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14. ABSTRACT

Epidemiological observations indicate that breast cancer risk is lower in visually impaired women compared to sighted women and that risk is inversely correlated with degree of visual impairment. A hypothesis to explain these findings is that blind people are less susceptible to suppression of melatonin by light exposure at night and therefore have higher levels of melatonin. Melatonin has oncostatic properties in vitro. In a survey of blind women, we found that blind women with no perception of light (NPL) have a reduced risk of breast cancer compared to blind women with light perception (LP) (OR = 0.45 [CI: 0.25, 0.80]). In adjusted analyses the effect was consistent, but attenuated (OR = 0.56, CI: 0.30, 1.02). When we stratified the data at age 50, we found a significantly lower risk among women over age 50 in adjusted analyses (OR = .40, CI: .22, .74). These differences could not be explained by differences in known reproductive risk factors for breast cancer. In contrast, NPL women appear to have risk factors consistent with an elevated risk, including an earlier reported menarche than LP women (NPL = 12.18 ±1.53 years vs. 12.46 ±1.57 years, P<0.01). These findings suggest that light may influence reproductive development in women and provides support for the hypothesis that light exposure at night is a risk factor for breast cancer.

15. SUBJECT TERMS
Breast Cancer, Melatonin, Estrogen, Light, Blindness, Circadian

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INTRODUCTION

Since 1940, breast cancer incidence rates have been steadily rising in the United States (1). There is growing evidence for possible effects of exposure to light at night (LAN) on cancer risk due to the increased use of modern electric lighting (2-8). Epidemiological observations indicate that breast cancer risk is lower in women who are visually impaired as compared to the sighted population and that the risk may be inversely correlated with degree of visual impairment (9-13). One hypothesis proposed to explain these findings is that blind people are less susceptible to suppression of melatonin by light exposure at night and therefore have higher circulating levels of melatonin. Melatonin has been shown to have oncostatic properties in vitro (14). Frequent light-induced melatonin suppression has been hypothesized as a cause of the higher breast cancer incidence observed in female shiftworkers and flight-attendants (3-6,15-17). Blindness is also associated with disorders of the circadian system (18) and changes in reproductive function (19-20) which may also contribute to breast cancer risk. The aim of this study is to investigate further the relationship between the severity of blindness and melatonin and estrogen production while simultaneously assessing how blindness and/or melatonin production are related to known risk factors for breast cancer.

BODY

The study design and approved Statement Of Work is divided into two parts; Part 1 is an epidemiological health survey of breast cancer risk in 1394 women and Part 2 is an assessment of melatonin and estrogen levels in a subset of 130 of the women.

Statement of Work progress report

Part 1 – Epidemiological Survey of Cancer in the Visually Impaired

Task 1 (Months 1-4). Task 1 has been completed as described in previous reports.

Task 2 (Months 5-12). Task 2 has been completed as described in previous reports.

Task 3 (Months 12-24) – Data collection.
a,b,c,e) Tasks 3 a-c and e have been completed, however, as described in our last report the response to the survey was lower than anticipated.

d) Given the lower than expected recruitment rate for the main survey, we have not initiated a repeat prospective study as the numbers would be too modest for meaningful prospective analysis.

Task 3 – Data analysis (Months 25-36).
a) Task 3 a has been completed as described in previous reports
b) Statistical analysis of the survey data has been completed for selected variables (see below).
c) Manuscripts describing major findings from the survey data are in progress.
d-h) As described above, our recruitment rates are substantially lower than initially anticipated and precluded the completion of a third call for volunteers, as planned, and establishment of a prospective cohort.

Part 2 – Assessment of Melatonin and Estradiol Levels in the Visually Impaired

Task 1 (Months 1-4). Task 1 has been completed as described in previous reports.

Task 2 (Months 5-12). Task 2 has been completed as described in previous reports.

Task 3 (Months 13-36). Task 3 has been completed as described in previous reports.

Task 4 (Months 12-36). Task 4 is ongoing. a-d) Data entry and plotting of sleep-wake times has been completed. Urinary assays for 6-sulphatoxymelatonin have been completed. Urinary assays for estrone-3-glucuronide have been completed in 46 subjects. Preliminary analysis has been completed on a subset of subjects (see report below).

Research findings for the period of the report

Part 1 – Epidemiological Survey of Cancer in the Visually Impaired

Methods

The Blind and Visually Impaired Women's Health Project (BVIWHP) consisted of a nationwide survey of women in the US and Canada with a visual acuity of legally blind (20/200 on the Snellen Scale) or less. At the launch of the study in April 2005, partnerships were established with the American Council of the Blind (ACB) and the National Federation of the Blind (NFB), who each published study advertising and articles detailing study procedures, which were distributed to their approximately 84,000 female and male members. Subjects were later recruited through informational letters sent directly from the Perkins Braille and Talking Book Library in Watertown Massachusetts, the ACB and the Canadian National Institute for the Blind (CNIB). Approximately 25,000 letters were sent through these initiatives. Other participants were recruited through advertisements placed on radio reading services, magazines for the blind, newsletters targeting blind consumers, radio interviews on programs targeting blind women, 'list servs', postings at guide dog schools and through postings at state rehabilitation centers. Women interested in participating in the survey were asked to call a toll-free number or complete the survey directly via a screen-reader accessible survey website (1998 US Rehabilitation Act, Section 508-compliant). All study participants were screened for gender, age, and blindness, over the telephone or via the website.

Ethical permission for the study was granted from the Institutional Review Board at Partners Healthcare (2003-P-000263) and the United States Department of Defense Human Subjects Research Review Board (HSRRB #A-12744). After obtaining informed consent, each subject was provided with the survey in the format of their choice. Survey formats included via e-mail, website, compact disc, computer disk, audio tape, large print, Braille, in person, or verbally over the telephone. The survey consisted of 120 questions and included demographic information, personal and parental medical histories, detailed information on eye conditions, history of blindness and visual acuity, reproductive history, medication use, the Harvard National Depression Screening scale (HANDS) (21), the Pittsburgh Sleep Quality Index (PSQI) (22), and additional questions about lifestyle factors of interest.

Research assistants entered survey data during telephone interviews or following receipt of written or audio surveys. Braille surveys were transcribed by a third party and entered into the database upon transcription. Survey entries were audited for accuracy on a weekly basis. Inconsistent records were manually checked and corrected on a weekly basis and again at the completion of the study. Data comprising the present analysis were exported on March 21, 2007. Test entries, duplicate subject survey entries, data from subjects who completed <30% of the survey, data from participants outside North America and data from male participants were removed from the analysis. The final data set included 1392 participants.

Statistical methods

Subjects were divided into two groups; 1) those with any light perception in either eye (LP); and 2) those who reported having no perception of light in both eyes (NPL). A participant was defined as LP if she indicated that she was able to detect any degree of light perception or if she indicated having any usable field of vision in either eye. A participant was defined as NPL if she reported having no light perception or no field of vision in both eyes or if she reported having removal of both eyes. NPL subjects were further subdivided into three ordinal categories by the age that light perception was lost in both eyes as 1) onset of NPL from birth; 2) onset of NPL from age one until two years prior to menarche; and 3) onset of NPL from two years prior to menarche and after. Subjects with LP were further categorized by visual acuity based on self reported level of best corrected vision. These

categories included 1) able to see the top letter on the vision chart, 2) unable to see the chart, but able to count fingers, 3) unable to count fingers, but able to see shadows and hand movement 4) light perception only. Participants who did not report their degree of light perception for both eyes were excluded from comparisons (n=34).

Menarche was defined as the age of one's first reported period. Subjects were asked to define their current menopausal status as pre-menopause, in menopause or post-menopause. 'Menopause start' was defined as the age when one started experiencing menopausal symptoms such as regularly missed periods, intermittent bleeding and/or hot flashes. 'Menopause stop' was defined as the age that one's periods stopped completely. The present analysis includes menopausal information for only those who experienced a natural (not surgical or medical) menopause. Those who reported experiencing a surgically or medically induced menopause were excluded from baseline comparisons.

Univariate summary measures were calculated for primary demographic characteristics and compared between groups of light perception. Between-group assessments were calculated using two-sided Student's two-sample *t* tests for continuous variables and chi-square tests for categorical variables. Multivariate odds ratios (OR) and 95% confidence intervals (CI) were estimated by logistic regression using light perception status (NPL/LP) or breast cancer history as the dependent variable (23). In examinations of breast cancer risk, data were stratified by age 50. In comparisons of breast cancer risk by degree of light perception, NPL was used as the referent group. Independent variables considered for inclusion in multivariate logistic regression were selected from variables with a hypothesized relationship to the outcome of interest where $P<0.10$ in univariate comparisons. Multivariate models examining menarche were ultimately adjusted for the continuous variables current age, BMI and BMI at age 18. In models of breast cancer risk, data were adjusted for current age, reporting at least one full term pregnancy and smoking history. Subjects with missing data for any included variable were excluded from the model. Tests for trend were conducted using linear regression to compare the continuous variable 'age of menarche' to the increasing category of age of loss of NPL used as a continuous variable. All *P*-values were two tailed. All statistical comparisons were made using SAS software (SAS Institute Inc., version 9.0, Cary, NC).

Results

Demographic Comparisons

Some degree of light perception was reported in 958 subjects, while 400 women reported being NPL and 34 did not report their degree of light perception. In Table 1, we present baseline demographic and reproductive characteristics of the study participants. Subjects ranged in age from 19 to 98 years at the time of the survey, with a mean age of 56.80 years (±17.68). The mean BMI for the cohort was overweight at 28.58 kg/m^2 (±7.37). Of all women, 53% reported a history of at least one full term pregnancy. In addition, 56% of all subjects reported earning a college degree and 28% reported earning a post-college degree, with slightly more NPL subjects reporting advanced degrees than LP subjects (32% vs. 26% respectively). Approximately 70% of all subjects reported ever being married, with LP subjects being significantly more likely to have reported a history of marriage. At the time of the survey, 58% of all women reported being post-menopausal, 11% reported being in menopause and 28% reported being pre-menopausal (3% did not report menopausal status).

NPL women differed from LP women for several demographic measures (Table 1). NPL women were significantly younger than LP women (mean age 54.24 years vs. 57.81 years, respectively) in this cohort. They were also shorter than LP women (mean height 63.08 inches vs. 63.70 inches; respectively) but were the same weight, resulting in a significantly higher current BMI for NPL versus LP women (mean BMI, 29.31 vs. 28.19 kg/m^2). This BMI difference appeared to not have existed at age 18, with NPL women reporting being significantly lighter at that age than LP women (Table 1). A significantly smaller proportion of NPL women reported a history of one full term pregnancy and NPL women reported having a significantly earlier menarche than LP women by 4 months (mean age at menarche, 12.16 years vs. 12.45 years, respectively) (Table 1). There were no significant differences in natural menopause measures, weight or the mean age of first full term pregnancy.

Table 1. Comparisons of selected demographic characteristics among blind women with (LP) and without (NPL) light perception. A. Univariate comparisons of continuous variables by light perception status. B. Univariate comparisons of dichotomous variables by light perception status.

	Cohort			LP			NPL			
Variable	**N**	**Mean**	**Std Dev**	**N**	**Mean**	**Std Dev**	**N**	**Mean**	**Std Dev**	**p-value**
Age	1379	56.80	17.68	959	57.87	19.00	413	54.14	13.85	**0.0001**
Height	1376	63.52	3.05	960	63.70	3.05	412	63.10	3.01	**0.0009**
Weight	1378	163.98	44.18	960	162.97	43.89	412	166.49	44.84	0.1763
Weight at 18	1332	125.04	24.83	930	126.64	25.63	396	121.54	22.53	**0.0003**
BMI	1369	28.58	7.37	956	28.23	7.28	409	29.40	7.51	**0.007**
BMI at 18	1323	21.78	4.01	925	21.94	4.15	394	21.43	3.64	**0.0262**
Menarche	1355	12.37	1.56	938	12.45	1.57	410	12.18	1.53	**0.0032**
Menopause Start (natural only)	479	47.37	5.39	316	47.21	5.59	160	47.62	4.98	0.4305
Menopause Stop (natural only)	461	50.10	5.14	308	50.07	5.63	149	50.09	3.99	0.9552
Age of 1st Term Pregnancy	717	25.36	5.30	536	25.24	5.22	175	25.69	5.43	0.3304
Age BC Diagnosis	81	57.09	13.48	68	57.00	13.74	13	57.54	12.51	0.896
PSQI	1238	8.05	4.21	860	7.77	4.22	372	8.74	4.12	**0.0002**

	Odds Ratio	p-value
History of at least 1 full term pregnancy	1.80 (1.42, 2.27)	**<0.0001**
College Graduate	0.75 (0.59, 0.97)	0.018
Ever Married	1.63 (1.27, 2.08)	**<0.0001**

BMI = Body Mass Index; BC = Breast Cancer; PSQI = Pittsburgh Sleep Quality Index; **p<.05

Menarche

In logistic regression models, the unadjusted odds ratio for each increasing year of menarche among NPL women compared to LP women was 0.89 (95% CI: 0.83, 0.96) i.e., for every year that a woman with LP attained menarche it was 0.11 times less likely that an NPL woman of the same age would have attained menarche. After adjustment for confounders, the odds ratio was virtually identical (OR: 0.89, 95% CI: 0.82, 0.97).

In order to assess the potential effects of light on age at menarche in more detail, we compared women with NPL from birth to all other participants. We found that women NPL from birth reported experiencing menarche approximately six months earlier than participants with LP at birth (11.94 ±1.67 vs. 12.40 ±1.55, *P*=0.0058). When comparisons on reported age of menarche were made between those who reported having NPL from birth compared to all others, the unadjusted and adjusted odds ratios were strengthened OR: 0.81, 95% CI: 0.70-0.94; OR: 0.79, 95% CI: 0.68-0.94, respectively). When logistic regression analysis was restricted to those with NPL at birth, compared to those with NPL after birth, the effect was consistent but attenuated in both unadjusted and adjusted models (OR: 0.87, 95% CI: 0.74-1.02; OR: 0.84, 95% CI: 0.70-1.00, respectively). When we examined the association between age at onset of NPL and age at menarche, we found a significant positive association with earlier menarche being associated with an earlier age category of loss of light perception (Table 2). We further found that the mean age of menarche in women who lost vision, but not light perception, was earlier in those who became legally blind at an earlier age (mean age at menarche when onset of legal blindness was from birth up to two years prior to menarche: 12.34 ±1.58, mean age at menarche when onset of legal blindness was lost from two years prior to menarche or after: 12.57 ±1.55, *P*=0.02).

Table 2. Mean age of menarche among blind women categorized by onset of having no perception of light (NPL) relative to onset of menarche and in blind women who retained light perception (LP).

Visual Category	N	Mean Age of Menarche	Standard Error
NPL from birth	93	11.94	0.17
Onset of NPL from age 1 until 2 years prior to menarche	64	12.13	0.13
Onset of NPL 2 years prior to menarche and after	253	12.28	0.01
LP from birth	938	12.45	0.05

Test for Trend (Linear Regression): P-value: 0.0005

Breast Cancer

There were 84 total cases of breast cancer reported in this cohort. The majority of women reporting a history of breast cancer also reported having some degree of light perception (83%). The majority of breast cancer cases were reported among women with the highest level of vision (27%). Among those with a history of breast cancer, 17% also reported being NPL.

In unadjusted logistic regression analyses, NPL women were found to have a significantly lower breast cancer risk compared to LP women (Figure 1; OR = 0.45 [95% CI: 0.25-0.80]). Current age and reporting at least one full term pregnancy were both significantly associated with an increased breast cancer risk (OR = 1.04, 95% CI: 1.03-1.06; OR = 1.77, 95% CI: 1.11-2.80, respectively). Current or past smoking history was modestly associated with an increased risk of breast cancer (OR = 1.50, 95% CI: 0.95-2.35). Body mass index (BMI), menarche, current alcohol consumption of one or more drinks per day, and breastfeeding were not associated with a reported history of breast cancer. When adjusted for current age, at least one full term pregnancy, and smoking history, the effect of NPL was similar to unadjusted results, but no longer statistically significant (OR = 0.56, 95% CI: 0.30, 1.02). Stratified unadjusted comparisons of NPL and breast cancer history among women with a current age under 50 were similar to grouped results, but were not statistically significant (OR = 0.56, CI: 0.06, 4.80) and included only 6 breast cancer cases. When adjusted for current age, history of full term pregnancy and smoking history, the relationship was only modestly different (OR = .54, 95% CI: 0.06-4.73). We found a significant association between NPL and reduced breast cancer risk among women over age 50 at the time of the survey (OR = .40, 95% CI: 0.22-0.74). When adjusted for current age, history of a full term pregnancy and smoking history, the association remained significant (OR = 0.48, 95% 95% CI: 0.25-0.91). When analyzed by degree of visual impairment, the ORs for breast cancer were 2.46 (95% CI: 1.13, 5.37), 2.55 (95% CI: 1.17, 5.56), 3.13 (95% CI: 1.55, 6.34) and 1.52 (95% CI: 0.77, 2.99), respectively, for each increasing category of visual acuity compared to NPL women (Table 3).

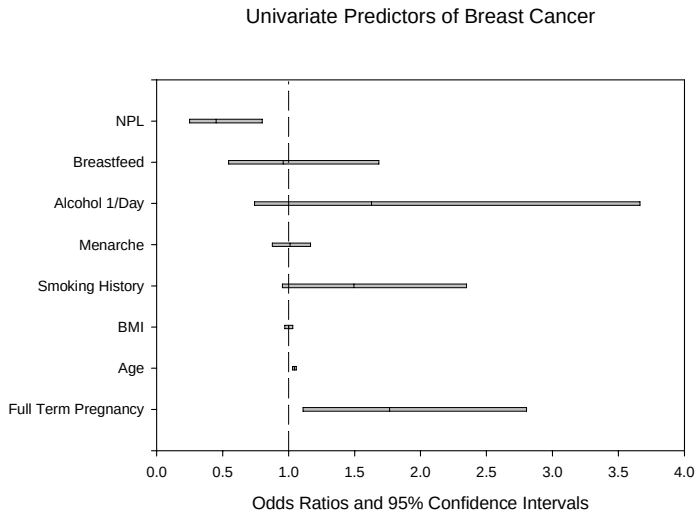

Figure 1. Odds ratios and 95% confidence intervals for uinvariate predictors of breast cancer.

Table 3. Cancer risk by level of vision.

Corrected Visual Acuity	Odds Ratio	95% CI	p-value
NPL (referent)	1		
Light Perception Only	2.46	(1.13, 5.37)	**0.0232**
Shadows and Hand Movement	2.55	(1.17, 5.56)	**0.019**
Counting Fingers	3.13	(1.55, 6.34)	**0.002**
Top Letter on Vision Chart	1.52	(0.77, 2.99)	0.228

Sleep Disorders

The mean PSQI score (range 0-21, with a score of ≥5 indicating a sleep disorder) was elevated in all groups, with a cohort mean of 8.05 (± 4.21) (Table 1). NPL women had a significantly higher mean PSQI score than LP women (Table 1). Analysis of sleep disorders in relation to other variables in the cohort is on going.

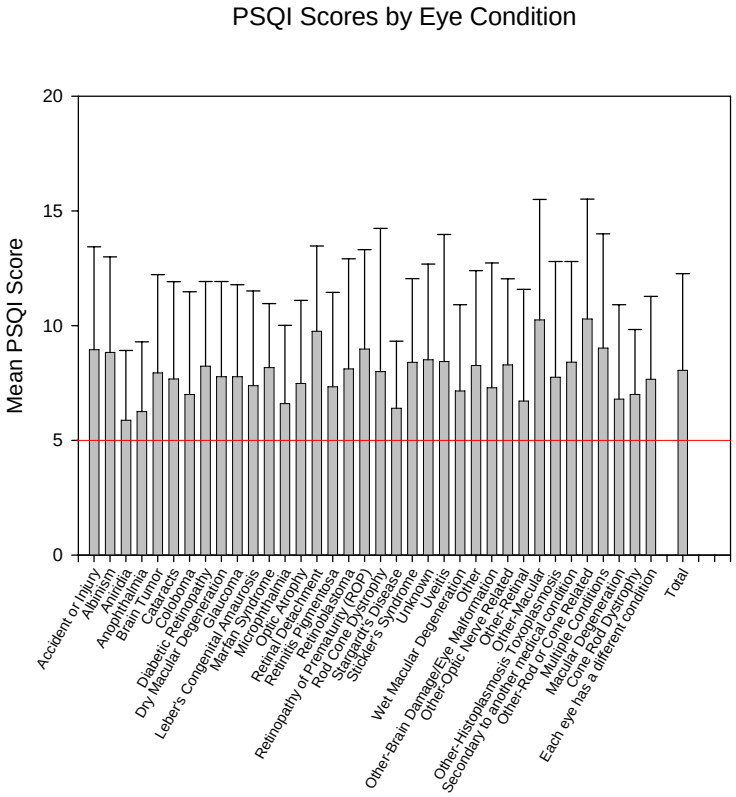

Figure 2. Mean PSQI score by condition causing visual impairment.

Part 2 – Assessment of Melatonin and Estrone Levels in the Visually Impaired

Methods

Sleep and urinary hormone data collection

A total of 130 subjects have completed a sleep, nap and (in pre and peri-menopausal women) menstrual cycle diary for eight weeks. Subjects also wore an activity monitor continuously during the eight week period. All subjects completed two or three 48 h sessions of urine samples. The first set of samples was collected after subjects completed the sleep diary for two to four weeks. The second set of samples was collected two to six weeks following the first set. The subjects were instructed to collect all urine over the course of each 48 h episode in four hourly bins throughout the waking period and eight hourly periods throughout the sleep period. Subjects were instructed to collect urine starting after the first morning void on the first day of collection. They were instructed to weigh each sample using speaking scales at the end of each sampling window and were instructed to pipette a small sample from each window into a 7ml tube and immediately freeze each sample. Subjects were asked to record the times of each void, the sample window and the total urine volume for each sample period. Subjects were not asked to alter any lifestyle habits throughout the study.

6-sulphatoxymelatonin (aMT6s) and estrone-3-glucuronide (e1g) assay

Urinary aMT6s concentrations were measured by Stockgrand Ltd., University of Surrey (Guildford, UK) RIA using the method of Aldhous and Arendt. Urinary e1g concentrations were also measured by Stockgrand Ltd, using a commercially available ELISA. All samples from an individual were measured in a single assay.

Statistical analyses

The mean 24h aMT6s and e1g outputs were calculated for each subject for each sample period. Data were grouped by degree of light perception and by menopausal status (pre, post-menopausal). For analysis of the circadian rhythms, data were converted into micrograms per hour for each 4-8 hourly collection period. Data for each subject and sample period were plotted and subjected to

cosinor analysis (software provided by Dr. D. S. Minors, University of Manchester, Manchester, UK) to provide the acrophase (peak) time and the amplitude of the urinary rhythm. Only results that showed a significant fit to the cosine curve (P<0.15) were used to assess entrainment. Subjects were considered normally phased if the mean of the two acrophase times for aMT6s fell within the normal range as described in Lockley et al., 1997 (range; 1.3-7.1)(18). Subjects were considered 'abnormally phased' if the mean of the two acrophase times fell outside the normal range. Mean 24 h output of aMT6s and e1g were calculated for each subject. Comparisons of 24 h values were made using Student's two-sample t tests and linear regression.

Results

Demographic comparisons of field study subjects

In Table 4 we present demographic characteristics of all women who participated in the field study. We found no significant differences between LP and NPL women on any variable examined.

Table 4. Comparisons of selected demographic characteristics among blind women who participated in the home based field study.

	Cohort			LP			NPL			
Variable	**N**	**Mean**	**Std Dev**	**N**	**Mean**	**Std Dev**	**N**	**Mean**	**Std Dev**	**P-value**
Age	129	51.00	13.46	87	50.39	14.88	42	50.90	10.56	0.818
Height	130	63.47	2.59	88	63.58	2.40	42	63.24	2.95	0.484
Weight	130	159.35	43.26	88	158.26	45.12	42	161.62	39.50	0.681
BMI	130	27.72	6.90	88	27.43	7.23	42	28.32	6.19	0.496
Menarche	125	12.26	1.63	86	12.12	1.54	39	12.56	1.80	0.156
Menopause Start (natural only)	51	45.90	6.41	29	45.97	5.72	22	45.82	7.37	0.936
Menopause Stop (natural only)	48	49.85	5.68	29	40.45	6.03	19	48.95	5.13	0.376
Age of 1st Term Pregnancy	65	25.31	5.68	47	24.89	5.47	18	26.39	6.21	0.346
PSQI score	124	8.05	4.25	84	7.56	4.12	40	9.08	4.41	0.063

6-sulphatoxymelatonin and estrone-3-glucoronide 24-h production in visually impaired women

We found no differences in 24 h aMT6s production in LP compared to NPL women (LP = 23.38 ±2.00 ug/24 h; NPL = 20.59 ±2.08 ug/24 h; p=0.34) or among pre-menopausal women compared to post-menopausal women (pre- = 23.82 ±2.71 ug/24 h; post- = 21.73 ±2.10 ug/24 h; p=0.54) (Figure 3). We also did not find any differences among post-menopausal women with LP compared to post-menopausal women with NPL (LP = 21.11 ±2.67 ug/24 h; NPL = 23.17 ±3.29 ug/24 h; p=0.66). However, we did find a significant difference in aMT6s production over 24 h in pre-menopausal women with LP compared to pre-menopausal women with NPL (LP = 26.20 ±3.39 ug/24 h; NPL = 14.72 ±2.66 ug/24 h; p=0.01).

Similarly, we found no differences in 24 h E1G production in LP compared to NPL women (LP = 0.02 ±0.003 ug/24 h; NPL = 0.01 ±0.002 ug/24 h; p=0.14) or among post-menopausal women with LP compared to post-menopausal women with NPL (LP = 0.001 ±0.008 ug/24 h; NPL = 0.007 ±0.001 ug/24 h; p=0.97) (Figure 4). As expected, we found a significant difference in 24 h E1G production among pre- compared to post-menopausal subjects (pre- = 0.03 ±0.003 ug/24 h; post- = 0.007 ±0.006 ug/24 h; p<.001). As in comparisons of 24 h aMT6s production, we also found a borderline significant difference in E1G production among pre-menopausal women with LP compared to pre-menopausal women with NPL (LP = 0.03 ±0.004 ug/24 h; NPL = 0.02 ±0.004 ug/24 h; p=0.06).

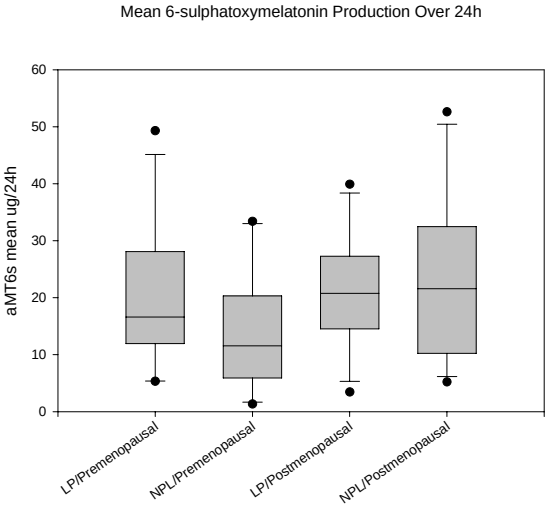

Figure 3. Mean aMT6s production over 24 hours by light perception and menopausal status. Box plots represent 50th, 25th and 75th percentiles.

When we compared 24 h aMT6s production to E1G production among all women analyzed, we found no relationship in all women combined (R^2 = 0.71 ±12.59; p=0.49). Similarly, we found no relationship among pre- or post-menopausal women (pre-menopausal

R^2 = 0.67 ±12.95, p=0.92; post-menopausal R^2 = 0.76 ±12.38, p=0.47). We also did not find a significant relationship among women with light perception (R^2 = 0.78 ±11.78; p=0.59) (Figure 5) regardless of menopausal status (pre-menopausal R^2 = 0.67 ±14.36, p=0.55; post-menopausal R^2 = 0.85 ±9.40, p=0.80). We did find a significant relationship between 24 h production of aMT6s and E1G among NPL women (R^2 = 0.73 ±12.69; p=0.04) (Figure 6). This relationship was attenuated among pre-menopausal NPL women (R^2 = 0.73 ±9.77; p=0.16) (Figure 7) and non-significant among post-menopausal women with NPL (R^2 = 0.69 ±15.51; p=0.48) (Figure 8). Additional analyses are underway to assess the relationship of E1G and aMT6s by menstrual phase and E1G rhythmicity.

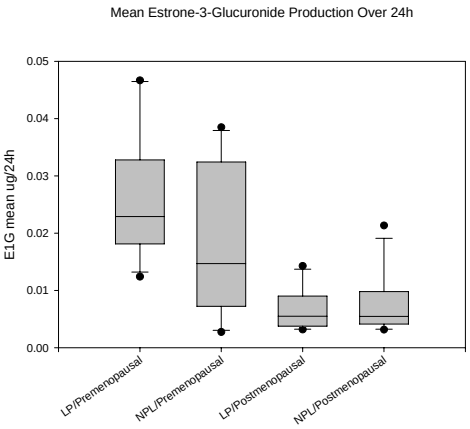

Figure 4. Mean E1G production over 24 hours by light perception and menopausal status. Box plots represent 50th, 25th and 75th percentiles.

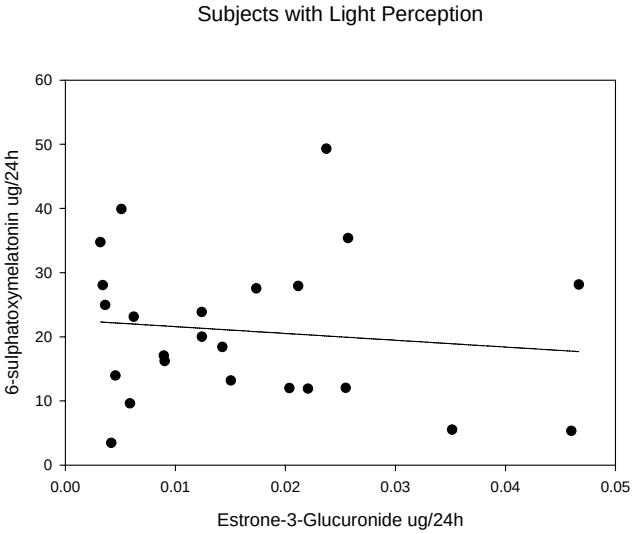

Figure 5. Mean E1G production over 24 h compared to mean aMT6s production over 24 h among all field study subjects with LP.

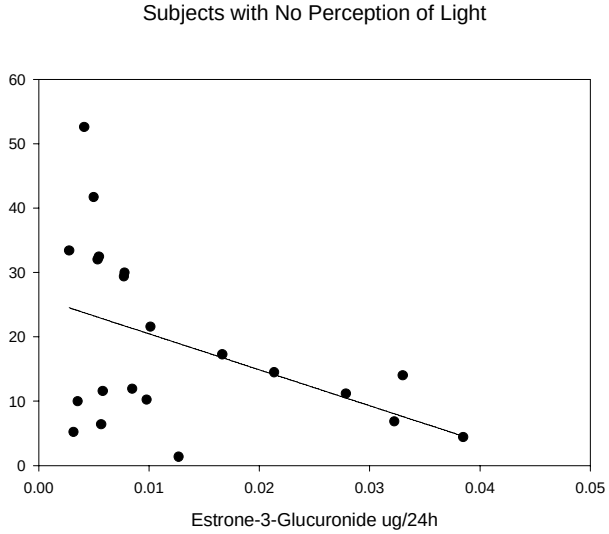

Figure 6. Mean E1G production over 24 h compared to mean aMT6s production over 24 h among all field study subjects with NPL.

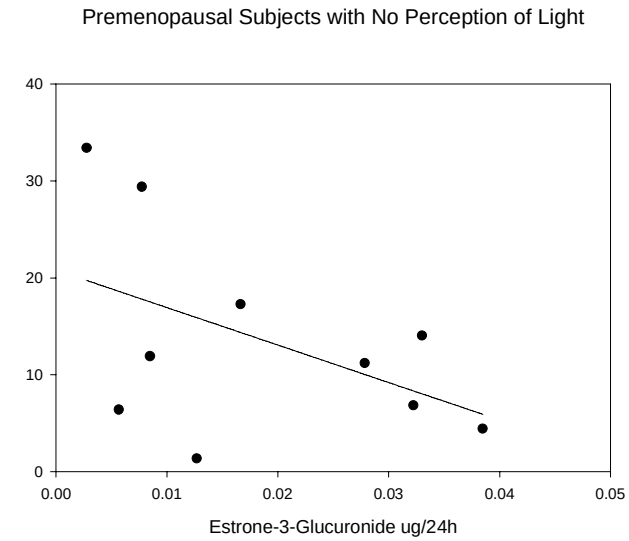

Figure 7. Mean E1G production over 24 h compared to mean aMT6s production over 24 h among pre-menopausal field study subjects with NPL.

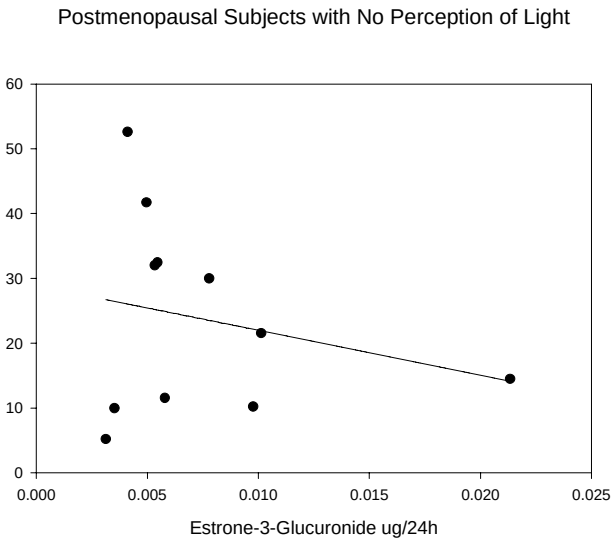

Figure 8. Mean E1G production over 24 h compared to mean aMT6s production over 24 h among post-menopausal field study subjects with NPL.

6-sulphatoxymelatonin circadian rhythmicity

When assessed by cosinor analysis, 75 subjects showed a significant aMT6s rhythm for at least two sample collection sessions. Of these subjects, 47 reported some degree of light perception and 28 reported having NPL. We found that 28 of the LP subjects and 12 of the NPL subjects were classified as normally phased (mean acrophase within normal range 1.3-7.1). We also found 19 of the LP subjects and 16 of NPL subjects were abnormally phased (mean acrophase outside the normal range). Figure 9 shows the distribution of sleep onset times among all LP subjects and Figure 10 shows the distribution of sleep onset times among NPL subjects. Figure 11 shows sleep diary plots for 20 subjects to illustrate the variability in the sleep timing of the field study cohort. Similar analyses are ongoing for comparisons of the timing of peak aMT6s acrophase time and sleep onset and offset by light perception status. Additionally these data will be examined to assess how total sleep time relates to degree of light perception, circadian entrainment and E1G production.

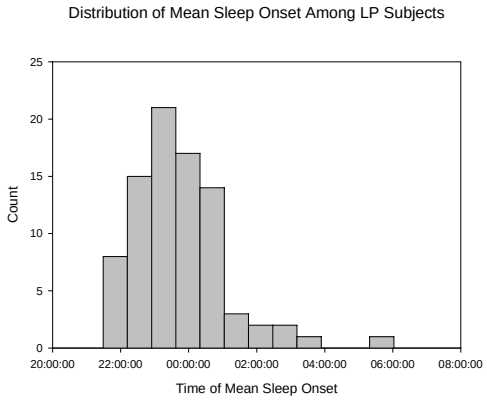

Figure 9. Mean sleep onset times among field study subjects with LP.

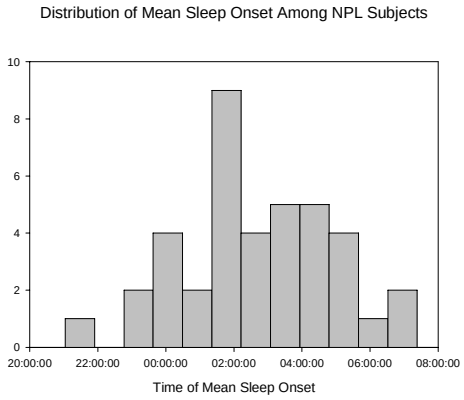

Figure 10. Mean sleep onset times among field study subjects with LP.

Discussion

We found several demographic and reproductive differences between LP and NPL blind women. We found NPL women to be significantly shorter than LP women, with a higher current BMI, but a lower weight and lower BMI at age 18. A smaller proportion of NPL women were parous and they reported a significantly earlier age of menarche than LP women. We also found a significantly lower reported history of breast cancer among NPL women. We further found some evidence that a reciprocal relationship may exist between estrogen and melatonin production over 24 hours in NPL women.

Our finding, that NPL women have a 55% lower risk of breast cancer compared LP women supports the melatonin hypothesis and is similar to prior reports. Hahn found that blind women had a lower than expected incidence of breast cancer, but the same incidence of heart attack and stroke (relative risk 0.57) when compared to sighted women in a study examining hospital medical records (9). Our results are consistent with this finding, as the highest OR we found among NPL women was 0.56. Three record linkage studies reported lower, but non-significant breast cancer risk among the NPL blind women with reported standardized incidence ratios of 0.82 among Swedish women, 0.47 among Finnish women and 0.64 among Norwegian women (10, 11, 13). We found lower ORs in our cohort, which is not unexpected considering our cohort was comprised of entirely blind women and prior reports compared blind women to the general sighted population. We found a modest effect of current or ever smoking and contrary to prior reports, we found that reporting at least one full term pregnancy was associated with an increased risk of breast cancer (24). It is unclear why a history of full term pregnancy would be associated with an increased breast cancer risk in our cohort, or why breastfeeding and menarche would have no effect on breast cancer risk. These findings suggest that blind women may differ from sighted women on some lifestyle variables, but these differences do not account for differences in breast cancer risk between LP and NPL women.

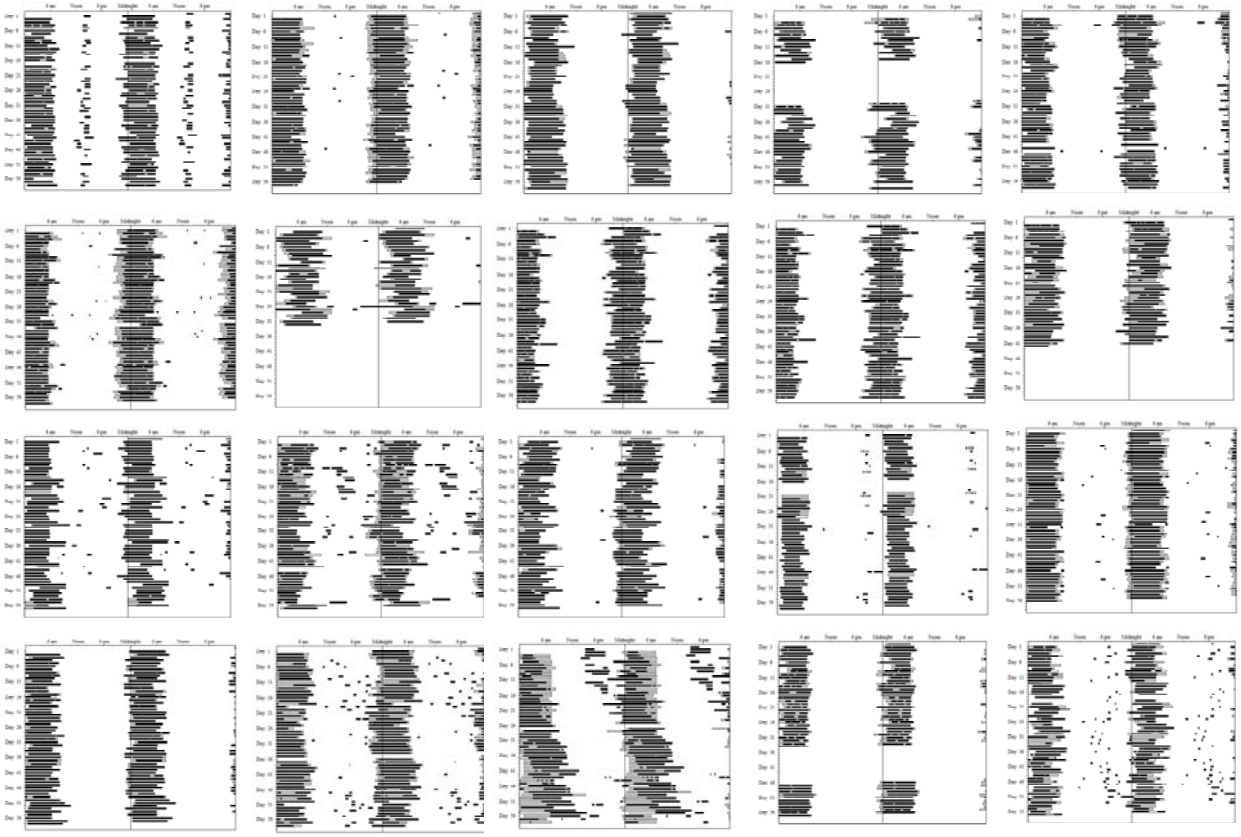

Figure 11. Sleep diary plots for 20 field study subjects with varying degrees of light perception. Each plot represents 56 days double plotted. Open bars represent time in bed awake and closed bars represent sleep.

As expected, older current age was strongly associated with reporting a history of breast cancer. When we adjusted our results for all significant predictors of breast cancer, including current age, the relationship of NPL with lower breast cancer risk was dampened and only bordered significance. When we stratified our results to remove the effect of current age, we found a significantly lower risk of breast cancer among NPL women in both unadjusted and adjusted analyses. Our analysis of women under age 50 was similar to our finding in women over age 50, but was not significant. We only found six breast cancer cases among women under 50 and only one case among NPL women under 50. We believe that the low sample size in women under 50 reduced our ability to detect a significant difference.

We found a significantly increased risk of breast cancer when we compared LP women by level of vision to NPL women. We found that women who could only perceive light, see shadows and hand movement, and count fingers had an increasing risk of breast cancer commensurate with increasing level of light perception when compared to NPL women. This finding is similar to the inverse association reported by Verkasalo and later by Pukkala (12, 25). However, we found significant results among women in the three lowest categories of visual acuity, but no difference among women with the highest level of vision. These differences could be due to differences in the definitions between prior studies and the present study. For example, the category with the highest visual acuity in the Finnish reports (moderate low vision) was defined using the World Health Organization classification system as 20/70 to 20/160 on the Snellen Scale. In the present study, women with a visual acuity better than 20/200 were excluded from participation in the study and our highest level of visual acuity was defined as being able to see only the top letter on the vision chart. It is unclear whether visual acuity relates to level of light perception, yet the stepwise differences in breast cancer

risk among the first three categories of visual acuity suggest that light input may be responsible for the differences in risk.

In multivariate analyses of menarche, adjusted for current age, current BMI and BMI at age 18, menarche remained significantly earlier in NPL women, compared to blind women with LP. In a further examination to assess the potential effects of light on menarche, we compared reported age at menarche in women NPL from birth to those with LP at birth. We found age of menarche to be even earlier in those women NPL from birth compared to the other women in the cohort. In addition we found a significant positive association between early loss of light perception and early onset of menarche. Our examinations of reproductive measures revealed an earlier menarche among NPL women compared to LP women by nearly four months. This finding confirms and extends earlier reports by Zacharias and Wurtman (19, 26) and Magee (27). Zacharias and Wurtman compared blind girls with retinopathy of prematurity (ROP) to prematurely-born sighted girls and found that menarche was advanced in girls with ROP. They found this effect to be accentuated among the NPL subset of girls with ROP. In a follow up study, they reported that NPL blind girls with ROP experienced menarche an average of seven months earlier than prematurely born sighted controls. They also found that among LP blind girls born at full term, menarche was an average of 4 months earlier than their sighted counterparts (19). Our findings were made in a cohort of women with a variety of eye conditions suggesting that differences in menarche are not due to a factor specifically related to ROP, but instead result from a direct effect of loss of light perception. We further found that women with NPL from birth and women who reported being NPL at least two years prior to menarche experienced a significantly earlier menarche compared to women who reported NLP occurring within the two years prior to onset of or after menarche, suggesting that timing of blindness is an important factor influencing the reproductive axis. Our findings differ from earlier reports in that all of the women in our cohort reported being at least legally blind, which likely accounts for the smaller differences in our findings. As in our study, Magee and colleagues examined only blind girls and found a significantly earlier age of menarche in girls with clinically confirmed minimal and NPL (12.0 years) compared to those with shadow vision and guiding sight (12.8 years) (27). Further, the mean menarche for our entire cohort occurred at 12.37 years while similar studies suggest that sighted women of the same age range experience menarche around 12.7 years (28).

In contrast to ours and others' findings, two reports found no differences in age at onset of menarche in blind as compared to sighted women. Thomas and Pizzarello found no differences in menarche in 26 institutionalized blind and 69 institutionalized sighted girls, and Lehrer found no differences in a comparison of 24 LP and 31 NPL women (29). There are several possible reasons for the conflicting results. The study by Thomas and Pizzarello did not account for degree of blindness, type of blindness or the reason for the institutionalization of the sighted girls, any of which may be important factors in the timing of menarche. Lehrer examined a small cohort of blind women and did not account for when each woman became blind.

In addition to differences in reproductive and cancer outcomes, we found NPL women to be shorter than LP women. This finding is consistent with that of Bellastella and colleagues, who reported that blind girls and adults with varying degrees of light perception had a shorter stature than age-matched sighted controls (30, 31). The mean BMI in our cohort was overweight and bordered on obese, and we found the BMI of NPL women to be statistically higher than in LP women due to decreased height. This finding is similar to that of the general US population for women in this age range, who reportedly have an average BMI of 29.2 (32). Our findings are also similar to those of Leger and colleagues, who found BMI to be marginally lower in blind people compared to sighted people (33). The increased current BMI in NPL women does not account for the earlier menarche observed in this group, as could be hypothesized (34), as their weight and BMI were significantly lower than LP women at age 18. Consistent with prior reports, we found no differences between LP and NPL women in age at natural menopause, which occurred around age 50 (33). We also found no differences in the age of first birth between LP and NPL women; however, only 42% of NPL women reported being parous compared to 57% of LP women.

Our cohort was highly educated with over half of all participants attaining a college degree and nearly a third attaining a post-graduate degree. In the general US population, only around 17% earn at least a college degree and only around 9% more go on to complete an advanced degree (35). It is

unclear why our cohort is different. One possibility is that educational programs aimed at blind women lead to a higher rate of achievement than in the general population. Another equally plausible explanation is that women who are highly educated were more interested in participating in this type of research.

Given the role that light plays in mediating seasonal reproduction in other mammals, our findings may have important implications for how light may affect the human reproductive system. Seasonal and daily circadian cycles are synchronized by light via specialized photoreceptors in the ganglion cell layer of the retina (36). One of the primary targets of this light signal is the endogenous circadian pacemaker, located in the SCN of the hypothalamus (37). This clock controls the timing of internal rhythms such as sleep-wake cycles, production of hormones (e.g. melatonin, cortisol, prolactin), core body temperature, and oscillations in alertness and performance throughout each day. In the absence of ocular light information reaching the SCN, as in bilaterally enucleated and most NPL subjects, the endogenous pacemaker reverts to its internal period (day-length) which is close to, but not exactly 24-hours (range 23.9-25.0 hours) (18, 38-41). This inability to entrain the internal clock to the environmental light–dark cycle induces a chronic cyclic sleep disorder called non-24-hour sleep-wake disorder characterized by cyclic episodes of good and bad sleep for many weeks or months (33, 42-44). In addition, the pineal hormone melatonin is acutely suppressed by ocular light exposure via the same pathway (45-47), and people with NPL lacking circadian photoreception do not experience light-induced melatonin suppression (45, 46).

It is unclear how attenuated light exposure and/or disordered circadian rhythms may play a role in human reproductive development. Importantly, however, photoperiodic timekeeping via melatonin duration signalling is a major mechanism for control of seasonal reproduction in seasonally breeding mammals (). Seasonal reproduction is maintained through light-mediated signalling of long or short night length, which is translated by the circadian system into long or short nocturnal melatonin duration, respectively. The duration of these melatonin pulses confers day/night length information to the reproductive axis to signal appropriately timed seasonal breeding (48). When an animal is blinded, night length information is removed, and in the absence of non-photic cues the animal is no longer able to synchronize breeding to the appropriate season (49). While data on the role of melatonin in reproductive development in humans are not available, it is possible that a similar mechanism is responsible for our observed differences in menarche in blind women. Humans can detect photoperiodic changes via their melatonin rhythm (50) and, given that light can alter melatonin duration and timing, it is conceivable that attenuation of circadian photoreception due to extensive ganglion cell damage may interfere with light-related reproductive development. The suggestion that ocular light exposure may influence reproductive development also has important implications for research that suggests exposure to light at night is associated with an increased risk of breast cancer. Recently, the World Health Organization categorized shift-work as a “probable carcinogen” (51). This decision was based on findings that female night shift workers and flight attendants have an increased risk of breast cancer (3, 52). We and others have reported that blind women have a reduced risk of breast cancer, possibly via similar albeit opposite mechanisms (9). The “protective effect” of blindness is reported to increase in a dose-dependent manner with decreasing visual acuity, suggesting that degree of light perception may be responsible for the differences in reported risk (12, 25). These studies, however, have not examined how other reproductive risk factors for breast cancer may contribute to the reduced risk observed in the blind. Our finding of earlier menarche in NPL women, for example, is inconsistent with reduced breast cancer risk; in sighted women, early menarche, late age at first term birth, and null parity are all associated with an increased risk of breast cancer (53).

The physiological changes that relate to degree of blindness are currently unknown. Our examinations comparing aMT6s and E1G production over 24 h provide encouraging preliminary evidence that a reciprocal relationship exists among NPL women. Analyses of these hormones are ongoing and examinations of estrogen rhythmicity over 24 hours and assessments of the relationship of estrogen and melatonin during different menstrual phases will be important.

One limitation of our study is that our categorization of blindness may suffer from some degree of misclassification. It is likely that a small subset (~5%) of the women who reported being totally functionally blind still retain circadian photoreception without realizing it, as the cells that transmit the light signal to the SCN are different from those used for sight (45, 46). This would mean that some

women whose melatonin rhythms are sensitive to light are included among the NPL group. Such misclassification would likely have lead to an underestimate of the association between NLP and age at menarche, when compared to women with LP. A further limitation of this study is that it may suffer from some degree of selection bias as we found that the women in our cohort tended to have a higher level of education than the general population. This bias would only hamper the generalizability of our results and it is unlikely that this could account for the differences observed between NPL and LP women given that education levels were similar between the groups. It is likely that there are other differences between the prior reports on breast cancer and the blind and the present study. Our study results are based on subjective data; in contrast, all prior reports were based on historical records that did not necessarily provide accurate data on each subject's visual acuity.

Problems encountered in accomplishing the Statement Of Work

We have not been able to achieve the anticipated recruitment rate to date for the epidemiological survey. We had hoped to establish a database with 12,000 participants but only 1400 have completed the study, despite reaching ~40,000 visually impaired women by conservative estimate based on several nationwide appeals. While this study still represents the largest and most comprehensive database of breast cancer risk factors in the visually impaired constructed to date, the relatively numbers preclude development of a prospective cohort as we had originally planned.

KEY RESEARCH ACCOMPLISHMENTS

- We have surveyed 1392 visually impaired women and established a database addressing a wide range of risk factors associated with breast cancer in this population
- We found that NPL blind women have a lower risk of breast cancer compared to LP blind women and that the differences in breast cancer risk cannot be explained by any known risk factor for breast cancer.
- We found that NPL blind women experience an earlier menarche compared to LP blind women. This finding suggests that light may play a role in reproductive development.
- We found encouraging preliminary evidence that suggests that melatonin and estrogen may be reciprocally related, however, these findings are currently limited to NPL women.
- Finally, we ran two summer undergraduate training programs in circadian biology and breast cancer and an ongoing undergraduate volunteer program that, combined, have been completed by nearly 30 undergraduate students.

REPORTABLE OUTCOMES

Databases

As described above, we have constructed a database of 1400 visually impaired women for the assessment of risk factors associated with breast cancer including visual impairment, reproductive function and history, diet and circadian rhythm desynchrony.

Abstracts and Presentations

2004 Lockley SW. Circadian rhythms in blind women. Circadian Disruption and Breast Cancer Meeting; 2004 Jul 9-11; Chapel Hill.

2005 Evans EE, Schernhammer ES, Silver ES, Stevens RG, Lockley SW. Reproductive and hormonal risk factors for breast cancer in blind women. Dana-Farber/Harvard Cancer Center Cancer Disparities Program, New Investigators Poster Session; 2005; Apr 15; Boston,

2005 Lecture Series: Reproductive and hormonal risk factors for breast cancer in blind women Summer undergraduate program (10 x 3-h lectures), Division of Sleep Medicine, Brigham and Women's Hospital; 2005; Jun 1-Aug 16; Boston.

2005 Evans EE, Schernhammer ES, Silver ES, Stevens RG, Lockley SW. Reproductive and hormonal risk factors for breast cancer in blind women. Era of Hope Department of Defense Breast Cancer Research Program Meeting; 2005; Jun 8-11; Philadelphia

2006 Evans EE, Schernhammer ES, Silver ES, Stevens RG, Lockley SW. Reproductive and hormonal risk factors for breast cancer in blind women. Division of Sleep Medicine Annual Poster Session, Harvard Medical School; 2006; Jun 13; Boston.

2006 Lockley SW. Circadian Rhythms in human health and disorders. National Institute of Environmental Health Sciences Workshop; 2006; Sep 14-16, Washington.

2006 Evans EE, Lockley SW. Reproductive and hormonal risk factors for breast cancer in blind women. Brigham and Women's Hospital Biomedical Research Institute: Cancer Research Center Annual Retreat poster session; 2006; Oct 13; Boston.

2008 Evans EE, Stevens RG, Lockley SW. Differences in breast cancer risk in blind women with and without light perception. Abstract. 11th meeting of the Society for Research in Biological Rhythms (SRBR); 2008 May 17-21; Destin, USA.

2008 Evans EE, Lockley SW. Urinary melatonin and estrogen production in pre- and post-menopausal blind women. Era of Hope Department of Defense Breast Cancer Research Program Meeting; 2008; Jun 25-28; Baltimore, USA.

2008 Evans EE, Stevens RG, Lockley SW. Effect of light perception on reproductive function in blind women. Era of Hope Department of Defense Breast Cancer Research Program Meeting; 2008; Jun 25-28; Baltimore, USA.

2008 Lockley SW, Hull JT, Gooley JJ. Effects of photic and non-photic stimuli on melatonin. Abstract. FASEB Summer Research Conference 'Melatonin receptors: Actions and therapeutics'; 2008; Aug 10-15 Snowmass, USA.

Publications

2007 Stevens RG, Blask DE, Brainard GC, Hansen J, Lockley SW, Provencio I, Rea MS, Reinlib LE. The role of environmental lighting and circadian disruption in cancer and other diseases. *Environmental Health Perspectives* 2007; 115:1357–1362.

2008 Flynn-Evans EE, Stevens RG, Tabandeh H, Schernhammer ES, Lockley SW. Effect of light perception on menarche in blind women. *Epidemiology*, 2008; under review.

CONCLUSIONS

In summary, our study is the largest cross-sectional examination of breast cancer risk, reproductive function and history, sleep disorders and health of blind women to date. These data will provide a rich resources for test multiple hypotheses and generate new scientific directions.

In the current study, we found differences in demographic and reproductive characteristics in women with and without light perception. We report that NPL women are somewhat shorter than LP women accounting for a slightly higher BMI. We found that a higher percentage of LP women report having at least one pregnancy than NPL women, though the average age of first birth between these groups was the same. Our findings extend and confirm prior reports that NPL blind women experience advanced menarche, with an even earlier effect among those women NPL from birth to two years prior to menarche. We found a lower risk of breast cancer among NPL women compared to LP women. We found that for the lowest three categories of visual acuity, cancer risk increased as vision increased. We further found that when stratified by age 50, NPL women over age 50 reported a significantly lower history of breast cancer compared to LP women, when adjusted for current age, history of at least one full term pregnancy and current or past smoking status. Our findings are consistent with the melatonin hypothesis and suggest that NPL blind women have a reduced risk of breast cancer that cannot currently be explained by lifestyle differences. Our ongoing analysis will examine specific differences in NPL and LP blind women to determine if physiological differences exist and how such differences might relate to differences in breast cancer risk.

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