Schernhammer Eva, MD, DrPH
Professor of Epidemiology
Lecturer of Medicine
Harvard Medical School
Harvard School of Public Health
Brigham and Women's Hospital
Channing Laboratory
181 Longwood Avenue
Boston, MA 02115
Phone: 617-525-4648
Email: eva.schernhammer@channing.harvard.edu

Institution to which the award was made:
Brigham and Women's Hospital
75 Francis Street
Boston, MA 02115

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Minimizing Cancer Risk in Shift Workers

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Co-investigators: Susan Hankinson, Bernard Rosner

Project director: Eva Schernhammer

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List of Terms and Abbreviations.

WHO	World Health Organization
IARC	International Agency for Cancer Research
M	Million
NHS2	Nurses' Health Study II
NHS	Nurses' Health Study
SCN	Supra-chiasmatic nuclei
aMT6	6-sulfatoxy melatonin
BMI	Body-mass Index
ER	Estrogen receptor
PR	Progesterone receptor
ERPR	Estrogen-progesteron receptors
TMA	Tissue micro-array

Abstract.

Occupational safety and health issues that were addressed. Observational studies have consistently associated rotating shift work with increases in cancer risk, prompting the WHO in December 2007 to classify night shift work a probable carcinogen – the main operating mechanism being assumed circadian disruption by means of melatonin suppression [1]. Still, while cancer is one of the bleakest disease endpoints, a broader range of outcomes is affected: increases in cardiovascular risk; peptic ulcer disease; chronic fatigue and various sleep problems; higher body weight due to abnormal eating habits and/or metabolism; a higher abortion and miscarriage rate as well as lower pregnancy rates; higher rates of substance abuse and depression; a greater number of vehicle accidents; and a higher divorce rate have been reported in shift workers. Further, chronic sleep deprivation leads to mistakes that also affect the health of others.

Importance of the problem. Almost 15 million Americans regularly work alternate shifts, including evening (4.7 M), night (3.2 M), and rotating shifts (2.5 M) or other employer-arranged irregular schedules [2]. Shift work is integral to the modern work force, which spans all age and ethnic groups. While fewer women than men work alternate shifts (12.4% vs. 16.7%), there is a substantially larger proportion of female night workers in health care and social assistance professions, including nursing. Because no definitive strategy currently exists to reduce the cancer risk associated with shift work-induced circadian disruption, determining what aspects of shift schedules are most detrimental to health is the next frontier in shift work and disease prevention. To date, no study has examined how specific aspects of shift schedules including length, frequency of rotation, and hours worked per week interact and relate to cancer risk. Moreover, whether there is an age range at which people are particularly vulnerable to the effects of circadian disruption also remains unclear. Future research would benefit from a clear and complete description of work schedules and their effects on human cancer risk.

Approach. In 2009, we have added a battery of shift work questions to the main NHS2 questionnaire. We newly assess specific aspects of the nurses' work schedule including types of shift schedules and changes throughout their professional career. In addition, other factors of potential relevance to circadian disruption were assessed, including morningness-eveningness and sleep duration during each of the various life time periods. To evaluate the hypothesis that certain aspects of shift work schedules are more strongly associated with breast cancer risk than others, we will use both a cohort and cross-sectional study approach. We estimate 1,108 newly diagnosed cases of breast cancer between 2009 and 2013. We will use these cases, as well as prevalent cases to enhance the power of subanalyses, for an estimated total of 4,295 women with a diagnosis of breast cancer through 2013. Because mechanisms that link shift work to breast cancer risk are similar for other cancers, the findings from this proposal are likely to affect not only the risk of breast cancer but that of other cancers and men as well. We expect that the impact of our results will extend well beyond the specific aims we currently propose. Ultimately, as time progresses and nurses become older, this data and the newly added battery of shift work questions will lay the foundation for a comprehensive and unique study of shift work on the health needs of older workers.

Key Findings. Over the past five years, our work using data from the Nurses' Health Study cohorts has produced landmark findings and generated provocative novel hypotheses related to the health effects of night work and sleep deprivation: We showed that night shift work increases the risk of a number of different cancers (most notably breast, but also colorectal, endometrial, and lung cancer), after extended periods of night work, and that risk appears to wane over time once night work stops. In addition, we have linked rotating night shift work – through its profound effects on metabolism and weight (i.e. obesity) to other major chronic diseases, including cardiovascular disease, metabolic syndrome, hypertension, stroke, endometriosis, and type 2 diabetes; we found effect variation by ethnicity,[3] and we developed melatonin as a biomarker for the circadian system, which we subsequently linked to cancer [4] and other chronic disease risk (e.g., diabetes,[5] hypertension [6]). We showed that these effects are mediated by variants in clock genes [women homozygous for the minor allele (AA) in the circadian gene NPAS2 Ala394Thr, with ≥ 24 months of shift-work having a 2.83-times higher breast cancer risk compared to homozygous AA women with < 24 months of shift-work (95% CI = 1.47-5.56)][7] and a variety of biomarkers including melatonin. We studied the precise associations between light exposure and the circadian system, delineating that, and how, consecutive number of night shifts matters, and how chronotype mitigates these effects. Lastly, we have established that shift work can affect

reproduction, demonstrating menstrual cycle changes and higher spontaneous abortion rates in night shift workers.

How the results can be utilized in the work place. This project's results will not have any direct impact on the NHS2 cohort, but the findings may benefit women who work rotating night shifts in the future. Ultimately, as outlined in detail in our proposal, they are geared towards providing a frame work for health policy makers on which they may chose to base decisions to change shift work schedules in order to minimize breast cancer risk in night workers. Our findings relating light to melatonin levels suggest that certain thresholds of light determine duration and intensity of light exposure needed to negatively affect the circadian system. Chronotype is an important factor, which should be considered in future studies of shift workers and health.

SECTION 1

Highlights/Significant Findings.

Aim 1: We described the baseline characteristics of the NHS2 cohort, with a special emphasis on their occupational history as it pertains to night shift work, including their sleep patterns. In addition, we examined associations between various aspects of occupational history and breast cancer risk. Perhaps the most interesting finding of these studies was that – in addition to the consistently elevated risk of breast cancer we observed after longer durations of shift work – the risk seemed to wane once women quit working night shifts.

Aim 2: We set out to study how various relevant aspects of shift work related to urinary melatonin levels – a marker of the circadian system if measured in first morning urine. The most important observation to that end was that immediacy of shift work more so than long-term shift work appears to influence the circadian system, and our results emphasized the importance of body mass index

Translation of Findings.

Our findings readily translate into field application as they confirm a previously observed higher risk of breast cancer among women with longer duration of night shift work. Most importantly, the updated long-term findings in the NHS and NHS2 cohorts have important implications for future IARC evaluations of the shift work and breast cancer association. Our results may serve to put the literature into the context of short-term versus long-term effects, and suggest that there may be a period of increased risk, that wanes with time.

Outcomes/Relevance/Impact.

We were funded to comprehensively describe the shift work profile of nurses participating in the NHS2 cohort and to evaluate in detail associations of various shift schedule characteristics with breast cancer risk. Breast cancer is the main cancer to which shift work has been related and reason for the WHO ruling describing shift work as a probable carcinogen [1]. With the exception of the melatonin aims, all aims are completed or the data are being analyzed and manuscripts will be submitted over the next year.

In NHS2, the younger age of the cohort as well as updated exposure information throughout follow-up allowed us to assess breast cancer risk with more recent shift work exposure. We found a strong positive association with breast cancer among the women who had accumulated 20+ years of rotating night shift work early in their careers, in their 20's and 30's. Those participants also contributed to the 20+ year shift work category in the cumulative shift work measure, but were mixed with women who had different patterns of shift work accumulation after baseline, likely attenuating this association. Nonetheless, the cumulative measure of shift work was consistent with a marginally significant increased risk of breast cancer. We saw no association between rotating night shift work and breast cancer incidence over the full 24 years of follow-up in the NHS cohort. Due to the older age range of the women in NHS, we may have captured primarily post-retirement time with the expansion of follow-up (only 3% were still working rotating night shifts in 1996) and likely very little additional shift work was accumulated. This may in part explain the lack of an association we observed in NHS with the additional 14 years of follow-up. To explore this further, we conducted analyses restricting to ever shift workers, and observed a significant trend with longer time since stopping shift work being associated with greater breast cancer risk in these women. In 2009, women in NHS2 were asked about their primary work schedule during the age ranges of 20-25, 26-35, 36-45 and 46+. For the person-time attributed to the >16 years of time since stopping shift work category, 94% reported being rotating night shift workers before age 35 (compared with 67% for current, 82% for <=8 years since stopping, and 89% for 9-16 years since stopping shift work). Hence, greater time since stopping shift work may be a marker for shift work performed at young adult ages.

SECTION 2

Scientific Report

Background.

Breast cancer is the most common cancer among women worldwide [8]. The noticeably higher prevalence in industrialized nations compared with developing countries suggests that environmental aspects of modern society may play an important role in breast cancer etiology [9]. Disruption of the circadian system with exposure to light during the environmental nighttime hours as with occupational night shift work schedules has been hypothesized to influence carcinogenesis through suppression of melatonin, modulation of sex hormones, or altered expression of peripheral clock genes [10-13]. Supporting epidemiologic studies as well as strong mechanistic data from animal studies led the International Agency for Research on Cancer (IARC) to classify night shift work that involves circadian disruption as probably carcinogenic to humans (group 2A) in 2007 [14].

Since the IARC report, five meta-analyses have been published in an effort to summarize the growing literature on the association between night shift work and breast cancer risk, with varying approaches and conclusions. He et al, Wang et al, and Jia et al found moderate increased risk of breast cancer with night shift work, reporting pooled estimates in the range of 1.19-1.20 [15-17]. The overall estimate from Kamdar et al was similar in magnitude but was marginally significant.[18] Based only on case-control studies, Ijaz et al reported a 9% increased risk of breast cancer for every 5 years of night shift work [19] and He et al reported a 16% increased risk of breast cancer for every 10 years of shift work [15]. Each of the meta-analyses cited significant heterogeneity across studies, with differing results by type and quality of study. For all, there was insufficient evidence from cohort studies alone to draw a conclusion about the relationship of shift work and breast cancer risk.

Among the three cohort studies published since the IARC decision, two found statistically significant positive associations [20, 21] and one found no evidence of an association [22]. However, they were limited by their small sample sizes (Knutsson et al N=4036, Akerstedt et al N=13,656) or short follow-up time (Pronk et al less than 5 yrs for self-reported shift work exposure). The Nurses' Health Study (NHS) and Nurses' Health Study II (NHS2) were among the few cohort study analyses with prospectively collected shift work exposure that informed the 2007 IARC decision [23, 24].

Mechanisms Linking Shift Work to Health: Circadian Disruption

The 'biological clock' in the suprachiasmatic nucleus (SCN) in the hypothalamus controls numerous functions that follow regular, daily patterns (or 'circadian' rhythms), including: the sleep/wake cycle; body temperature; blood pressure; renal function; hormone secretion; and immune function. In addition, the SCN appears to be crucial for behavioral and physiological adaptation to 24-hour food availability. Environmental lighting powerfully influences this circadian system in humans. Light entrains the biological clock, as the SCN receives environmental dark/light information directly from the retina. This mechanism appears to have evolved to detect changes in day length/season (for functions like migration and hibernation). Melatonin (5-methoxytryptamine) is a hormone produced primarily by the pineal gland, which follows an approximately 24-hour rhythm. Hence it is widely regarded as a marker of circadian rhythmicity intimately linked to the circadian system, which, in addition, demonstrates cancer-protective properties [25]. Melatonin secretion is controlled by the SCN and its levels may be considered the main humoral reflection of SCN function. The intrinsic period of SCN in humans is slightly greater than 24 hours [26] and is modulated by the temporal pattern of light and dark on the retina as the main stimulator. Direct light exposure at night suppresses melatonin production (34), which as a proxy of circadian disruption [27], has been proposed as the main explanation for the health effects associated with night-shift work [28, 29]. Mechanisms for the cancer-protective activity of melatonin relate to its influence on other hormones, as well as melatonin's antioxidant and immune-modulatory effects which appear to also slow down the aging process and, more generally, delay mortality. Melatonin production is acutely sensitive to light exposure. It is currently thought that nightly exposure to artificial light erodes and shifts the natural melatonin peak, thereby increasing disease risk. Not surprisingly, this complex circadian system has a genetic component: Mutations in the fruit fly *Drosophila melanogaster* that affect the circadian clock [30], and

later, human twin studies of abnormal circadian phenotypes showing that much of morning and evening preference is heritable [31], led to the identification and characterization of clock genes responsible for circadian behavior [32].

Melatonin

Melatonin (5-methoxytryptamine) is a hormone with cancer-protective properties [33], which can also enhance endothelial function [34, 35] and reduce inflammation [36]. It is secreted predominantly during darkness (i.e., at night), with little production throughout the day [37]; however, its nocturnal production is also suppressed within minutes if light reaches specialized retinal photoreceptors at night (e.g., during night shift work).[38] In the general population, measurements of melatonin's primary urinary metabolite, 6-sulfatoxymelatonin (aMT6s), in first morning urine samples closely correlate with plasma melatonin levels measured during the previous night [39, 40], and some epidemiologic studies have associated lower levels of aMT6s with an increased risk of breast cancer [41-43] and several cardiovascular disease markers [5, 44, 45] [46]. In addition, body size throughout life (beginning at birth and including adult body mass index; BMI) has been related to premenopausal breast cancer risk [47, 48] and cardiovascular disease [49, 50]. Moreover, inverse associations between adult BMI and aMT6s levels have previously been observed [5, 51, 52]. Still, previous studies have not examined the relation of earlier-life body size on melatonin levels in adulthood.

Because no definitive strategy currently exists to reduce the cancer risk associated with shift work-induced circadian disruption, determining what aspects of shift schedules are most detrimental to health is the next frontier in shift work and disease prevention. To date, no study has examined how specific aspects of shift schedules including timing of meals, napping behavior, and individual chronotype interact and relate to actual light exposure.

In this study we explored the effects of rotating night shift work as modifiable risk factors for melatonin secretion in a sample of women from the NHS2 cohort and studied associations between this occupational exposure and breast cancer risk. With double the follow-up time and twice as many breast cancer cases compared to our previous publication, we were now also able to investigate timing of risk and as well as breast cancer tumor markers.

Specific Aims. The following specific aims were proposed in the original application:

Specific Aim 1 (Shift work aims):

a. We will comprehensively describe the shift work profile of nurses participating in the NHS2 cohort; they comprise 70% of all registered nurses from 14 selected states within the US.
b. Night and early morning shifts are associated with a higher risk of breast cancer than are day and evening shifts.
c. The association between number of night shifts per month and breast cancer risk is linear.
d. The association between number of years on rotating night- or early morning shifts is linear, independent of age.
e. Exposure to shift work (as defined above) earlier in life is associated with a higher risk of breast cancer.
f. These associations are strongest in individuals with fewer hours of average sleep and those who describe themselves as morning persons.

Specific Aim 2 (Melatonin aim):

a. We propose to investigate, in a subset of women in whom melatonin has already been measured (funded through CA050385), the associations between various shift work aspects and melatonin levels, to provide a biologic rational for the observations that will emerge in Aim 1. We expect melatonin levels to be lowest in women who followed the shift rotation that was most strongly related to breast cancer risk.

Procedures and Methodology.

Study Population

Eligible study participants included women from the NHS, which was established in 1976 when 121,701 female registered nurses, ages 30-55, returned a mailed questionnaire with detailed information about their lifestyles, occupational and environmental exposures, medication use, and medical conditions. Further, the NHS2 was established in 1989 when 116,430 female registered nurses, ages 25-42, returned a similar questionnaire. Participants in both cohorts have provided updated information biennially thereafter, and cumulative follow-up in the cohorts is >90%. Both studies are currently ongoing. The Institutional Review Board of Brigham & Women's Hospital (Boston, MA) approved both studies, and all participants provided informed consent through the return of the initial questionnaire.

In NHS2, a first morning urine collection occurred between 1996-1999; collection methods and laboratory measurement of melatonin secretion have been described elsewhere [11, 53, 54]. Briefly, urine samples were assayed for aMT6s concentrations as part of previous nested case-control studies of breast cancer[41] and hypertension [53, 55], with aMT6s levels creatinine standardized (aMT6s concentrations divided by concentration of creatinine) to account for differences in urine sample concentrations [51].

Study Design and Study Procedures
All study materials were mailed to participants. The study was approved by the Harvard School of Public Health and the Brigham and Women's Hospital Human Subjects Committee, and written informed consent was obtained from subjects. Rotating night shift work duration was assessed through self-reported answers to the following question: "What is the total number of years during which you worked rotating night shifts (at least 3 nights/month in addition to days/evenings in that month)?" in 1988 for NHS and in 1989 for NHS2. In NHS2, a cumulative shift work measure was determined by adding baseline history to subsequently updated shift work information (from a question asking about the total number of months having worked rotating night shifts in the prior years), collected in 1991, 1993, 1997, 2001, 2005 and for a subset or women with email addresses who were sent an online questionnaire in 2007 (N=35,418, 34% of participants active in 2007). In addition, the 2001 questionnaire asked about shift work in the period 1995-1997. Answers were very similar to those given on the 1997 questionnaire (Pearson's $r=0.53$, $p<0.0001$), indicating that recall of shift work information was reasonably comparable to real-time collected information. Of those asked about current shift work exposure in 2007, only 8% were still working rotating night shifts. Therefore, for 2009 and subsequent cycles when shift work duration was not assessed, zero shift work was assumed.

Outcome assessment
Breast cancer cases were identified as having occurred during the period June 1, 1988 to June 1, 2012 (NHS) and June 1, 1989 to June 1, 2013 (NHS2). Nurses who reported 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 subjects were included in the analysis if they were corroborated by a phone interview or written confirmation 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 non-respondents from each two-year questionnaire. Only confirmed invasive breast cancers (i.e. excluding breast cancer in situ) were used in these analyses.

For secondary analyses of breast cancer by hormone receptor status, estrogen receptor (ER) and progesterone receptor (PR) status were determined by immunohistochemical staining of tumor tissue. The breast cancer tissue collection, tissue microarray (TMA) construction, and staining and reading for tumor markers has been described in detail elsewhere [56]. When TMA results were unavailable, medical record documentation of ERPR status was used instead.
Study population for analysis

At baseline (1988 in NHS and 1989 in NHS2), there were 103,415 participants in NHS, and 116,430 women in NHS2. Of these, participants with prior cancers except non-melanoma skin cancer (NHS: 7,957 (8%); NHS2: 1,050 (1%)) and those who did not answer the initial shift work history question (NHS: 16,942 (16%); NHS2: 581 (<1%)) were excluded. The remaining datasets for analysis comprised 78,516 women, ages 42-67, in NHS and 114,559 women, ages 25-42, in NHS2.

Results.

Aim 1:

a. Shift work patterns among nurses in NHS2 (Ramin C et al. [57]). In our comprehensive statistical analyses based on all respondents to the 2009 NHS2 questionnaire, aimed at describing the lifetime occupational history of this unique cohort of US nurses, as it relates to their detailed shift work and sleep behavior as well as chronotype (i.e. eveningness-morningess), we found that targeted lifestyle interventions for night shift workers should potentially take into account age when shift work is performed, as risk factors may vary over the course of a woman's shift work career. Specifically, we examined the association of night shift work history and age when night shift work was performed, with cancer and cardiovascular disease risk factors among 54,724 women in NHS2, 72% of whom had ever worked night shifts. We further examined these associations by age (20-25, 26-35, 36-45, and 46+ years) at which shift work was performed. We found that women who had ever worked night shifts were more likely to be obese (OR=1.37, 95%CI, 1.31-1.43), to drink more coffee (≥131 mg/day) (OR=1.16, 95%CI, 1.12-1.22) and to consume more total calories (≥1715 kcal/day) (OR=1.09, 95%CI, 1.04-1.13). They were also more likely to currently smoke (OR=1.30, 95%CI, 1.19-1.42) and to experience shorter sleep durations (≤7 hours of sleep/day) (OR=1.19, 95%CI, 1.15-1.24) compared to never night shift workers. These estimates varied depending on age at which night work was performed, with a suggestion that night shift work before age 25 was associated with fewer risk factors compared to night shift work at older ages. Hence, in sum, our results indicate that night shift work may contribute to an adverse chronic disease risk profile, and that *risk factors may vary depending on the age at which night shift work was performed*. These findings require further investigation, which we will be able to do in the current proposal.

b. Chronotype, shift work, and chronic disease risk (Ramin C et al. [58] and Vetter C et al. [59]). We examined associations between chronotype and breast cancer risk in the NHS2,[58] and found that participants who self-reported as neither morning nor evening chronotype had a 27% increased risk of breast cancer (multivariable-adjusted OR= 1.27; 95% CI, 1.04-1.56), compared to definite morning types. None of the other chronotypes were significantly associated with breast cancer risk. However, there was suggestive effect modification of the association between chronotype and breast cancer risk by history of night-shift work: among women without a history of rotating night shift work, those who were neither morning nor evening chronotype had a 46% increased risk of breast cancer compared to definite morning types (number of cases=534; multivariate-adjusted OR=1.46, 95% CI 1.01-2.12); among women with neither chronotype who had 1-9 years of shift work history, the relative risk of breast cancer was increased by 26% above definite morning types (number of cases=1119; multivariable-adjusted OR=1.26, 95% CI=.97-1.64), and among women with neither chronotype and ≥10 years of shift work history, breast cancer risk decreased by 8% when compared to women with definite morning type (number of cases=170; multivariable-adjusted OR=.92, 95% CI=.45, 1.86). Interestingly, in a recent analysis with type 2 diabetes (T2D) as the endpoint (also NHS2, Vetter et al. [59]), we, too, see that *chronotype modulates chronic disease risk, and this effect varies strongly depending on working times*. Specifically, compared to intermediate chronotypes, morning types had a significantly reduced risk of T2D (OR=0.74, 95%CI 0.66-0.83), while the risk of T2D was significantly elevated among evening types (OR=1.39, 95%CI 1.20-1.61); these associations were attenuated in multi-variable adjusted models, accounting for demographic and lifestyle factors, and family history of diabetes (morning types: OR=0.87, 95%CI 0.77-0.98; evening types: OR=1.04, 95%CI 0.89-1.21). By contrast, among women who never worked night shifts, evening types had a significantly elevated risk of T2D (OR=1.51, 95%CI 1.13-2.02), while no significant association was observed for morning types (OR=0.81, 95%CI 0.63-1.04). With increasing years of night shift work exposure, the association reversed: evening chronotypes were less likely (1-10 yrs: OR=0.93, 95%CI 0.76-1.13; 10+ yrs: OR=0.87, 95%CI 0.56-1.34), while morning chronotypes were more likely to have T2D (1-10 yrs: OR=0.84, 95%CI 0.72-0.98, 10+ yrs: OR=1.15, 95%CI 0.81-1.63; $P_{interaction}$<0.001).

c. Various aspects of shift work and breast cancer risk (Gu et al. [60]; Wegrzyn LR et al., submitted). We initially focused on exploring associations between duration and timing of rotating nightshift work and breast cancer risk and *confirmed our hypothesis that earlier life exposure to shift work is associated with a higher risk of breast cancer*. Specifically, among the 116,343 nurses participating in NHS2, we documented 2,869 incident invasive breast cancer cases. We find that baseline 20+ years of rotating nightshift work is associated with a significantly increased risk of breast cancer (RR_{20+yrs} =2.11, 95%CI 1.21-3.66; p_{trend}=0.22).

Updated rotating night shift work exposure appears to also be associated, albeit non-significantly, with a modest increase in risk of breast cancer (RR_{20+yrs}=1.33, 95%CI 0.93-1.89; p_{trend}=0.68). We put these data into the context of recent analyses form the NHS cohort; compared to our initial analysis within that cohort, which was based only on 10 years of follow-up and showed that 30+ years of rotating nightshift work was associated with a 36% significant increase in breast cancer risk, we added 12 years of follow-up, which were accrued for the most part after nurses' retirement. In these extended analyses, 30+ years of rotating night shift work was no longer associated with breast cancer risk (multivariable RR_{30+yrs}=0.95, 95%CI 0.77-1.17; p_{trend}=0.95), and only insignificantly associated with breast cancer mortality (multivariable HR_{30+yrs}=1.50, 95%CI, 0.95-2.36). Taken together, our results suggest that long-term rotating night shift work particularly early in career may be associated with an increased risk of breast cancer, which appears to diminish after nightshift work ceases.

Aim 2:

d. Melatonin, shift work, and breast cancer risk (Wegrzyn LR et al., in preparation; Ramin C et al. [61], submitted; Brown et al. [62]). Using first morning urine samples, we determined melatonin levels and conducted a range of analyses pertaining to the aims of our previous application. For example, and of relevance to the current proposal, we evaluated associations of birth weight, body shape (or somatotype) at ages 5 and 10, BMI at age 18 and adulthood, weight change since age 18, waist circumference, waist to hip ratio, and height with creatinine-adjusted morning urinary melatonin (6-sulfatoxymelatonin, aMT6s) levels among 1,343 healthy women (aged 32-53 at urine collection, 1996-1999) in the NHS2 (Ramin C et al. [61]). We found that adult BMI was inversely associated with aMT6s levels (mean aMT6 levels=34 vs. 50 ng/mg creatinine, comparing adult BMI ≥30 vs. <20 kg/m^2; P_{trend} <0.0001); however, other measures of body size were not related to aMT6s levels after accounting for adult BMI. Night shifts worked prior to urine collection, whether recent or cumulatively over time, did not modify the association between adult BMI and aMT6s levels (e.g., $P_{interaction}$=0.72 for night shifts worked within two weeks of urine collection). While our results suggest that adult BMI, but not earlier measures of body size, is associated with urinary aMT6s levels in adulthood, and that these observations did not vary by night shift work status, they do point out *the need to study these associations in younger individuals*. Further, in a case-control study nested within NHS2, among 600 predominantly premenopausal women with incident breast cancer and 786 matched controls, while overall, we found that melatonin levels were not significantly associated with the risk of total breast cancer (top (Q4) vs. bottom (Q1) quartile of aMT6s, OR=0.91, 95% CI: 0.64, 1.28, P_{trend}=0.38) or subtypes including invasive, in situ, or ER+/PR+ breast cancer, or findings varied modestly by time between urine collection and diagnosis: the OR of total breast cancer among women with ≤5 years between urine collection and diagnosis was 0.74 (Q4 vs. Q1, 95% CI: 0.45, 1.20, P_{trend}=0.09), and 1.20 with >5 years (Q4 vs. Q1, 95% CI: 0.72, 1.98, P_{trend}=0.70). Hence, our data do not support an overall association between urinary melatonin levels and breast cancer risk, although *the potential short-term protective effect of melatonin warrants further exploration*.

Discussion and Conclusion.

Shift work is an indispensable part of modern life. In the United States, almost 15 percent of full-time wage and salary workers work alternative shifts [63]. A growing body of literature provides evidence linking night-shift work with increased risk of several malignancies including breast cancer [64-73], endometrial cancer [74], prostate cancer [75, 76], colorectal cancer [77], and lymphoma [78]. Night-shift work is also believed to be associated with other health problems such as cardiovascular disease, diabetes, gastro-intestinal disorders, and sleep disorders [79-81]. One hypothesis is that night shift work impacts the health of shift workers through disruption of circadian rhythm [82].

Short of abolishing alternate working schedules from modern society, several strategies could be envisioned to reduce the health risk associated with shift work-induced circadian disruption. Such strategies could entail changing light sources or filtering short wavelength (blue) light (the most disruptive to our circadian system) by having night workers wear goggles could offer another prevention strategy, albeit perhaps the least practical. Further, determining which factors relate to shift schedules and what aspects of shift schedules are most detrimental to health are the next frontiers in shift work and disease prevention. Future research would benefit from a clear and complete description of work schedules and their effects on human health. We have taken an important step towards that direction by essentially providing the most thorough description of the effect of light at night on melatonin and interrelated factors in a population of shift workers.

In our study we found that breast cancer risk is consistently elevated among women with long durations of rotating night shift work history. These risks did not seem to vary depending on ER/PR status of the breast tumor We also found suggestive evidence in support of a reduced risk after women quit working night shifts and that circadian markers are Our findings relating light to melatonin levels, finally, suggest that adult BMI, not earlier-life body size, may be an important mediator of the circadian system (i.e., melatonin) and influence later-life chronic disease risk. Lastly, even though chronotype is an important factor which merits consideration in future studies of shift workers and health, we found that overall, chronotype was not associated with breast cancer risk. However, we observed a modestly increased risk among neither morning nor evening chronotypes, which may indicate circadian disruption as a potentially underlying mechanism.

Publications.

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Wegrzyn RL, Tamimi RM, Rosner BA, *et al*. Urinary 6-sulfatoxymelatonin and mammographic breast density in premenopausal and postmenopausal women in the Nurses' Health Study II. (in preparation; Aim 2).

Wegrzyn RL, Rosner BA, Tamimi RM, *et al.* Rotating night shift work and mammographic breast in premenopausal and postmenopausal women in the Nurses' Health Studies. (in preparation; Aim 2)

Inclusion of gender and minority study subjects.

All subjects in this study are women. This is appropriate because the cohort is already established and was originally limited to women as breast cancer was the primary interest; breast cancer in men is very rare while breast cancer is a leading cause of death among U.S. women. The population is also predominantly white, reflecting the ethnic background of women entering nursing in the U.S. in the 1970's and 1980's. There were no exclusions based on race in the original enrollment.

According to the May 1997 supplement to the Current Population Survey (CPS), a greater proportion of blacks (20.9 percent) worked alternative shifts than either whites (16.1 percent) or Hispanics (16.0 percent) [83]. Furthermore, even on the same job, it is usually the case that more blacks than whites work on alternative shift. Thus, despite the lack of minorities within this cohort, our research will serve to a great extent members of minority groups, particularly blacks, based on the assumption of no ethnic differences in the underlying patho-physiology.

Inclusion of children.

By the nature of this project, only adults were eligible to participate. Moreover, the ultimate goal of this project is to reduce breast cancer risk in night workers. Breast cancer is extraordinarily rare in children, thus studying breast cancer in children is virtually impossible.

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