cancer cases. Logistic regression models were used to calculate relative risks (RRs) and 95% confidence intervals (CIs), adjusted for confounding variables and breast cancer risk factors. All statistical tests were two-sided.** ***Results:*** **We observed a moderate increase in breast cancer risk among the women who worked 1–14 years or 15–29 years on rotating night shifts (multivariate adjusted RR = 1.08 [95% CI = 0.99 to 1.18] and RR = 1.08 [95% CI = 0.90 to 1.30], respectively). The risk was further increased among women who worked 30 or more years on the night shift (RR = 1.36; 95% CI = 1.04 to 1.78). The test for trend was statistically significant (*P* = .02).** ***Conclusions:*** **Women who work on rotating night shifts with at least three nights per month, in addition to days and evenings in that month, appear to have a moderately increased risk of breast cancer after extended periods of working rotating night shifts. [J Natl Cancer Inst 2001;93:1563–8]**

The suprachiasmatic nucleus in the hypothalamus, one of the most important physiologic determinants of alertness and performance, drives a circadian pacemaker in mammals, with an intrinsic period averaging 24 hours. Light is the primary stimulus to disrupt and reset this pacemaker, which is expressed in changing melatonin rhythms. Light exposure at night may, therefore, be related to a variety of behavioral changes and associated health problems not yet well explored. Studies *(1)* have suggested an increased risk of coronary heart disease among rotating night shift workers, not fully explained by an increased prevalence of coronary risk factors. Others have linked night work to an increased breast cancer risk among women *(2)*.

Melatonin, the "hormone of the darkness," has only recently gained substantial attention from the scientific community with regard to its potential oncostatic actions and its possible effect on breast cancer risk *(3–10)*. Melatonin serum levels in humans decrease when people are exposed to light at night *(11)*. Suppressed serum melatonin levels might enhance tumor development *(12)*. Observational studies *(2,13–15)* are compatible with an effect of melatonin on breast cancer risk, reporting meaningful increases in breast cancer risk among postmenopausal women exposed to shiftwork. Recently, a tumor-promoting effect of light exposure was demonstrated on chemically induced tumors in rodents *(16)*. To date, melatonin has been shown to be oncostatic for a variety of tumor cells in experimental carcinogenesis *(17–26)*. The evidence of a relation between melatonin and oncogenesis in humans is conflicting *(27)*, but the majority of reports indicate protective action *(28)*.

Several mechanisms have been hypothesized to explain an association between melatonin and breast cancer. Cohen et al. *(29)* proposed that loss of pineal function and the resulting decreased melatonin serum levels may increase reproductive hormone levels and, in particular, estradiol levels, thereby increasing the growth and proliferation of hormone-sensitive cells in the breast. More recent research focuses on potential mechanisms through which melatonin is directly oncostatic. Melatonin is believed to have antimitotic activity by affecting directly hormone-dependent proliferation through interaction with nuclear receptors *(4)*. Another explanation is that melatonin increases the expression of the tumor suppressor gene p53 *(3)*. Cells lacking p53 have been shown to be genetically unstable and thus more prone to tumors *(30)*.

Breast cancer is the most common cancer among women in the United States. To date, the relationship between night work and breast cancer risk has not been evaluated in prospective cohort studies. A causal link between the two would be of public health importance, because small changes in shift patterns may create a substantial decrease of disease burden among women.

In this report, we evaluate the relationship between night work, as a surrogate for light exposure at night, and breast cancer risk in a large prospective cohort of premenopausal and postmenopausal women. Our analysis is based on 10 years of follow-up in 78 562 women participating in the Nurses' Health Study.

Subjects and Methods

In 1976, a total of 121 701 female registered nurses 30–55 years of age and living in 11 large U.S. states were enrolled in the Nurses' Health Study. Since baseline, they have completed biennial-mailed questionnaires that comprise items about their health status, medical history, and known or suspected risk

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factors for cancer *(31)* and heart disease *(32)*. The questions include age, age at menarche, parity, age at first birth, weight, height, menopausal status, family history of breast cancer, and personal history of benign breast disease and cancer. Every 2 years, follow-up questionnaires have been sent to cohort members to update the information on potential risk factors and to identify newly diagnosed case subjects with cancer and other major medical events. In 1980, the questionnaire was expanded to include an assessment of diet *(31,33)* and alcohol consumption. Follow-up data are available for more than 90% of the cohort. Further details of the Nurses' Health Study are described elsewhere *(34)*.

Ascertainment of Night Shift Working Status

In 1988, the study participants were asked how many years in total they had worked rotating night shifts with at least three nights per month in addition to days or evenings in that month. Information on lifetime years worked on rotating night shift was gathered in eight prespecified categories: never, 1–2, 3–5, 6–9, 10–14, 15–19, 20–29, and 30 or more years. Of the 103 613 nurses who responded to the 1988 questionnaire, 85 197 answered the shiftwork question.

Documentation of Breast Cancer and Deaths

Breast cancer cases were defined as having occurred during the period from June 1988 through May 1998. Nurses who reported the occurrence of breast cancer were asked for permission to review their medical records, and breast cancer was confirmed through review of these records. When medical records were unavailable, breast cancer cases were defined as probable and included in the analysis if they were corroborated by an interview or a letter from the subject. Approximately two thirds of the deaths among cohort members were reported to us by next of kin or the postal system in response to follow-up questionnaires. In addition, we searched the National Death Index to identify deaths among the nonrespondents to each 2-year questionnaire; the computerized National Death Index is a highly sensitive method for identifying deaths in this cohort *(34)*. Data on mortality were more than 98% complete *(34,35)*. For all deaths possibly attributable to breast cancer, we requested permission from family members (subject to state regulation) to review the medical records. Breast cancer was considered to be the cause of death if the medical records or autopsy report confirmed a fatal breast cancer, if the breast cancer was listed as the underlying cause of death without another, more plausible cause, and if the nurse was known (from hospital records, a family member's report, or another source) to have had breast cancer before death. In no case was the cause listed on the death certificate used as the sole criterion for death due to breast cancer. All interviews and reviews of medical records were conducted by investigators without knowledge of exposure. A total of 2441 case subjects with breast cancer were diagnosed in the base population from June 1988 through May 1998, and pathology records were obtained for 93% of the case subjects. Although these 2441 case subjects included 92 women whose pathology reports had not yet been obtained, we based our analyses on the total, because the accuracy of the self-reporting was extremely high *(36)*. In addition, an analysis limited to case subjects confirmed by pathology reports yielded the same association with night work.

Study Population

A total of 103 613 of the women returned the 1988 questionnaire, which included the question about night work. The population for this study consisted of 85 197 (82.2%) of the respondents who answered the question on night work. Women who did not answer the shiftwork question on the 1988 questionnaire did not differ substantially from respondents in terms of their risk profile *(1)*. We excluded women who reported breast cancer or any other cancer other than nonmelanoma skin cancer on the 1988 questionnaire or any previous questionnaire. A total of 78 562 women remained to form the baseline population for this analysis, and 736 015 person-years of follow-up were accrued from June 1988 through May 1998.

Statistical Analysis

Women were first categorized according to their night work status; the groupings were selected to provide equal 15-year categories: having worked rotating night shifts either never or 1–14, 15–30, or 29 or more years. In some analyses, we collapsed the data into only two categories; in others, we went back to the original eight categories. Information about breast cancer and established risk factors for breast cancer was updated according to the biennial follow-up questionnaire. Information on alcohol consumption was updated every 4 years—1986, 1990, and 1994. For each participant, person-months were allocated to categories of years having worked on rotating night shifts, according to the 1988 data. The primary analysis was based on incidence rates, with person-months of follow-up used as the denominator. We used relative risk (RR) as the measure of association; the RR was defined as the incidence rate of breast cancer among women in various categories of years working on rotating night shifts divided by the incidence rate among women who never worked on rotating shifts. Mantel–Haenszel summary RRs were calculated, adjusting for age in 5-year categories *(37)*. All statistical tests were two-sided. Tests of trends across categories of exposure were calculated by treating the levels of exposure as a continuous, ordinal variable in the regression model. Pooled logistic regression models were used to calculate RRs with adjustment for age, age at menarche (≤12, 13, and ≥14 years), age at menopause (≤43, 44–46, 47–49, 50–52, 53–55, 56–58, and >58 years), parity (nulliparous, 1–2, 3–4, and ≥5), age at first birth (<25, 25–29, and ≥30 years), weight change between age 18 years and menopause (<2, 2–9, 10–20, and ≥20 kg) for menopausal women only, body mass index (weight in kilograms divided by the square of the height in meters) at age 18 years in five categories (<21, 21–22.9, 23–24.9, 25–28.9, and ≥29 kg/m^2), current alcohol consumption (nondrinkers <90 and ≥90 g/week), height in eight categories (≤150, 151–155, 156–160, 161–165, 166–170, 171–175, 176–180, and >180 cm), oral contraceptive use (ever/never), use of postmenopausal hormones (never, past user <5 years, past user ≥5 years, current user <5 years, and current user ≥5 years), menopausal status, benign breast disease (yes/no), and family history of breast cancer (yes/no). For all factors, indicator variables were created for missing values and included in the analyses. With short intervals between questionnaires and the low rate of events, this approach yields results similar to those of a Cox regression analysis with time-varying covariates *(38)*.

Results

We documented 2441 incident breast cancer cases. Women who had never worked on rotating night shifts accounted for 40.4% of the person-years of follow-up, those who worked for 1–14 years on shifts accounted for 52.2%, those who worked for 15–29 years accounted for 5.6%, and those who worked for 30 or more years accounted for 1.8%. Women who had ever worked on rotating shifts were similar in their baseline characteristics to those who had not. However, there were slightly fewer women who had not given birth among the never night shift workers, and they tended to be somewhat leaner (Table 1). Night shift workers were older and thus more likely to be postmenopausal than those who had never worked on rotating night shifts.

Table 2 shows the relationship between total years on rotating night shifts and breast cancer. Higher duration of working shiftwork was modestly associated with an increased breast cancer risk (P_{trend} = .02). Women who had worked 30 or more years on rotating night shifts had a 36% greater risk of breast cancer compared with never workers (multivariate-adjusted RR = 1.36; 95% CI = 1.04 to 1.78).

In analyses stratified by menopausal status, the relation of duration of night work and breast cancer was slightly different in premenopausal and postmenopausal women (Table 3). Among postmenopausal women, we observed an association in the highest (≥30 years) shift group (multivariate-adjusted RR = 1.36; 95% CI = 1.04 to 1.78), and the test for trend was statistically significant (P = .05). Similarly, we observed an increased breast cancer risk for the highest shift group (≥20 years) of premenopausal women (RR = 1.66; 95% CI = 0.81 to 3.40) but also a modest association (RR = 1.23; 95% CI = 0.97 to 1.55) among those who had worked 1–14 years on rotating night shifts (Table 3): more specifically, those who worked only 1–2 years on rotating night shifts (data not shown). The trend was not statistically significant (P = .12).

Table 1. Age and age-standardized* characteristics according to rotating shiftwork status in 1988 among 78 562 women in the Nurses' Health Study

Characteristic	Value of indicated characteristic by years worked on rotating night shifts			
	Never (n = 31 761)	1–14 y (n = 40 993)	15–29 (n = 4426)	>30 y (n = 1382)
Mean age, y (SD)†	54.3 (7.2)	54.7 (7.1)	56.1 (6.9)	60.4 (4.6)
Menarche before age 12 y, %	21.8	22.8	23.3	26.3
Nulliparous, %	5.7	7.3	6.8	5.6
Parity ⩾5 children, %	14.5	13.4	16.2	13.9
Age at first birth ⩾30 y, %‡	6.9	9.0	8.0	7.4
First-degree family history of breast cancer, %	11	11	11	12
History of benign breast disease, %	37	38	34	29
Ever use oral contraceptives, %	48.5	48.4	44.4	41.1
Postmenopausal in 1988, %	71.1	71.7	75.5	82.8
Mean age at menopause, y (SD)†	46.3 (6.6)	46.3 (6.7)	46.0 (6.7)	47.0 (6.6)
Age at menopause ⩾55 y, %§	4.6	4.6	3.8	5.2
Current PMH‖ use ⩾5 y, %	10.9	11.3	9.9	9.1
BMI¶ in 1988 ⩾25, %	40.1	42.6	52.5	42.7
BMI¶ at age 18 y ⩾25, %	26.6	27.9	33.3	25.8
Weight change >10 kg, age 18 y to menopause, %§	15.6	15.9	20.9	18.5
Mean current alcohol consumption, g/day (SD)†	6.3 (11.01)	6.5 (11.1)	5.5 (11.2)	5.7 (11.0)
Mean height in inches (SD)†	64.4 (3.2)	64.5 (3.3)	64.4 (3.0)	64.2 (3.6)
Socioeconomic status (husband's education beyond high school), %	41.8	42.2	29.5	28.4
Nurse's education higher than a bachelor's degree, %	9.2	9.2	5.6	2.6

*Age standardized according to eight categories of age (<44, 45–49, 50–54, 55–59, 60–64, 65–69, 70–74, and ⩾75 years) as of the 2-year period when participants first entered follow-up.

†SD = standard deviation.

‡Among the parous women only.

§Among the postmenopausal women only.

‖PMH = postmenopausal hormone.

¶BMI = body mass index.

Table 2. Adjusted relative risks (RRs) and 95% confidence intervals (CIs) of breast cancer by rotating night shift work in four categories among 78 562 women in the Nurses' Health Study, with prospective follow-up from 1988 through 1998 and with a total of 2441 breast cancer case subjects

Years on rotating night shift	No. of case subjects	Person-years	Age-adjusted RR (95% CI)	Multivariate RR* (95% CI)
Never†	925	298 815	1.0	1.0
1–14	1324	383 882	1.12 (1.03 to 1.22)	1.08 (0.99 to 1.18)
15–29	134	40 759	1.08 (0.90 to 1.29)	1.08 (0.90 to 1.30)
⩾30	58	12 559	1.54 (1.18 to 2.01)	1.36 (1.04 to 1.78)
P_{trend}			.00	.02‡

*Relative risk adjusted for age, in eight categories (<44, 45–49, 50–54, 55–59, 60–64, 65–69, 70–74, and ⩾75 years), age at menarche (⩽12, 13, and ⩾14 years), parity (nulliparous, 1–2, 3–4, and ⩾5), and age at first birth (<25, 25–29, and ⩾30 years) combined, weight change between age 18 years and menopause (<2, 2–9, 10–20, and ⩾20 kg), body mass index at age 18 years in five categories (<21, 21–22.9, 23–24.9, 25–28.9, and ⩾29), family history of breast cancer in sister or mother (yes/no), benign breast disease (yes/no), oral contraceptive use (yes/no/missing), current alcohol consumption (none, and <90 and ⩾90 g/week), time period (June 1988 through May 1990, June 1990 through May 1992, June 1992 through May 1994, June 1994 through May 1996, and June 1996 through May 1998), age at menopause in seven categories (⩽43, 44–46, 47–49, 50–52, 53–55, 56–58, and >58 years), use of postmenopausal hormones (never, past user for <5 years, past user for ⩾5 years, current user for <5 years, and current user for ⩾5 years) and menopausal status (yes/no) combined, and height in seven categories (⩽150, 151–155, 156–160, 161–165, 166–170, 171–175, 176–180, and >180 cm).

†Reference categories in all analyses.

‡*P* value (Wald test) for continuous linear term.

Night work was only weakly associated with physical activity, region, and dietary variables such as lifetime alcohol consumption. However, to address the possibility that these variables could account for the observed relation between shiftwork and breast cancer, we conducted additional analyses, including these variables as well as the nurses' and their husband's educational levels (as markers of socioeconomic status) in our multiple logistic regression models. We did not include them in the final model because they did not alter our estimates (data not shown). Because of the observed age difference between ever and never rotating night shift workers, we modeled subsequent analyses with age as a continuous variable and by 2-year categories. However, modeling age in different ways did not lead to substantial changes in the estimates of the RR.

We attempted to explain the slight differences in the association of shiftwork duration and breast cancer risk between premenopausal and postmenopausal women by examining whether the effects of shiftwork varied in specific subgroups. Because hormone receptor-positive tumors are more likely to be found in older women *(39)*, we examined breast cancers according to their hormonal receptor status and conducted further analyses for premenopausal and postmenopausal women separately. As with total breast cancer, for the estrogen receptor-positive breast cancer case subjects, longer duration in rotating night shifts was associated with a moderate increase in risk, particularly for premenopausal women, and we observed slightly elevated risks with shorter durations of shiftwork. The risk of hormone receptor-negative breast cancer was not elevated after 30 or more years of rotating night shifts (data not shown).

DISCUSSION

In this large and, to our knowledge, first prospective cohort study of shiftwork

Table 3. Adjusted relative risks (RRs) and 95% confidence intervals (CIs) of breast cancer in the Nurses' Health Study by rotating night shift work in four categories and prospective follow-up from 1988 through 1998 among 54 980 postmenopausal women with 2125* breast cancer case subjects and among 23 436 premenopausal women with 309 breast cancer case subjects

Years on rotating night shift	No. of case subjects	Age-adjusted RR (95% CI)	Multivariate RR† (95% CI)
Premenopausal women			
Never‡	121	1.0	1.0
1–14 y	174	1.23 (0.98 to 1.56)	1.23 (0.97 to 1.55)
⩾15 y§	14	1.30 (0.75 to 2.26)	1.34 (0.77 to 2.33)
P_{trend}		.13	.12‖
Postmenopausal women			
Never‡	801	1.0	1.0
1–14	1146	1.09 (1.00 to 1.20)	1.06 (0.97 to 1.16)
15–29	120	1.02 (0.84 to 1.24)	1.05 (0.87 to 1.27)
⩾30	58	1.45 (1.11 to 1.90)	1.36 (1.04 to 1.78)
P_{trend}		.02	.05‖

*Women with dubious menopause excluded.

†RR adjusted for age in eight categories (<44, 45–49, 50–54, 55–59, 60–64, 65–69, 70–74, and ⩾75 years), age at menarche (⩽12, 13, and ⩾14 years), parity (nulliparous, 1–2, 3–4, and ⩾5), and age at first birth (<25, 25–29, and ⩾30 years) combined, weight change between age 18 years and menopause (<2, 2–9, 10–20, and ⩾20 kg), body mass index at age 18 years in five categories (<21, 21–22.9, 23–24.9, 25–28.9, and ⩾29), family history of breast cancer in sister or mother (yes/no), benign breast disease (yes/no), oral contraceptive use (yes/no/missing information), current alcohol consumption (none, and <90 and ⩾90 g/week), time (June 1988 through May 1990, June 1990 through May 1992, June 1992 through May 1994, June 1994 through May 1996, and June 1996 through May 1998), age at menopause in seven categories (⩽43, 44–46, 47–49, 50–52, 53–55, 56–58, and >58 years), use of postmenopausal hormones (never, past user for <5 years, past user for ⩾5 years, current user for <5 years, and current user for ⩾5 years) and menopausal status (yes/no) combined, and height in seven categories (⩽150, 151–155, 156–160, 161–165, 166–170, 171–175, 176–180, and >180 cm).

‡Reference categories in all analyses.

§Collapsed categories.

‖*P* value (Wald test) for continuous linear term.

and breast cancer, the risk of breast cancer was statistically significantly elevated in postmenopausal women who worked for 30 or more years on rotating night shifts, compared with those who never worked rotating night shifts. Among premenopausal women, we observed an increased breast cancer risk of 23% (RR = 1.23; 95% CI = 0.97 to 1.55) after 1–14 years of shiftwork.

Earlier work from Tynes et al. *(13)* showed an elevated breast cancer risk among postmenopausal radio and telegraph operators exposed to shiftwork. The authors observed no association for women aged 50 years or less but reported an increased breast cancer risk among postmenopausal women more than age 50 years (odds ratio [OR] = 4.3; 95% CI = 0.7 to 26.0) in their small study with 50 case subjects and 259 control subjects. Pukkala et al. *(14)* found a similar increased incidence of breast cancer among flight attendants (standardized incidence ratio [SIR] = 1.87; 95% CI = 1.15 to 2.23). In a population-based, case–control study conducted among 7035 Danish women with breast cancer and their matched control subjects, Hansen *(2)* estimated an OR of 1.5 (95% CI = 1.2 to 1.7) for breast cancer among women who predominantly worked at night for at least 6 months, after adjustment for socioeconomic status, age at birth of first and last child, and number of children.

Light is known to be a potent stimulus for regulating the pineal gland's production of melatonin and the broader circadian system in humans *(11,40–42)*. Light not only suppresses nocturnal melatonin secretion but also does so in a characteristic dose–response manner: the brighter the photic stimulus, the greater the suppression of nocturnal melatonin *(40)*. A recent observation among 10 935 visually impaired women *(43)* underlines a dose-related relationship between visible light and breast cancer risk. The investigators found SIRs for breast cancer of 1.05 (95% CI = 0.84 to 1.3), 0.96 (95% CI = 0.59 to 1.46), 0.79 (95% CI = 0.44 to 1.29), 0.66 (95% CI = 0.24 to 1.44), and 0.47 (95% CI = 0.01 to 2.63) among women with moderate low vision, severe low vision, profound low vision, near-total blindness, and total blindness, respectively. Our own data did not provide sufficient information on intensity of light exposure during night work, but future epidemiologic investigations could define such dose–response estimates in humans.

Several mechanisms have been hypothesized to explain the association of decreased melatonin levels and increased cancer risk. Although the presence of specific melatonin membrane receptors, MT1 (a high-affinity receptor) and MT2 (a low-affinity receptor), has been demonstrated for some time *(44,45)*, nuclear receptors also have been found (RZR α [retinoid Z receptor α] and RZR β [retinoid Z receptor β]). Only recently, an attempt was successfully undertaken to clarify whether melatonin is able to influence MCF-7 cell proliferation by modulating cell cycle kinetics in MCF-7 human breast cancer cells *in vitro (3)*. Melatonin increases the expression of p53. A receptor interaction with RZR nuclear melatonin receptors may cause an arrest of MCF-7 cells in the G_0/G_1 phase of the cell cycle pathway that is mediated by the p53 pathway. Such receptor-mediated effects on hormone-dependent cancers had been proposed before, yet these are the first important steps toward clarification. As a potential free-radical scavenger, melatonin may also protect against cancer by shielding DNA from oxidative damage *(46)*. Other recent work *(23)* suggests that melatonin acts as an immune-modulating agent, since it affects thymic endocrine activity and interleukin 2 by means of metabolic zinc pool turnover in mice. Finally, disturbances in sleep rhythm can directly promote chemically induced liver carcinogenesis in rodents *(16)*. This is the first rodent model in which light-induced circadian clock suppression directly exerted a cancer-promoting effect on the liver.

The results from our study are compatible with a possible oncogenic effect of nighttime light exposure through the melatonin pathway. Although we did not validate self-reported duration of rotating nightshifts, it is likely that our results are accurate, because other self-reports have been highly accurate in this cohort *(47)*, and previous validations of similar questions (e.g., electric blanket use) *(48)* have shown reasonable reproducibility. Moreover, the prospective design of our study eliminates recall bias. On the other hand, assessment of exposure status with regard to working on rotating night shifts can only be a rough estimate, and misclassification is likely to occur. Since there are

more than two comparison groups, even random misclassification may bias the study results in any direction *(49)*. We are concerned that the way we asked for lifetime night work on the 1988 questionnaire may have misled some of the nurses. In the United States, a substantial portion of nurses worked on permanent night shifts during the period of our investigation *(50)*. These nurses may not have classified themselves as working on rotating shifts, but instead as never-rotating workers, because they may have perceived permanent night work as nonrotating, as opposed to rotating night work. Measurements of melatonin profiles in night workers follow an unidentifiable rhythm and show great variability in the timing of melatonin secretion, thus suggesting that no uniform adaptation of the melatonin rhythm can be achieved in permanent night shift work *(51,52)*. Because permanent night workers do not completely entrain to their circadian shift rhythm *(53)*, the average serum melatonin levels among these women would be lower than those of never workers. According to the "melatonin hypothesis," which states that certain aspects of modern life, such as light at night, may increase breast cancer risk *(12,54)*, the permanent night worker would, therefore, be at higher breast cancer risk than a never worker. However, rotating shift workers would still remain at the highest overall risk, because they cannot entrain to their circadian shift rhythm at all and, therefore, would have the lowest melatonin levels. Thus, such misclassification would bias our results toward the null.

Reports about a reduction of plasma melatonin concentration as a general characteristic of healthy aging are conflicting *(4,8,55,56)*. We controlled for age in various ways, but our results did not change substantially in any of these analyses.

Another potential limitation in our study is that women who work more frequently on night shifts may differ from women who do not in a way that influences risk of breast cancer for which we were not able to control. Even though we controlled for known potential confounding factors, there may still be uncontrolled confounding, such as hormone levels, stress, or other differences in lifestyle. Yet whether to treat factors, such as hormone levels or stress, as confounding factors or rather as intermediate factors that represent a step in the causal chain between exposure and disease would need to be considered.

In conclusion, working on rotating night shifts was associated with a moderately increased breast cancer risk among the female nurses in our cohort. The findings from our study, in combination with the results of earlier work, reduce the likelihood that this association is solely due to chance. Since breast cancer constitutes a huge disease burden in the United States and since a substantial portion of workers engage in shiftwork, it will be necessary to further explore the relationship between light exposure and cancer risk through the melatonin pathway.

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