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Associations among Salivary Cortisol, Melatonin, Catecholamines, Sleep Quality and Stress in Women with Breast Cancer and Healthy Controls

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Dysregulations in several biological systems in breast cancer patients have been reported, including abnormalities in endocrine and sympathetic nervous system indices, as well as psychological disturbances and sleep disorders. The purpose of this exploratory study was to compare women with breast cancer to healthy control women on measures of salivary cortisol, urinary catecholamines, overnight urinary melatonin, and self-reported sleep quality, symptoms of stress, depression, anxiety and mood disturbance, to determine if discernable patterns of dysregulations across systems were apparent. Thirty-three women were tested in each group, with an average age of approximately 52 years, primarily Caucasian and well-educated. Forty percent of the women with breast cancer had stage 2 disease and they were an average of 1.36 years post-diagnosis. Women with breast cancer had significantly higher levels of disturbance on all the psychological indices, but there were no differences between groups on any of the biological measures, with the exception that the control women had higher dopamine values than the participants with breast cancer. None of the psychological scores were correlated with the biological measures. These results are consistent with other studies of early-stage breast cancer and highlight the importance of considering disease characteristics when investigating endocrine and sympathetic nervous system functioning.

KEY WORDS: breast cancer; cortisol; melatonin; catecholamines; stress; depression; anxiety.

Women with breast cancer have been documented to have dysregulation in several important circadian systems, including hormonal, sleep and autonomic rhythms (Bovbjerg, 2003; Touitosu *et al.*, 1996; Sephton and Spiegel, 2003). Such dysregulation has been associated with shorter survival time in some cases (Sephton *et al.*, 2000). Stress and mood disorders may contribute to these abnormal biological rhythms, and thus may be indirect contributors to the poorer outcomes (Spiegel and Sephton, 2001; Antoni, 2003; Lutgendorf and Costanzo, 2003). Associations between dysregulation in these different, yet inter-related, systems remain unclear. The current study aimed to examine these associations in women with breast cancer, in order to test hypotheses generated via a novel biobehavioral model of the causes and consequences of circadian dysregulation. This study is unique in that it investigated salivary cortisol, urinary catecholamines and melatonin as well as self-report measures of stress, sleep and mood in both breast cancer patients and healthy controls of equivalent age and education. By better defining the factors involved in circadian dysregulation among breast cancer patients, the proposed research aimed to (i) pave the way for effective biobehavioral interventions (e.g., pharmacological therapy, sleep therapy, stress management training) aimed at improving circadian rhythms; and (ii) help

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increase public awareness of behaviors that may help improve the circadian profile (and thus a myriad of concomitant problems).

Biobehavioral Model of Altered Dysregulation in Circadian Systems

Previous research demonstrates the importance of circadian systems in the well-being and quality of life of women with breast cancer (Spiegel *et al.*, 2001; Sephton *et al.*, 2003). Nevertheless, the mechanisms contributing to circadian dysregulation are poorly understood from a biobehavioral perspective. We evaluated the hypothesis that the degree of dysregulation in the endocrine system is related to; (i) sympathetic (SNS) nervous system activity (as measured by urinary catecholamines), (ii) self-reported sleep quality, and (iii) psychosocial stress. The model illustrated in Fig. 1 shows the relationships of interest between the constructs that were measured in this study. The boxes with thin arrows in the last level indicate the operational measures of functioning in each system that were assessed. In general, the model postulates potential reciprocal connections among dysregulated salivary cortisol slopes and increased SNS activity, as well as between lower melatonin levels and increased SNS activity and sleep disturbance. The literature review will briefly outline the research that supports the importance of each of these measures in a breast cancer population.

Cortisol

Cortisol is the primary stress hormone secreted from the adrenals. A large body of evidence has associated excessive release with suppression of the immune system (for reviews see Andersen *et al.*, 1994; Cohen and Williamson, 1991; Spiegel *et al.*, 1998) and it is largely responsible for the downregulation of immune function as a result of stress. Its hypersecretion also results in depressed mood (Sikes and Lasley, 1989; Wolkowitz, 1994). Cortisol levels have been reported to be elevated and overall diurnal profiles flatter in breast cancer patients compared to control women (Abercrombie *et al.*, 2004; van der Pompe *et al.*, 1996; Porter *et al.*, 2003), but the supportive data stems primarily from women with metastatic, rather than earlier stage, cancers. For example, abnormal patterns of cortisol secretion have been reported in up to 75% of a sample of metastatic breast and ovarian cancer patients (Touitou *et al.*, 1996). Further, the slope of the rate of change of cortisol levels measured four times a day for three

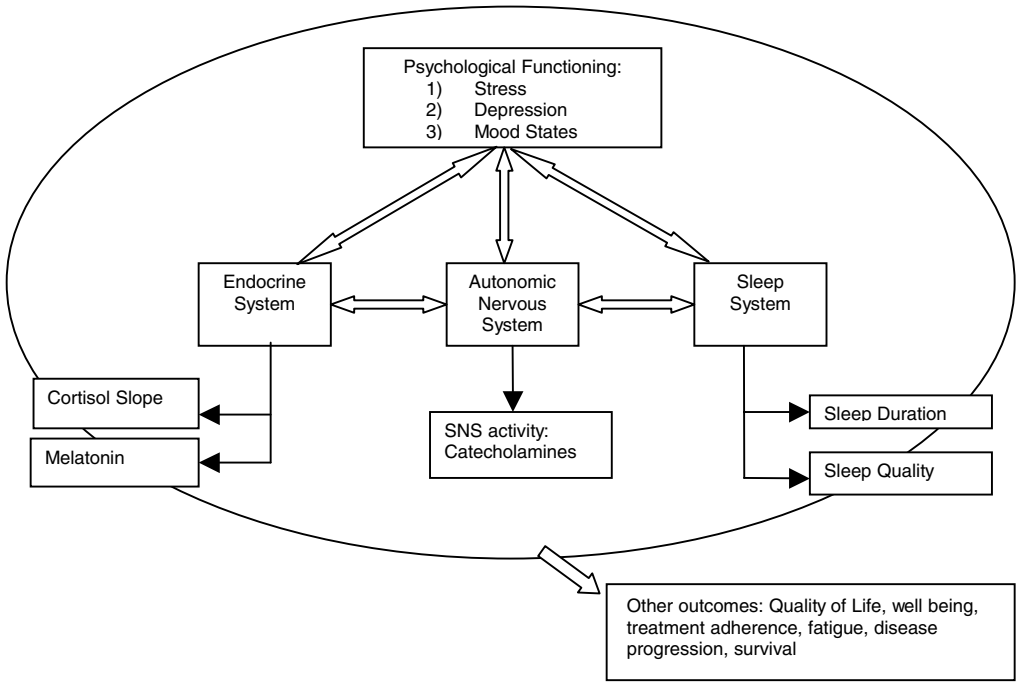

Fig. 1. Conceptual model.

consecutive days was associated with survival time in a group of 104 women with metastatic breast cancer. Those women who displayed less variation in salivary cortisol levels, expressed as a flatter slope and indicating a lack of normal diurnal cortisol variation, experienced earlier mortality over a 7-year follow-up period, with a hazard ratio of 464.9; (95% CI = 47.5 – 28 953.0)(Sephton *et al*., 2000).

When these patients were split at the median cortisol slope for descriptive purposes, 77% of those with flat rhythms had died after surviving an average of 3.2 years. In contrast, 60% of the patients with relatively steep rhythms had died, with an average survival of 4.5 years. Hence, the women with steeper slopes survived more than 1 year longer on average. This relationship held even when other prognostic medical variables were taken into account, such as markers of disease status (e.g., location of metastases, estrogen receptor status), medical treatment (e.g., chemotherapy drugs) and psychosocial variables (e.g., stress levels and marital satisfaction) (Sephton *et al*., 2000). The authors speculate that these abnormal circadian rhythms of cortisol secretion represent compromised hypothalamic-pituitary-adrenal (HPA) axis functioning, which may be responsible for earlier mortality. Indeed other studies have reported circadian abnormalities in the secretion of 12 hormones in women at high risk for developing occurrences of breast cancer (Ticher *et al*., 1996), as well as associations with later stages of cancer development and other prognostic indicators such as poorer performance status and more metastatic involvement (Mormont and Levi, 1997; Touitou *et al*., 1996).

A recent study of similar design to this project compared women with newly diagnosed primary breast cancer to healthy matched controls on cortisol levels and psychological measures (Vedhara *et al*., 2006). The primary purpose was to compare the validity of different techniques for calculating diurnal cortisol production, but they also compared cortisol levels between the two groups but found no significant differences except on distress levels, which were higher in the breast cancer patients. This research stands in contradistinction from that reviewed in metastatic populations above.

Melatonin

The pineal hormone melatonin has been implicated in the treatment of many types of cancers and other diseases (Saez *et al*., 2005; Bubenik *et al*., 1998). Proposed mechanisms of action include its effects as a free radical scavenger, an antioxidant, as well as an immunomodulatory agent and through the promotion of apoptosis of cancer cells in animal and human models (Vijayalaxmi *et al*., 2002). In both in vitro and in vivo investigations, melatonin protected healthy cells from radiation- induced and chemotherapeutic drug-induced toxicity (Vijayalaxmi *et al*., 1999; Vijayalaxmi *et al*., 1998). In humans, a series of clinical trials using melatonin in conjunction with standard treatment found superior survival response in patients with advanced cancer receiving adjuvant melatonin therapy (Lissoni *et al*., 1999a), and higher tolerance of standard chemotherapy regimes (Lissoni *et al*., 1999b; Lissoni *et al*., 1997). A review of the animal and human literature concluded that converging evidence supports large transnational research-based clinical trials of melatonin therapy for a wide variety of cancers (Vijayalaxmi *et al*., 2002). For years reports have indicated that women with breast cancer have suppressed or absent nocturnal melatonin peaks (Tamarkin *et al*., 1982). Epidemiological studies have also shown increased risk of breast cancer in women who work night shifts (Davis *et al*., 2001; Schernhammer *et al*., 2001). One biologically plausible explanation for this association is that these women have blunted melatonin secretion rhythms, and lack the nocturnal melatonin peak that is associated with normal sleep cycles. We sought to determine if melatonin production was different in breast cancer patients compared to healthy matched controls, and whether it was related to self-reported sleep quality.

Autonomic Nervous System

There is preliminary evidence suggesting that autonomic control may sometimes be impaired among breast cancer patients (Bettermann *et al*., 2001). Women at high familiar risk for breast cancer showed a greater catecholamine response to laboratory stressors than healthy women with normal risk levels (Gold *et al*., 2003), and they also had higher urinary levels of epinephrine during the work day (James *et al*., 2004). During sleep there is usually a reduction of sympathetic nervous system activity and an increase in parasympathetic nervous system function, but the quality of sleep contributes to these autonomic changes. Deep-wave sleep is characterized by markedly reduced SNS activity, in that both norepinephrine and epinepherine levels decline (Linsell *et al*., 1985). Hence, sleep quality can impact

production of SNS catecholamines, or vice versa (higher SNS arousal can negatively impact sleep quality). These observations are consistent with the mechanisms in Fig. 1, depicting the inter-relationship between autonomic nervous system activity and sleep. If sympathetic nervous system activity is not reduced sufficiently during sleep, other systems may also suffer dysregulation. Hence, we sought to determine if epinepherine and norepinepherine production was related to sleep quality and melatonin release.

Sleep System

In the general North American population 1/3 of adults experience intermittent insomnia, and 10% suffer chronic insomnia (Hossain and Shapiro, 2002; Kushida *et al*., 2000). The prevalence of chronic insomnia is much higher in breast cancer, with anywhere from 30–50% of patients reporting sleep difficulties (Savard *et al*., 2001; Savard and Morin, 2001) that often persist well into the post-treatment period. In metastatic breast cancer patients, 63% reported serious sleeping problems (Koopman *et al*., 2002). Suggested causes of sleep disturbance range from the physiological (e.g., effects of cancer and its treatments) to the psychological (e.g., stress, depression and worry), with factors relating to anxiety and stress emerging as one of the most important concomitants of sleep complaints in general (Hall *et al*., 2000). Breast cancer patients who suffer from insomnia or other sleep disturbances may experience many adverse effects as a consequence of the psychological trauma of cancer diagnosis, and during the course of illness and treatment. Individuals with insomnia have been characterized by increased SNS activity, increased 24-hour metabolic rate, and elevated cortisol and norepinepherine. A general state of physiological hyperarousal is considered to be symptomatic of these poor sleepers, and insufficient sleep duration has been shown to be associated with all-cause mortality (Kripke *et al*., 2002). In order to address these issues we compared self-reports of sleep activity between breast cancer patients and controls.

Psychosocial Stress and Related Psychosocial Factors

A number of observations have shown that stress is highly associated with quality of life, mood disturbance and fatigue in breast cancer patients (Shapiro *et al*., 2003; Shapiro, 2001; Carlson *et al*., 2003). For example, in our ongoing work evaluating a stress-reduction intervention in breast and prostate cancer patients (Carlson *et al*., 2004), scores on stress measures are consistently and highly correlated with measures of quality of life and total mood disturbance, both before and after intervention participation. Interestingly, as noted above, several lines of evidence show psychological stress may adversely affect sleep quality, endocrine function, and sympathetic nervous system function. Indeed, reported decreases in symptoms of stress after a stress-reduction program were significantly correlated with both reductions in fatigue and in sleep disturbance in a mixed group of cancer patients (Carlson and Garland, 2005). In that study improvements in sleep were more strongly related to reductions in somatic rather than psychological stress symptoms: this may hint at the importance of activating the physiological relaxation response and decreasing SNS arousal in order to impact the sleep system. Thus it is possible that the impact of stress on overall quality of life, mood and fatigue levels in breast cancer patients might be through its effects on various circadian systems. Combined, these observations suggest that anxiety, depression and stress may be associated with several circadian systems important for the well-being of breast cancer patients.

Hence, taking into account the background literature which documents the relationships between each biological system and breast-cancer related outcomes, we assessed measures of salivary cortisol, overnight urinary melatonin, measures of 24-hour urinary catecholamines (norepinepherine, epinepherine and dopamine) as SNS markers, and self-reported sleep, mood, depression, anxiety and stress levels.

Study Objectives

1. To compare, between women with breast cancer and healthy controls, measures of psychological status.
2. To compare, between women with breast cancer and healthy controls, measure of functioning of the following biological systems: a) endocrine; b) autonomic and; c) sleep.
3. To measure the degree of association among these biological systems within each group.
4. To measure associations between biological and psychological measures of stress and mood disturbance within each group and across groups.

METHODS

Participants

Inclusion criteria

Patients. Inclusion criteria in the breast cancer patient group included: 1) over 18 years of age; 2) Completion of primary treatment at least three months previously (except hormonal therapy); 3) Pre- or postmenopausal status. Women who report being amenorrheic at least 12 months, or report a surgical menopause, were classified as postmenopausal.

Controls. Inclusion criteria for the control group included: 1) over 18 years of age attending the Screen Test program for routine mammography (with negative results); 2) no current or prior diagnosis of any type of cancer; 3) pre- or postmenopausal status.

Exclusion Criteria

Exclusion criteria for both patients and controls included: 1) concomitant Axis I disorder (evaluated with a structured clinical interview for DSM-IV-R); 2) presence of any of the following medical conditions: diabetes mellitus, previously diagnosed obstructive sleep apnea, pacemakers/defibrillators, atrial fibrillation, myocardial infarction, percutaneous transluminal coronary angioplasty or coronary artery bypass graft within 6 months of enrollment, congestive heart failure, uncorrected primary valvular disease, uncorrected thyroid heart disease, renal or hepatic dysfunction, dementia, multiple sclerosis, alcohol or drug abuse within 12 months, current pregnancy, primary sleep disorders including insomnia; 3) Medication use that would affect hormone levels or alter autonomic function or sleep including: hydrocortisone, anxiolytics, oral contraceptives, hormone replacement therapy, benzodiazepines, non-benzodiazepine hypnotics, barbiturates, Selective Serotonin Reuptake Inhibitors (SSRIs), MAO-inhibitors.

Recruitment

Patients. Participants were recruited from the Tom Baker Cancer Centre (Calgary, AB, Canada). With the approval and co-operation of the breast tumor group staff, eligible patients were invited to participate in the study and given a one-page pamphlet summarizing the research protocol during their clinic visit. If patients were interested in participating, their name was placed on a waiting list administered by the research assistant. Patients were also recruited with pamphlets and posters around the centre, and were able to self-refer. The study was approved by the Conjoint Health Research Ethics Board of the University of Calgary Faculty of Medicine and Tom Baker Cancer Centre. Participants were not paid for their participation in the study, but parking expenses were covered and all equipment supplied.

Controls. Women who were attending for routine mammography screening were recruited after all the breast cancer patients had been tested. This allowed for selective recruitment in order to create groups that were similar to the patients in terms of key demographic variables such as age, menopausal status and parity. Women were not contacted until after they were aware that their mammogram had come back negative (no cancer present). Women with cancer were also able to refer their eligible friends as control participants, which proved to be a useful strategy to find women matched on important demographic features.

Measures

Psychological Measures

Stress. Symptoms of Stress Inventory (SOSI) (Leckie and Thompson, 1979): The SOSI was designed to measure physical, psychological, and behavioral responses to stressful situations. Predictive and concurrent validity has been demonstrated. In a mixed chronic-illness sample of malignant melanoma and myocardial infarction patients, manifest symptom distress as measured by the SOSI was directly related to functional alterations due to disease and inversely related to cognitive adaptation and perceived quality of life (Cowan *et al*., 1992). Cronbach's alpha for the SOSI total score was 0.97.

Depression. Centre for Epidemiological Studies—Depression Inventory (CES-D) (Radloff, 1977). The CES-D is a 20-item self-report scale designed to measure depressive symptomatology in the general population. It assesses the primary symptoms used to make a clinical diagnosis of depression. Each response is scored from zero to three

depending on the frequency of symptom occurrence. Higher total scores indicate more depressive symptoms. A score of 16 or higher has been used extensively as the cut-point for high depressive symptoms. It serves as a reliable and valid tool for screening symptoms of a major depressive episode (Radloff, 1977).

Anxiety. Spielberger State-Trait Anxiety Inventory (STAI) (Speilberger, 1983). This 40-item inventory was used primarily to assess trait anxiety. This scale has internal consistency reliabilities $> .80$ and test-retest reliabilities ranging from .73 to .86 for intervals up to 103 days (Speilberger, 1983). The STAI has also been related to daytime drowsiness (Claghorn *et al*., 1981), insomniacs' sleep latency (Chambers & Kim, 1993) and altered sleep patterns assessed with polysomnography (Kajimura *et al*., 1998).

Mood. Profile of Mood States (POMS)(McNair *et al*., 1971): The POMS is a 65-item scale which assesses six affective dimensions: tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue inertia, and confusion-bewilderment. It has been widely used in the assessment of mood changes resulting from a variety of interventions due to its responsiveness, and has been used extensively with cancer populations (Cassileth *et al*., 1985). Kuder-Richardson internal consistency of the six subscales range from .84 (Confusion) to .95 (Depression) in two studies, with test retest stability of .65 (vigor) to .74 (depression) over a period of 20 days on average.

Sleep

Pittsburgh Sleep Quality Index (PSQI) (Buysse *et al*., 1989). This is a self-rated questionnaire which assesses sleep quality and disturbances over a 1-month time interval. Nineteen individual items generate seven "component" scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. The sum of scores for these seven components yields one global score, referred to as the Global Sleep Quality score. Acceptable measures of internal homogeneity, consistency (test-retest reliability), and validity have been demonstrated. A global PSQI score greater than 5 yielded a diagnostic sensitivity of 89.6% and specificity of 86.5% (kappa $= 0.75$, p less than 0.001) in distinguishing good and poor sleepers.

Biological Measures

Salivary Cortisol. Saliva sampling has been selected in order to prevent the stress-inducing effects of blood sampling on cortisol levels (Kirschbaum and Hellhammer, 1994). Determination of cortisol in saliva provides a reliable measure of the free unbound fraction of cortisol, and by measuring at four time periods it is possible to account for the large variation of cortisol levels throughout the day which results from the circadian rhythm of cortisol secretion (Kirschbaum *et al*., 1994). Cortisol levels present in saliva were assayed using solid-phase ELISAs according to the manufacturer's instructions (ALPCO Diagnostics, Widham, NH). Saliva was collected at waking, 1200, 1700 and 2200 h using Sali-Savers (ALPCO Diagnostics, Widham, NH) and stored at 4°C overnight. Saliva was then extracted from the Sali-Savers by centrifugation at $300 \times g$ for 10 min. The saliva extracted was immediately frozen and subsequently stored at -20°C. All assays were run in one batch on the thawed samples at the conclusion of data collection.

Urinary Catecholamines. Urine samples were collected in one container over a period of 24-hours, commencing at the second void after awakening, finishing with the first void the following morning—times of beginning and completing collection were recorded on the container. Urine samples were kept cold by storage in a portable cooler throughout the day of the 24-hour sample period, in the refrigerator overnight, and returned the following day. Samples were assayed for norpinepherine, epinepherine, dopamine and creatinine. Urinary levels of the catecholamines were determined by high-pressure liquid chromatography (HPLC) with electrochemical detection. Urine creatinine was determined using the Jaffe method as modified by Slot, with kits supplied by Sigma Chemical Company (St. Louis, MO). Catecholamine levels were expressed as urine concentration (μg/ml) per urine concentration of creatinine (mg/ml), yielding values of μg per mg creatinine for each sample; this provides catecholamine excretion indices that are corrected for individual differences in body size and urine volume (White *et al*., 1995). To guard against the possibility of occasional poor compliance with 24-hour urine collection, 24-hour creatinine excretion was compared against normative ranges, using published algorithms based on gender, ethnicity and body size (James *et al*., 1988).

Urinary Melatonin. In order to adequately capture the peak in melatonin secretion and the bulk

of the circadian curve which is elevated overnight (Geoffriau *et al.*, 1998), melatonin was measured from urine samples collected from the time of the last void of the evening before bed, up to and including the first void of the following morning. Participants were instructed to avoid exposure to light sources during the time of urine collection, including in the morning until after the first void. This included refraining from turning on artificial lighting if they awakened in the night to urinate. Samples were kept cold by storage in a portable cooler overnight during the collection period, in the refrigerator the following day while urine for catecholamines was being collected in a different container, and then returned the following day.

Melatonin was extracted from the urine using the Extraction Reagent Set (B-EKDSM-ERS) for pre-treatment before subsequent analysis in the Buhlmann direct Saliva Melatonin ELISA (EK-DSM) according to the manufacturer's instructions (ALPCO Diagnostics, Windham, NH).

Procedures

Participants visited the lab on a weekday morning at 8:00 AM. After providing fully informed consent, they completed questionnaires and were instructed how to collect a 12-hour overnight urinary sample for measurement of melatonin that evening, and a separate 24-hour urine sample for measurement of catecholamines beginning the following morning. The time of starting and finishing each collection period was recorded. Participants were also given four cortisol salivettes for collecting saliva samples the following day. Times that samples were collected were recorded on each salivette as it was collected. Approximately 48 hours later, patients returned to the lab to return the saliva and urine samples, and complete the post-questionnaires. All testing occurred during weekdays.

Data Analysis

Descriptive statistics were used to describe central tendency (mean, median), and variation (SD, range) of the biological measures in each group. Any values that were significantly skewed were transformed appropriately. Values were compared to one another between groups using independent-samples t-tests. Associations between variables were investigated using Pearson product-moment correlation coefficients. A significance value of $p < .05$ was used without correction for multiple comparisons, as this data is exploratory in nature. Associations between the biological outcome measures and the psychological measures were also calculated using Pearson product-moment correlations. The same cut-off values were used to maximize the probabilities of detecting relationships of interest for hypothesis generation.

Since the cortisol data was not normally distributed, log transformations were performed and subsequent analysis completed on transformed data. Mean daily cortisol level was calculated by summing the four daily measures and calculating a mean for each participant. Because previous work had shown the rate of change of cortisol levels throughout the day to be a potentially important measure (Turner-Cobb *et al.*, 2000; Sephton *et al.*, 2000), the slope of diurnal change in salivary cortisol levels was calculated as an estimate of diurnal variability for each patient. The cortisol slope was calculated by regressing the cortisol values as the dependent variable on the time of day that each sample was collected for each individual.

The mean cortisol slopes were then compared between groups using the Chow test, which determines whether the slopes and intercepts of two regression equations are equal (Davidson and MacKinnon, 1993). The error sums of squares of each of the individual slopes in each group were summed and divided into the pooled error sums of squares resulting from performing the regression on the pooled data from both groups (all of which were adjusted for degrees of freedom). The resultant ratio (Q-statistic) was compared to the F value for the overall regression, and if it was smaller than this value the null hypothesis of equality of the slopes was accepted.

Another common method for analyzing cortisol data is to calculate the Area under the Curve (AUC). Two different AUC measurements have been defined by Pruessner *et al* (Pruessner *et al.*, 2003): 1) AUC ground (AUCg) which captures the total hormonal output from a baseline of zero, and hence the basal activity of the HPA axis, and; 2) AUC increase (AUC_i), which calculates AUC from the lowest measurement of the day, or nadir, and is meant to capture the reactivity of the HPA axis. In this case the final value before bedtime was used as the baseline for the AUC_i calculation. Differences in the time intervals between collections were taken into account in the calculations using the actual recorded

times of sample collection. AUC was calculated on the transformed cortisol values using both these methods, and the mean AUC values compared between groups using independent samples t-tests.

RESULTS

Participants

A sample of 33 women in the breast cancer group and 33 in the control group provided full data. Demographic characteristics of both groups are presented in Table I. The majority of women with breast cancer were diagnosed with stage 2 cancer (39.4%) and were an average of 1.36 (SD = .60) years post-diagnosis. Women with breast cancer did not differ from the controls in terms of age, ethnicity, education, marital status, or body mass index (BMI).

Objectives 1 & 2: Between Group Comparisons

Psychological

The scores on the psychological questionnaires for both groups are presented in Table II. Overall, there was greater variability in the amount of psychological distress reported by the women with breast cancer, with some women reporting very poor adjustment and other women adjusting relatively well. This inequality was considered in all analyses performed. Levene's test for equality of variances was significant for the CES-D total score ($F = 11.35$, $p = .001$) and the Trait Anxiety scale of the STAI ($F = 8.59$, $p = .005$), indicating that equal variances could not be assumed. After controlling for unequal variances, the women with breast cancer reported significantly more depression ($t = 3.00$, $p = .005$) and anxiety ($t = 2.22$, $p = .03$) relative to the controls. Levene's test for equality of variances was significant for the SOSI subscales of peripheral manifestations ($F = 5.44$, $p = .023$), cardiopulmonary symptoms ($F = 6.65$, $p = .012$), depression ($F = 4.50$, $p = .038$), cognitive disorganization ($F = 6.90$, $p = .011$), and the total symptoms of stress score ($F = 6.57$, $p = .013$), indicating that equal variances could not be assumed. After correcting for unequal variances there were significant differences between the groups, with the women with breast cancer reporting more peripheral manifestations ($t = 2.82$, $p = .007$), cardiopulmonary symptoms ($t = 2.17$, $p = .034$), depression ($t = 2.22$, $p = .033$), anxiety/fear ($t = 3.24$, $p = .002$), cognitive disorganization ($t = 3.06$, $p = .004$) and total symptoms of stress ($t = 2.95$, $p = .005$). No significant differences were found on measures of central neurological symptoms, gastrointestinal symptoms, muscle tension, habitual patterns, and emotional irritability.

The women with breast cancer also endorsed more negative symptomotology relative to the controls as measured by the POMS. Levene's test for equality of variances was significant for the POMS subscales of anxiety ($F = 6.57$, $p = .013$), depression ($F = 12.69$, $p = .001$), fatigue ($F = 4.57$, $p = .036$), confusion ($F = 9.01$, $p = .004$), and total mood disturbance, ($F = 9.33$, $p = .003$), indicating that equal variances could not be assumed. After controlling for unequal variances, the women with breast cancer had significantly more anxiety ($t = 2.10$, $p = .041$), depression ($t = 2.48$, $p = .017$), fatigue ($t = 2.21$, $p = .031$), confusion ($t = 2.66$, $p = .01$), and total mood disturbance ($t = 2.49$, $p = .017$). No significant differences were found on measures of anger and vigor.

Table I. Demographics Between Groups

	Breast cancer N = 33		Control N = 33	
Variable	Mean	*SD*	Mean	*SD*
Age	51.25	10.19	53.41	5.98
Body mass index	26.65	5.60	26.68	4.87
Ethnicity	N	Percent	N	Percent
Caucasian	29	88	32	97
Other	4	12	1	3
Education				
College +	20	61	22	67
High school	10	30	11	33
< High school	3	9	0	0
Marital status				
Married	20	61	27	82

Table II. Psychological Test Scores Between Groups

	Breast cancer		Control	
Variable	Mean	*SD*	Mean	*SD*
CES-D	11.18	10.43	5.30**	4.25
STAI-T	35.45	12.60	29.70*	7.91
SOSI				
Peripheral Manifestation	7.39	5.87	4.06**	3.41
Cardiopulmonary	8.45	7.11	5.18**	4.94
Central neurological	2.27	2.25	1.97	1.90
Gastrointestinal	5.21	5.01	3.76	3.97
Muscle tension	9.85	7.17	7.94	5.44
Habitual patterns	15.42	7.86	13.42	8.41
Depression	9.64	15.60	3.52*	2.79
Anxiety/Fear	11.09	8.46	5.45	5.30
Emotional irritability	4.36	3.86	2.70	3.40
Cognitive disorganization	4.79	4.47	2.15**	2.11
Total symptoms of stress	78.48	47.40	50.15**	28.28
POMS				
Anxiety	5.45	7.94	2.09*	4.69
Depression	8.88	11.35	3.67*	4.11
Anger	7.42	7.80	5.21	5.31
Vigor	17.24	6.59	18.94	4.83
Fatigue	8.58	7.25	5.18*	5.01
Confusion	2.55	4.77	0.03**	2.59
Total mood disturbance	15.64	38.50	−2.76*	18.06

Note. Group differences, $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$.

Sleep

Scores on the PSQI are presented in Table III. There were significant differences between the breast cancer and control groups on measures of sleep disturbances ($t = 3.07$, $p = .003$) and global sleep quality ($t = .037$, $p = .037$). Measures of subjective sleep quality ($t = 1.67$, $p = .10$), sleep latency ($t = 1.83$, $p = .072$) and daytime dysfunction ($t = 1.59$, $p = .12$) were not significantly different, although trends were evident. There were no differences between the groups on measures of sleep duration, sleep efficiency, and use of sleeping medications.

Biological

The raw cortisol data was significantly skewed (Skewness > 2.0) at each time point, so natural log (Ln) transformations were made on all data points. After the transformation cortisol data was not significantly skewed (< 2.0). All statistical analyses were subsequently performed on the transformed data. Cortisol samples were collected at median times of 7:25, 12:10, 17:09 and 22:00 hrs for the breast cancer group and 6:32, 12:02, 17:03 and 22:15 hrs for the control group. Time of melatonin collection initiation was a median of 21:45 hrs for the breast cancer

Table III. Sleep Scores Between Groups

	Breast cancer		Control	
Variable	Mean	*SD*	Mean	*SD*
PSQI				
Subjective sleep quality	1.30	0.77	1.00	0.71
Sleep latency	1.73	0.98	1.27	1.04
Sleep duration	1.00	0.95	0.88	0.96
Sleep efficiency	1.27	1.23	0.97	1.26
Sleep disturbances	1.79	0.55	1.39**	0.50
Use of sleeping medications	0.52	1.03	0.36	0.82
Daytime dysfunction	0.94	0.79	0.67	0.60
Global sleep quality	8.45	3.73	6.55*	3.55

Note. Group differences, $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$.

Table IV. Biological Measures Between Groups

	Breast cancer		Control	
Variable	Mean	*SD*	Mean	*SD*
CRT Wake (nmol/L)	14.26	6.84	13.92	8.99
CRT 12:00 (nmol/L)	5.85	2.55	4.95	2.65
CRT 17:00 (nmol/L)	3.09	1.23	4.40	3.56
CRT Bed (nmol/L)	1.86	0.89	2.71	3.22
CRT Daily mean (nmol/L)	25.18	7.85	25.82	11.89
CRT Slope	−0.89	0.13	−0.77	0.38
CRT AUC ground	83.70	24.49	92.21	38.95
CRT AUC increase	56.43	30.40	51.10	42.33
Norepinephrine (NE) (nmol/mmol, corrected)	26.24	8.04	27.23	11.24
Epinephrine (E) (nmol/mmol, corrected)	2.97	1.29	2.63	1.40
Dopamine (D) (nmol/mmol, corrected)	122.27	46.61	148.70*	44.42
Melatonin (pg/mL)	52.08	50.85	50.83	46.89

Note. Group differences, $^{*}p < .05$.

and 22:00 hrs for the control group, and the last collection was taken at 7:27 and 6:30 hrs. Similarly, for catecholamine collection, the median time collection began was 7:20 and 6:30 AM, for breast cancer and control groups, respectively. The control group were earlier risers than the breast cancer patients, resulting in earlier collection of the last melatonin sample, the start of the catecholamine collection and the first salivary cortisol.

Comparisons of the biological outcomes between groups are presented in Table IV (untransformed values are presented for ease of interpretation) and cortisol values across the day in Fig. 2. The women with breast cancer were not significantly different from the control women on measures of cortisol production at any time of the day, on mean daily cortisol level, on slopes using the Chow test, or either AUC_g or AUC_i. The groups also did not differ significantly in terms of overnight urinary melatonin levels, or 24 hour urinary norepinephrine and epinephrine levels. There only group differences was on 24 hour urinary dopamine levels ($t\,(61) = -2.30$, $p = .025$), where the control women had higher dopamine values than the participants with breast cancer.

Objectives 3 & 4: Relationships Among Outcomes

There were no significant correlations between psychological and sleep measures (total scores of the SOSI, POMS, STAI, CES-D and PSQI) and the biological outcomes (cortisol, melatonin, catecholamines) or health behaviors (BMI, caffeine and alcohol consumption) within either group or when both groups were combined. However, across groups BMI was correlated to levels of both epinephrine and dopamine, but in opposite directions: higher BMI was associated with lower epinephrine ($r = -416$, $p < .001$) and higher dopamine ($r = 353$, $p < .003$). Greater BMI was also associated with higher caffeine consumption ($r = .243$, $p < .05$).

Due to the heightened interest in the associations between cortisol slopes and psychological functioning, women within each group were classified on the basis of their transformed cortisol slopes into "steep" or "flat" slope categories using a median split, and compared on the basis of their scores on the psychological outcomes within each group. Women with flatter slopes were not statistically significantly different than those with steeper slopes on any of the other measures within each group of breast cancer patients or controls. When the groups were analyzed together on the basis of steep vs. flat slopes, there were still no differences on psychological scores. Hence, having a flatter cortisol slope was not associated with more psychological or sleep disturbance in either of the groups.

DISCUSSION

This study evaluated hypotheses that women with a history of breast cancer would have dysregulations in endocrine, autonomic and sleep markers and psychological measures of stress and mood disturbance compared to healthy control women, that would be related to one-another. Indeed, the women who had completed treatment for primary breast cancer an average of 16-months previously

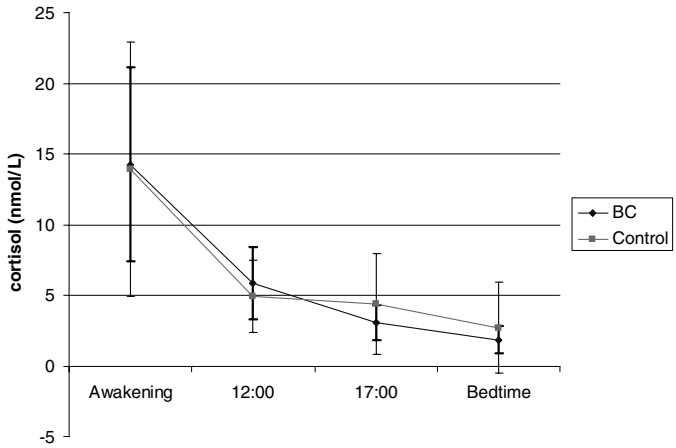

Fig. 2. Mean salivary cortisol slopes (± SD) between groups.

had significantly higher levels of depressive symptoms, anxiety, fatigue, confusion, cardiopulmonary symptoms of stress and sleep disturbance than the comparison women; however, despite these disturbances there were no group differences on any of the biomarkers, including salivary cortisol, urinary catecholamines and melatonin, with the exception of higher dopamine levels in the control participants.

These results are contrary to those of other researchers who have found dysregulations in cortisol secretion patterns in women with breast cancer compared to controls — for example in women with metastatic breast and ovarian cancer (van der Pompe *et al*., 1996; Abercrombie *et al*., 2004; Touitou *et al*., 1996). However, the women in the previous studies had advanced metastatic breast cancer, whereas our group was diagnosed stages I-III, primarily stage II, with no metastatic spread. This may account for the failure to find differences in any of the endocrine measures, as earlier work has shown that progression of breast cancer is associated with physiological adaptations that result in increased cortisol production, that may not be seen in earlier developmental stages of the disease (Drafta *et al*., 1981; Hays and O'Brian, 1989). Our results are consistent with a recent report of a comparison between breast cancer patients and matched controls (Vedhara *et al*., 2006). In that study, 85 newly diagnosed breast cancer patients were compared to 59 healthy control women, but no differences were found on any of the cortisol measures, including AUC_i or AUC_g, daily slope or mean cortisol levels. Although stage of disease was not reported in that study, participants were newly diagnosed with no previous cancer history and expected survival greater than 15 months. That, and the lack of significant psychological disturbance, may account for the absence of associations between diagnosis and cortisol indices. In the case of Bower *et al* who reported flatter cortisol slopes in breast cancer patients (Bower *et al*., 2005), the women were also experiencing significant fatigue, which our sample was not. Indeed, an earlier study by that group found fatigued survivors had lower levels of morning serum cortisol than non-fatigued survivors (Bower *et al*., 2002). This research collectively suggests that disturbances in HPA axis functioning may be related to the process of fatigue and disease progression in breast cancer patients, rather than breast cancer survivorship in general.

In terms of the psychological outcomes, these results are similar to other reports that show elevated symptomatology in cancer survivors compared to controls; often well after treatment completion (Hewitt *et al*., 2003; Pollack *et al*., 2005). The question arises in this sample as to whether the differences were due to above normative distress scores in the women with breast cancer coupled with normal scores in the controls, or if the breast cancer patients were functioning normally but the controls had very low levels of psychological disturbance. Comparing to normative samples on the CES-D, both groups fell into the normative range (scores below 16) although the women with breast cancer scored just above 11, sometimes considered to reflect mild mood disturbance. Similarly, for anxiety on the STAI, both groups were in the normal range. Compared to a mixed group of patients during treatment from a similar subject pool, the women

with breast cancer in this study had minimal amounts of mood disturbance, but comparably high levels of symptoms of stress (Speca *et al*., 2000). In terms of sleep disturbance, both groups of women had problems, with the controls scoring over the cut-off for bad sleep in normal samples of five (Buysse *et al*., 1989), and the breast cancer patients with scores over the cut-off suggested for cancer patients, of eight (Carpenter and Andrykowski, 1998). This is consistent with reports of elevated sleep disturbance in patients, often long after cancer treatment (e.g., Carlson *et al*., 2005). Even though the women reported poor sleep, no associations between sleep and melatonin or catecholamines were found.

The failure to find any correlations between biological measures and psychological scores is again consistent with Vedhara (Vedhara *et al*., 2006) and other studies of breast cancer patients (Porter *et al*., 2003) as well as other participant groups (Marshall, Jr. *et al*., 1998; Edwards *et al*., 2003). One study found a relationship between indices of change in cortisol levels and stress scores, but not on absolute levels at each time point or for AUC (Vedhara *et al*., 2003), as was measured in this study. Despite the well-known association between stress and cortisol reactivity (Kirschbaum *et al*., 1994), the evidence for basal cortisol or dysregulated diurnal cortisol secretion patterns being associated with self-reported stress or mood disturbance in breast cancer patients remains equivocal.

Some of the limitations of this study include lack of objective measures of sleep such as polysomnography, and possible reactivity to the data collection procedures. It is possible that collection of urine and saliva samples may have provoked reactivity that may have obscured differences between groups. Such a one-time assessment may be either universally stressful for both groups, or distract the cancer patients from their rumination or stress about their situation and decrease reactivity—in both cases this can obscure a physiological effect. In this regard a one-time assessment is not ideal: longer data collection periods are probably necessary to adequately capture differences in biological rhythms if they exist. A period of time for habituation to the procedures may help to minimize any effects of reactivity. Minimally, a sleep log for a week in duration would be optimal in order to better characterize baseline sleep, and assessments done at several time periods would increase their reliability. In addition, we collected only one day of saliva samples for cortisol assessment- ideally one would collect at least three days of samples and determine the average values (Kirschbaum *et al*., 1994).

In addition, no statistical corrections for multiple comparisons were conducted, which increases the odds of discovering spurious associations by chance (Type I error). However, our findings tended to be more in the direction of finding no associations, so the influence of this type of error on the outcomes is likely minimal, and the study may indeed be underpowered (Type II error). This study may be considered valuable as a pilot or feasibility study which demonstrates that participants are willing and able to follow a demanding procedure of 36 hours of urine collection as well as salivary swabs four times daily. The next step may be to collect data for longer periods of time in samples of women with metastatic disease, in which case it may be possible to capture variations in stress over multiple assessments.

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REFERENCES

Abercrombie, H. C., Giese-Davis, J., Sephton, S., Epel, E. S., Turner-Cobb, J. M., and Spiegel, D. (2004). Flattened cortisol rhythms in metastatic breast cancer patients. *psychoneuroendocrinology* 29: 1082–1092.

Andersen, B. L., Kiecolt-Glaser, J. K., and Glaser, R. (1994). A biobehavioral model of cancer stress and disease course. *Am. Psychol.* 49: 389–404.

Antoni, M. H. (2003). Psychoneuroendocrinology and psychoneuroimmunology of cancer: Plausible mechanisms worth pursuing? *Brain Behav. Immun.* 17(1): S84–S91.

Bettermann, H., Kroz, M., Girke, M., and Heckmann, C. (2001). Heart rate dynamics and cardiorespiratory coordination in diabetic and breast cancer patients. *Clin. Physiol.* 21: 411–420.

Bovbjerg, D. H. (2003). Circadian disruption and cancer: sleep and immune regulation. *Brain Behav. Immun.* 17(1): S48–S50.

Bower, J. E., Ganz, P. A., Aziz, N., and Fahey, J. L. (2002). Fatigue and proinflammatory cytokine activity in breast cancer survivors. *Psychosom. Med* 64: 604–611.

Bower, J. E., Ganz, P. A., Dickerson, S. S., Petersen, L., Aziz, N., and Fahey, J. L. (2005). Diurnal cortisol rhythm and fatigue

in breast cancer survivors. *Psychoneuroendocrinology* 30: 92–100.
Bubenik, G. A., Blask, D. E., Brown, G. M., Maestroni, G. J., Pang, S. F., Reiter, R. J., *et al*. (1998). Prospects of the clinical utilization of melatonin. *Biol. Signals Recep.* 7: 195–219.
Buysse, D. J., Reynolds, C. F., Monk, T. H., and Berman, S. R. (1989). The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. *Psychiatr. Res.* 28: 193–213.
Carlson, L. E., and Garland, S. N. (2005). Impact of mindfulness-based stress reduction (MBSR) on sleep, mood, stress and fatigue symptoms in cancer outpatients. *Int. J. Behav. Med.* 12: 278–285.
Carlson, L. E., Speca, M., Patel, K. D., and Goodey, E. (2003). Mindfulness-based stress reduction in relations to quality of life, mood, symptoms and stress and immune parameters in breast and prostate cancer outpatients. *Psychosom. Med.* 65: 571–581.
Carlson, L. E., Speca, M., Patel, K. D., and Goodey, E. (2004). Mindfulness-based stress reduction in relation to quality of life, mood, symptoms of stress and levels of cortisol, dehydroepiandrosterone-Sulftate (DHEAS) and melatonin in breast and prostate cancer outpatients. *Psychoneuroendocrinology* 29: 448–474.
Carpenter, J. S., and Andrykowski, M. A. (1998). Psychometric evaluation of the Pittsburgh Sleep Quality Index. *J. Psychosom. Res* 45: 5–13.
Cassileth, B. R., Lusk, E. J., Strouse, T. B., Miller, D. S., Brown, L. L., and Cross, P. A. (1985). A psychological analysis of cancer patients and their next-of-kin. *Cancer* 55: 72–76.
Chambers, M. J., and Kim, J. Y. (1993). The role of state-trait anxiety in insomnia and daytime restedness. *Behav. Med.* 19: 42–46.
Claghorn, J. L., Mathew, R. J., Weinman, M. L., and Hruska, N. (1981). Daytime sleepiness in depression. *J. Clin. Psychiatr.* 42: 342–343.
Cohen, S., and Williamson, G. M. (1991). Stress and infectious diseases in humans. *Psychol. Bull.* 109: 5–24.
Cowan, M. J., Graham, K. Y., and Cochrane, B. L. (1992). Comparison of a theory of quality of life between myocardial infarction and malignant melanoma: a pilot study. *Progr. Cardiovasc. Nurs.* 7: 18–28.
Davidson, R., and MacKinnon, J. G. (1993). *Estimation and Inference in Econometrics.* Oxford University Press Oxford, England.
Davis, S., Mirick, D. K., and Stevens, R. G. (2001). Night shift work, light at night, and risk of breast cancer. *J. Nati. Cancer Inst.* 93: 1557–1562.
Drafta, D. S., Stroe, E., Schindler, E. E., Teodosiu, T., Gozariu, L., and Drafta, D. (1981). Adrenal function in early and metastatic breast cancer: dexamethasone suppression of plasma cortisol. *Endocrinologie* 19: 115–121.
Edwards, S., Hucklebridge, F., Clow, A., and Evans, P. (2003). Components of the diurnal cortisol cycle in relation to upper respiratory symptoms and perceived stress. *Psychosom. Med.* 65: 320–327.
Geoffriau, M., Brun, J., Chazot, G., and Claustrat, B. (1998). The physiology and pharmacology of melatonin in humans. *Horm. Res.* 49: 136–141.
Gold, S. M., Zakowski, S. G., Valdimarsdottir, H. B., and Bovbjerg, D. H. (2003). Stronger endocrine responses after brief psychological stress in women at familial risk of breast cancer. *Psychoneuroendocrinology* 28: 584–593.
Hall, M., Buysse, D. J., Nowell, P. D., Nofzinger, E. A., Houck, P., Reynolds, C. F., III, *et al*. (2000). Symptoms of stress and depression as correlates of sleep in primary insomnia. *Psychosom. Med.* 62: 227–230.
Hays, J. H., and O'Brian, J. T. (1989). Endocrine and metabolic function in patients with neoplastic disease. In Herberman, R. B. (Ed.), *Influence of the Host on Tumor Development* (pp. 530–543). Kluwer Academic Publishers, Dordrecht.
Hewitt, M., Rowland, J. H., and Yancik, R. (2003). Cancer survivors in the United States: age, health, and disability. *J. Gerontol. A Biol. Sci. Med. Sci.* 58: 82–91.
Hossain, J. L., and Shapiro, C. M. (2002). The prevalence, cost implications, and management of sleep disorders: an overview. *Sleep Breath.* 6: 85–102.
James, G. D., Berge-Landry, H. H., Valdimarsdottir, H. B., Montgomery, G. H., and Bovbjerg, D. H. (2004). Urinary catecholamine levels in daily life are elevated in women at familial risk of breast cancer. *Psychoneuroendocrinology* 29: 831–838.
James, G. D., Sealey, J. E., Alderman, M., Ljungman, S., Mueller, F. B., Pecker, M. S., *et al*. (1988). A longitudinal study of urinary creatinine and creatinine clearance in normal subjects. Race, sex, and age differences. *Am. J. Hypertens* 1: 124–131.
Kajimura, N., Kato, M., Sekimoto, M., Watanabe, T., Takahashi, K., Okuma, T., *et al*. (1998). A polysomnographic study of sleep patterns in normal humans with low- or high-anxiety personality traits. *Psychiatr. Clin. Neurosci.* 52: 317–320.
Kirschbaum, C., and Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology* 19: 313–333.
Koopman, C., Nouriani, B., Erickson, V., Anupindi, R., Butler, L. D., Bachmann, M. H., *et al*. (2002). Sleep disturbances in women with metastatic breast cancer. *Breast J.* 8: 362–370.
Kripke, D. F., Garfinkel, L., Wingard, D. L., Klauber, M. R., and Marler, M. R. (2002). Mortality associated with sleep duration and insomnia. *Archives Gen. Psychiatr.* 59: 131–136.
Kushida, C. A., Nichols, D. A., Simon, R. D., Young, T., Grauke, J. H., Britzmann, J. B., *et al*. (2000). Symptom-Based Prevalence of Sleep Disorders in an Adult Primary Care Population. *Sleep Breath.* 4: 9–14.
Leckie, M. S., and Thompson, E. (1979). *Symptoms of Stress Inventory.* University of Washington. Ref Type: Serial (Book,Monograph), Seattle, WA.
Linsell, C. R., Lightman, S. L., Mullen, P. E., Brown, M. J., and Causon, R. C. (1985). Circadian rhythms of epinephrine and norepinephrine in man. *J. Clin. Endocrinol. Metab* 60: 1210–1215.
Lissoni, P., Barni, S., Mandala, M., Ardizzoia, A., Paolorossi, F., Vaghi, M., *et al*. (1999a). Decreased toxicity and increased efficacy of cancer chemotherapy using the pineal hormone melatonin in metastatic solid tumour patients with poor clinical status. *Eur. J. Cancer* 35: 1688–1692.
Lissoni, P., Cazzaniga, M., Tancini, G., Scardino, E., Musci, R., Barni, S., *et al*. (1997). Reversal of clinical resistance to LHRH analogue in metastatic prostate cancer by the pineal hormone melatonin: Efficacy of LHRH analogue plus melatonin in patients progressing on LHRH analogue alone. *Eur. Urology* 31: 178–181.
Lissoni, P., Tancini, G., Paolorossi, F., Mandala, M., Ardizzoia, A., Malugani, F., *et al*. (1999b). Chemoneuroendocrine therapy of metastatic breast cancer with persistent thrombocytopenia with weekly low-dose epirubicin plus melatonin: a phase II study. *J Pineal Res.* 26: 169–173.
Lutgendorf, S. K., and Costanzo, E. S. (2003). Psychoneuroimmunology and health psychology: an integrative model. *Brain Behav. Immun.* 17: 225–232.
Marshall, G. D., Jr., Agarwal, S. K., Lloyd, C., Cohen, L., Henninger, E. M., and Morris, G. J. (1998). Cytokine dysregulation associated with exam stress in healthy medical students. *Brain Behav. Immun.* 12: 297–307.
McNair, D. A., Lorr, M., and Droppelman, L. F. (1971). *Profile of Mood States . Educational and Industrial Testing Service*, San Diego.

Mormont, M. C., and Levi, F. (1997). Circadian-system alterations during cancer processes: a review. *Int J Cancer*, 70 : 241–247.
Pollack, L. A., Greer, G. E., Rowland, J. H., Miller, A., Doneski, D., Coughlin, S. S., *et al.* (2005). Cancer survivorship: a new challenge in comprehensive cancer control. *Cancer Causes Control* 16(1): 51–59.
Porter, L. S., Mishel, M., Neelon, V., Belyea, M., Pisano, E., and Soo, M. S. (2003). Cortisol levels and responses to mammography screening in breast cancer survivors: a pilot study. *Psychosom. Med.* 65: 842–848.
Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., and Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* 28: 916–931.
Radloff, L. S. (1977). The CES-D Scale: A self-report depression scale for research in the general population. *Appl. Psychol. Measurement* 1: 385–401.
Saez, M. C., Barriga, C., Garcia, J. J., Rodriguez, A. B., Masot, J., Duran, E., *et al.* (2005). Melatonin increases the survival time of animals with untreated mammary tumours: neuroendocrine stabilization. *Mol. Cell Biochem.* 278: 15–20.
Savard, J., and Morin, C. M. (2001). Insomnia in the context of cancer: a review of a neglected problem. *J. Clin. Oncol.* 19: 895–908.
Savard, J., Simard, S., Blanchet, J., Ivers, H., and Morin, C. M. (2001). Prevalence, clinical characteristics, and risk factors for insomnia in the context of breast cancer. *Sleep* 24: 583–590.
Schernhammer, E. S., Laden, F., Speizer, F. E., Willett, W. C., Hunter, D. J., Kawachi, I., *et al.* (2001). Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. *J. Nati. Cancer Institute* 93: 1563–1568.
Sephton, S., and Spiegel, D. (2003). Circadian disruption in cancer: a neuroendocrine-immune pathway from stress to disease? *Brain Behav. Immun.* 17: 321–328.
Sephton, S. E., Sapolsky, R. M., Kraemer, H. C., and Spiegel, D. (2000). Diurnal cortisol rhythm as a predictor of breast cancer survival. *J. Nati. Cancer Inst.* 92: 944–1000.
Shapiro, S. L., Bootzin, R. R., Figueredo, A. J., Lopez, A. M., and Schwartz, G. E. (2003). The efficacy of mindfulness-based stress reduction in the treatment of sleep disturbance in women with breast cancer: an exploratory study. *J. Psychosom. Res.* 54: 85–91.
Shapiro, S. L. (2001). Quality of life and breast cancer: Relationship to psychosocial variables. *J. Clin. Psychol.* 57: 501–519.
Sikes, C. R. A., and Lasley, B. J. (1989). Cognitive sequelae of hypothalamic-pituitary-adrenal (HPA) dysregulation in depression. *Biol. Psychiatr.* 25: 148A–149A.
Speca, M., Carlson, L. E., Goodey, E., and Angen, M. (2000). A randomized, wait-list controlled clinical trial: the effect of a mindfulness meditation-based stress reduction program on mood and symptoms of stress in cancer outpatients. *Psychosom. Med.* 62: 613–622.
Speilberger, C. D. (1983). *Manual for the State-Trait Anxiety Inventory: STAI (Form Y)*. Consulting Psychologists Press, Palo Alto, CA.
Spiegel, D., and Sephton, S. E. (2001). Psychoneuroimmune and endocrine pathways in cancer: effects of stress and support. *Semin. Clini. Neuropsychiatr.* 6: 252–265.
Spiegel, D., Sephton, S. E., Terr, A. I., and Sittes, D. P. (1998). Effects of psychosocial treatment in prolonging cancer survival may be mediated by neuroimmune pathways. *Ann. New York Acad. Sci.* 840: 674–683.
Tamarkin, L., Danforth, D., Lichter, A., DeMoss, E., Cohen, M., Chabner, B., *et al.* (1982). Decreased nocturnal plasma melatonin peak in patients with estrogen receptor positive breast cancer. *Science* 216: 1003–1005.
Ticher, A., Haus, E., Ron, I. G., Sackett-Lundeen, L., and Ashkenazi, I. E. (1996). The pattern of hormonal circadian time structure (acrophase) as an assessor of breast-cancer risk. *Int. J. Cancer* 65: 591–593.
Touitou, Y., Bogdan, A., Levi, F., Benavides, M., and Auzeby, A. (1996). Disruption of the circadian patterns of serum cortisol in breast and ovarian cancer patients: relationships with tumour marker antigens. *Br. J. Cancer* 74: 1248–1252.
Turner-Cobb, J. M., Sephton, S. E., Koopman, C., Blake-Mortimer, J., and Spiegel, D. (2000). Social support and salivary cortisol in women with metastatic breast cancer. *Psychosom. Med.* 62: 337–345.
van Der Pompe, G., Antoni, M. H., and Heijnen, C. J. (1996). Elevated basal cortisol levels and attenuated ACTH and cortisol responses to a behavioral challenge in women with metastatic breast cancer. *Psychoneuroendocrinology* 21: 361–374.
Vedhara, K., Miles, J., Bennett, P., Plummer, S., Tallon, D., Brooks, E., *et al.* (2003). An investigation into the relationship between salivary cortisol, stress, anxiety and depression. *Biol. Psychol.* 62: 89–96.
Vedhara, K., Stra, J. T., Miles, J. N., Sanderman, R., and Ranchor, A. V. (2006). Psychosocial factors associated with indices of cortisol production in women with breast cancer and controls. *Psychoneuroendocrinology*, 31: 299–311.
Vijayalaxmi, Meltz, M. L., Reiter, R. J., and Herman, T. S. (1999). Melatonin and protection from genetic damage in blood and bone marrow: whole-body irradiation studies in mice. *J. Pineal Res.* 27: 221–225.
Vijayalaxmi, Reiter, R. J., Meltz, M. L., and Herman, T. S. (1998). Melatonin: possible mechanisms involved in its 'radioprotective' effect. *Mutation Res.* 404: 187–189.
Vijayalaxmi, Thomas, C. R., Jr., Reiter, R. J., and Herman, T. S. (2002). Melatonin: from basic research to cancer treatment clinics. *J Clin. Oncology* 20: 2575–2601.
White, I. R., Brunner, E. J., and Barron, J. L. (1995). A comparison of overnight and 24 hour collection to measure urinary catecholamines. *J. Clin. Epidemiology* 48: 263–267.
Wolkowitz, O. M. (1994). Prospective controlled studies of the behavioral and biological effects of exogenous corticosteroids. *Psychoneuroendocrinology* 3: 233–255.