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Effects of Light at Night on the Circadian System in Nurses

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List of Terms and Abbreviations.

WHO	World Health Organization
RPI	Rensselaer Polytechnic Institute
NHS2	Nurses' Health Study II
aMT6	6-sulfatoxy melatonin
NS	Night shift worker on night shift
DS	Day shift worker
OS	Night shift worker off shift
P	P-value
H-LEA	Harvard Light Exposure Assessment
SCN	Suprachiasmatic nucleus
LAN	Light at night
LAD	Light at day
DNA	Desoxyribonucleic acid

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.

Approach. During the initial phase of this project, the Lighting Research Center at RPI (under the direction of Mark Rea) developed in a collaborative effort a Daysimeter, which was refined in three major steps until it was found acceptable by nurses. The Daysimeter represents an improvement compared to conventional lightmeters because its blue sensor is calibrated toward short-wavelength visual light and because it is head-mounted (rather than wrist-mounted) to avoid significant errors in weighting retinal flux. Further, the Daysimeter has an internal clock unlike any other conventional illuminance meter and can, therefore, characterize both the time and the duration of circadian light exposure. Applying this new device, together with 4-hourly urine collections over three days for melatonin measurements in a large scale prospective cohort study like the NHS2 is unprecedented in literature and vital for progress in light research. Overall, 148 women participating in NHS2 were recruited and successfully completed a 7-day light and urine collection scheme.

Key Findings. 24-hour light exposure was lower among rotating-shift workers on night shift (NS) compared to day shift (DS) workers. Average light exposure over night was significantly higher at NS compared to DS and night workers off shift (OS). Compared to DS or OS, in NS, the amplitude of melatonin's primary urinary metabolite, aMT6, was significantly lower and its acrophase was delayed by one hour. Both intensity of light exposure overnight and activity index were significantly and inversely associated with aMT6 ($P<0.01$). Duration of light exposure above a certain lux threshold was also positively associated with melatonin levels, e.g., each hour exposed to more than 20 lux light was associated with 0.06 decrease in log-transformed aMT6. Next we found that, in multivariate analyses, chronotype was statistically significantly associated with aMT6 level ($P < 0.001$). We further observed that definite morning types were 2.7 hours phase advanced and had the highest melatonin amplitude compared to definite evening types (P trend < 0.001). Taking daytime naps was associated with lower overnight melatonin secretion. However, this association was not statistically significant after adjustment for possible cofounders. Late-night meal also was not independently associated with aMT6 level. Number of night shifts during the past 30 days and number of years having worked rotating shifts over the past 20 years were not statistically significantly associated with aMT6. We observed no statistically significant association between aMT6 and alcohol use, smoking, non-steroidal anti-inflammatories, ß-blockers, anti-depressants, and hormone therapy. Lastly, using the light measures obtained from the Daysimeter, we validated a newly developed paper-based questionnaire for self-reported light exposure (H-LEA).

How the results can be utilized in the work place. The Daysimeter is a readily applicable instrument which has been tested in the field in ours as well as other applications since its initial conception. The H-LEA questionnaire is a practical and readily applicable method of assessing light exposure in large scale epidemiologic studies to study the effects of shift work on health. 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: The Daysimeter was successfully developed and implemented, thereby providing the basis for our investigation. This lightmeter represents an improvement compared to conventional lightmeters primarily because it is calibrated toward short-wavelength visual light and because it is worn head-mounted (rather than wrist-mounted) to avoid significant errors in weighting retinal flux. In addition to spectral, spatial and intensity calibrations, the circadian light meter has an internal clock unlike any other conventional illuminance meter and can, therefore, characterize both the time and the duration of circadian light exposure.

Aim 2: As hypothesized, overall 24-hour light exposure was lower among the rotating-shift workers on a NS compared to the DS workers. Further, average light exposure over night was significantly higher at NS compared to DS and OS. In addition, the amplitude of aMT6 was significantly lower at NS compared to DS and OS we observed a one-hour shift delay in aMT6 associated with NS. The activity-index phase was significantly disrupted at NS with an 8-hour phase difference and an amplitude of 8.6 compared to 2.4 and 2.7 for OS and DS, respectively. Intensity of circadian light exposure was significantly associated with aMT6 ($P < 0.0001$). When we stratified this analysis by time of light exposure, this association remained significant only for overnight light exposure category ($P = 0.0078$). Activity index was also significantly associated with melatonin level ($P < 0.0001$). This association was significant regardless of timing of physical activity. Duration of light exposure above certain lux thresholds was also positively associated with melatonin levels. For example, each hour exposed to more than 20 lux “circadian” light was associated with 0.06 decrease in log-transformed aMT6.

As a **secondary objective** of this aim we developed and successfully validated a paper-based questionnaire for self-reporeted light exposure (the H-LEA questionnaire, which we validated with actual light measures obtained from the Daysimeter).

Aim 3: We observed a significant trend towards higher aMT6 levels associated with morning chronotype, and there was a 2.7 hours phase advancement comparing definite-morning type and definite-evening type (P trend < 0.001). The amplitude of aMT6 was significantly higher with increase in morning-type trait, with highest amplitude observed in the definite-morning category. In multivariate analyses, chronotype was statistically significantly associated with aMT6 level ($P < 0.001$). Taking naps in daytime was associated with lower overnight melatonin secretion. However, this association was not statistically significant after adjustment for possible cofounders. Late-night meal was not independently associated with aMT6 level.
The number of night shifts in the past 30 days and number of years in rotating shifts in the past 20 years were not statistically significantly associated with aMT6. We observed no statistically significant association between aMT6 and alcohol use, smoking, non-steroidal anti-inflammatories, ß-blockers, anti-depressants, and hormone therapy. Restricting this analysis to DS workers did not change our results.

Translation of Findings.

Our findings can be readily translated into field applications on several levels. Firstly, the Daysimeter is designed to be used as a practical and efficient measure of light, which has a lightmeter incorporated that is calibrated towards short-wave length visual light, thought to be most disruptive of the circadian system. Secondly, our H-LEA is a newly developed paper-questionnaire aimed at large-scale observational studies to assess light exposure for subsequent association studies of chronic diseases. The results from our studies linking light measurements to melatonin cannot yet be fully applied to the work place, but are extremely useful in that they address fundamental associations which had been demonstrated in sleep laboratories (i.e., controlled environments) but needed to be established in the field. We have done so in what constitutes one of the most detailed ways possible by 1) applying superior technology and 2) conducting an extremely extensive data collection to explore these associations in a night working population. Our data allow us to discriminate between day and night workers, which is important to delineate the effects of light exposure in rotating night workers. In sum, we anticipate that our findings and resulting publications will be broadly used and referenced

to guide future investigative activities. Finally, the importance of chronotype in the observed associations between light exposure and melatonin has not been described before let alone in such detail as in the current data set and, if replicated suggests that this factor needs to be taken into account in future studies of disease risk in night workers.

Outcomes/Relevance/Impact.

A portable, light-weight lightmeter calibrated towards short-wavelength visual light, the Daysimeter, was developed as part of this project and successfully applied within the framework of a large, prospective cohort study, the NHS2, demonstrating both the usefulness and ease of applying technical devices in observational studies. Our data collection entailed 7-day continuous light measures and 3-day repeat urine collections among 148 women from within the NHS2. The high participation rate and low drop-out rate as well as excellent data quality demonstrate feasibility of such extensive protocols in a setting like ours (i.e., large scale observational study). In our evaluation of the collected data we found – as hypothesized – that 24-hour light exposure was lower among rotating-shift workers on night shift (NS) compared to day shift (DS) workers. Further, also as predicted, average light exposure over night was significantly higher during NS compared to DS and among night workers off shift (OS). Compared to DS or OS, in NS workers, the amplitude of aMT6 was significantly lower and its acrophase was delayed by one hour. Both intensity of light exposure overnight and activity index were significantly and inversely associated with aMT6 ($P<0.01$). Interestingly, duration of light exposure if above a certain lux threshold level appeared to be positively associated with melatonin levels: for example, each hour exposed to more than 20 lux light was associated with a decrease of 0.06 in log-transformed aMT6. As a secondary aim of our proposal, we used the the light measures obtained from the Daysimeter and validated a newly developed paper-based questionnaire for self-reported light exposure (H-LEA). The correlation between actual light measures and self-reported light levels was remarkably high, suggesting that such a paper-based instrument could be used in large-scale prospective observational studies that are needed to study chronic disease outcomes. New in our results is that we found chronotype (i.e., whether a person is a morning or evening type, more a “lark” or an “owl”) to be an important predictor of melatonin secretion. More specifically, we observed that definite morning types were 2.7 hours phase advanced and had the highest melatonin amplitude compared to definite evening types (P trend < 0.001). In multivariate analyses, chronotype was statistically significantly associated with aMT6 level ($P < 0.001$). We also observed that taking daytime naps was associated with lower overnight melatonin secretion. However, this association was not statistically significant after adjustment for possible cofounders. Late-night meal was not independently associated with aMT6 level. Number of night shifts during the past 30 days and number of years having worked rotating shifts over the past 20 years were not statistically significantly associated with aMT6. Finally, we observed no statistically significant association between aMT6 and alcohol use, smoking, non-steroidal anti-inflammatories, ß-blockers, anti-depressants, and hormone therapy.

SECTION 2

Scientific Report

Background. 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.

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 carcinogen class 2A (probable carcinogen) – the main operating mechanism being assumed circadian disruption by means of melatonin suppression (1). Based on results from the Nurses' Health Study II (NHS2), in March 2009, Denmark as the first country worldwide began paying monetary compensation to women who developed breast cancer after 20 or more years of night shift work. 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.

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 (3). 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 (4) 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 (5), has been proposed as the main explanation for the health effects associated with night-shift work (6, 7). 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 (8), and later, human twin studies of abnormal circadian phenotypes showing that much of morning and evening preference is heritable (9), led to the identification and characterization of clock genes responsible for circadian behavior (10).

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 effect of light exposure, activity, and shift schedules as modifiable risk factors for melatonin secretion in a sample of women from the NHS2 cohort using a novel circadian light meter.

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

Specific Aim 1: To implement a newly developed concept for which the biologic basis is established and the principal design has already been completed and tested: a circadian light meter that can be worn as a lightweight headset close to the eye and that measures daily patterns of exposure to light among the study participants. Design considerations are limited to ergonomics and data management issues. In contrast to other commercially available light meters, the system will measure and record temporal patterns of exposure to the short-wavelength portion of the visible spectrum, which is believed to have the largest impact on non-image forming functions such as circadian phase shifting or melatonin suppression. This meter will be used to accomplish specific aims 2 and 3.

Specific Aim 2: To further understand the acute effects of light exposure on the circadian system. Using the circadian light meter together with a light related questionnaire, we will obtain light meter recordings continuously over 7 days, and we will conduct urinary melatonin measurements as markers of circadian phase, in addition to yielding information about melatonin levels. We will explore the following hypothesis:

Hypothesis 1: When assessed simultaneously, higher levels of light exposure will be associated with lower melatonin levels within an individual subject.

Hypothesis 2: The diurnal pattern of light exposure will vary significantly between women who work night shifts and women who do not work night shifts: Women who work at night will have significantly higher light exposure at night and lower 24-hour total melatonin production compared to women who sleep at night and work during the day.

The ***secondary objective*** of this component is to assess whether questionnaire data can be used to predict light exposure and to test the reproducibility of the questions on night work on the biennial NHS II questionnaires.

Specific Aim 3: To explore the characteristics of shift schedules that affect melatonin production, e.g., duration and frequency of night work. The population of women from the ongoing NHS II selected for this study will be stratified according to these characteristics.

Hypothesis 3: Variations in light-induced melatonin suppression among night workers can be described by a model based on the factors that interact with a worker's exposure, e.g., job and work setting factors that determine mean light exposure, number of night shifts per month, and total duration of shift work in years. We hypothesize that a higher number of night shifts per month (excluding women who work permanent night shifts) over extended periods enhances the effect of light at night (LAN) on melatonin suppression more strongly than fewer night shifts and shorter periods of time.

Procedures and Methodology.

Study Population

Eligible study participants included women from the Nurses' Health Study II (NHS2) cohort, which was established in 1989 when 116, 609 female nurses between 25 and 42 years old completed a mailed questionnaire. The women have since been followed by mailed questionnaires every two years to update exposure and disease information. In 2005, 90,051 participants were still alive and being followed. Volunteer participants for the light measurement validation study were recruited between November 2006 and April 2008, based on predefined eligibility criteria from the overall cohort, as outlined below.

Eligibility and Recruitment

Women who, at enrollment, presently worked or had worked rotating night shifts as well as women who never worked night shifts were identified from the NHS2 cohort. Roughly 60% of the nurses in the NHS2 cohort worked on rotating night shifts (alternating among day-, afternoon-, evening-, and night shifts; from this point forward the term "night workers" will be used when referring to these rotating night shift workers). Eligible participants were randomly selected from all women within the NHS2 cohort who met the following

inclusion/exclusion criteria: returned the 2005 questionnaire; answered the night work questions in the NHS2 questionnaires, from 1989 (baseline) onwards; were cancer-free in 2005 (with the exception of nonmelanoma skin cancer); did not report having cardiovascular disease up to 2005; presently (in 2005), did not take any hormonal preparations (oral contraceptives or postmenopausal hormones); were not pregnant or lactating; and were presently (in 2005) in the work force. After these exclusions, 18,044 women remained eligible, and of these, participants were randomly chosen, in two waves, using a computer-based random number generator. They were contacted initially by mail, with follow-up by email and telephone (see Figure 1). The first wave of these invitations was mailed in September 2006 to 720 participants Because the majority of participants from this first wave were day workers, and the remaining nurses from the first wave who were not contacted were also day workers, a second wave of invitation letters was mailed in November 2007 in order to collect more night workers who would be willing to participate in the study. These invitation letters were mailed to NHS2 cohort members who met the following inclusion/exclusion criteria: answered the 2007 online questionnaire to determine current night work status; answered the night work questions in the NHS2 questionnaires, from 1989 (baseline) onwards; were cancer-free in 2005 (with the exception of nonmelanoma skin cancer); did not report having cardiovascular disease up to 2005; presently (in 2005) did not take any hormonal preparations (oral contraceptives or postmenopausal hormones); were not pregnant or lactating; answered the question about rotating night work in the 2007 online questionnaire ("Since June 2007, how many months have you worked rotating night shifts – none, 1-4 months, …, 20+ months) and did not answer with "none"; and were not in the prior selection for the first wave of having a history of night work. To maximize enrollment of night workers, we selected all 242 current night workers who passed all exclusion criteria, were not part of the first wave, and worked at least 15 months of night work between 2005 and 2007. Further details regarding enrollment from these two recruitment waves are offered in Figure 1. Based on our initial target enrollment of 150 women, combining waves one and two, we accrued a total of 148 study participants until the end of the study.

Horne Ostberg Morningness-Eveningness Questionnaire
Morningness-eveningness preference was assessed with the Horne-Ostberg Morningness-Eveningness Questionnaire (MEQ) (11), a 19-item questionnaire with a total score ranging from 16 to 86, previously used extensively in adults and adolescents (12-15). A higher score is indicative of morning types, a lower score of evening types. We added the MEQ only halfway through our study, hence we obtained MEQ scores to define morningness-eveningness preference only in a subgroup of 88 participants.

"Circadian" Light Meter
The Daysimeter was developed by the Lighting Research Center (LRC) at Rensselaer Polytechnic Institute (RPI), Troy, NY (USA), as a personal circadian light exposure and activity meter to measure circadian light-dark and activity-rest patterns (16). It is a self-contained, battery-operated data logger worn as a lightweight headset. It has two optical sensors placed near the plane of one cornea; the first (photopic) sensor detects optical radiation and closely matches the standard photopic luminous efficiency function; the second (blue) sensor has an intrinsic long-wavelength response cut-off at approximately 580 nm together with a UV blocking filter creating a spectral response peaking at approximately 460 nm. From both the photopic and blue sensors, the Daysimeter stores a light value proportional to the logarithm of the short circuit current of the cell. An accelerometer within the Daysimeter is used to detect the subject's activity by measuring acceleration in both horizontal and vertical directions. The Daysimeter stores the root mean square value of the acceleration data for each axis over 30 seconds. Current through a reference diode in the Daysimeter is used to approximate the temperature. When data logging, the Daysimeter takes a sequence of ten readings once a second, spread out over one cycle of 60 Hz. The maximum run time of the Daysimeter is 72 days, but in practice this is limited by battery life to 30 days of active logging. Further technical details about Daysimeter have been described elsewhere (17).

Measures obtained from the Daysimeter were summarized by calculating hourly averages of the 30-second data points of light exposure for each day. Mean photopic illuminance values (lux) were derived from the photopic sensor on the Daysimeter, and mean spectrally weighted illuminance values as a measure for circadian light were obtained from the blue sensor. These circadian light levels were approximated using post-processing algorithms based upon the model of circadian phototransduction by Rea et al.(18) and reported in lux, as outlined in more detail in Miller et al. (17). Mean corneal illuminance was approximated from the light

questionnaire (H-LEA questionnaire) using estimated average illuminance values (lux) for each light source offered on the questionnaire. Values for these approximations were obtained from the Lighting Research Center (LRC) at RPI (Table 1). In the H-LEA questionnaire, we assigned "other" light sources a value of 10 lux based on our observation that nurses tended to report in their logs almost exclusively low light levels such as TV in a dark room, or PC, or candle light, as "other" light sources. If a woman noted more than one light source for an individual hour on the H-LEA questionnaire, the average illuminance for these light sources was calculated; for example, an "F" (representing fluorescent lamp) and "H" (representing halogen lamp) would amount to 100 lux + 20 lux = 120 lux / 2 = 60 lux.

Harvard Light Exposure Assessment (H-LEA) Questionnaire

The initial version of the H-LEA questionnaire used in the present study was developed by investigators from the Division of Sleep Medicine at Harvard Medical School and subsequently modified for our validation study (see Figure 2). Light exposure was grouped into 7 types of light sources: halogen lamp, fluorescent lamp, incandescent light, other artificial light source, indoor natural light, sunlight/outdoor natural light, and darkness. Participants were asked to record the light source(s) they were exposed to hourly for seven consecutive days.

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. Each participant completed the self-administered H-LEA while simultaneously wearing the Daysimeter for seven consecutive days and received $100 compensation upon successful completion of the study. Efforts to increase compliance rates included maintaining ample contact and communication with all participants. Investigator-initiated telephone contact was established prior to enrollment. During the seven day study period, study investigators supplied email and phone number information in case subjects had technical issues or questions related to the rather complex study procedures and materials. If a problem with the Daysimeter arose, a replacement Daysimeter was shipped to the participant as soon as possible, and the study dates were changed in accordance with the study protocol to a new seven day study period. Study material was accompanied by detailed instructions and thorough study information, including a precaution that study investigators were going to assess the recorded Daysimeter data to check for adherence to study guidelines, and if the participant was determined to be noncompliant, she would not receive the full $100 compensation.

Participants were instructed to wear the Daysimeter for a seven-day period, during all waking hours, except when in water. We did not record whether participants wore sunglasses when outdoors, but assumed that outdoor light levels that required them to wear sunglasses would still accurately be reflected by Daysimeter recordings much higher in relation to any measurement obtained from indoor light exposure. To protect the light meter while in, for example, the shower or a swimming pool, they were asked to place the Daysimeter nearby, on a bathroom counter or any surface away from the water, but in the same light environment. They were instructed to keep it next to their bed while sleeping. The Daysimeter is suited for use in working environments, including sterile operating rooms.

Exclusions Based on Faulty Data

Of 148 participants, 16 (10.8%) were excluded because of either technical issues or gross noncompliance with wearing the Daysimeter, leaving a total of 132 women for our analyses. Six of the women that were excluded used a particular Daysimeter for which it was detected only in hindsight that after its use in May 2007, a technical problem with the photopic sensor rendered the recorded data less useful. For four other women, the Daysimeter was either started incorrectly or it malfunctioned such that no data were recorded. It was possible to identify nurses who did not comply with the protocol by using data from the Daysimeter's on-board temperature sensor and accelerometer; room-temperature readings, as opposed to elevated temperatures when the Daysimeter is in closer contact with the body, and extended periods of inactivity were certain signs that the nurse did not wear the device when required. Based on these criteria, three women were excluded because they wore the Daysimeter for less than three days, and another three women were excluded because their recorded data showed flagrant noncompliance with wearing the Daysimeter over the course of the seven days, for example, only wearing the Daysimeter for a couple of hours each day. Of the remaining 132 women,

ten others, whose recorded data indicated they were compliant less than 100% of the study time, but was not sufficient to conclude they 'barely' (i.e., less than 50% of the time) wore the Daysimeter, were still included for the analysis. A sensitivity analysis was performed excluding these ten subjects.

Results.

Aim 1:

During the initial phase of this project, the Lighting Research Center at RPI (under the direction of Mark Rea) developed a Daysimeter, which has been refined several times over the course of the first two years to be acceptable for wear by nurses. Three major developments were completed. First, the device was pilot tested subsequent to which the size of the Daysimeter was significantly reduced. Second, the smaller design was implemented into a new form-factor so that it could be worn comfortably on inexpensive and widely available headbands. Finally, the Daysimeter was upgraded to prevent system failure from electrostatic discharge, a problem identified during some of the early field tests of the Daysimeter (see Figure 11, below). After completion of all refinements the Daysimeter has been applied in our study and was widely accepted by the nurses. Only one system failure occurred subsequently, which lead to dysfunction of one of the light measures, but otherwise no system failures in the field occurred.

Figure 11: Final version of the Daysimeter used in this project

The Daysimeter represents an important improvement compared to conventional lightmeters in part because its blue sensor is calibrated toward short-wavelength visual light [which, as science has evolved over the last years, however, is only partly what contributes to circadian disruption; in fact, the spectrum used to define 'circadian' light exposure herein has been modeled from prior data (17, 18) but may be subject to revision based on new data (19) or alternative models (20)]. Secondly, it represents an important improvement compared to previously used devices to measure light in that potential errors in characterizing circadian light dosages can be quite large with conventional photometric instrumentation such as standard light meters (21): spectral errors approaching 300% are common with broadband sources such as daylight, fluorescent and incandescent light sources. More dramatically, errors well over 1000% are associated with light exposures from narrowband light sources such as red and blue LEDs, which are becoming more common in work and living environments. Distribution errors will also contribute to improper assessment of circadian light exposures. A standard light meter has a spatial sensitivity approaching a cosine distribution. That is, the maximum sensitivity to light is orthogonal to the detector plane and falls off as the cosine from that angle. The spatial sensitivity of the human pupil is approximately, but not exactly, like a cosine detector due to the eye's location below the brow and adjacent to the nose (22). Thus, the spatial distribution of a conventional illuminance meter located parallel to the plane of the pupil would be approximately correct for weighting retinal flux entering the eye, except for the fact no one would be willing to hold an illuminance meter next to their face for extended periods of time, particularly in the hospital work environment. Consequently, our circadian light meter was designed to be lightweight and head-mounted (rather than wrist-mounted) to avoid significant errors in weighting retinal flux. In addition to spectral, spatial and intensity calibrations, the circadian light meter has an internal clock unlike any other conventional illuminance meter and can, therefore, characterize both the time and the duration of circadian light exposure. In sum, this new device will provide information, which, together with our melatonin measurements, is unprecedented in literature and vital for progress in light research.

Aim 2:

The characteristics of study population are provided in Table 21. Current night-shift workers were significantly more likely to have worked in the rotating-shift in the past 15 years as well as in the past 30 days prior to the study. There was no significant difference in age, BMI, smoking status, and alcohol use between rotating-shift

and day-shift workers. Rotating-shift workers reported a statistically nonsignificant higher rate of antidepressants use (42% vs 28%) and sleeping pills (46% vs. 30%) use.

Phase shift
In the phasor analysis of melatonin secretion, we observed a one-hour shift delay in aMT6 associated with NS (Figure 21a, supplementary Table 21). The amplitude of aMT6 was significantly lower at NS compared to DS and OS. Although the amplitude of light exposure was significantly lower at NS, the phase was similar to DS and OS (Figure 21b). Activity-index phase was significantly disrupted at NS with an 8-hour phase difference and an amplitude of 8.6 compared to 2.4 and 2.7 for OS and DS, respectively (Figure 21c).

Intensity
Overall, we observed a significant association between light exposure and activity index and melatonin level (Figure 22). In both continuous models and categorical models (using quintiles of light exposure), the weighted average intensity of circadian light exposure in each collection period was significantly associated with aMT6 ($P < 0.0001$) (Table 22). When we stratified this analysis by time of light exposure, this association remained significant only for overnight light exposure category ($P = 0.0078$). Activity index was also significantly associated with melatonin level ($P < 0.0001$). This association was significant regardless of timing of physical activity.

Duration
To evaluate the effect of duration of light exposure, we included number of hours in each urine collection time period above 20 lux threshold (Table 22). When we restricted this analysis to overnight exposure we used 60 lux as the threshold. Regardless of time of exposure, number of hours exposed above the threshold was significantly associated with melatonin level ($P < 0.0001$). Each hour exposed to more than 20 lux light was associated with 0.06 decrease in log-transformed aMT6.

Shift-work
The details of light exposure by shift status are provided in Table 23. Overall 24-hour light exposure was lower among the rotating-shift workers on a NS compared to the DS workers. Average light exposure over night was significantly higher at NS compared to DS and OS (86.7 vs. 9.3 and 11.6, respectively). The LAD to LAN ratio was similar in OS (3.28) compared to DS (3.29) and both were significantly higher than NS (1.09).

Total 24-hour melatonin secretion was not affected by NS (Table 24). Average log-transformed aMT6 was 10.04 on DS and 9.94 and 9.95 on OS and NS, respectively. However, first-morning urine melatonin concentration and rate was significantly lower after a NS compared to DS and OS. We observed a 35% decrease in aMT6 level associated with NS. NS workers had slightly higher aMT6 levels during the day, which compensates for lower melatonin secretion overnight and resulted in an equal total 24-hour aMT6 among all the shift categories. In the multivariate analysis, after adjustment for age and BMI, type of shift work was statistically significantly associated with aMT6 level. NS was associated with significantly lower aMT6 level ($P<0.001$). This association was not statistically significant after adjustment for light exposure and the activity index.

Overall, on a NS, a nurse spent an average of 6.4 hours above 20 lux overnight compared to 1.2 hours on OS and 0.8 hours in DS. In contrast, in the first 8 hours of day, NS workers spent 5.3 hours above this threshold compared to 6.9 hours for OS and 7.7 hours for DS. Similarly, activity index was significantly lower during daytime and higher over night among the NS workers (Table 24).

Interaction of light and activity
We did not observe a significant interaction between light exposure and activity. In the multivariate analysis that included both variables, light exposure and activity index remained statistically significant. In this model, higher activity index and lower light exposure were associated with a lower aMT6 levels (Figure 24).

Secondary Objective (Validation of H-LEA Questionnaire):

After adjustment for age, BMI, collection day, and night work status, the overall partial Spearman correlation between self-report of light exposure and actual photopic light measurements was 0.72 (P<0.001; Kendall τ =0.57) and 0.73 (P<0.0001; Kendall τ =0.58) when correlating circadian light measurements. There were only minimal differences in accuracy of self-report of light exposure and photopic or circadian light measurement between day (r=0.77 and 0.78, respectively) and rotating night shift workers (r=0.68 and 0.69, respectively). In sum, the results of this study provide evidence of the criterion validity of self-reported light exposure using the H-LEA questionnaire. Impact: This questionnaire is a practical method of assessing light exposure in large scale epidemiologic studies.

Aim 3:

Chronotype and timing of meal intake

The results of phasor analysis are provided in Table 32 and Figure 31. We observed a significant trend towards higher aMT6 levels associated with morning chronotype. Morning-type was associated with statistically significant higher mesors (P trend < 0.001). There was a 2.7 hours phase advancement comparing definite-morning type and definite-evening type (P trend < 0.001). The amplitude of aMT6 was significantly higher with increase in morning-type trait, with highest amplitude observed in the definite-morning category. The results of phasor analysis of circadian light and activity index were similar to aMT6 (Figure 31).

In the multivariate analysis (Figure 32), after adjusting for age, BMI, circadian light exposure, activity-index and shift-work schedule, chronotype was statistically significantly associated with aMT6 level (P < 0.001). Taking naps in daytime was associated with lower overnight melatonin secretion. However, this association was not statistically significant after adjustment for possible cofounders. Late-night meal was not independently associated with aMT6 level.

Other baseline characteristics

To evaluate the effect of baseline characteristics on melatonin secretion, we performed a multivariate regression analysis with total 24-hour aMT6 and also first-morning aMT6 as outcome. We excluded from this analysis the days that a nurse was on night shift. The number of night shifts in the past 30 days and number of years in rotating shifts in the past 20 years were not statistically significantly associated with aMT6. We observed no statistically significant association between aMT6 and alcohol use, smoking, non-steroidal anti-inflammatories, ß-blockers, anti-depressants, and hormone therapy. Restricting this analysis to DS workers did not change our results.

<u>Discussion and Conclusion.</u>

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 (23). A growing body of literature provides evidence linking night-shift work with increased risk of several malignancies including breast cancer (24-33), endometrial cancer (34), prostate cancer (35, 36), colorectal cancer (37), and lymphoma (38). Night-shift work is also believed to be associated with other health problems such as cardiovascular disease, diabetes, gastro-intestinal disorders, and sleep disorders (39-41). One hypothesis is that night shift work impacts the health of shift workers through disruption of circadian rhythm (42).

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, i.e., in the field.

In our study we found that the Daysimeter is a readily applicable instrument which has been tested in the field in ours as well as other applications since its initial conception. We generated evidence that the H-LEA questionnaire is a practical and readily applicable method of assessing light exposure in large scale epidemiologic studies to study the effects of shift work on health. Our findings relating light to melatonin levels, finally, suggest that certain thresholds of light determine duration and intensity of light exposure needed to negatively affect the circadian system, and that chronotype is an important factor which merits consideration in future studies of shift workers and health.

Publications.

Bierman, A, Klein, TR, and Rea, MS: [2005] The Daysimeter: A device for measuring optical radiation as a stimulus for the human circadian system. Measurement Science and Technology;16*:* 2292-2299. (Aim 1)

Rea MS, Bierman A, Figeuiro MG, Bullough JD: [2008] A new approach to understanding the impact of circadian disruption on human health. Journal of Circadian Rhythms; 6:7. (Aim 1)

Rea MS, Figueiro MG, Bierman A, Bullough JD: [2010] Circadian light. Journal of Circadian Rhythms; 8:2. (Aim 1)

Miller D, Bierman A, Figueiro M, Schernhammer E, Rea, MS: [2011] Ecological measurements of light exposure, activity, and circadian disruption in real-world environments. Lighting Research and Technology, in press. (Aim 1)

Bajaj A, Rosner B, Lockley S, Schernhammer ES: [2011] Validation of a light questionnaire with real life photopic lux measurements: The Harvard Light Exposure Assessment Questionnaire. Cancer Epidemiol Biomarker Prev, in press. (Aim 2)

Razavi P, Bajaj A, Lockley SW, Rea MS, Ricciuti V, Schernhammer ES: [2011] Effects of light at night on melatonin production in night workers, in preparation. (Aim 2 and 3)

Razavi P, Bajaj A, Lockley SW, Rea MS, Ricciuti V, Schernhammer ES: [2011] The impact of sleep, timing of meals, and chronotype on circadian disruption in night workers, in preparation. (Aim 3)

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) (58). 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.

Materials available for other investigators.

Daysimeter

Figure 1: Final version of the Daysimeter used in this project

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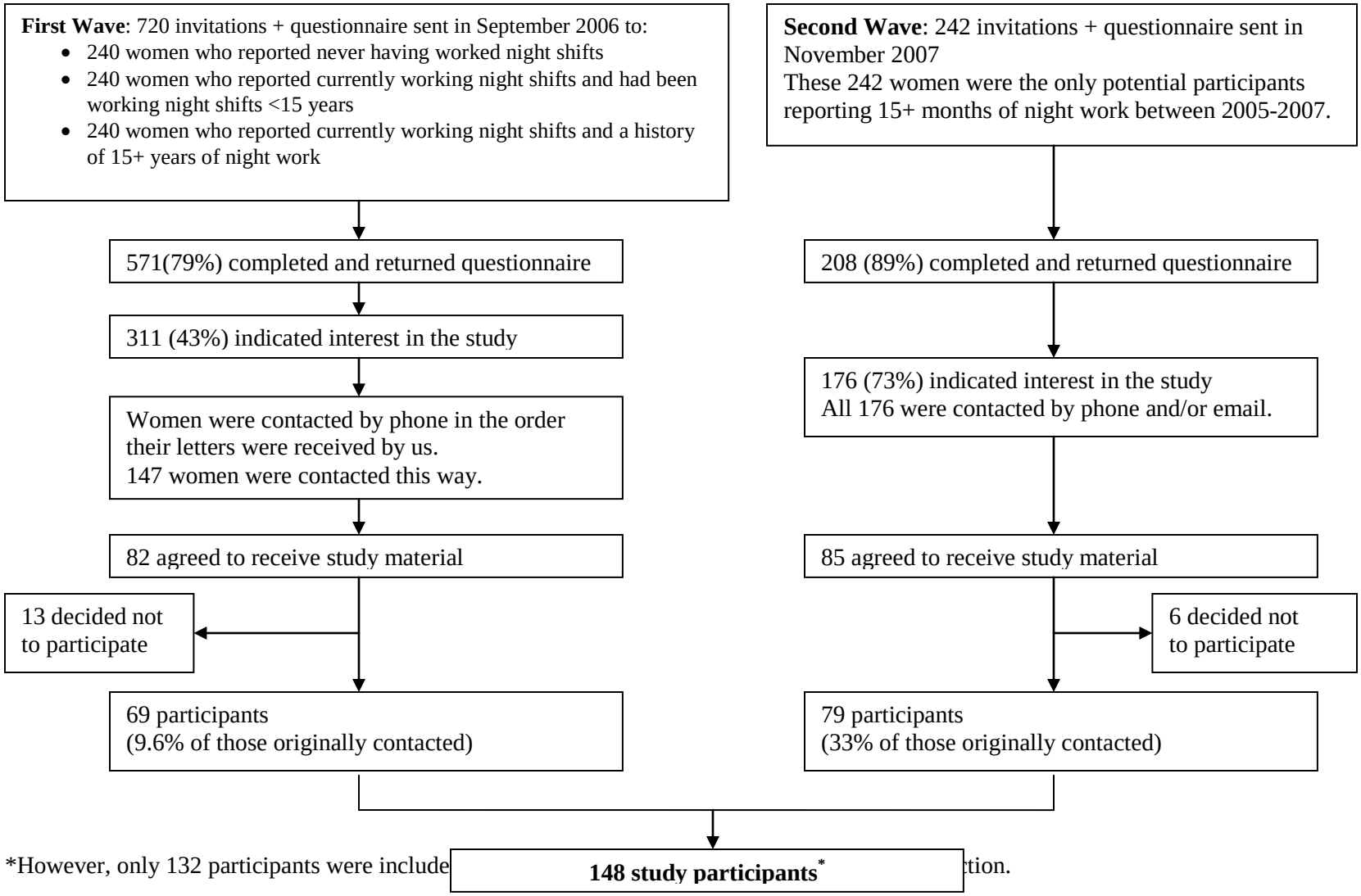
Figure 1. Flow diagram: Subject recruitment.



*However, only 132 participants were include ction.

Figure 2. Harvard Light Exposure Assessment (H-LEA) Questionnaire.

During a typical day, describe your exposure to the below specified light sources. Please fill in as applies, for each single day of your 7-day trial, and circle the hours at which you had a meal, like indicated in the example below.

H…Halogen Lamp F…Fluorescent Lamp I…Incandescent light O…Other Artificial Light Source
N...Natural Light (Indoors) S…Sunlight, Natural Light (Outdoors) D…Darkness

EXAMPLE:

DATE 01/01/2007 □ **NIGHT SHIFT WORKHOURS FROM ___ TO ___** OR □ **DAYSHIFT WORKHOURS FROM ___ TO ___** OR □ **OFF WORK**																							
1am	2am	3am	4am	5am	6am	7am	8am	9am	10am	11am	noon	1pm	2pm	3pm	4pm	5pm	6pm	7pm	8pm	9pm	10pm	11pm	12am
D	D	D	D	D	D	I	N	F	F	F	S	F	F	F	F	H	F	F	F	I	I	H	H

Table 21. Characteristics of 46 day-shift workers and 84 rotating-shift workers randomly selected from Nurses Health Study II participants

Variable	Day-shift N=46	Rotating-shift N=84	P value*
Age (years), mean (SD)	53.7 (4.5)	52.1 (4.9)	0.063
BMI (Kg/m^2), mean (SD)	26.3 (6.3)	27.9 (6.8)	0.21
Rotating night shift (1989-2005), N (%)			<.001
Never	24 (52.2)	16 (19.1)	
1-4 years	6 (13.0)	9 (10.7)	
5-9 years	4 (8.7)	16 (19.1)	
10+ years	9 (19.6)	29 (34.5)	
Rotating night shifts in past 30 days, N (%)			<.001
Never	37 (80.4)	2 (2.4)	
1-4 nights	6 (13.0)	15 (17.9)	
5-9 nights	2 (4.4)	31 (36.9)	
10+ nights	0 (0.0)	35 (41.7)	
Alcohol, 2+ drinks/week, N (%)	16 (34.8)	26 (30.9)	0.17
Never smoked, N (%)	44 (95.7)	81 (96.4)	0.29
Exercise (hours/week), N (%)			0.35
0-1	16 (34.8)	20 (23.8)	
1-3	12 (26.1)	28 (33.3)	
3-6	9 (19.6)	22 (26.2)	
6+	9 (19.6)	14 (16.7)	
Pregnant/breast feeding, N (%)	1 (2.2)	3(3.6)	-
HRT use, N (%)	4 (8.7)	4 (4.8)	0.37
Sleeping pills use, N (%)	14 (30.4)	39 (46.4)	0.09
NSAID use, N (%)	13 (28.3)	35 (41.7)	0.18
B-Blocker use, N (%)	4 (8.7)	6 (7.1)	0.74
Benzodiazepin use, N (%)	1 (2.2)	1 (1.2)	-
Anti depressant use, N (%)	4 (8.7)	10 (11.9)	0.76

* P-values based on t-test for continuous variables and chi-square test and ANOVA for categorical variable.
Abbreviations: SD: Standard deviation; HRT: hormone replacement therapy; NSAID: non-steroidal anti-inflammatory drug;

Table 22. Association of circadian light and activity index with urine aMT6 level in 46 day-shift workers and 84 rotating-shift workers based on mixed models adjusted for age, BMI, type of shift work, and night-shift status

Variables	Duration	Intensity	Quintiles					
	Per hour exposure	Average Log-transformed	Q1	Q2	Q3	Q4	Q5	P trend*
Circadian light (lux)								
24 hour								
Range			< 11	11-49	50-116	117-333	> 333	
Slope (SE)	-0.059 (0.010)	-0.057 (0.010)	Referent	-0.19 (0.06)	-0.34 (0.07)	-0.25 (0.07)	-0.34 (0.07)	
P value	<.0001	<.0001	-	0.0019	<.0001	0.0003	<.0001	**<.0001**
11pm-6am								
Range			< 1	1-3	4-21	22-62	> 62	
Slope (SE)	-0.047 (0.013)	-0.043 (0.016)	Referent	0.14 (0.11)	0.03 (0.11)	-0.06 (0.12)	-0.45 (0.13)	
P value	0.0005	0.0078	-	0.23	0.76	0.63	0.0005	**0.0008**
7am-10pm								
Range			< 25	25-75	76-158	159-457	> 457	
Slope (SE)	-0.062 (0.017)	-0.016 (0.014)	Referent	-0.12 (0.07)	-0.16 (0.08)	-0.11 (0.08)	-0.12 (0.08)	
P value	0.0005	0.24	-	0.11	0.044	0.19	0.16	**0.23**
Activity index (Δg-force)								
24 hour								
Range			<0.036	0.036-0.114	0.115-0.171	0.172-0.226	>0.226	
Slope (SE)	-0.053 (0.010)	-0.096 (0.014)	Referent	-0.25 (0.06)	-0.43 (0.06)	-0.31 (0.07)	-0.49 (0.07)	
P value	<.0001	<.0001	-	<.0001	<.0001	<.0001	<.0001	**<.0001**
11pm-6am								
Range			<0.005	0.005-0.019	0.020-0.040	0.041-0.185	> 0.185	
Slope (SE)	-0.053 (0.014)	-0.055 (0.028)	Referent	0.03 (0.12)	-0.06 (0.13)	-0.17 (0.13)	-0.39 (0.13)	
P value	0.0002	0.048	-	0.83	0.66	0.18	0.0029	**0.0010**
7am-10pm								
Range			<0.073	0.073-0.135	0.136-0.182	0.183-0.230	>0.230	
Slope (SE)	-0.041 (0.020)	-0.062 (0.021)	Referent	-0.12 (0.07)	-0.25 (0.08)	-0.12 (0.08)	-0.32 (0.08)	
P value	0.048	0.0038	-	0.12	0.0014	0.14	0.0001	**0.0009**

Abbreviations: aMT6: 6-sulfatoxymelatonin; SE: standard error

* Based on likelihood ratio test

Table 23. Circadian light and activity-index among 46 day-shift workers and 84 rotating-shift workers for the 3-day study period

	Day-shift		Rotating-shift			
	Day-shift (N days=138) Mean (SD)		Off-shift (N days=110) Mean (SD)		Night-shift (N days=142) Mean (SD)	
Variable*	**Measured**	**Log transformed**	**Measured**	**Log transformed**	**Measured**	**Log transformed**
Average circadian light 24hr [a, b]	312.1 (375.7)	5.28 (0.95)	281.1 (406.1)	5.01 (1.12)	254.4 (415.4)	4.90 (1.08)
Circadian light (lux)						
7am to 10am [a, b]	430.1 (749.3)	5.41 (1.04)	389.5 (1115.0)	4.85 (1.67)	413.8 (1286.8)	4.50 (1.80)
11am to 2pm [b, c]	710.8 (1118.6)	5.86 (1.15)	750.7 (1776.1)	5.41 (1.73)	530.3 (1424.3)	3.66 (3.07)
3pm to 6pm [b, c]	651.1 (1080.3)	5.60 (1.37)	488.1 (763.1)	5.21 (1.52)	332.9 (805.9)	4.40 (1.88)
7pm to 10pm [a, b, c]	65.1 (108.8)	3.34 (1.31)	35.0 (46.5)	2.91 (1.20)	77.2 (94.2)	3.68 (1.47)
11pm-6am [a, b, c]	9.3 (16.1)	1.06 (1.71)	11.6 (27.4)	0.60 (2.11)	86.7 (87.1)	3.81 (1.58)
LAD / LAN [b, c]	74 (132)	3.28 (1.43)	71 (123)	3.29 (1.43)	15 (45)	1.09 (1.75)
Hours exposed to >20 lux						
7am to 10am [a, b, c]	3.8 (0.5)	-	3.3 (1.1)	-	2.7 (1.2)	-
11am to 2pm [a, b, c]	3.9 (0.3)	-	3.6 (0.9)	-	2.6 (1.7)	-
3pm to 6pm [b, c]	3.5 (0.9)	-	3.5 (0.9)	-	3.0 (1.2)	-
7pm to 10pm [b, c]	2.1 (1.3)	-	1.8 (1.4)	-	2.9 (1.5)	-
11pm-6am [b, c]	0.8 (0.8)	-	1.2 (2.2)	-	6.4 (2.7)	-
Activity index (Δg-force)						
7am to 10am [a, b, c]	0.181 (0.072)	-1.86 (0.70)	0.153 (0.074)	-2.14 (1.06)	0.118 (0.104)	-2.57 (1.19)
11am to 2pm [a, b, c]	0.201 (0.074)	-1.74 (0.73)	0.164 (0.084)	-2.23 (1.41)	0.111 (0.123)	-3.58 (2.30)
3pm to 6pm [b, c]	0.194 (0.071)	-1.73 (0.47)	0.176 (0.070)	-1.87 (0.62)	0.142 (0.075)	-2.26 (1.08)
7pm to 10pm [b, c]	0.116 (0.066)	-2.51 (1.17)	0.129 (0.065)	-2.29 (0.93)	0.184 (0.090)	-1.96 (1.00)
11pm-6am [b, c]	0.018 (0.017)	-4.69 (1.38)	0.037 (0.059)	-4.56 (1.76)	0.173 (0.117)	-2.25 (1.44)
AAD / AAN [a, b, c]	9.3 (24.5)	1.53 (0.88)	5.6 (14.1)	1.10 (0.89)	1.3 (3.5)	-0.31 (0.93)

* a: P<0.05 comparing day-shift with off-shift; b: P<0.05 comparing day-shift with night-shift ; c: P<0.05 comparing off-shift with night-shift
Abbreviations: LAD: light at day; LAN: light at night; AAD: activity at day; AAN: activity at night; SD: standard devviation

Table 24. aMT6 level among 46 day-shift workers and 84 rotating-shift workers for the 3-day study period

	Day shift		Rotating shift			
	Day-shift (N days=138) Mean (SD)		Off –shift (N days=110) Mean (SD)		Night-shift (N days=142) Mean (SD)	
Variable*	**Measured**	**Log transformed**	**Measured**	**Log transformed**	**Measured**	**Log transformed**
Total aMT6 (ng/24 hr)	27790 (16014)	10.04 (0.70)	27980 (24591)	9.94 (0.79)	27962 (24670)	9.95 (0.75)
Total Cr-corrected aMT6 (ng/mg/24hr)	515 (536)	5.90 (0.82)	445 (472)	5.76 (0.80)	405 (312)	5.74 (0.75)
aMT6 concentration (ng/ml)						
7am to 10am	16.4 (21.9)	2.27 (1.00)	22.9 (37.0)	2.48 (1.11)	19.0 (34.4)	2.27 (1.13)
11am to 2pm	6.6 (7.1)	1.45 (0.94)	7.2 (10.6)	1.32 (1.35)	11.7 (25.3)	1.58 (1.21)
3pm to 6pm	6.5 (8.6)	1.35 (1.03)	5.2 (5.4)	1.28 (0.98)	8.6 (20.0)	1.43 (1.09)
7pm to 10pm	10.3 (14.0)	1.83 (0.95)	13.4 (26.8)	1.80 (1.14)	12.3 (16.8)	1.94 (1.10)
11pm-6am [b, c]	37.3 (34.2)	3.20 (1.01)	39.5 (47.2)	3.10 (1.13)	26.3 (28.5)	2.78 (1.01)
aMT6 rate (ng/hr)						
7am to 10am	1308 (1319)	6.78 (0.96)	1630 (2834)	6.87 (0.96)	1481 (2640)	6.67 (1.06)
11am to 2pm [c]	574 (575)	6.00 (0.85)	638 (972)	5.86 (1.18)	937 (1493)	6.20 (1.08)
3pm to 6pm	477 (543)	5.70 (0.97)	396 (422)	5.61 (0.85)	758 (1778)	5.82 (1.14)
7pm to 10pm	653 (611)	6.19 (0.76)	791 (1133)	6.18 (0.94)	977 (1340)	6.27 (1.09)
11pm-6am [b]	1994 (1428)	7.25 (1.00)	1961 (2110)	7.12 (1.03)	1566 (1516)	6.98 (0.91)
Cr-corrected aMT6 rate (ng/mg/hr)						
7am to 10am	28.5 (30.4)	2.82 (1.12)	30.2 (72.5)	2.82 (0.97)	25.2 (34.5)	2.62 (1.12)
11am to 2pm	14.1 (17.9)	2.07 (1.06)	15.2 (25.5)	2.04 (1.18)	15.3 (16.5)	2.25 (1.03)
3pm to 6pm	9.8 (13.4)	1.65 (1.10)	8.7 (11.6)	1.61 (1.02)	12.9 (24.9)	1.75 (1.17)
7pm to 10pm	16.8 (70.9)	2.03 (0.97)	11.7 (15.6)	1.98 (0.99)	12.8 (17.4)	1.92 (1.13)
11pm-6am [b]	30.6 (39.0)	2.92 (1.05)	26.1 (29.4)	2.81 (0.96)	19.9 (17.3)	2.62 (0.93)

* a: P<0.05 comparing day-shift with off-shift; b: P<0.05 comparing day-shift with night-shift ; c: P<0.05 comparing off-shift with night-shift
Abbreviations: aMT6: 6-sulfatoxymelatonin; Cr: creatinine; SD: standard devviation

Supplementary Table 21: Phasor analysis of magnitude and phase of circadian light, aMT6 and activity index among 46 day-shift workers and 84 rotating-shift workers for the 3-day study period based on coisinar analysis of log-transformed variables

	Day shift		Rotating shift			
	Day/Off (N days=138)		Day/Off (N days=110)		Shift (N days=142)	
	Amplitude*	Acrophase (hr)	Amplitude*	Acrophase (hr)	Amplitude*	Acrophase (hr)
Creatinine-corrected aMT6 (ng/mg/hr)	0.70	-0.10	0.67	0.08	0.49	0.73
aMT6 (ng/hr)	0.85	-0.87	0.83	-0.78	0.60	-0.66
Circadian light (lux)	3.37	8.88	3.23	9.04	0.33	8.64
Activity index (Δg-force)	2.76	9.23	2.39	10.03	0.67	-6.00

* Log-scale

Abbreviations: aMT6: 6-sulfatoxymelatonin; Cr: creatinine

Figure 21. Phasor analysis of magnitude and phase of circadian light, aMT6 and activity index among 46 day-shift workers and 84 rotating-shift workers for the 3-day study period based on cosinar analysis of log-transformed variables.

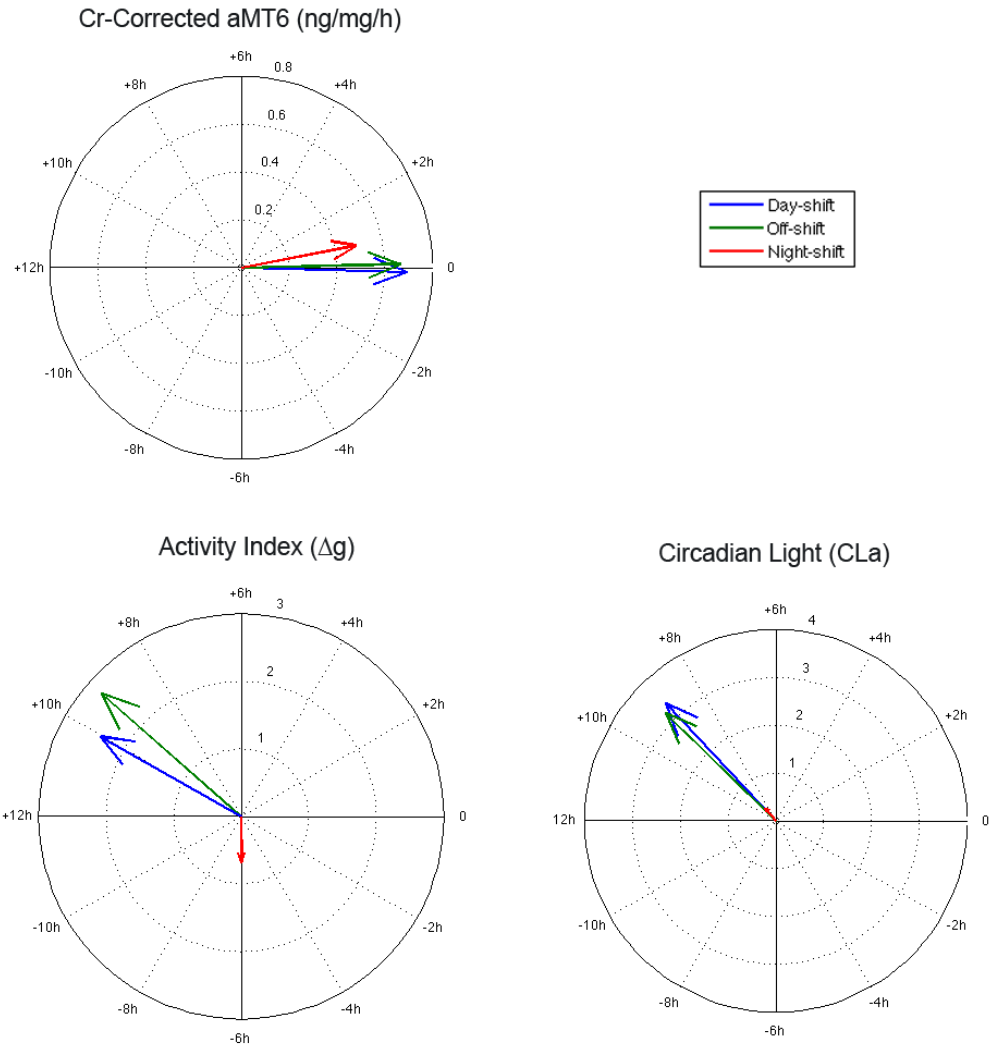
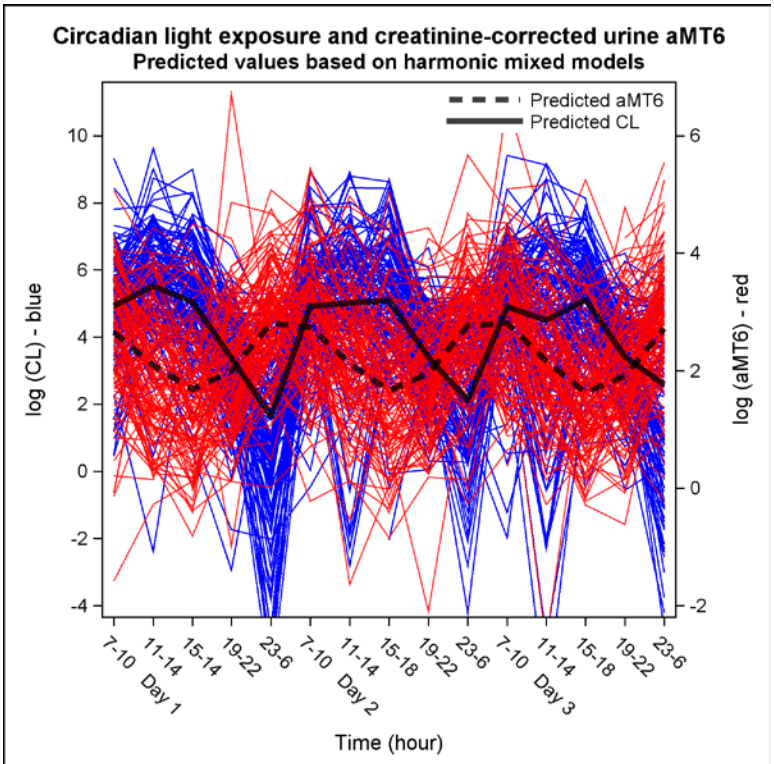


Figure 22. Circadian light and activity index and predicted values among 46 day-shift workers and 84 rotating-shift workers based on harmonic mixed models



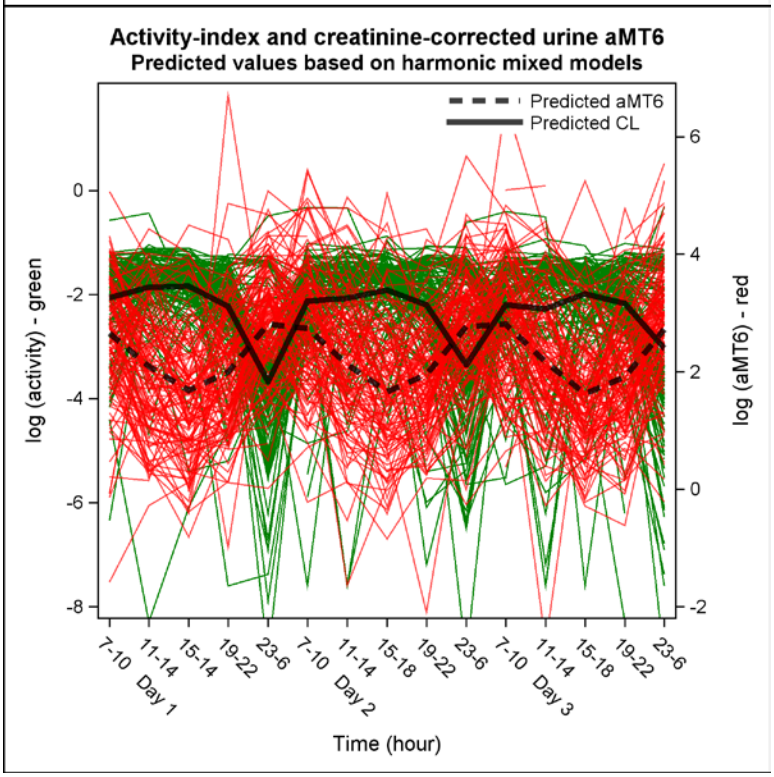


Figure 23. Circadian light, activity-index and aMT6 level by type of shift-work based on mixed models for repeated measurements.

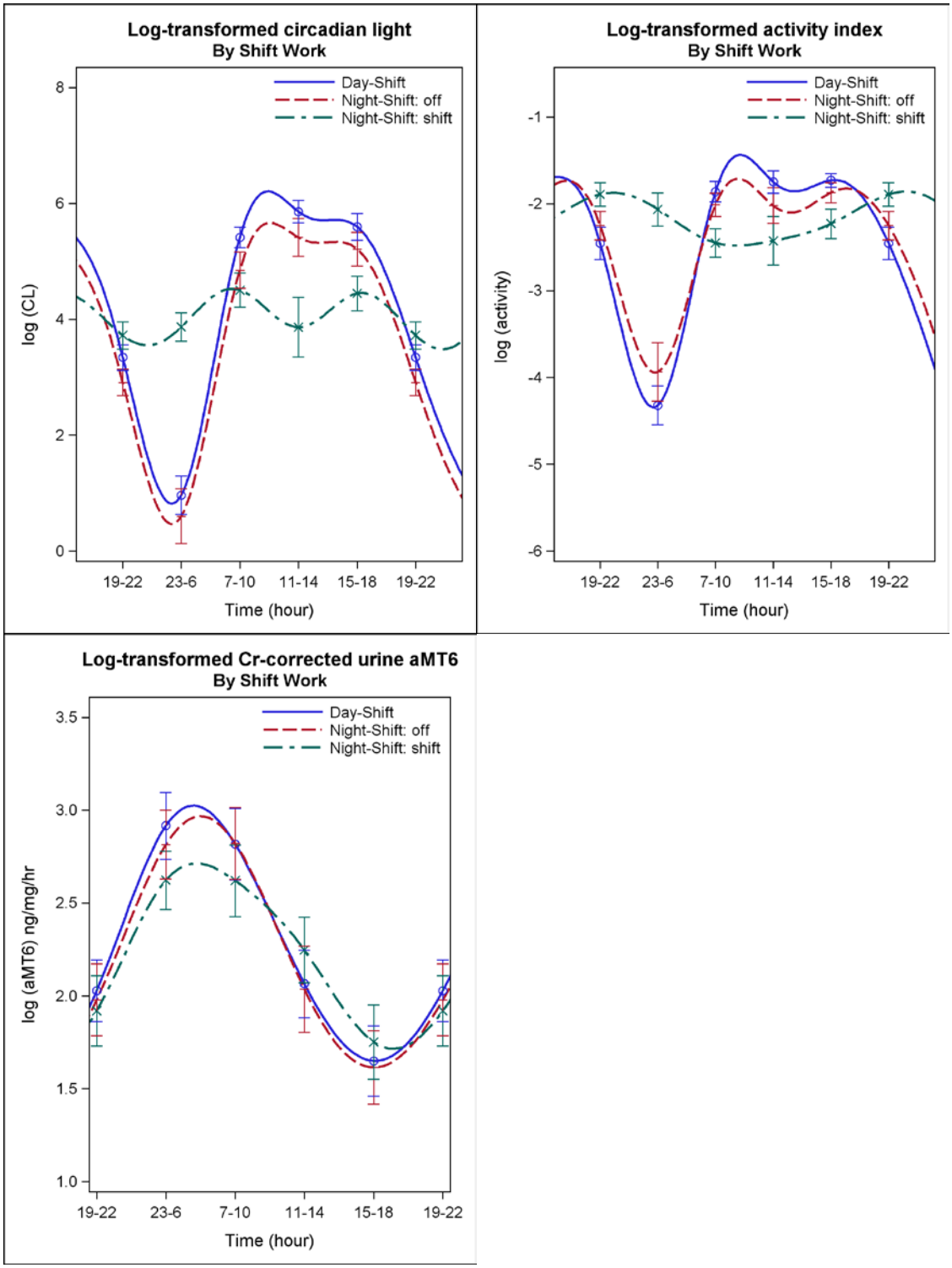


Figure 24. Contour fit plot of circadian light, activity index and aMT6 level

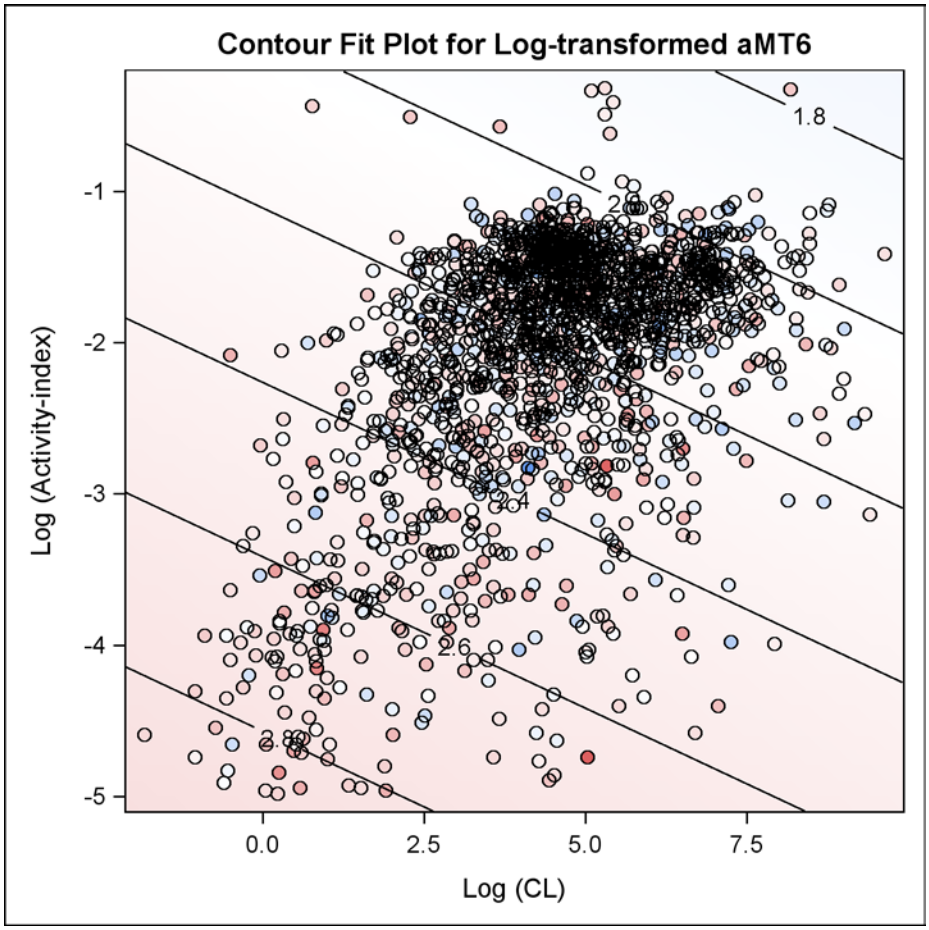


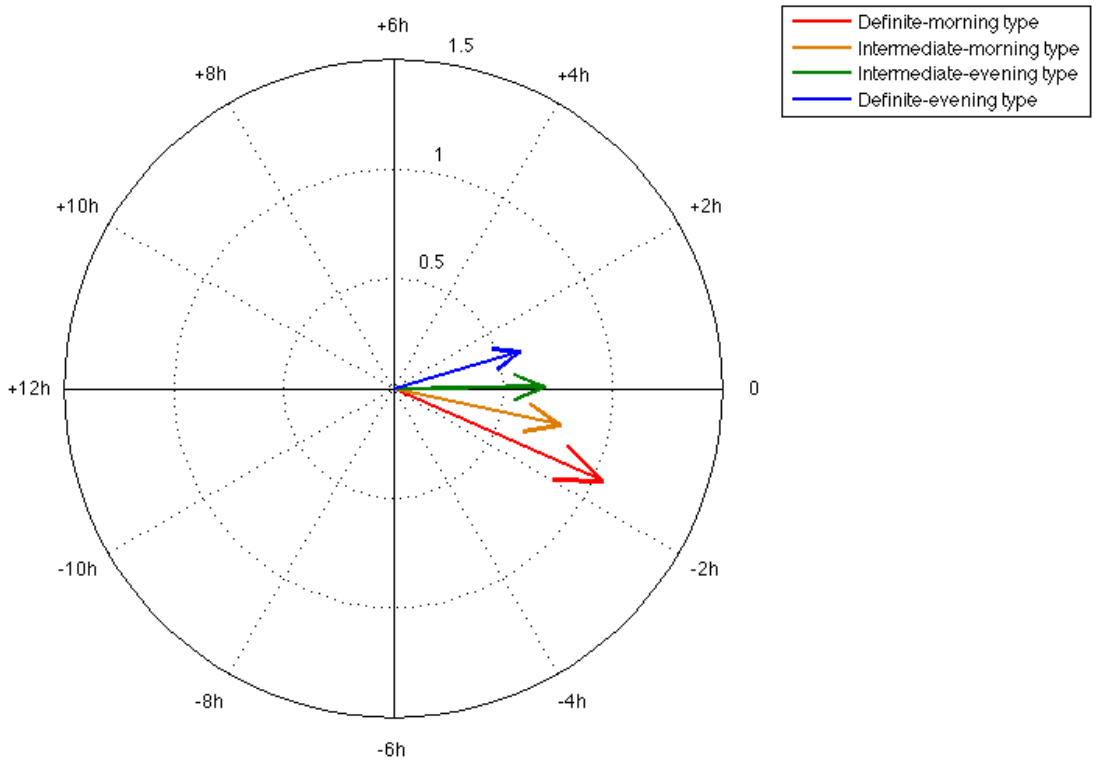
Table 32. Phasor analysis of magnitude and phase of circadian light, aMT6 and activity index by chronotype among 46 day-shift workers and 84 rotating-shift workers for the 3-day study period based on coisinar analysis of log-transformed variables

Variable	Chronotype	Mesor*	Amplitude*	Acrophase (hr)
aMT6 (ng/hr)				
	Definite-morning	6.63	1.04	-1.59
	Intermediate-morning	6.52	0.78	-0.82
	Intermediate-evening	6.48	0.69	0.04
	Definite-evening	6.24	0.60	1.10
Circadian light (lux)				
	Definite-morning	3.13	3.54	8.45
	Intermediate-morning	3.33	3.54	8.83
	Intermediate-evening	3.44	3.04	9.36
	Definite-evening	2.92	2.88	9.53
Activity index (Δg-force)				
	Definite-morning	-3.46	2.59	9.22
	Intermediate-morning	-3.57	2.89	9.23
	Intermediate-evening	-3.25	2.33	9.95
	Definite-evening	-3.68	2.26	10.63

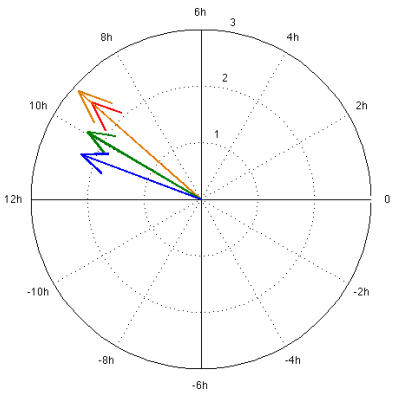
* In log-scale
Abbreviations: aMT6: 6-sulfatoxymelatonin

Figure 31: Phasor analysis of aMT6, circadian light, and activity index among 46 day-shift workers and 84 rotating-shift workers on day-shift/off-shift

aMT6



Circadian light



Activity index

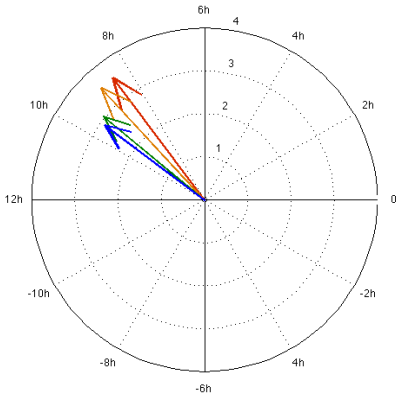
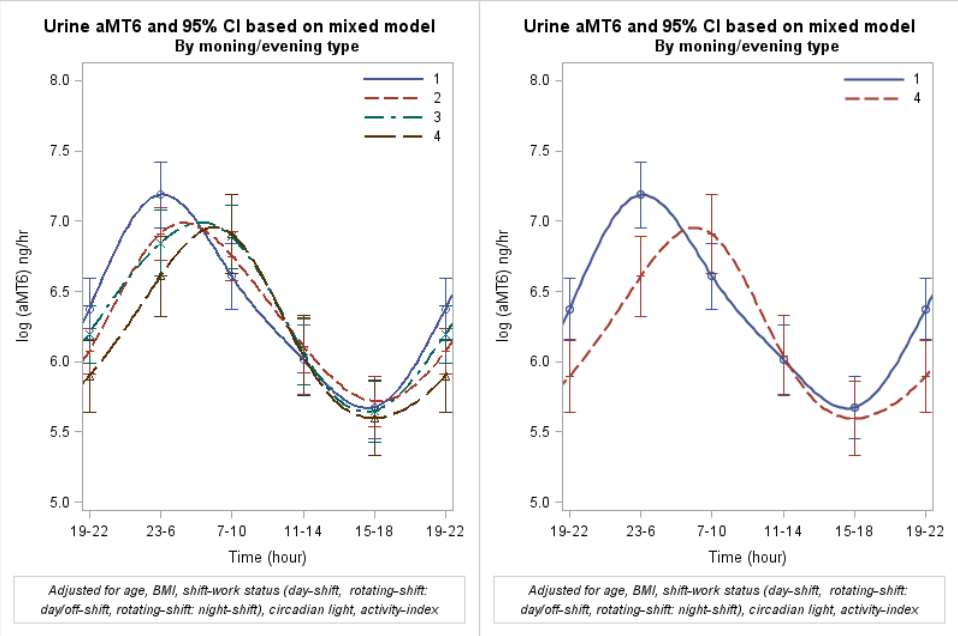


Figure 32: Association of chronotype, naps in daytime with urine aMT6 level among 46 day-shift workers and 84 rotating-shift workers based on mixed models for repeated measurements adjusted for age, BMI, shift-work schedule, circadian light exposure and activity index.

A. chronotype



B. Naps in daytime